PHYSICO-CHEMICAL AND BIOLOGICAL TREATMENT OF EFFLUENT FROM NATURAL RUBBER LATEX CENTRIFUGING UNITS

Thesis submitted to
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by

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February 2008

Declaration

I hereby declare that the thesis entitled: "**PHYSICO-CHEMICAL AND BIOLOGICAL TREATMENT OF EFFLUENT FROM NATURAL RUBBER LATEX CENTRIFUGING UNITS**" is an authentic record of the research work carried out by me under the supervision and guidance of Dr. N. Radhakrishnan Nair, Scientist, Rubber Research Institute of India, Kottayam and Dr. G. Madhu, Professor and Head, Division of Safety and Fire Engineering, School of Engineering, Cochin University of Science and Technology, Cochin. The work presented in this thesis has not been submitted for any other degree or diploma earlier.

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CERTIFICATE

This is to certify that the thesis entitled: **"PHYSICO-CHEMICAL AND BIOLOGICAL TREATMENT OF EFFLUENT FROM NATURAL RUBBER LATEX CENTRIFUGING UNITS"** is an authentic record of the research work carried out by Mrs.Vimalamma T. Abraham, under my supervision and guidance for the fulfilment of the requirements of the degree of Doctor of Philosophy under the Faculty of Science of the Mahatma Gandhi University. The work presented in this thesis has not been submitted for any other degree or diploma earlier. It is also certified that Mrs.Vimalamma T. Abraham has fulfilled the course requirements for the Ph.D. Degree of this University.

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ABSTRACT

The growth of natural rubber latex processing industries has resulted in an increase in the quantum of effluent being discharged into the environment. It is essential to develop cost-effective and efficient treatment methods for the removal of pollutants from these effluents. Among the different rubber processing industries, latex centrifuging units produce serum effluent which is highly polluting. The thrust of this work is to minimise the pollutant load of the serum effluent using environmentally friendly methods. To have an understanding of the pollutant load, characteristics of the serum were studied and it was found that the effluent from latex centrifuging units is acidic and contains high amount of organic as well as inorganic pollutants. Lime and sodium hydroxide were added to raise the pH. Efficiency of few metal coagulants were assessed to remove colloidal and suspended particles. The effect of natural and synthetic polyelectrolytes as coagulant and coagulant aid was also studied. After coagulation, the effluent was treated using UASB reactor for different organic loading and hydraulic retention time. The influence of trace metals like cobalt, nickel and molybdenum in the anaerobic treatment was investigated using a batch reactor. The effluent from the UASB reactor was treated by electrochemical oxidation in the presence of Fenton's reagent and it was found that this process could enhance the removal of pollutants from anaerobically treated effluent. Solar radiation was also used as an alternative source of power for electrochemical oxidation with the help of photovoltaic cell. The effluent after the electrochemical oxidation was further purified by adsorption. Different forms of activated carbon, furnace black and nano clays were used to purify the effluent. The effect of gamma irradiation from a Co-60 gamma radiation source in the presence of Fenton's reagent on the treatment of raw and anaerobically treated skim serum effluent was also investigated. The effectiveness of the different types of treatment was assessed in terms of contaminant removal as indicated by COD, BOD, total Kjeldahl nitrogen, ammoniacal nitrogen, sulphide, total solids and dissolved solids. Biochemical and microbiological analysis was also used to assess the treatment efficiency. Through coagulation, anaerobic treatment using UASB reactor, electrochemical oxidation/gamma irradiation and adsorption, it is possible to bring down the pollutant load of the serum effluent to the levels prescribed by the statutory authorities.

Key words: Natural rubber latex processing industries, latex centrifuging units, serum effluent, pollutant load, metal coagulant, UASB reactor, hydraulic retention time, anaerobic treatment, electrochemical oxidation, Fenton's reagent, gamma irradiation, COD, BOD, total Kjeldahl nitrogen, adsorption.

PREFACE

Hevea brasiliensis is one of the major sources of natural rubber. India is the fourth largest producer of natural rubber in the world, producing 852895 tonnes in the year 2006-07. Kerala enjoys a near monopoly by producing 785620 tonnes (92% of the total production) in 2006-07. Raw natural rubber is harvested from the tree in the form of latex which needs to be processed to avoid putrefaction and to improve its properties. Different forms of processed rubber, prepared from latex are ribbed smoked sheet, estate brown crepe, pale latex crepe, technically specified rubber and latex concentrate (centrifuged latex (cenex)/cream). Among these processing units, sheet rubber is mostly produced by individual growers in their own holdings. Processing natural rubber especially into latex concentrate produces effluent containing organic content.

To have a sustainable growth and development, it is necessary to reduce pollution, especially pollution of water which is a major problem facing mankind. Therefore, it is absolutely essential to treat effluent from natural rubber processing units before being discharged into the water bodies; hence, the study was undertaken. Though the science underlying the treatment of effluent especially biological treatment is highly complex, the main objective of the study is to develop a treatment system to reduce the concentration of the pollutants from latex centrifuging units.

This thesis is structured into eight chapters. The first chapter includes a brief description of natural rubber latex, possible sources of effluent, existing treatment practices and the objectives of the study. The second chapter gives an account of the characteristics of skim serum effluent from latex centrifuging units and important physical, chemical and biochemical methods of analysis. Chapter 3 is divided into Part A and Part B. Part A explains the efficacy of metal coagulants and Part B discusses the effectiveness of natural and synthetic polyelectrolytes as coagulant and coagulant aid. Part A of Chapter 4 evaluates the performance of anaerobic treatment using UASB reactor and Part B explains the effect of trace metals on anaerobic treatment. Chapter 5 deals with the electrochemical treatment of raw and anaerobically treated effluent and Chapter 6 gives the details of further purification of anaerobically and electrolytically pre-treated effluent using various adsorbents. Chapter 7 investigates the effect of gamma radiation on raw as well as anaerobically treated serum effluent. The last chapter summarises the overall results and proposes a treatment system for skim serum effluent.

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LIST OF ABBREVIATIONS

‘γ’	Gamma
μg	Microgram
μm	Micro metre
^{0}C	Degree Celsius
AN	Ammoniacal nitrogen
ATE	Anaerobically treated effluent
BOD	Biochemical oxygen demand
COD	Chemical oxygen demand
DRC	Dry rubber content
Fig	Figure
HRT	Hydraulic retention time
kg	Kilogram
kg /m^3/d	Kilogram per cubic metre per day
mg/L	Milligram per litre
mL	Millilitre
nm	Nanometre
NR	Natural rubber
OLR	Organic loading rates
PLC	Pale latex crepe
RE	Raw effluent
rpm	Revolutions per minute
RPUs	Rubber processing units
RRIM	Rubber Research Institute of Malaysia
RSS	Ribbed smoked sheet
TCA	Trichloroacetic acid
TDS	Total dissolved solids
TKN	Total Kjeldahl nitrogen
TMTD	Tetramethyl thiuram disulphide
TOC	Total organic content
TS	Total solids
TSR	Technically specified rubber
TSS	Total suspended solids
UAHR	Upflow anaerobic hybrid reactor
UASB	Upflow anaerobic sludge blanket
UV	Ultraviolet
VFA	Volatile fatty acid

CHAPTER 1

INTRODUCTION AND OBJECTIVES

1.1. General

The concern for the ever increasing pollution of water, land and air is growing all over the world. One of the major challenges facing mankind today is to provide clean water to a vast majority of the population around the world. All forms of life on earth depend upon water and each human being needs to consume several litres of fresh water daily to sustain life. Today India is amongst the water stressed countries with a deficit of 25 percent and by the year 2025, it will be amongst the water scarce countries with a deficit of 33 percent[1]. This is an average figure, but for a large fraction of the Indian population, water scarcity is already a reality. For a secure future, it is necessary to improve water quality and its availability. Rivers, canals, estuaries and other water bodies are constantly polluted due to the indiscriminate discharge of industrial effluents, other anthropogenic activities and natural processes. The increasing population, accelerating urbanisation and climate changes have made it imperative to treat wastewater and reuse it in water stressed countries.

With the development of economy, pollution, particularly water pollution, caused by industries has turned out to be serious. The increasing awareness about the potential health hazards of insufficient or inappropriate handling of wastewater has stimulated the search for effective treatment alternatives. Hence, there is an urgent need to develop innovative, more effective and economical methods for the treatment of wastewater.

1.2. Natural rubber

Hevea brasiliensis, a forest tree, is one of the most domesticated crop species in the world and is the major commercial source of natural rubber (NR). In India rubber cultivation is mainly concentrated in Kerala, Kanyakumari region of Tamil Nadu, Karnataka, North Eastern region, Andaman and Nicobar Islands, Goa and Maharashtra. It has been recently introduced in Orissa, Andhra Pradesh, Madhya Pradesh and West Bengal. However, Kerala enjoys a near monopoly by holding 82 (501700 hectares) percent of the area under the crop in the country of which 23 percent of the area is in Kottayam district. Today, India is the fourth largest producer of natural rubber in the world, having 615000 hectares under cultivation in 2006 – 07. India is also the fourth largest consumer of natural rubber after China, USA and Japan[2]. The total production of NR in India in the year 2006 - 07 was 852895 tonnes. Out of it Kerala's contribution was 785620 tonnes (92 percent of total production in India) and Kottayam district alone accounts for 179910 tonnes (23 percent of total production in Kerala).

1.3. Composition of NR latex

NR latex is the milky white or slightly yellowish opaque plant fluid of the rubber tree (*Hevea brasiliensis*). Latex is obtained from the bark of the tree by tapping and it is collected by fixing a spout and collection cup. NR is a high molecular weight polymer with viscoelastic properties. Structurally it is cis-1, 4-polyisoprene (C_5H_8). The diameter of the rubber particles in the latex is widely distributed from several tens of nm to several hundreds of nm[3]. The NR latex is a colloidal system comprising of, lyophilic negatively charged spherical or pear shaped rubber globules dispersed in an aqueous serum. The dispersed rubber particles are strongly protected by a complex film made of protein, lipids and phospholipids. This complex film renders lyophilic colloidal nature to latex. The stability of latex is due to the negative charge present on the protective layer [4, 5]. The field latex contains a variety of non-rubber constituents both organic and inorganic, in addition to rubber. The proportion of these constituents varies with clones, nutrition, climate etc. General composition of the latex is given in the Table 1.1

Table 1.1. General composition of natural rubber latex

Constituents	% Composition
Rubber	30-40
Resin	1-2
Protein	2-2.5
Sugar	1-1.5
Ash	0.7-0.9
water	55-65

(Source- N.Usha Nair in "Natural Rubber, Agromanagement and crop processing", P.J. George, C.K. Jacob, Eds., RRII, Kerala, India, 2000, pp 250-253)

During coagulation of NR latex, a large amount of liquid called natural rubber serum (NRS) is obtained as a byproduct. The serum contains primarily water and various non-rubber substances. The non-rubber substances include nitrogenous materials like ammonium salts, proteins and amino acids, sugars and pseudo-sugars (quebrachitol, glucose, fructose and galactose), ash (K, P, Zn, Mg, Na, Ca, Fe, Al, Rb), and fatty acids (lauric, palmitic, stearic, oleic, linoleic)[6]. Various amino acids found in NRS are glutamic acid, aspartic acid, alanine, glycine, leucine, lysine, cystine, serine, valine, tyrosine, proline, tryptophan, threonine, histidine and arginine[6]. The second largest group of compounds found in NRS is sugar and pseudo-sugar. The presence of large amount of reducing sugars leads to the quick putrefaction of latex by bacteria if it is not properly preserved. Hence, the amount of such sugars found in NRS depends on the extent of bacterial decomposition. Most of the ash compounds and fatty acids do not occur naturally in NR latex but result from the addition of various chemicals before and during the course of processing the latex[6].

1.4. Processing of NR

Fresh latex, as it comes out of the tree is slightly alkaline or neutral and microorganisms contaminate it. They metabolise the non-rubber constituents of the latex and produce volatile fatty acids such as formic, acetic and propionic acids. These acids neutralise the negative charge on rubber particles and the latex gradually thickens and gets coagulated within 6 to 12 hours of its collection[7]. Therefore, anticoagulants (or preservatives) like ammonia, sodium sulphite or formalin (depends on the type of processing) are added to keep it for longer periods before it is processed[7, 8]. Among the various chemicals used as preservatives, ammonia is of prime importance. NR latex harvested from plantations needs to be processed soon after harvesting to maintain its quality. Processing of this latex is essential also for easy storage, transportation, better uniformity in quality and utilization by manufacturing industries. A large number of rubber processing units (RPUs) have been established in Kerala for the processing of latex and the field coagulum into different marketable forms of NR.

The major categories of processed rubber are:

(a) Ribbed smoked sheets (RSS)

(b) Pale latex crepe (PLC)

(c) Technically specified rubber (Block rubber) (TSR)

(d) Preserved field latex and latex concentrate [centrifuged latex (cenex /cream)]

In these four types of processing, the first category (i.e. ribbed smoked sheets) is carried out mostly by individual growers in their own holdings. The different types of processed rubber produced during the year 2005-2006 comprised latex concentrates 10.6%, solid block rubber 11.3%, RSS 70.9% and others 7.2% [2].

Table 1.2 gives the statistics (on March 31st 2007) of different types of NR processors in India.

Table 1.2. Number of different types processors of NR in India

Type of units	No
Centrifuged Latex Concentrating Units	68
Creamed Latex Units	15
Ammoniated Latex	8
Pale Latex Crepe	9
Estate Brown Crepe	15
Technically specified rubber	52

A brief description of various processing methods and sources of effluents are given in the following sections

(a) Ribbed smoked sheets (RSS)

It is the oldest and simplest method of processing latex into a marketable form. Latex collected from the field is first sieved to remove foreign materials. It is then bulked for uniform property and diluted to standard dry rubber content (DRC) of 12.5 per cent to improve the quality of coagulum. Chemicals like sodium bisulphite and paranitrophenol are added to prevent discolouration/mould growth. They also act as preservatives. The latex is then coagulated using formic acid or acetic acid. After completion of coagulation, the serum is drained out and the coagulum is obtained in the form of blocks. Washed coagulum is then rolled to sheets using a pair of plain rolls and another set of grooved ones. The sheets are then allowed to drip in shade and dried in smoke houses for producing ribbed smoked sheet.

Water is used in the process for diluting the latex and washing the coagulum during sheeting operations. This water forms part of the effluent. The water consumption is in the range of 20-30 litre/kg of dry rubber.

Fig. 1.1 depicts the sources of effluent generation from ribbed smoked sheet producing units from latex

(b) Pale latex crepe (PLC)

When coagulated latex or any form of field coagulum or RSS cuttings, after necessary preliminary treatments, are passed several times through a set of creping machines, lace-like rubber is obtained. This lace like rubber when air-dried is called crepe rubber.

Different stages in the manufacture of pale latex crepe (PLC) are shown in Fig.1.2. The field latex containing anticoagulants like sodium sulphite is received at the factory. The latex is then sieved, bulked, standardised by dilution and chemically treated. The chemically treated latex is then subjected to fractional coagulation using acetic acid for removing colouring materials[9]. It is then sieved through a 60 mesh sieve. The latex after fractional coagulation and bleaching is coagulated using formic acid. About 4 mL of formic acid per kg of dry rubber is used. The coagulum obtained in the form of block is milled in creping machines. Water is used for washing coagulum during milling to wash out the serum and other non-rubber materials. The crepe is then dried at ambient or slightly elevated temperature in drying sheds and packed.

In a pale latex crepe unit, water is required for diluting the latex, washing the coagulum and for cooling the rollers. Wastewater is released from the latex coagulation section and crepe milling section[10]. Water consumption per kg DRC of the product is about 35-50 litres. The effluent discharged marginally exceeds the quantity of water consumed, because of the addition of serum in the process of coagulation.

(c) Technically specified rubber (TSR)

TSR or crumb rubber is mostly produced from field coagulum[11]. The stages in the process are collection of field coagulum, soaking and pre-cleaning, blending, milling and size reduction, drying, pressing and packing. When crumb rubber is produced from field latex, the additional stages required are bulking, coagulation and pre-machining. Sodium bisulphite at the rate of 1 g/kg DRC rubber is added as 1 % solution to the bulking tank to prevent surface darkening of the coagulum. Coagulation of latex is done using either formic or acetic acid. Individual slabs of latex coagula are cut into smaller pieces before feeding to an extruder or one or two passes are given through a creper before feeding to a hammer mill for final size reduction. Water is required for scrap soaking, ,milling, size reduction and crumb collection. The water consumption and effluent generation is estimated to be 35-55 litres per kg of DRC. The flow diagram of the process is shown in Fig.1.3.

(d) Preserved field latex and latex concentrate

Field latex preserved with suitable preservatives is termed as preserved field latex. Processing into preserved field latex consists of adding the preservative, bulking, settling and blending into consignments of suitable size for despatch.

Latex concentrate is an important raw material having wide uses. The process of latex concentration removes substantial quantity of serum from field latex, thus making the cream richer in rubber content. The major processes in a latex concentrating unit are ammoniation, desludging, latex concentration and skim recovery.

The latex from the field is sieved and bulked and ammonia is bubbled through it so as to get a concentration of 0.7 to 1.0 per cent by weight of latex. Ammonia inhibits bacterial growth, acts as an alkaline buffer and raises the pH and neutralises the free acid formed in latex[12]. The degree of ammoniation depends on the period, which has elapsed since collection and ranges from 0.25% for immediate centrifuging to 0.8% for two days or more. Only the minimum required quantity of ammonia should be added since most of it goes to the skim which makes coagulation of the skim difficult. Ammonia deactivates some metal ions which tend to coagulate latex by forming insoluble compounds or complexes with them. For the complete precipitation of magnesium as magnesium ammonium phosphate, calculated quantity of diammonium hydrogen orthophosphate is added if the phosphate ions already present in the latex are not sufficient.

After ammoniation, latex is left undisturbed for about 15-20 minutes to allow separation of sedimentable impurities and reaction product (magnesium ammonium phosphate).

Preserved field latex is unsuitable for most latex applications as its rubber content is low. For most product manufacture, latex of minimum 60 per cent dry rubber content is essential. The important methods for the concentration of preserved field latex are (i) centrifuging and (ii) creaming. These methods involve partial removal of non-rubber constituents. The particle size distribution of the concentrate differs from that of the initial latex. A portion of the smaller particles escapes to the serum. At present, more than 90 per cent of the latex concentrate is obtained by centrifuging.

1. Centrifuging

Different types of centrifuging machines are used for concentration of latex and the most widely used machine is of Alfa Laval make. In the centrifuging process of latex, the centrifugal force brings about separation of rubber particles from the serum. The rotating mass of ammoniated field latex is broken up into a number of thin conical shells within the bowl rotating around 6000 rpm whereby individual rubber particles tend to separate into a layer surrounding the axis of rotation leaving behind an outer layer (skim) having a comparatively lower rubber content. The DRC of the centrifuged latex is around 60 per cent. About 85 to 93 per cent of the total rubber in the field latex will be separated into the cream or concentrated fraction. The cream is separately collected in a bulking tank, its ammonia content estimated and made upto 0.7% on latex and packed in drums.

LATZ latex

Centrifuged latices are commercially available as high ammonia (HA- minimum 0.6% ammonia), and low ammonia (LA- maximum 0.3% ammonia), types. The former is preserved solely with ammonia and the latter contains one or more preservatives in addition to ammonia and the most popular LA type latex is low ammonia-TMTD-Zinc oxide(LA-TZ) which contains 0.2% ammonia, 0.013% tetramethyl thiuram disulphide (TMTD), 0.013% ZnO and 0.05% lauric acid. Major advantages of LATZ are reduced cost of production through, savings in preservatives, acid and effluent treatment and improvement in the quality of output. It also ensures better working environment in the factory.

Double centrifuged latex is a purer form of concentrated latex obtained by a second stage of centrifuging ordinary centrifuged latex after dilution to 60% DRC.

Skim latex and serum effluent

When field latex is centrifuged /creamed, in addition to the concentrate containing most of the rubber, an equal fraction in volume, containing a very small proportion of rubber is also obtained. This is known as skim latex. 3.5 to 6 per cent rubber will be lost into the skim fraction. It contains about two thirds of the total serum from the field latex. To recover the rubber in skim latex, it is coagulated with sulphuric acid and the serum left out is drained off and is known as **skim serum effluent.** In 2005-06, India produced about 802,625 tonnes of natural rubber of which 10.6 % was in the form of latex concentrate[2].

In-depth study conducted in RPUs reveal that the volume of water consumed by latex concentrating units is much less when compared to that of other processes. The water requirement in centrifuging process is in the range of 3.5 to 6.0 litres per kg of dry rubber as latex concentrate. The quantity of effluent generated is found to vary widely from unit to unit and depends on the processing capacity of the unit. Apart from the water consumed for processing, the serum left out after coagulation of the skim latex is also discharged from industries which have adopted centrifuging process. Hence, the quantity of effluent discharged is much higher than that of the water consumed[13]. The water requirement is mainly for cleaning the latex storage tanks once a week, washing the barrels and floor, washing the bowls of the centrifuging machine twice in a shift and coagulation of skim latex. Apart from the skim serum, all the water used in the process comes out as effluent.

The flow diagram of the process with effluent generation is shown in Fig.1.4.

2. Creaming

Field latex is ammoniated to 1 % and kept for a few days for ageing. Ammonium alginate or cooked tamarind seed powder is usually used as the creaming agent. A 3% solution of the seed powder is prepared by boiling the required quantity in water. The calculated quantity of the creaming agent solution which has been sieved to remove uncooked materials and a 10 per cent soap solution is added to latex in order to get a final concentration of 0.3 per cent in latex. The latex is stirred for one hour. After stirring, the latex is allowed to remain undisturbed for a minimum period of 48 hours to obtain the desired level of creaming[11].

Although a minimum period of 48 hours is usually required for creaming, fixed time can be set for all conditions. After the creaming agent is added there is an induction period of several hours before any creaming is visible. For the first 24 - 40 hours, creaming is rapid and then it becomes slower. The gravitational force brings about the separation of rubber particles during creaming. When the desired level of creaming is obtained, the skim layer is drained off. Latex with 55-58 per cent DRC is obtained by the creaming method. The skim serum left behind will contain 2-3 per cent rubber. The water requirement in a latex creaming unit is for operations like cleaning the reception tanks and floor of the factory. This water goes out as effluent.

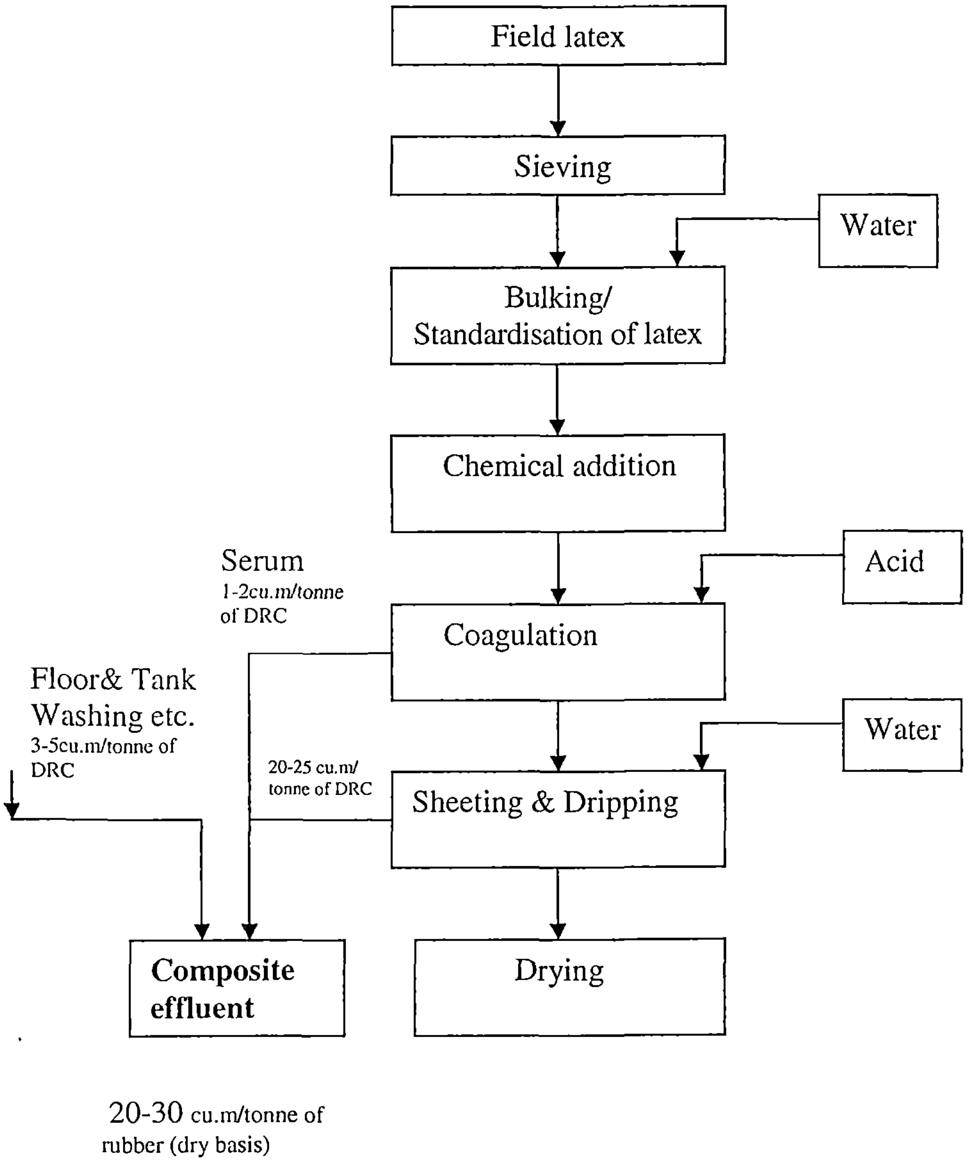

Fig.1.1. Process flow chart for ribbed smoked sheet manufacture

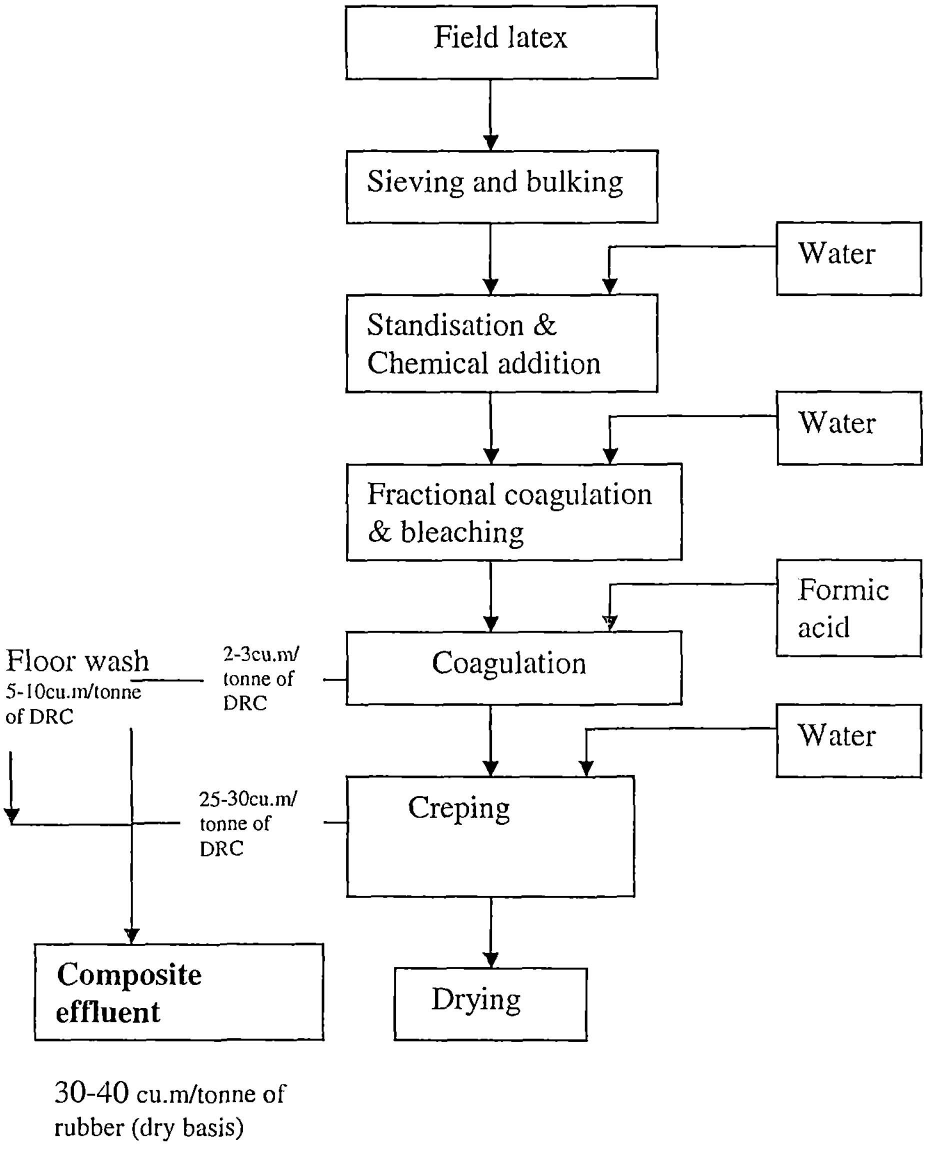

Fig.1.2. Process flow chart of pale latex crepe unit.

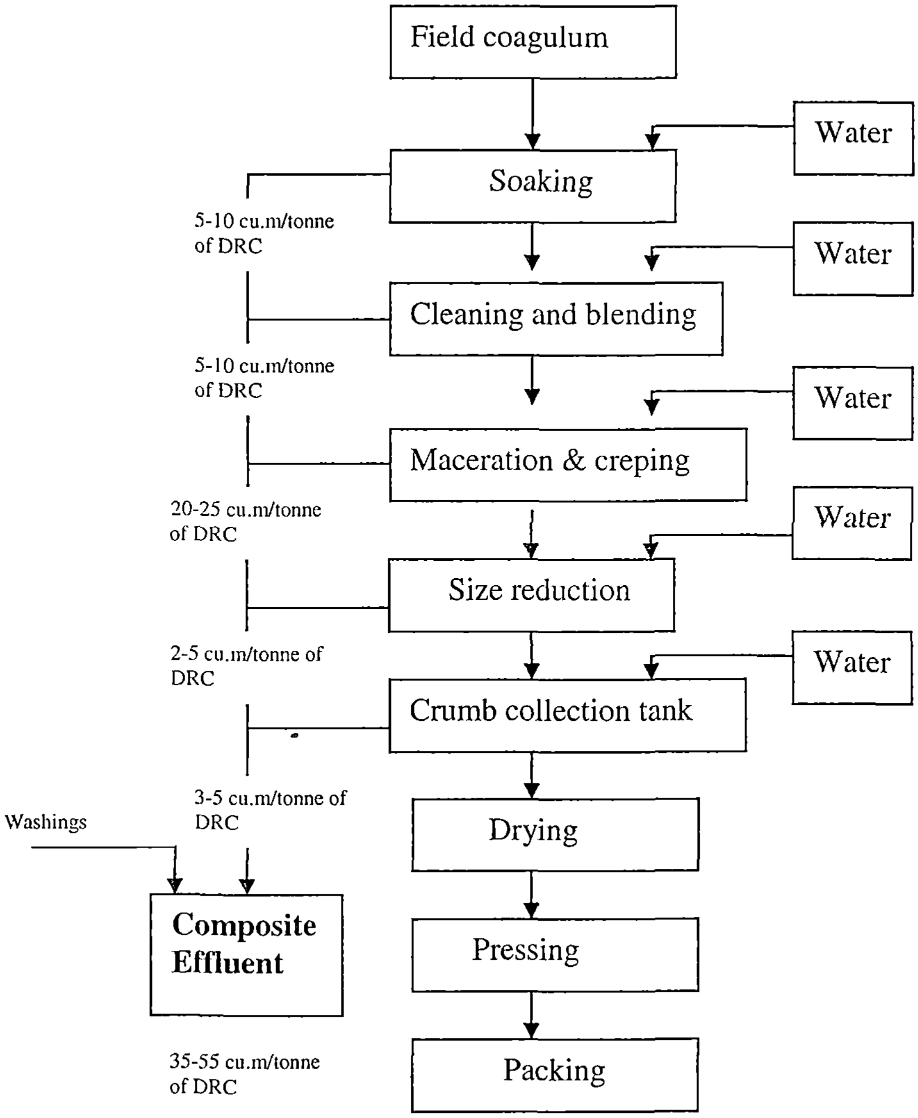

Fig.1.3. Process flow chart for technically specified rubber factory

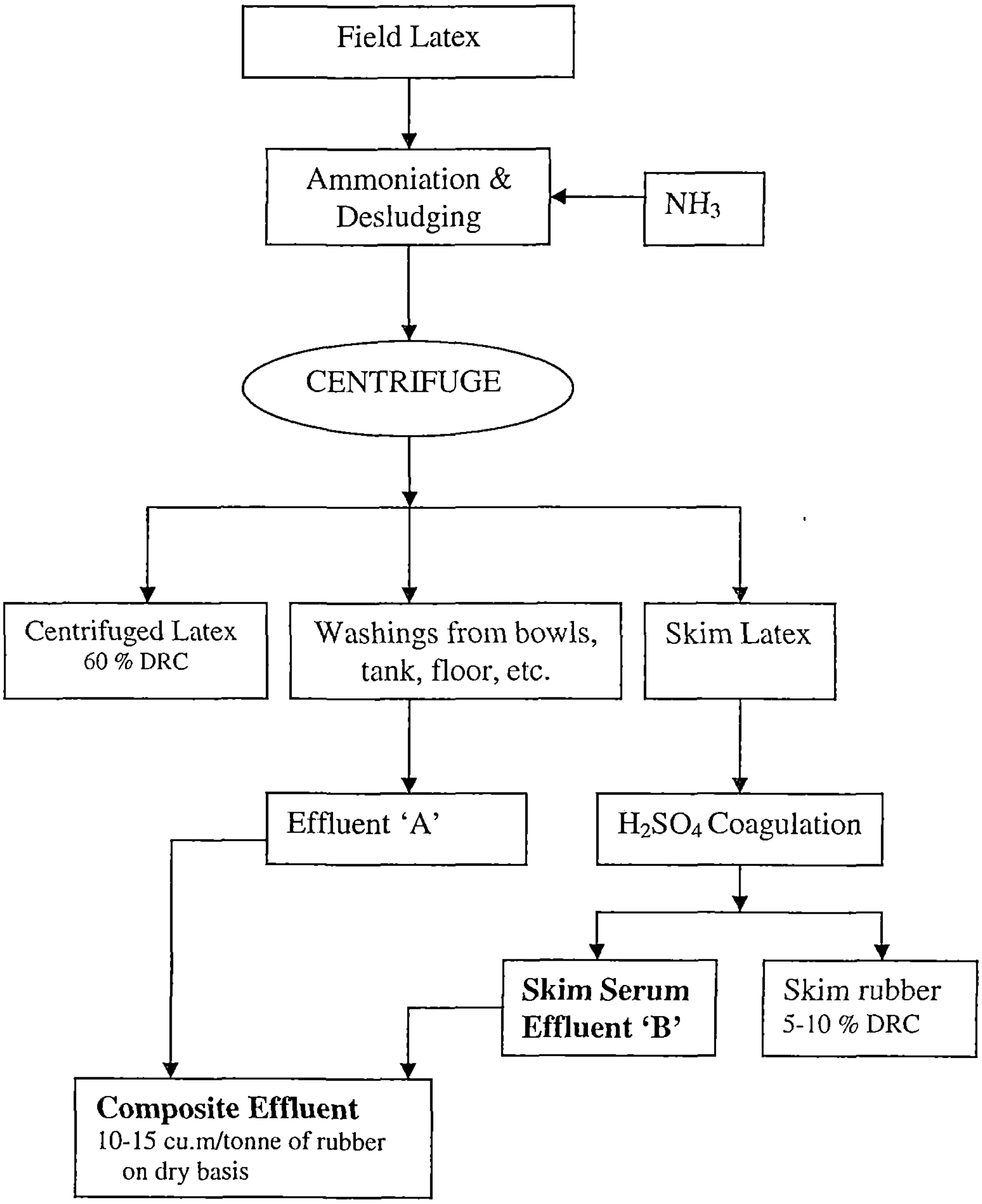

Fig.1.4. Process flow chart of latex centrifuging unit

1.5 General wastewater characteristics

A general idea of the physical, chemical, biological and biochemical characteristics of the wastewater is essential to assess the water quality, pollutant load, efficiency of wastewater treatment method and disposal facilities. The pollution potential of wastewater, (no matter it be domestic or industrial) is expressed in terms of these characteristics.

1.5.A. Physical characteristics

The most important physical characteristics of wastewater are colour, odour, temperature, total solid content and turbidity[14].

(1) Colour

Colour in water may result from the presence of natural metallic ions, humus, industrial waste, organic components, turbidity, suspended matter etc. Colour changes to black as organic compounds are broken down by bacteria resulting in the depletion of dissolved oxygen in wastewater[15]. In this condition, the wastewater is said to be septic (or stale). Some industrial wastewaters are coloured in nature and the colour is related to pH.

(2) Odour

Odour and taste depend on the actual contact of the stimulating cell and it varies from person to person. Acceptability of drinking water is determined by its taste and odour. Organic as well as inorganic matter contributes taste and odour. Many inorganic salts produce taste without odour. Uncontaminated water has neither odour nor taste. Odours in wastewater usually are caused by gases produced by the decomposition of organic matter. The most characteristic odour of industrial wastewater or septic wastewater is that of hydrogen sulphide, which is produced by anaerobic microorganisms that reduce sulphates to sulphides during the process of wastewater treatment. Odour and taste are useful as a check on the quality of raw and finished water, and as means of tracing the source of contamination[16].

(3) Temperature

Elevated temperatures resulting from hot water discharges may have significant ecological impact. The temperature of water is a very important parameter because of its effect on aquatic life, the chemical reactions and reaction rates, and the suitability of water for beneficial uses[17]. Water temperature as a function of depth is required for limnological studies. Identification of the source of water supply, such as deep wells, is possible by temperature measurements alone.

(4) Total solids

Any solid matter suspended or dissolved in water or wastewater is known as residue. The total solids content of wastewater is the sum total of floating matter, matter in suspension, colloidal matter, and matter in solution. Analytically, the total solids content of a wastewater is defined as all the matter that remains as residue upon evaporation at 103 to 105°C. The total solids can be classified as either suspended solids or filterable solids by passing a known volume of liquid through a filter. The filter is commonly chosen so that the minimum diameter of the suspended solids is about 1 micron[18]. The filterable-solids fraction consists of colloidal and dissolved solids. The colloidal fraction consists of particulate matter with an approximate diameter ranging from 1 milli micron to 1 micron. The dissolved solids consist of both organic and inorganic molecules and ions that are present in true solution in water.

(5) Turbidity

Turbidity, a measure of the light-transmitting properties of water, is used to indicate the quality of wastewaters and natural waters with respect to colloidal matter. Colloidal matter will scatter or adsorb light and thus prevent its transmission[19]. Turbidity in water is caused by the presence of suspended matter, such as clay, slit, finely divided organic and inorganic matter, plankton, and other microscopic organisms. Turbidity is usually measured using nephelometric turbidimeter.

1.5. B. Chemical characteristics

In chemical characteristics, the components of importance are organic matter, inorganic matter, nitrogen, phosphorus, and gases.

(1) Organic matter

Most of the suspended solids and filterable solids are organic in nature[14]. These solids are derived from both animal and plant kingdoms and the activities of man as related to the synthesis of organic compounds. Organic compounds are normally composed of a combination of carbon, hydrogen, and oxygen, together with nitrogen in some cases. Other important elements such as sulphur, phosphorus and iron, may also be present. The principal groups of organic matter found in wastewater are proteins, carbohydrates, fats and oils.

The commonly used indices for the measurement of organic content in wastewater are biochemical oxygen demand (BOD) and chemical oxygen demand (COD). BOD test is an empirical test used to determine the relative oxygen requirement of wastewaters, effluents, and polluted waters. The most widely used parameter of organic pollution is the 5-day BOD (BOD_5). Its determination involves the measurement of the dissolved oxygen used by microorganisms in the bio-chemical oxidation of organic matter. Biochemical oxidation is a slow process and theoretically takes infinite time to complete[20]. The oxidation is about 95 to 99 per cent complete within 20 days of incubation which is too long for practical purposes. For this reason, the 5-day period at 20°C has been accepted as standard[16].

The COD test is employed to measure the content of organic matter of both wastewater and natural waters. It also measures the inorganic substances that can react with and are oxidised by the dichromate. COD is in fact the total oxygen demand while BOD is the oxygen demand for biological oxidation. In this test, the oxygen equivalent of the organic matter that can be oxidised is measured by using a strong chemical oxidising agent in acidic medium, at an elevated temperature. Potassium dichromate is an excellent oxidising agent for this purpose. Compared to the BOD test which takes 5 days, the COD can be determined within 3 hours. The total organic content (TOC) of the wastewater is also widely used as an index of organic matter.

(2) Inorganic matter

Some of the parameters of importance are pH, metals, nitrogen and phosphorus.

(a) pH

The pH of a solution refers to its hydrogen ion activity and is expressed as the logarithm of the reciprocal of the hydrogen ion concentration in moles per litre at a given temperature. Generally water is slightly basic in nature because of the presence of carbonates and bicarbonates. Departure from neutral state is caused by the influx of acidic or alkaline industrial waste. In wastewater treatment, measurement and regulation of pH is required for water pollution control. It is the pH which is more important than acidity or alkalinity since the latter measures resistance to pH change or buffering capacity of a sample. Wastewater with high concentration of hydrogen ion is difficult to treat by biological means.

(b) Metals

Metals are classified as macronutrients and micronutrients. Excess concentration of metal causes toxicity in living system and the presence of metals is a matter of serious concern in potable water, domestic wastewater, industrial wastewater and receiving waters due to its toxic effect. Presence of metals may be determined by atomic absorption spectrophotometer.

(c) Nitrogen

Since nitrogen is an essential building block in the synthesis of protein, nitrogen content of the wastewater will be required to evaluate its treatability by biological processes. Insufficient nitrogen may necessitate the addition of nitrogen to make the wastewater treatable. Where control of algal growth in the receiving water is necessary to protect beneficial uses, removal or reduction of nitrogen in wastewaters prior to discharge may be desirable[14].

(d) Phosphorus

Like nitrogen, phosphorus is also essential for the growth of algae and other biological organisms. The usual forms of phosphorus that are found in aqueous solutions include the orthophosphate, polyphosphate, and organic phosphate[21]. Excessive concentrations of phosphates cause noxious algal blooms in surface waters. Hence, there is a need for controlling the amount of phosphorus compounds that enter surface waters through domestic and industrial waste discharges and natural runoff.

(3) Gases

Some of the gases present in the untreated wastewater include nitrogen, oxygen, carbon dioxide, ammonia, hydrogen sulphide and methane. The first three are common gases of atmosphere and will be found in all waters exposed to air. The last three are derived from the decomposition of the organic matter present in wastewater. Ammonia is found in the untreated wastewater as ammonium ion or free ammonia. Hydrogen sulphide is formed from the decomposition of organic matter containing sulphur or from the reduction of sulphites and sulphates. It is not formed in the presence of an abundant supply of oxygen. The blackening of wastewater and sludge usually results from the formation of hydrogen sulphide that has combined with the iron present to form ferrous sulphide[21]. One of the principal by-products of the anaerobic decomposition of organic matter in wastewater is methane gas. Methane is a colourless, combustible hydrocarbon of high fuel value. It is also one of the most important green house gases.

1.5. C. Biochemical characteristics

The organic matter that may be present in wastewater belongs to major groups like proteins, amino acids, reducing and non reducing sugars, phenols, lipids, fats and oils etc. Estimation of these groups gives an indication about the different types of organic pollutants and the method of treatment to be adopted for their removal.

1.5. D. Biological characteristics

Protista and plants are the principal groups of organisms found in wastewater. The category protista includes bacteria, fungi, protozoa and algae. Among them, bacteria play a fundamental role in the decomposition and stabilisation of organic matter, both in nature and in treatment plants[14]. Seed plants, ferns, mosses and liverworts are classified as plants. Viruses are also found in wastewater and are classified according to the host infected.

Pathogenic organisms found in wastewater may be discharged by human beings who are infected with disease or who are carriers of a particular disease. The usual bacterial pathogenic organisms that may be excreted by man cause diseases of the gastrointestinal tract, such as dysentery, diarrhea, cholera, typhoid and paratyphoid fever[22].

Identification of pathogenic organisms in water and wastewater is both extremely time-consuming and difficult. The coliform group of organisms is now used as an indicator of the presence of feces in wastewater and hence pathogenic organisms[23]. Coliform organisms are the countless rod-shaped bacteria present in the intestinal tract of man. Each person discharges 100 to 400 billion coliform organisms per day.

1.6. Water consumption and wastewater generation in rubber processing units

Quantity of water consumed and the wastewater generated depend upon the processing of NR. Processing of NR requires large quantity of water for various purposes such as dilution of latex, cleaning of utensils and machinery used, floor and bowl washings, cooling etc. In the latex processing unit wastewater generated is higher than the water consumed due to the generation of serum during coagulation whereas in the field coagulum they are almost same[24]. In a centrifuging unit the effluent generated depends on the processing capacity of the unit. The average quantity of effluent generated by each centrifuging machine is around 12,600 litres per day.

1.7. Characteristics of effluents from natural rubber processing units

An understanding of the characteristics of various types of effluent is necessary for developing proper environmental management practices in rubber processing units.

Table 1.3. Characteristics of effluents from natural rubber processing units

Type of units	Parameters						
	pH	COD	BOD	TS	DS	Total Kjeldahl nitrogen	Amm. nitrogen
Latex centrifuging (Composite)	3.2-4.2	3800-10200	2500-5200	6050-26500	4500-23000	1150-3950	900-3850
Technically specified rubber	6.5-7.1	850-4850	400-2450	800-5000	500-3200	50-250	20-230
Pale latex crepe units	5.5-6.3	3000-7000	1800-2800	1900-3500	1810-2800	10-30	8-25
Ribbed smoke sheets	4.9-5.0	4590-8080	2030-4080	3600-4150	3550-4020	18- 30	10-15

(All values are expressed in mg/L except pH)

Table 1.4. Characteristics of effluents from individual sections of latex centrifuging units

Sections in Latex centrifuging units	Parameters						
	pH	COD	BOD	TS	DS	Total Kjeldahl nitrogen	Amm. nitrogen
Bowl washings + Floor washings	6.7-8.1	500-12850	300-3400	500-10500	250-5000	100-600	60-400
Skim serum	3.6-4.7	27000-38800	10500-23280	59000-70000	56000-68000	7000-11000	2500-5000

(All values are expressed in mg/L except pH)

Earlier studies carried out in India, Malaysia, Indonesia, Sri Lanka, Vietnam and Thailand on pollution from rubber processing factories accentuate the fact that effluent from RPUs are highly polluting [25-34]. Table 1.3. shows the characteristics of effluent generated from different processing units and Table 1.4. shows the characteristics of effluent from different sections of latex centrifuging units[23, 26, 27, 28, 31, 35, 36]. The presence of a number of viable and indicative bacteria in the skim serum effluent makes it a highly oxygen absorbing liquid[36]. The bacterial population is comparatively low in the effluent from centrifuging latex unit due to the high acidity of the effluent[25]. The ammoniation of field latex and acid coagulation of skim latex during the processing of latex concentrate contribute to the high pollution load[38]. The skim serum effluent contains high concentration of proteins, carbohydrates, carotenoids, sugars, lipids, inorganic and organic salts[39, 40]. These substances form excellent substrates for the proliferation of microorganisms which in turn leads to high BOD and objectionable odour[29, 40]. Natural rubber serum contains micro and macronutrients and therefore could be used as a fertilizer for young plants and as a feed supplement for microorganisms, fish and animals[32,41-46]. It can also be used for deriving pharmaceutical-grade quebrachitol[45].

The acidic nature of the effluent is attributed to the use of formic, acetic, phosphoric or sulphuric acid in the process line. The high biochemical oxygen demand (BOD) and chemical oxygen demand (COD) of the latex concentrate and ribbed sheet factory effluent indicate that the total solids in the effluents are mainly of organic origin with high oxygen demand for their oxidation. The high ammoniacal and total nitrogen of latex concentrate effluent is due to the use of substantial quantity of ammonia in the preservation of latex. Hence, the two potential pollutants in rubber effluent are organic carbon and ammoniacal nitrogen. The characteristics of individual sections of effluent from latex centrifuging units show that skim serum effluent from the coagulation section has the highest pollution load.

1.8. Impact of effluent discharge on water bodies

In Kerala there are 41 rivers flowing towards west. Most of them originate in the Western Ghats and fall into the Arabian Sea. Besides, there are 3 rivers originating in Kerala, which flow towards east through the states of Karnataka and Tamil Nadu. In addition, Kerala is rich with 29 fresh water reservoirs and lakes. The entire state is divided into 32 river basins with reference to the catchment area of the major rivers[9]. Most of the RPUs in the country is in Kerala and Kottayam district alone accounts for high concentration of these units. The factors which contribute to the regional concentration of RPUs are the availability of raw material and water for processing. The pollution load of the effluent from RPUs varies depending upon the type, size and quantity of water used in the factory operation.

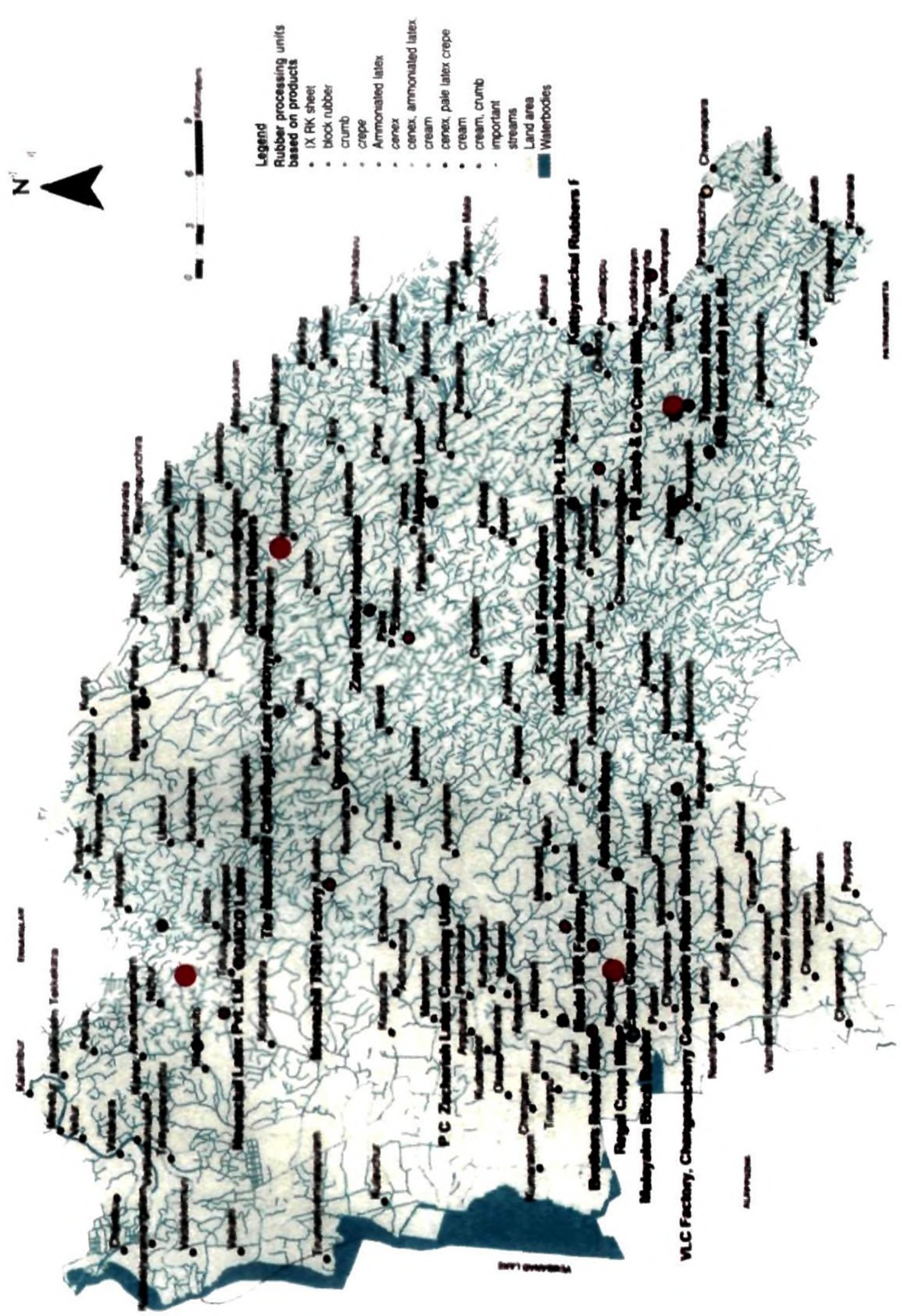

Fig.1.5. Clusters of RPUs in Kottayam district[47]

The clusters of RPUs in Kottayam district shows (Fig. 1.5) that there is a wide net work of streams near to the RPUs[47]. Most of the RPUs are established in river basins and some of them are on the banks of the major rivers. These units discharge treated as well as untreated effluents directly or indirectly into the water bodies or open land[9, 47]. The pollution of water bodies and landmass is a very serious problem facing Kerala today. The discharge of raw effluent into the environment, if goes on unchecked, will adversely affect the quality of the receiving water bodies. If the untreated effluents from rubber latex centrifuging units are discharged into any stream or river, it will deplete the dissolved oxygen concentration of the water body due to the high organic content, thus affecting the survival of aquatic life. The acidic nature of the effluent also may affect the growth of fish and other aquatic life. The presence of ammonia, nitrogen, and phosphate in the effluent leads to a phenomenon called 'eutrophication' which supports the unchecked growth of aquatic weeds and algae causing annihilation of aquatic animal life[48]. The foul smell and dirty appearance of the polluted water is unhealthy even for bathing. The menace of water-borne diseases and epidemics still looms large on the populace of developing countries like India.

Effective control and corrective measures, by adopting appropriate treatment methods, are to be taken on a war footing to reduce the impact of the pollution load on the environment, lest the long term damages to the environment and living organisms in this part of the country will be beyond any stretch of imagination. The destiny of Kerala, the ‘Gods Own Country’, may not be so promising unless people and authorities take cognisance of the severity of the hazards of pollution and enforce effective control and regulatory measures.

1.9. Existing treatment practices

Based on pollution potential, industries are categorized into red/orange/green. Among the rubber processing units, latex centrifuging /creaming units come under red category. Crepe, crumb and RSS units belong to the orange category. Environment (Protection) Rules[48] has prescribed standards for the discharge of effluents from natural rubber industry. Statutory standards are fixed for the discharge of effluents into inland surface waters and on land for irrigation. The standards and guidelines given by the Kerala State Pollution Control Board[24] are presented in the Table 1.5.

The earlier practice was to discharge the effluents indiscriminately to the surroundings without treatment. Some of these units have provided rubber traps to detain rubber particles and one or two settling tanks before discharging the effluent to public waterways[9]. Some factories discharge the effluent into their own estates where it is allowed to percolate in the soil or used for irrigation purposes. The growing environmental awareness among people and the introduction of various environmental legislations have forced the rubber processing units in India, mostly situated in the South Indian states of Kerala, Tamil Nadu and Karnataka, to adopt suitable effluent treatment systems.

Table 1.5. The standards given by the Kerala State Pollution Control Board

Parameters	Standards			
	Inland surface waters	On land for irrigation	Inland surface waters	On land for irrigation
	Centrifuging and Creaming Units		Crepe and Crumb units	
pH	6 to 8	6 to 8	6 to 8	6 to 8
BOD	50	100	30	100
COD	250	***	250	***
Oil and grease	10	20	10	20
Total Kjeldahl nitrogen	100	***	50	***
Ammoniacal nitrogen	50	***	25	***
Sulphides	2	***	2	***
Total Dissolved Solids (TDS)	2100	**	2100	**
Suspended Solid (SS)	100	200	100	200

(All values except pH are expressed in mg/L)

** Not prescribed in case the effluent is used for rubber plantations of their own

*** Not specified

The early investigations carried out by the Rubber Research Institute of Malaysia (RRIM) suggested trickling filter method, aeration systems and sequencing batch reactor method, oxidation ditch method, biological oxidation using rotating biodisc, algal pond method, anaerobic reactor and anaerobic-facultative ponding system for the treatment of effluent from rubber processing units[31,37,38,49-60]. All these systems are found suitable for the rubber processing factory effluents under Malaysian conditions [38, 61]. Isa[58] used a high rate anaerobic reactor to treat diluted sulphate-rich serum and found that the digestion process was inhibited.

In India, Kumaran et al[62] investigated flocculation followed by oxidation, Doraisamy[63] applied upflow anaerobic sludge blanket (UASB) and upflow anaerobic hybrid reactor (UAHR) methods for rubber latex processing wastewater and Mathew et al used anaerobic immobilised growth digester for treatment of RSS effluent and biological treatment for crumb rubber effluent[64,65]. Investigations had been made in the treatment of composite effluents from centrifuge latex concentrate units using coagulation and laboratory scale anaerobic treatment methods, and UV radiation treatment methods[27, 34].

1.10. Objectives and scope of the work

Among the rubber processing units, centrifuge latex concentrate units produce acidified skim serum effluent, which causes deleterious effects on environment. Treatment of the skim serum is a difficult task, since it is highly acidic and contains large amount of sulphate [29, 37]. Very few studies have been conducted on the treatment of acidified skim serum effluent and most of the treatment systems were developed under Malaysian conditions. However, a detailed systematic study on the efficiency of different treatment systems for the natural rubber serum effluent under Indian conditions is not available[66]. Hence, there is an urgent need for carrying out detailed and systematic studies on highly polluted skim serum effluent to develop an eco-friendly and cost effective treatment scheme.

The present study was initiated to develop a treatment system consisting of physico-chemical, electrochemical, biological and radiation methods for the effective treatment of acidified skim serum effluent from natural rubber centrifuging units. The treatment methods evaluated were a combination of different methods which have been successfully applied for sewage and effluents from other industries like, pulp and paper mills, slaughterhouses, pharmaceutical units, citric acid units, distilleries, tanneries, textiles, dye industry, sugar industry, food processing, etc. [67-85].

The endeavour of this project is to employ these methods for the complete and systematic treatment of skim serum effluent from latex centrifuging units.

The objectives of the study are as follows:

1. Characterisation of skim serum effluent from latex centrifuging units.
2. Evaluation of metal coagulants in treating skim serum effluent from latex centrifuging units.
3. Evaluation of the effectiveness of natural and synthetic polyelectrolytes as coagulant and coagulant aid.
4. The performance evaluation of an upflow anaerobic sludge blanket reactor for the treatment of skim serum effluent.
5. Studies on the influence of trace metals in anaerobic treatment of serum effluent.
6. Investigations on the effectiveness of electrochemical technique for the treatment of raw and anaerobically treated serum effluent.
7. Evaluation of adsorption efficiency of activated carbon and carbon black in purifying electrochemically treated serum effluent.

8. Evaluation of adsorption efficiency of clay and nano clays in purifying electrochemically treated serum effluent.

9. Investigations on the effect of gamma radiation in treating raw and anaerobically treated serum effluent.

10. To evolve a treatment system for skim serum effluent using a combination of different methods namely coagulation, anaerobic treatment using UASB reactor, electrochemical /gamma radiation and purification by adsorption.

1.11. Organisation of the thesis

The thesis is divided into eight chapters.

In Chapter 1, a general introduction on natural rubber and its composition, an overview of the various methods of natural rubber processing and sources of effluent generation are presented. The physical, chemical and biological characteristics of effluent have been discussed in brief. The impact of effluent discharge on receiving waters and the present treatment practices in natural rubber processing units have been described. The scope and objectives of the present work are defined in this chapter.

In Chapter 2, the methods for characterisation of acidified skim serum effluent from latex centrifuging units are described.

Chapter 3 is divided into two parts: Part A and Part B. Part A delineates the results of coagulation studies using five metal coagulants. The coagulants used are potash alum, ferric alum, aluminium sulphate, aluminium chloride, and ferrous sulphate. The efficiency of metal coagulants was evaluated in terms of the removal of turbidity, COD, total Kjeldahl nitrogen, ammoniacal nitrogen and phosphate.

Part B explains the results of coagulation studies using three natural polyelectrolytes and two synthetic polyelectrolytes. The natural polyelectrolytes used are drum stick seed powder, goose-berry powder and tamarind seed powder. The synthetic polyelectrolytes used for the study are polyacrylamide based cationic and polyamine based anionic polyelectrolytes. The effectiveness of the polyelectrolyte as primary coagulant and coagulant aid has been evaluated.

Chapter 4 is divided into two parts: Part A and Part B. Part A deals with the anaerobic treatment of skim serum effluent using an upflow anaerobic sludge blanket (UASB) reactor at various hydraulic retention times and different organic loadings. The effectiveness of the reactor has been evaluated in terms of the removal of COD, BOD, pH, volatile fatty acid, total solids, dissolved solids, volatile suspended solids, total Kjeldahl nitrogen, ammoniacal nitrogen, phosphate, and heavy metals. The formation of sulphide and biogas also has been assessed. Biochemical parameters like soluble proteins, amino acids, sugars and phenols have been applied to confirm the efficiency of the UASB reactor.

Part B deals with the effect of two different concentrations of trace metals like cobalt, nickel and molybdenum individually and in combination on the anaerobic treatment of skim serum effluent using batch type upflow anaerobic sludge blanket reactor. The influence of these trace metals was assessed in terms of variation in pH, sulphide, turbidity, COD, BOD, total Kjeldahl nitrogen and ammoniacal nitrogen.

The effect of anodic oxidation of raw and anaerobically treated skim serum effluent using different electrodes like aluminium, stainless steel, mild steel and cast iron is explained in Chapter 5. The efficacy of solar radiation using photovoltaic cell as well as the presence of Fenton's reagent in treating the effluents has also been explained. The effects of operating factors such as supporting electrolyte, solar radiation, direct current, pH, pollutant concentration of the effluent, duration of electrolysis, and presence of chemical oxidant on the removal of COD, BOD, total Kjeldahl nitrogen (TKN), ammoniacal nitrogen (AN), turbidity, sulphides and phosphates were investigated. The influence of these factors on the biochemical constituents and population of total bacteria was also studied.

Chapter 6 describes the results of the study carried out to purify anaerobically and electrolytically treated skim serum effluent using different adsorbents. Adsorbents like commercial activated carbon and activated carbon prepared from different natural sources like rice husk, coconut shell, sawdust, furnace carbon black and silica based adsorbents like clays and nanoclays (bentonite and montmorillonite) were used. The effectiveness of treatment was evaluated in terms of the removal of COD, BOD, TKN, AN, and phosphate along with the biochemical and microbiological parameters.

Chapter 7 gives an account of the investigations using gamma radiation for the treatment of raw and anaerobically treated skim serum effluent at various pH, radiation dose and concentrations of Fenton's reagent. The treatment efficiency was evaluated in terms of effective organic contaminant decomposition as indicated by the measurements of COD, BOD, turbidity, total Kjeldahl nitrogen (TKN), ammoniacal nitrogen (AN), total solid (TS), dissolved solid (DS) and sulphide. The impact of these factors on the biochemical constituents like protein, free amino acids, phenol, total sugar, reducing and non-reducing sugars and population of total bacteria were also studied.

Finally the Chapter 8 summarises the results and conclusions of the work done.

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CHAPTER 2

CHARACTERISATION OF SKIM SERUM EFFLUENT

2.1. Introduction

Centrifuging involves separation of field latex into two fractions, one containing the concentrated latex of more than 60% of dry rubber and the other containing 4-6 % dry rubber, using a centrifuging machine. To recover the rubber from the skim latex containing ammonia, latex is coagulated using 98% sulphuric acid and the serum left out is drained off. This is known as **skim serum effluent**. It is collected in skim rubber traps for recovery of rubber before going to wastewater treatment. Apart from the skim serum, the water used for cleaning the latex storage tanks once a week, washing the barrels and floor and that for washing the bowls of the centrifuging machine twice in a shift form part of the effluent. The washings are collected in separate rubber trap for rubber recovery. The water requirement in centrifuging process is in the range of 4-6 litres per kg of dry rubber as latex concentrate. Since the serum left out after coagulation of the skim latex is discharged, together with the water used in the processing, the quantity of effluent discharged is much higher than the quantity of water consumed for the processing[1]. The skim serum effluent contains significant amount of non-rubber particles like proteins, sugars, lipids, carotenoids and organic and inorganic salts originating from the latex and very little amount of uncoagulated latex[2]. These constituents are excellent substrates for the proliferation of micro organisms, which generate high BOD and obnoxious odour.

To develop an effective treatment scheme, it is necessary to have an exhaustive knowledge about the basics of physico-chemical, biochemical and bacteriological characteristics of the effluent. This chapter delineates the evaluation of the characteristics of skim serum effluent. The methods used for the characterisation are also included in this chapter.

2.2. Experimental

The samples used for the study and the methods followed for analyses are explained in the following section.

2.2. A. Sample collection

The skim serum effluent samples were collected from a centrifuge latex concentration unit in Kottayam district of Central Kerala. The sources of effluents from a centrifuging unit are shown in Fig.1.4

Ammoniated field latex containing 30 to 33% rubber is concentrated by centrifuging to 60% rubber and skim latex containing 4-6 percent rubber. High speed centrifuges are used in this process. The concentrated latex is stored in drums and marketed. The skim latex, which contains about 0.8 per cent ammonia, is coagulated with 98% sulphuric acid to recover rubber. The **skim serum** produced after coagulation of rubber is stored in a separate trap. Samples of the serum were collected at periodical intervals for the study.

2.2.B. Physico-chemical parameters of the effluent

The physico-chemical characteristics of the effluent samples were analysed as per standard methods[3] for the following parameters

1. pH
2. Turbidity
3. Chemical oxygen demand (COD)
4. Biochemical oxygen demand (BOD)
5. Sulphates
6. Total solids
7. Total dissolved solids (TDS)
8. Total suspended solids (TSS)
9. Total Kjeldahl nitrogen (TKN)
10. Ammoniacal nitrogen (AN)
11. Phosphate
12. Volatile fatty acids (VFA)
13. Sulphide
14. Nitrate
15. Free ammonia
16. Oil and grease
17. Chloride

Procedure

Determination of chemical oxygen demand

The chemical oxygen demand (COD) is a measure of oxygen equivalent of that portion of organic matter in a sample that is susceptible to oxidation by a strong chemical oxidant. Most types of organic matter are destroyed by boiling the mixture of chromic acid and sulphuric acid. The sample was refluxed with a known amount of potassium dichromate and sulphuric acid along with silver sulphate as catalyst, and the excess dichromate was titrated with ferrous ammonium sulphate using ferroin indicator to get a sharp end point. The straight-chain compounds were oxidized more effectively when silver sulphate was used as catalyst. Mercuric sulphate was added to the samples before refluxing to mask chlorides. The amount of the organic matter, measured as oxygen equivalent, was proportional to the potassium dichromate consumed. A blank (with out sample) with distilled water and all other reagents was refluxed in the same manner. The result was calculated from the equation:-

$$\text{COD (mg/L)} = \frac{(a-b)N \times 8000}{v}$$

where a = volume in mL of ferrous ammonium sulphate used for blank.

b = volume in mL of ferrous ammonium sulphate used for sample.

N = normality of ferrous ammonium sulphate, and

v = volume in mL of the sample taken for the test

Determination of biochemical oxygen demand

The biochemical oxygen demand (BOD) determination is an empirical test in which standardised laboratory procedures are used to determine the relative oxygen requirements for polluted waters.

Amount of oxygen demand in the sample will depend on the degree of dilution. It provides a measure of the dissolved oxygen consumed by the aerobic microbiological oxidation of the sample under defined conditions over a specific period. Normally BOD was determined by measuring the loss in dissolved oxygen of the sample by incubating it for five days at 20^0C and has been accepted as standard.

The dissolved oxygen oxidises the manganous hydroxide to manganic hydroxide, which in turn oxidises iodide to free iodine in the acid medium. The iodine thus liberated is determined by titrating with standard sodium thiosulphate using starch as indicator. BOD (5 days at 20^0C) mg/L = $\frac{(D_1 - D_2)}{p}$

where D_1 = Initial dissolved oxygen content (mg/L)

D_2 = Dissolved oxygen content after incubation.

p = decimal fraction of the sample used

Determination of total solids.

Total solids (TS) are the materials left in the vessel after evaporation of a sample and its subsequent drying in an oven at defined temperature. Total solids include non filterable solids and filterable solids.

A well mixed sample was evaporated in a weighed dish and dried to constant weight in an oven at 103^0C to 105^0C. The increase in weight over that of the empty dish represents the total solids.

$$\text{Total solids (mg/L)} = \frac{(A - B) \times 1000}{v}$$

where A = weight in mg of the residue and dish

B = weight in mg of the dish, and

v = volume in mL of the sample taken for the test.

Determination of total dissolved solids

Total dissolved solids (TDS) is the material that passes through a standard glass fiber filter and remains after evaporation, and drying to constant weight at 103^0C to 105^0C.

A well mixed sample was filtered through a standard fiber filter and the filtrate was evaporated to dryness in a weighed dish to constant weight at 103^0C to 105^0C. The increase in dish weight represents the total dissolved solids.

$$\text{Dissolved solids (mg/L)} = \frac{(A-B)\times 1000}{v}$$

where A = weight in mg of dish with dried residue

B = weight in mg of the dish, and

v = volume in mL of sample taken for the test.

Determination of total suspended solids.

The total suspended solids (TSS) are the retained materials on a glass filter after filtration of a well mixed sample. The well mixed sample was filtered through a weighed standard glass fiber filter and the residue retained on the filter was dried to a constant weight at 103^0C to 105^0C. The increase in weight of the filter represents the total suspended solids.

$$\text{Total suspended solids (mg/L)} = \frac{(A-B)\times 1000}{v}$$

where A = weight in mg of the filter with dried residue

B = weight in mg of the filter, and

v = volume in mL of the sample taken for test.

Determination of sulphides

A known quantity of well mixed sample was titrated with an excess amount of acidified iodine solution and the amount of untreated iodine was measured by titrating with a standard thiosulphate solution using starch indicator. A blank (without sample) with distilled water and all other reagents including same quantity of iodine is also to be conducted.

Suiphides (as H_2S) (mg/L) = $\frac{17000(A - B) \times N}{v}$

where A = volume in mL of standard sodium thiosulphate for blank.

B = volume in mL of standard sodium thiosulphate for sample.

N = Normality of thiosulphate solution, and

v = volume in mL of the sample taken for test.

Determination of phosphates

In a dilute orthophosphate solution, ammonium molybdate reacted under acid conditions to form molybdophosphoric acid. In the presence of vanadium the vanadomolybdophosphoric acid yellow colour was formed. The intensity of the colour was proportional to the phosphate concentration in the solution. The developed colour was measured using spectrophotometer at 470 nm. A blank was conducted with distilled water and all the reagents.

The calibration curve was prepared using standard phosphate solution. Phosphates (as P), (mg/L) = $\frac{(w \times 1000)}{v}$

where w = weight in mg of phosphates (P) as read from the calibration curve, and

v = volume in mL of the sample taken for test.

Determination of total Kjeldahl nitrogen

The total kjeldahl nitrogen (TKN) is a measure of organic nitrogen and ammonia.

In the presence of sulphuric acid, potassium sulphate and mercuric sulphate catalyst, amino nitrogen of many organic materials were converted to ammonium sulphate $(NH_4)_2SO_4$. Free ammonia and ammonium-nitrogen also were converted to $(NH_4)_2SO_4$. During digestion mercury ammonium complex was formed and then decomposed by sodium thiosulphate. After decomposition the ammonia was distilled from an alkaline medium and absorbed in boric acid. The amount of ammonia was determined by titrating with standard mineral acid using methyl red-methylene blue mixed indicator for a sharp end point. A blank (without sample) with all the reagents was also conducted.

$$\text{Total Kjeldahl nitrogen (as N) mg/L} = \frac{(A - B) \times N \times 14000}{v}$$

where A = volume in mL of the acid used for sample.

B = volume in mL of the acid used for blank,

N= normality of the acid, and

v = volume in mL of the sample taken for test

Determination of ammoniacal nitrogen

A well mixed sample was buffered at pH 9.5 with a borate buffer to decrease hydrolysis of cynates and organic nitrogen compounds and distilled into solution of boric acid. The ammonia in distilled water was measured by titrating with standard mineral acid using methyl red - methylene blue mixed indicator. A blank (without sample) was also conducted with all the reagents and distilled water

Ammoniacal nitrogen (as N), mg/L = $\frac{(A-B)\times N\times 14000}{v}$

where A= volume in mL of standard sulphuric acid used for sample.

B = volume in mL of standard sulphuric acid used for blank.

N = normality of the sulphuric acid, and

v = volume in mL of the sample taken for the test.

Determination of free ammonia

On heating the free ammonia was released and the same can be got absorbed in boric acid. It was measured by titrating with standard mineral acid using methyl red - methylene blue indicator.

Free ammonia (as N), mg/L = $\frac{(A-B)\times N\times 14000}{v}$

where A= volume in mL of standard acid used for titration.

B = volume in mL of standard sulphuric acid used for blank.

N = normality of the acid, and

v = volume in mL of the sample taken for the test.

Determination of nitrate nitrogen

The effluent sample was treated with sodium salicylate in an acid medium, the mixture was made alkaline and the yellow colour obtained was measured using a spectrophotometer at 420 nm. A blank (without sample) with all the reagents and distilled water was also conducted.

A calibration curve is determined with standard nitrate solution.

Nitrate nitrogen (as N) mg/L = $\frac{w}{v}$

w = weight in mg of nitrogen (as N) as read from the curve, and

v = volume in mL of the sample taken for test.

Turbidity was measured using digital Nephelo Turbidity Meter (Range1-1000, No.24), pH was determined using digital pH meter 335-systronics and phosphate by using Geneys 20 spectrophotometer (Thermo Electron corporation, Thermospectronic).

Heavy metals were estimated using Atomic Absorption Spectrophotometer (AAS, Model-Avanta., GBC.) at Rubber Research Institute of India (RRII), Kottayam.

2.2. C. Biochemical analysis

(1) Estimation of soluble protein

Protein was estimated by Lowry's method[4]

In this method folin ciocalteus phenol reagent is reduced by the copper treated protein giving a blue colour. The colour yield of this reaction is considered to arise mainly from the tyrosine, tryptophan and phenyl alanine and to some extent from the sequence of certain amino acids bearing functional side group such as arginine, glutamic acid and histidine. The method is sensitive and there is negligible interference from non-protein substance.

Reagents.

A: - 2% Na_2CO_3 in 0.1N NaOH;

B: - 0.5% $CuSO_4$ in 1% Rochelle salt (sodium potassium tartarate)

C:- Alkaline copper reagent was prepared on the day of use by mixing 50 mL A + 1mL B

D:- Folins' reagent: - 1: 2 diluted

E:- 10 % Trichloro acetic acid (TCA)

BSA: Albumin standard- 10 mg Bovine serum albumin in 20 mL of 0.1N. NaOH

Procedure: 0.2, 0.5 mL aliquots were taken from the sample. An equal volume of 10% TCA was added and kept over night in refrigerator. The tubes were centrifuged at 5000 rpm for 30 minutes, decanted and the precipitate was dissolved in 1 mL of 0.1 N NaOH. 5 mL of reagent C was added to the above solution and kept for 10 minutes. Then 0.5 mL diluted Folins' reagent was added, and the optical density was measured at 660 nm in a UV spectrophotometer after keeping it for 30 minutes. A blank containing 1mL of 0.1N NaOH, 5mL of reagent C and 0.5 mL diluted Folins' reagent was also prepared along with the sample. BSA was used as standard.

(2) Estimation of total sugars

This method was suggested by Scott and Melvin[5]

Reagents:

1. 0.1 g Anthrone was dissolved in a mixture of 29 mL water and 100 mL concentrated H_2SO_4 under ice cold conditions and kept in ice bath.
2. Sucrose (standard)

Procedure: To 0.1 mL sample, 0.4 mL 2.5% TCA was added and kept the tubes in ice bath and then added 3 mL cold anthrone reagent. Standards were also treated in the same way. It was heated in boiling water bath for

15 to 20 minutes and cooled in an ice bath. The absorbance was measured at 627 nm in a UV spectrophotometer. Sucrose was used as standard.

(3) Estimation of reducing sugars

Reducing sugar was estimated according to the procedure suggested by Nelson[6]

Reagents:

Alkaline copper reagent: - copper reagent A and B in the ratio 25: 1 by volume

Copper reagent A: - 25 g Na_2CO_3, 25 g sodium potassium tartarate, 20 g $NaHCO_3$ and 200 g Na_2SO_4 were accurately weighed and dissolved in 800 mL water and made up the volume to 1000 mL.

Copper reagent B: - 15% $CuSO_4$ in water containing 1 or 2 drops of conc. H_2SO_4. Copper reagent was prepared on the day of use by mixing reagent A and reagent B in the proportion 25:1 by volume

Arsinomolybdate: - 25 g ammonium molybdate in 450 mL water was prepared. Added 21 mL conc.H_2SO_4 and mixed well. Added 3 g sodium arsenate dissolved in 25 mL water and added to the above solution. The solution was mixed well, filtered and kept at 37^0C. Glucose was used as standard.

Procedure:- Pipetted out 0.5 mL aliquot into a test tube and evaporated it completely. 0.5mL distilled water and 1mL cupper reagent (freshly prepared 25 mL A and 1mL B) were added to this and heated on boiling water bath for 20 minutes. It was cooled and added 1mL arsenomolybdate and kept for 15 minutes to develop colour and then made up the volume to 12.5 mL. Optical density was measured at 520 nm in a UV spectrophotometer. A blank with

1mL of distilled water and all the reagents were also prepared along with the sample. Concentration of non reducing sugar was estimated from the difference between total sugar and reducing sugar.

(4) Estimation of amino acids

Amino acids were estimated according the procedure described by Moore and Stein[7]

Ninhydrine method:- When amino acids are heated with ninhydrine, they are quantitatively deammoniated and a blue colour appears. The blue coloured substance (Ruheman's purple) is formed by the reaction of some of the ninhydrine with its reduction product hydrin and ammonia. Optical density was measured at 570 nm in a UV spectrophotometer. Leucine was taken as the standard.

Reagents

1. 0.2 g of reagent grade $SnCl_2.2H_2O$ was dissolved in 125 mL citrate buffer. Added this solution to 5 g of ninhydrine dissolved in 125 mL of methyl cellosolve.
2. 0.2 M. citrate buffer - pH 5. 10.507 g of reagent grade citric acid monohydrate was dissolved in 100 mL of 1N NaOH and diluted to 250 mL.
3. Diluent solvent. It was prepared by mixing equal volumes of water and reagent grade n-propanol.

Procedure: - Added 1 mL of ninhydrine reagent to 0.5 mL sample and standard solutions. Mixed well and heated for 20 minutes in boiling water bath. 5 mL of diluent was added to each tubes and the content were mixed and optical density was measured at 570 nm after 15 minutes. A blank was also prepared along with the sample. The colour is stable for at least one hour.

(5) Estimation of phenol

Phenol was estimated according the procedure described by Swain and Hills[8]

Reagents: 1N Folins' reagent and saturated Na_2CO_3.

Procedure: - To 0.5 mL sample 0.5 mL distilled water was added to make the volume to 1 mL. To this solution 0.5 mL 1N Folins' reagent and 1 mL saturated Na_2CO_3 were added, made the volume to 5 mL with distilled water. The mixture was incubated for 1 hour and optical density was measured at 725 nm in a UV spectrophotometer. A blank was also prepared along with the sample. Catechol was used as standard.

The Spectrophotometer used for measuring optical density is UV–visible Recording spectrophotometer, UV-240.

(6) Total lipids

Total lipids were extracted according the procedure described by Bligh and Dyer[9]. 100 mL of the effluent sample was concentrated by evaporation on a water bath at 100^0C. To this 25 mL of methanol was added and kept over night. Then 50 mL of chloroform was added and shaken well. It was then filtered to a stoppered conical flask and washed with chloroform and methanol (2:1, 2 mL each) at least three times. To the filtrate distilled water was added (20% of total volume) and allowed to stand overnight at 4^0C for phase separation. The aqueous layer was removed with the help of a Pasteur pipette. Bottom layer was evaporated to reduce the volume, transferred it to a previously weighed beaker using 5mL of solvent and evaporated to dryness. The beaker was kept in a vacuum dessicator over KOH until constant weight was obtained. The difference between the two weights was taken as the weight of total lipids present. The process was duplicated.

2.2. D. Microbiological analysis

The population of total bacteria in the raw effluent was enumerated using appropriate media. The standard serial dilution plate technique of Pramer and Schmidt[10] was employed for the enumeration of microbiological population. The sample of wastewater to be tested was diluted serially. A small amount of each dilution (1mL) was then mixed with warm agar liquid culture medium poured into a culture dish, allowed to solidify, and incubated under controlled conditions. The separate distinct bacterial colonies formed on the plates after incubation was counted, and the results reported as colony-forming units (cfu) per unit volume of the sample. The total number of bacteria was determined using appropriate dilutions[11].

The medium was prepared as follows:- peptone - 5 g, glucose - 5g, NaCl - 5g, beef extract - 3g, agar - 15g, water - 1 litre, pH - 6.8. All these compounds were accurately weighed and dissolved in one litre of water. It was then transferred to (250 mL each) 4 conical flasks, corked and kept in autoclave for 1 hour.

2.3. Results and discussion

2.3. A. Physico-chemical characteristics

Table 2.1 shows the values of physico-chemical characteristics of skim serum effluent collected at various intervals of time and their average values. It is apparent from Table 2.1 that the values in most cases vary widely. The average of these values is shown in column (8).

Table 2.1 Physico-chemical characteristics of skim serum effluent from latex centrifuging units

No	Parameters	Effluent 1	Effluent 2	Effluent 3	Effluent 4	Effluent 5	Average values
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
1	pH	3.8	4.15	4.3	4.7	3.6	4.11
2	Turbidity (NTU)	140	170	160	153	157	156
3	COD	27500	37216	27000	27500	38800	31603
4	BOD	10500	22500	14000	13500	23280	16756
5	Sulphates	16500	17000	17000	16000	17000	16700
6	TS	69000	70000	59000	59000	60000	63400
7	TDS	67500	65000	56000	56000	58100	60600
8	TSS	1500	5000	3000	3000	1900	2800
9	VSS	1470	4170	2950	2965	1770	2745
10	TKN	7000	11000	7000	8000	7000	8000
11	AN	2500	5000	2500	4500	5000	3900
12	Phosphate	1400	1500	3000	4000	2900	2560
13.	VFA	1300	1500	1600	2000	1200	1520
14.	Sulphide	20.00	31.00	25.00	25.00	14.00	23
15	Nitrate	Trace	Trace	Trace	Trace	Trace	Trace
16	Oil and Grease	5.5	19.5	17.5	Trace	24	16.625
17	Chloride	55.5	56	69	60	Trace	60.125

(All values expect pH and turbidity are expressed in mg/L)

The general trend observed for various physico-chemical parameters of skim serum effluent are discussed below.

(a) pH

The pH values of skim serum effluent varied between 3.6 and 4.7 with an average value of 4.11. This shows that serum effluent is highly acidic and the high acidity of the effluent can be attributed to the use of sulphuric acid for the coagulation of skim latex.

(b) Organic matter

Organic compounds are normally made up of a combination of carbon, hydrogen, oxygen and nitrogen. COD is used to measure the oxygen equivalent of the organic material in wastewater that can be oxidised chemically using dichromate in acid solution. BOD determination involves the measurement of dissolved oxygen used by microorganisms in the biochemical oxidation of organic matter.

The organic matter in serum effluent was estimated in terms of COD and BOD. The COD and BOD values of serum effluent were in the range of 27000-38800 mg/L and 10500-23280 mg/L respectively. The average values of COD and BOD were 31603 and 16756 respectively. These figures indicated the presence of high concentration of organic compounds in the serum effluent. The high pollutant effect of serum effluent was mainly due to its high organic nature. The relationship between BOD and COD is shown in the Table 2.2. Typical values for the ratio BOD/COD for untreated municipal wastewater are in the range from 0.3 to 0.8. If the BOD/COD ratio for untreated wastewater is 0.5 or greater, the waste is considered to be easily treatable by biological process. If the ratio is below 0.3 the waste may have some toxic components in it and acclimated microorganism may be required for its stabilization. For the final effluent the ratio should be 0.1 to 0.3[11].

Table 2.2. Relationship between BOD and COD

Sample No.	1	2	3	4	5	Average value
BOD/COD	0.38	0.49	0.51	0.6	0.6	0.516

The average value of BOD/COD ratio of the serum effluent is 0.516 which shows that major pollutant is organic in nature and could be removed by biological methods.

(c) **Solids**

The solid content of the wastewater is expressed in terms of turbidity, total solids, dissolved solids, total suspended solids and volatile suspended solids.

Measurement of turbidity is based on the comparison of the intensity of light scattered by a sample to the light scattered by a reference suspension under the same conditions. The results of turbidity are reported as nephelometric turbidity (NTU) [11]. The turbidity values of the serum effluent varied between 140 and 170 NTU with an average value of 156 NTU. The turbidity value shows that serum effluent does not contain too much colloidal or residual suspended matter.

TS is obtained by evaporating a sample of wastewater to dryness at 100-105^{0}C and measuring the mass of residue. Serum effluent contains 59000-70000 mg/L of TS with an average value of 63400 mg/L. TDS by definition, is the solids contained in the filtrate that passes through a filter with a nominal pore size of 2.0 μm or less[3]. The TDS content in serum effluent was in the range of 56000-68000 mg/L having an average value of 60600 mg/L. This shows that the serum effluent from the latex concentration unit contains high concentration of dissolved solids and the major fraction of total solids are in the dissolved form which may vary from 92.8 to 98.5 per cent of the total solids showing an average value of 95.5 percent. The high concentration of TDS indicates the presence of high amount of organic matter in the wastewater[2]. TSS is the portion of TS retained on a filter with a specified pore size, measured after drying at a specified temperature of 105^0C. It is somewhat arbitrary because it depends on the pore size of the filter paper used for the test. TSS of serum effluent was in the range of 1500-5000 mg/L and the average value was 2880 mg/L. Volatile suspended solids (VSS) are those solids that can be volatilized and burnt off when the TSS are ignited at 500 to 550^0C and are considered as organic matter. VSS of serum effluent was in the range of 1470-4170 mg/L and the average value was 2745 mg/L. The major fraction of total suspended solid is considered as organic matter since 95.3 % of total suspended solid is volatile in nature.

(d) **Nitrogen**

TKN content of serum effluent varied between 7000 mg/L and 11000 mg/L with an average value of 8000 mg/L whereas the AN content varied between 2500 and 5000 mg/L with an average value of 3900 mg/L. At pH levels below 7, ammonium ion is the predominant species whereas at pH above 7, the predominant species is free ammonia. Therefore, ammonia is determined by raising the pH and distilling off the ammonia into a solution of boric acid and estimated titrimetrically with standard sulphuric acid.

The TKN includes ammonia and organic nitrogen but does not include nitrite and nitrate nitrogen. Hence, the TKN content monitored in the samples include the ammoniacal nitrogen and organic nitrogen present in the proteinaceous matter which is a constituent of natural rubber latex. The presence of ammoniacal nitrogen in the effluent is attributed to the fact that the field latex is preserved with ammonia to prevent coagulation and microbial action. From Table 2.1, it is evident that the ammoniacal nitrogen content of the effluents from the skim serum section is very high. The skim which is coagulated with sulphuric acid contains about 0.8 per cent ammonia and this should be the reason for the higher ammoniacal nitrogen content in the skim serum effluent.

(e) **Phosphates**

The usual forms of phosphates that are found in aqueous solutions include orthophosphate, polyphosphate and organic phosphates. Phospholipids in the original latex get hydrolysed into phosphates. Dihydrogen orthophosphate is also added during processing of rubber latex. The total concentration of phosphate in the serum effluent was in the range of 1400 to 4000 mg/L and its average value was 2560 mg/L.

(f) **Volatile fatty acids (VFA)**

Concentration of VFA was in the range of 1200 to 2000 mg/L and the average value is 1520 mg/L. For soluble and easily degradable substrates, such as sugars and soluble starches, the acidogenic reactions can be much faster and may increase the volatile fatty acid and hydrogen concentrations and depress the pH[11].

(g) **Sulphates and sulphides**

The concentration of sulphate is very high since sulphuric acid is added to coagulate skim latex. Sulphur is also required in the synthesis of proteins and is released during their degradation. Sulphate content of serum varied between 16000-17000 mg/L and with an average value of 16700 mg/L. Sulphate is gradually converted to sulphides in anaerobic conditions which in turn combine with hydrogen to form hydrogen sulphide. Concentration of sulphide in the serum effluent was 14 to 31 mg/L and the average value was 23 mg/L.

(h) Oil and grease

Oil and grease includes fats, oils, waxes and other allied constituents. It was estimated by extracting with petroleum ether. The concentration of oil and grease in serum effluent ranges from trace amount to 24 mg/L. The average value was 16.625 mg/L.

(i) Nitrate and chloride

Concentration level of nitrate was not significant enough to detect. Chloride concentration in the serum effluent varied between trace to 69 mg/L and the average value was 60.125 mg/L. Very small amount of chlorine is associated with natural rubber latex and the rest may originate during processing.

2.3.B. Metallic constituents

Many metals in trace quantities are necessary for the growth of biological life and the absence of sufficient quantities of these could limit biological growth. But higher concentration of these will lead to toxicity[11, 13]. The concentration of metals is high in the effluent compared to the original serum[14]. High concentration of these metals may come from the processing of rubber latex since preservatives like TMTD-zinc oxide (Tetramethyl thiuram disulphide) are added in addition to ammonia. Serum effluent contained 7.099 ppm of iron, 0.025 ppm copper, 204.5 ppm zinc, 0.037 ppm manganese, 3.44 ppm calcium and 1.61 ppm magnesium (Table 2.3) when analysed using atomic absorption spectrophotometer (AAS).

Table 2.3 Concentrations of metal ions estimated using AAS

Element	Concentration in ppm
Fe	7.099
Cu	0.025
Zn	204.500
Mn	0.037
Ca	3.440
Mg	1.610

2.3.C. Biochemical constituents

Biochemical analysis of serum effluent showed the presence 865 mg/L of proteins (soluble), 589 mg/L phenol, 1250 mg/L total sugars, 1095 mg/L reducing sugar, 155 mg/L non-reducing sugar, 15952 mg/L free amino acid and 540 mg/L lipids (Table 2.4). The high concentration of proteins, sugars and amino acids contributes to the pollutant effect.

Table 2.4 Biochemical constituents of serum effluent

Parameters	Concentration in mg/L

Soluble protein	865
Phenol	586
Total sugar	1250
Reducing sugar	1095
Non-reducing sugar	155
Free amino acid	15952
Lipids	540

2.3.D. Bacteriological properties

The total viable bacterial population present in skim serum effluent was determined by pour and spread plate method. This method was used to culture, identify, and enumerate bacteria[11]. The colony forming units per unit volume (cfu/mL) of the sample is 22 x 10^4. The high acidity of the effluent from latex concentrate units adversely affects growth of bacteria and hence the bacterial population is comparatively low[15].

2.4. Conclusion

- The skim serum effluent discharged from a latex centrifuging unit is strongly acidic in nature. The average pH value of the effluent is 4.11. The high acidity is due to the use of sulphuric acid in skim latex coagulation.
- The average values of COD and BOD are 31603 mg/L and 16756 mg/L respectively. The average value of BOD/COD ratio is 0.516 which shows that the major pollutant in the serum effluent from

latex centrifuging unit is organic in nature which could be removed by biological methods.

- The turbidity values of the serum effluent (average value of 156 NTU) shows that serum effluent does not contain too much colloidal or residual suspended matter. The average value of TDS content in serum effluent is 60600 mg/L showing that the serum effluent from latex concentration unit contain high amount of dissolved solids. A major fraction of total solids is present in the dissolved form showing an average value of 95.5 per cent of total solids.
- The average value of TKN content of serum effluent is 8000 mg/L whereas the AN content is 3900 mg/L. The TKN includes ammonia and organic nitrogen.
- Phosphates, VFA, sulphates and sulphides, oil and grease, nitrate and chloride were also estimated. The concentration of sulphates is very high (16700 mg/L) since sulphuric acid is added to coagulate skim latex.
- Metallic constituents of serum effluent were also estimated using AAS. It contains 7.099 ppm of iron, 0.025 ppm copper, 204.5 ppm zinc, 0.037 ppm manganese, 3.44 ppm calcium and 1.61 ppm magnesium.
- Biochemical analysis of serum effluent shows the presence 865mg/L of soluble protein, 586 mg/L phenol, 1250 mg/L total sugar, 1095 mg/L reducing sugar, 155 mg/L non-reducing sugar, 15952 mg/L free amino acid and 540 mg/L lipids. High

concentration of proteins, sugars and amino acids contributes to its pollutant load.

- The colony forming bacterial units per unit volume (cfu/mL) of the sample is 22 x 10^4. The high acidity of the effluent from latex concentrate units adversely affects the growth of bacteria and hence the bacterial population is comparatively low.

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CHAPTER 3

TREATMENT USING COAGULANTS

PART A:
METAL COAGULANTS

3. A. 1 Introduction

A viable pre-treatment technology for reducing the pollution problems is the physico-chemical treatment using conventional coagulants and this has been successfully applied to various wastewaters. This process transforms the dissolved contaminants into an insoluble solid, facilitating the contaminants' subsequent removal from the liquid phase by sedimentation or filtration. This process usually uses pH adjustment, addition of a chemical precipitant and flocculation. Precipitation, coagulation/flocculation and sedimentation are consecutive treatments to the same stream in wastewater treatment process. Precipitation removes a substance in solution and transforms it into a second phase, often in the form of solid particles that may be small or even colloidal. Coagulation transforms small suspended particles into larger suspended particles so that they can be more easily removed. Sedimentation removes the suspended particles from the liquid [1].

When particles in the liquid suspension are in the low or sub-micron range they either remain in suspension or settle too slowly for subsequent filtration. Unless individual primary particles are aggregated into larger units appreciable settlings cannot be observed[2]. Aggregation may result from one or more of the following mechanisms[3]. (a) Charge neutralization by double layer (b) The bridging action by added materials of high molecular weight (c) Entrapment of smaller particles within a loose fibrous structure of a floc formed.

In the precipitation process, chemical precipitants, coagulants and flocculants are used to increase particle size through aggregation. The precipitation process can generate very fine particles that are held in suspension by electrostatic surface charges. These charges cause clouds of counter-ions to form around the particles, giving rise to repulsive forces that prevent aggregation and reduce the effectiveness of subsequent solid-liquid separation processes. Therefore, chemical coagulants are often added to overcome the repulsive forces of the particles. The three main types of coagulants are inorganic electrolytes (such as alum, lime, ferric chloride and ferrous sulphate), organic polymers, and synthetic polyelectrolytes with anionic or cationic functional groups. Coagulation is a process in which colloidal and suspended particles, organic matter imparting turbidity and colour, and bacterial and algal cells are removed efficiently by a group of chemicals known as 'coagulants'. Coagulation is the term used to describe the aggregation of very small particles, usually colloidal in size, often encountered in water and wastewater solid-liquid separation process[4]. Coagulation may be defined as the process in which chemicals are added to an aqueous system to create rapidly settling aggregates out of colloidal matter present[5]. The addition of coagulants is followed by low-shear mixing in a flocculator to promote contact between particles that coalesce or flocculate, during sedimentation operation. As coalescence or flocculation occurs, the particles increase in mass and settle at a faster rate.

3. A. 2. Fundamentals of coagulation

(a) General

Colloidal dispersions in water consist of discrete particles held in suspension by their extremely small size (1- 200 nm), state of hydration and surface electrical charge. Surface charge develops most commonly through preferential adsorption, structural imperfections, ionization, and isomorphous replacement[6]. To settle easily, it is necessary to overcome the stability of colloidal dispersion through aggregation into larger particles. According to Stokes' law, particles will settle far more quickly the heavier they are. Colloids can be divided into two:-hydrophilic and hydrophobic. Hydrophilic colloids are readily dispersed in water, and their stability depends upon a marked affinity for water rather than upon the slight negative charge that they possess. Hydrophobic colloids have no affinity for water and owe their stability to the electric charge they possess. Metal oxide colloids, most of which are positively charged are examples of hydrophobic sols. A charge on the colloid is gained by adsorbing positive ions from the water solution[7]. Electrostatic repulsion among the charged colloidal particles produces a stable sol.

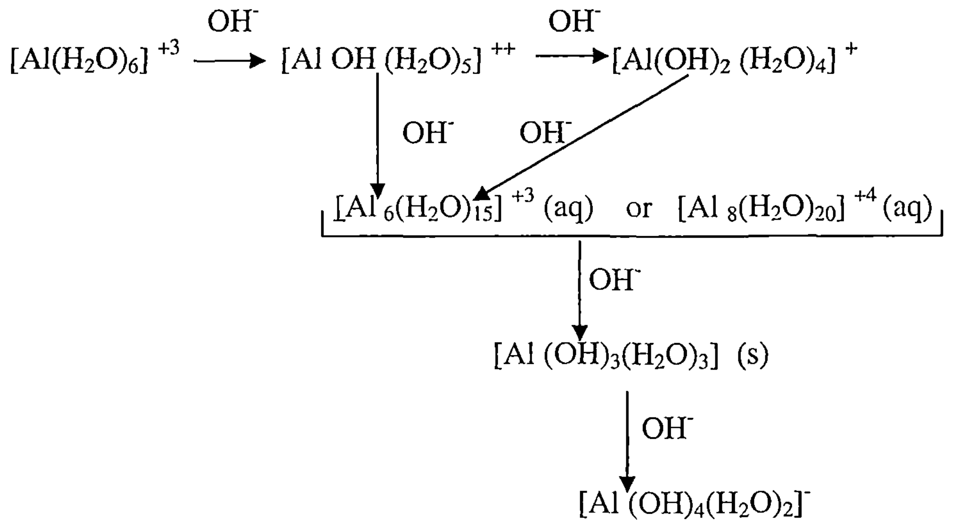
A colloidal suspension is said to be stable when the dispersion shows little or no tendency to aggregate. The repulsive force of the charged double layer disperses particles and prevents aggregation, thus particles with high zeta potential produce a stable sol. Factors tending to destabilize a sol are van der Waals' forces of attraction and Brownian movement[8]. van der Waals' forces are the molecular cohesive forces of attraction that increase in intensity as particles approach each other. These forces are negligible when the particles are slightly separated but become dominant when they come in contact. Brownian movement has a destabilizing effect on a sol because aggregation may result.

Destabilization of hydrophobic colloids can be accomplished by adding electrolytes to the solution. Counter ions of the electrolyte suppress the double layer charge of the colloids and lower the zeta potential permitting the particles to contact closely[9]. Upon meeting, van der Waals' forces of attraction become dominant and aggregation results. The most effective electrolytes are multivalent ions of opposite charge to that of the colloidal particles. As most of the counter ions in aqueous systems are positively charged, destabilization is done in practice by the use of aluminium and iron salts. Highly charged hydrolysis products of these metal salts reduce the repulsive forces between the colloids by compressing the double-layer charge, bringing in coagulation. Hydrolysed metal ions are also adsorbed on the colloids, creating bridges between the particles.

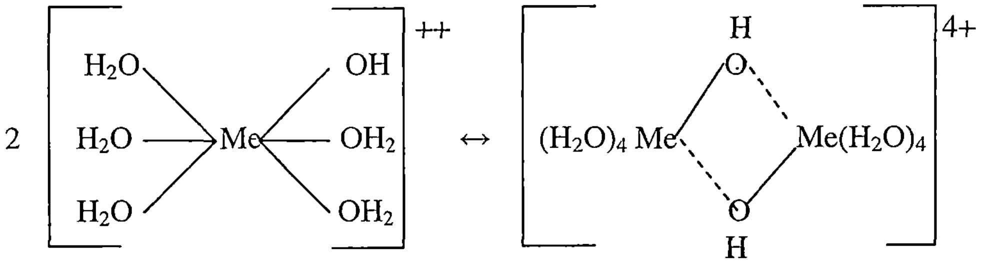
Colloids in domestic wastes or inorganic wastes are hydrophilic[10]. Their affinity for water arises from the presence of polar groups like -OH, -COOH, and $-NH_4$ on the particle surface. These groups are water soluble. Thus they acquire a sheath of water firmly around the particle. The primary charge on hydrophilic colloids arises from the ionization of the chemical groups present at the surface of the particles. Extent to which these surface groups ionize, determines the charge: and therefore, charge is pH dependent[11]. Most of the particles in wastewaters are stabilized by negative charges. Therefore, salts of polyvalent cations such as Al^{+3}, Fe^{+2}, Fe^{+3} and Ca^{+2} are used as coagulants in conformity with the Schulze Hardy rule[12].

(b) Hydrolysis of metal salts

When salts of metals of like aluminium or iron are added to water or wastewater, they hydrolyse into polyvalent cations Al^{+3} and Fe^{+3} which reduce the zeta potential and facilitate coagulation. In the past it was thought that free Al^{+3} and Fe^{+3} were responsible for the effects observed during particle aggregation; however it is now known that their complex hydrolysis products are responsible[13, 14]. A typical hypothetical model for Al^{+3} is shown below[15]. A number of alternative formation sequences have also been proposed[1, 16]



Before the reaction proceeds to a point where a negative ion is produced, polymerization as depicted in the following equation will usually take place[17].



$+ 2\,H_2O$

The complex compounds given above are known as coordination compounds, which are defined as a central metal ion attached to a group of surrounding molecules or ions by coordinate covalent bonds. The metal ions are called ligands, and the atoms attached directly to the metal ion are called ligand donor atoms[18]. A number of coordination compounds are also amphoteric in that they can exist both in acids and in strong bases[18]. In acid solutions aluminium hydroxide forms Al^{+3} and in basic solutions it forms $Al(OH)_4^-$

Amritharajan and Mills[19], Stumm and Morgan[13], Stumm and O'Melia[14] provided a good overview of the subject. The metal ions such as Fe (III) and Al (III) are hydrolyzed in water and wastewater and present as aqua complexes. It was thought that numerous hydrolysis intermediates are formed prior to the precipitation of aluminium hydroxide or iron hydroxide like $Al(OH)^{+2}$, $[Al_8(OH)_{20}]^{+4}$, $[Al_6(OH)_{15}]^{+3}$, $Al_{13}(OH)_{34}]^{+4}$, $[Al_7(OH)_{17}]^{+4}$, $Fe(OH)^{+2}$, $Fe(OH)_2^+$, $Fe(OH)_3$. Some of these are monomeric and dimeric hydroxo-complexes and they have a dramatic role in the interaction of colloids. One or more of the hydrolysis processes may be responsible for the coagulation action of metal salts. These hydrolysis reactions are sensitive to pH and hence pH may have to be adjusted[20]. Laboratory analysis can be performed to find out the concentration of metal salt just sufficient to cause coagulation in solutions of various pH. These critical values can then be plotted as boundaries between stable and unstable zones, thus establishing domains of stability[21]. The coagulation region defines the conditions of pH and coagulant concentrations that produce rapid clarification. Turbidity removal in water treatment practice is accomplished by applying the coagulant dosage within the region where it is most efficient. Several investigations have indicated that the most important parameters to be considered in coagulation are pH, concentration of metallic ions and electrolyte concentration [22, 23].

Stumm and O' Melia[14] have defined coagulation as a time-dependant process including the following reaction steps:

1. hydrolysis of multivalent metal ions and subsequent polymerization to multinuclear hydrolysis species;
2. adsorption of hydrolysis species at the solid-solution interface to accomplish destabilization of the colloids;
3. aggregation of destabilized particles by interparticle bridging involving particle transport and chemical interactions;
4. aggregation of destabilized particles by particle transport and van der Waals' forces;
5. "ageing" of flocs, accompanied by chemical change in the structure of metal-OH-metal linkages, concurrent change in the floc sorbability and in the extent of hydration ; and
6. precipitation of metal hydroxide.

Some of these steps occur sequentially, some overlap (step 1 and 2), and several may occur concurrently under certain conditions (steps 3 and 4 or step 6 with steps 1-5).

Several studies have been reported on the use of metal coagulants in the treatment of industrial wastewaters. In some cases sedimentation and precipitation are the only treatments to which the wastewater is subjected[20]. The study revealed that the coagulant demand depends on characteristics of wastewater such as pH, solids content, phosphates etc. Loehr[24] reported that the reduction of BOD, COD and suspended solids of agricultural wastewaters is a function of the amount and types of chemical coagulant added, pH of the effluent and type of wastewater. Smith[25] has shown that most of the suspended solids and part of the BOD are removed by the addition of alum or ferric chloride with or without polyelectrolyte. Weber[26] states that coagulation of wastewater can be accomplished by any of the coagulants including lime, iron and aluminium salts and synthetic polymers. The choice is to be made on the basis of suitability, cost and sludge treatment and disposal considerations.

Several coagulants like alum, ferric chloride and ferrous sulphate have been tried for the chemical coagulation of tannery effluents[27]. Ferrous sulphate is reported to be the best coagulant for the removal of sulphides and may be used for the effective removal of colour, BOD and suspended solids from chrome-tan wastes. Kothandaraman et al [28] reported that calcium chloride is very effective in the treatment of wool-scouring wastes from textile mills. Bhole and Dhabadgaonkar[29] evaluated the efficiency of a number of coagulants to remove colour and COD of textile mill waste. Aluminium chloride was found to be the best coagulant for the removal of both colour and COD. Commonly used inorganic coagulants are alum, poly aluminium chloride, sodium aluminates and lime[30]. Thomas et al[31] explained the effect of precipitation and flocculation on the removal of phosphates from industrial effluents.

Rao and Sastry[32] reported that several coagulants like lime and Nirmal seed extract could be effectively used for the clarification of coal washery wastes. Wastewaters from antibiotic manufacturing units can neither be clarified in settling tanks nor can be chemically coagulated to reduce BOD[33]. The poor response of the waste to coagulation is due to the fact that most of the substances contributing to BOD appear in solution. Thakur et al [34] studied the chemical treatment of sewage using alum, ferric chloride, ferrous sulphate and lime.

Coagulation using metal salts has been investigated for the treatment of composite effluents from latex centrifuging units [35,36] No systematic study has been reported in literature on the use of chemical coagulants in the treatment of skim serum effluent from natural rubber latex processing units. The aim of the present study is to evaluate the effectiveness of a few metal coagulants in the treatment of skim serum effluent from centrifuge latex concentration units. The effectiveness of coagulants was assessed in terms of the reduction of COD, turbidity, phosphate, TKN and AN. The influence of pH on the action of coagulants and sludge settling characteristics were also studied.

3.A. 3. Experimental

The coagulants used for the study were of commercial grade. The compositions of the coagulants were as follows:

1. Potash alum, $KAl(SO_4)_2.12H_2O$ (MERCK). M = 474.39 g/mol
2. Ferric alum, $NH_4Fe(SO_4)_2.12H_2O$ (MERCK). M = 482.19 g/mol
3. Aluminium sulphate, $Al_2(SO_4)_3.16H_2O$ (MERCK).M = 630.39 g/mol with product strength of 17 percent as Al_2O_3
4. Aluminium chloride, $Al_2Cl_6.12H_2O$ (MERCK). M =482.7 g/mol
5. Ferrous sulphate, $FeSO_4.7H_2O$ (MERCK). M =278.02 g/mol.

The skim serum effluent samples were collected from a natural rubber latex concentration factory situated in Kottayam district of central Kerala. The serum effluent was analyzed for pH, turbidity, COD, BOD, total solids, dissolved solids, suspended solids, TKN, AN, sulphate, and phosphate as per standard methods[37].

Coagulation experiments were carried out in a jar test apparatus provided with four stirrers having speed regulator. Different coagulants were compared for their ability to remove turbidity, COD, BOD, TKN, AN, and phosphate. Required dosages of the coagulants were added to the serum effluent samples. The contents were stirred thoroughly at 100 rpm for 1 minute and then at a speed of 30 rpm for 15 minutes for flocculation. The contents of the beakers were allowed to settle for 30 minutes. The supernatants were siphoned off for the analysis of pH, turbidity, COD, BOD, TKN, AN, and phosphate.

To find out the optimum pH needed for effective coagulation, the pH of the samples were adjusted to optimum levels using sodium hydroxide and sulphuric acid. 400 mg/L of each of the coagulants were added to the effluent samples having different pH.

Sludge settling characteristics were studied in an imhoff cone of one litre capacity by adding the optimum dosage of the coagulants and adjusting the pH to the optimum value. The samples in the imhoff cone were stirred well for 4 to 5 minutes. The rate of settling of the floc formed was measured by noting the position of the sludge at regular intervals until further settling was negligible.

3.A.4. Results and discussion

3.A.4.1 Optimisation of pH on the coagulation efficiency of metal coagulants

To determine the optimum pH needed for the coagulation of the five metal coagulants, pH of the serum effluent samples were adjusted to 3, 5, 7, 9 and 11 using 1N sodium hydroxide and sulphuric acid. After 30 minutes of settling, the coagulation efficiency was estimated in terms of percentage removal of various parameters like turbidity, COD, BOD, TKN, AN and phosphate achieved for a coagulant dosage of 400 mg/L.

(i) Potash alum

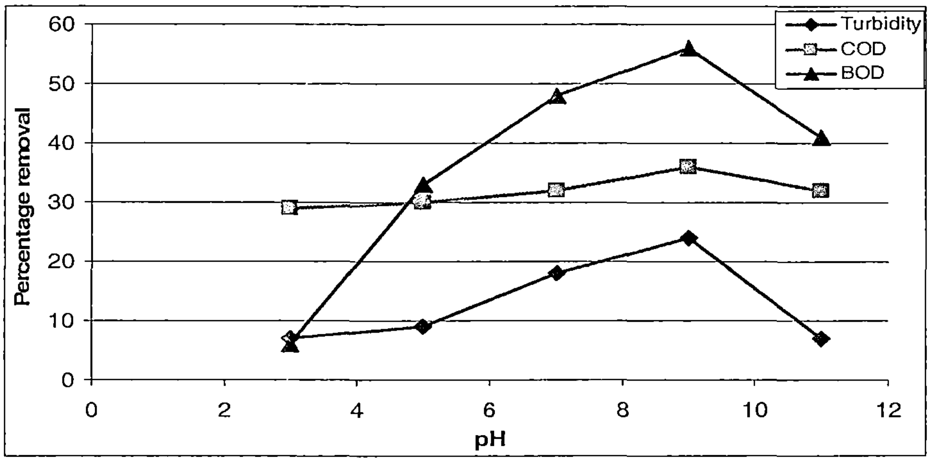


Fig. 3.A. 1. Optimisation of pH on coagulation of potash alum

For potash alum, pollutant removal efficiency (turbidity, COD and BOD) increased steadily on increasing the pH from 3 to 9. But on increasing the pH further, the efficiency of potash alum in removing pollutants was dropped. Maximum percentage removal of turbidity, COD and BOD are 24, 36 and 56 respectively at pH 9 (Fig.3.A. 1). Therefore pH 9 could be taken as the optimum pH when potash alum is used as coagulant.

(ii) Ferric alum

The effect of pH on the coagulation efficiency of ferric alum is illustrated in Fig. 3.A.2. As the pH increased from 3 to 7, a gradual increase in the efficiency to remove turbidity, COD, BOD, TKN, and AN were observed. Maximum amount of pollutants were removed at pH 7. For further increase in pH to 9 and 11, a steady decline in the percentage removal was observed. Therefore, pH 7 could be taken as the optimum pH.

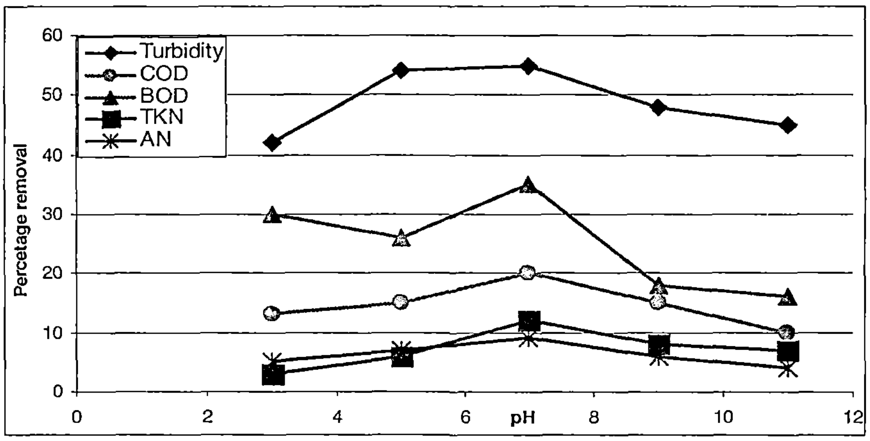


Fig. 3.A. 2. pH Optimisation curve for ferric alum as coagulant

(iii) Aluminium sulphate

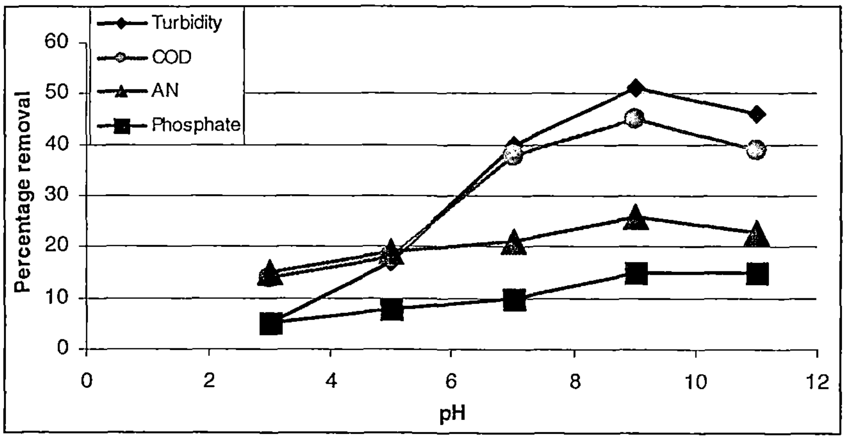


Fig. 3.A. 3. pH Optimisation curve for aluminium sulphate as coagulant

After adjusting the pH of the serum effluent to 3, 5, 7, 9 and 11, 500 mg/L of $Al_2(SO_4)_3$ was added, shaken, allowed to settle and analyzed for the efficiency of treatment in terms of turbidity, COD, AN and phosphate. Maximum percentage removal of all these parameters took place at pH 9 and hence this pH could be taken as the optimum for the coagulation using aluminium sulphate (Fig. 3.A. 3).

(iv) Aluminium chloride

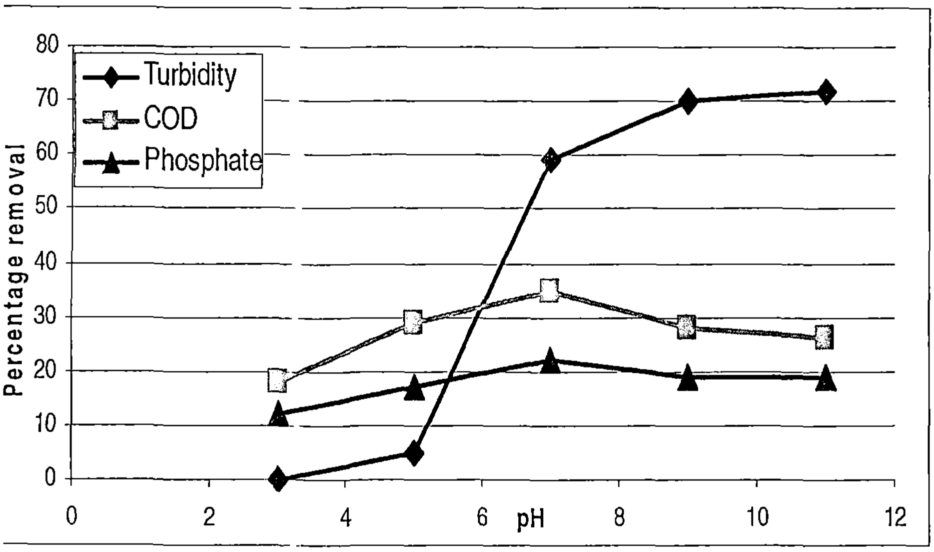


Fig. 3.A.4. pH Optimisation curve for aluminium chloride as coagulant

pH of the effluent was adjusted to 3, 5, 7, 9 and 11 and 400 mg/L of aluminium chloride was added in each of the samples. The results are given in the Fig. 3.A. 4. As the pH increases from 3 to 11 turbidity value also increased and reached a maximum of 72 percent. Maximum COD (35%) and phosphate (22%) removal took place at pH 7. Therefore, pH 7 could be taken as the optimum pH for aluminium chloride.

(v) Ferrous sulphate

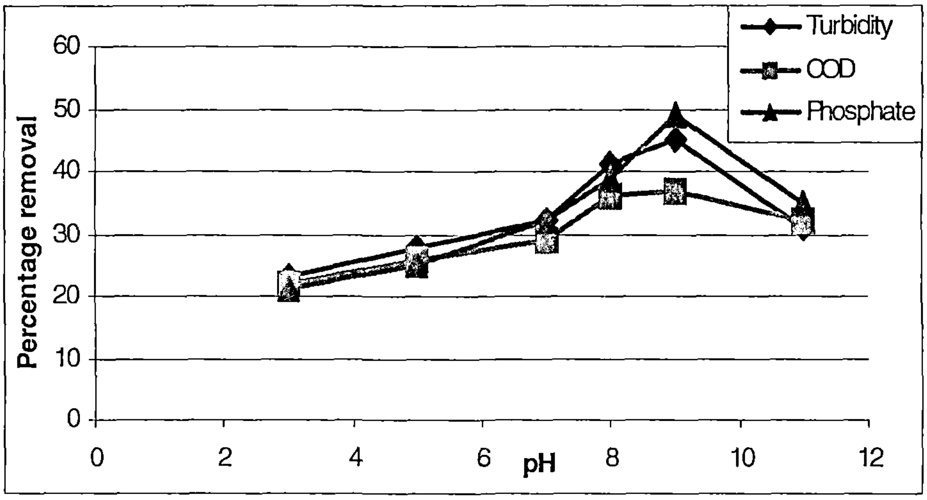


Fig. 3.A.5. pH Optimisation curve for ferrous sulphate as coagulant

The optimum pH was assessed in terms of the removal efficiency of turbidity, COD and phosphate. Since the highest percentage removal of these parameters is observed at pH 9, this could be taken as optimum for effective coagulation using ferrous sulphate (Fig. 3.A. 5).

Due to the presence of polar groups like -OH, -COOH, and $-NH_4$ on surface of colloid particles are hydrophilic[10] and they are water soluble. The primary charge on hydrophilic colloids arises from the ionization of the chemical groups present at the surface of the particles. Extent to which these surface groups ionize, determines the charge: and therefore, charge is pH dependent[11]. A number of coordination compounds are amphoteric in that they can exist in both acids and in strong bases. The acid and base properties of the hydroxides and nature of the covalent bond will depend on the position of the element in the periodic table. By the addition of potential determining ions like strong acids or bases to promote coagulation, reduce the charge of metal oxides or hydroxides near to zero so that coagulation can occur[6]. Therefore extent of coagulation depends on the charge neutralisation which in turn depend on the pH of the solution.

3.A.4.2 Effect of concentration of coagulant

To study the effect of concentrations of coagulants, different concentrations viz: 200, 400, 600, 800 and 1000 mg/L of coagulants were added. After shaking, the precipitate was allowed to settle for thirty minutes and the clear solution was taken for analysis. The percentage removal of turbidity, COD, BOD, TKN and AN were estimated.

(a) Potash alum

Percentage removal of turbidity increased as the concentration of potash alum increased and the highest percentage removal of 56 was achieved at 1000 mg/L. From the graph (Fig.3.A. 6) it is clear that maximum percentage removal of COD, BOD, TKN and AN are 34, 30, 25 and 30 respectively for a concentration of 400 mg/L and this could be taken as the optimum concentration for the removal of pollutants. When alum was used, Al^{3+} ions combines with the OH^- ions to form a precipitate of $Al(OH)_3$ which settled down at the bottom[38]. Double salts break up in solution into their constituent ions, $[K(H_2O)_6]^+$, $[Al(H_2O)_6]^{3+}$ and two $SO4^{-2}$. Aggregation of destabilized particles by interparticle bridging with Al^{3+} involves particle transport and chemical interactions that enhanced the removal of dissolved organics from the effluent[14].

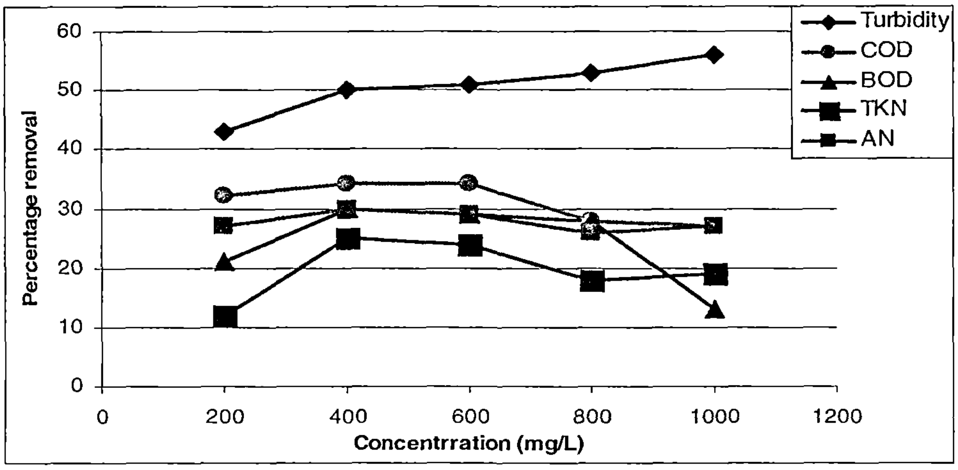


Fig. 3.A. 6. Effect of concentration of potash alum as coagulant

(b) Ferric alum

Turbidity removal increased as the concentration of ferric alum increased and the highest percentage removal achieved was 77 percent for a concentration of 1000 mg/L. For a ferric alum concentration of 200 mg/L, the COD removal efficiency was 24 percent and the value dropped to 17 percent when the concentration of ferric alum increased to 400 mg/L (Fig. 3.A. 7). Further increase in the concentration increased COD removal efficiency from 21 percent to 27 percent. In the case of TKN, the removal efficiency increased from 3 percent to 15 percent as the concentration of the ferric alum increased from 200 mg/L to 1000 mg/L. Only 9 percent of AN was removed when the concentration changed from 200 mg/L to 600 mg/L. A further increase in the concentration of ferric alum to 800 mg/L showed no influence in the removal of AN and for 1000 mg/L, AN removal efficiency showed negative value (-3). 600 mg/L could be taken as the optimum concentration for ferric alum since after this concentration removal of pollutants was not so significant.

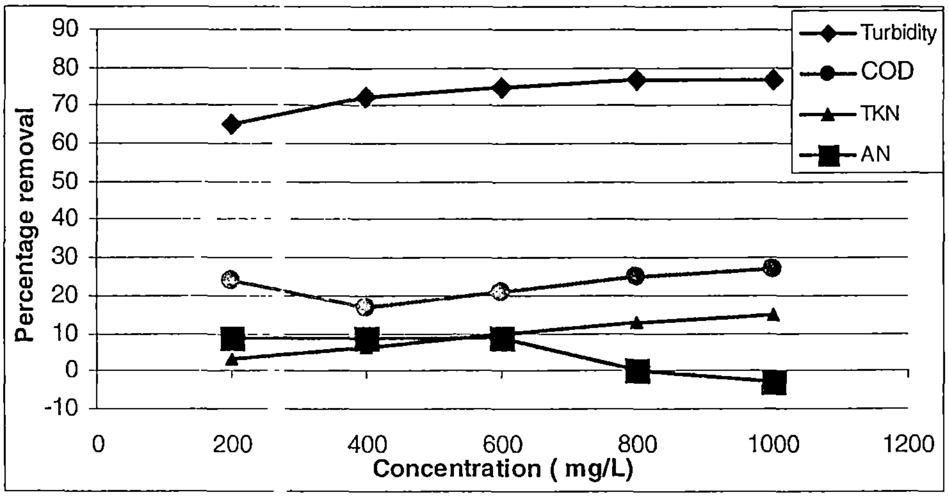


Fig. 3.A. 7. Effect of concentration of ferric alum as coagulant

Ionization of ferric alum might have produced $NH4^+$ ions at higher concentrations. This resulted in an increase in the concentration of $NH4^+$ ions and thus a negative percentage removal of AN at higher concentrations of ferric alum.

(c) Aluminium sulphate

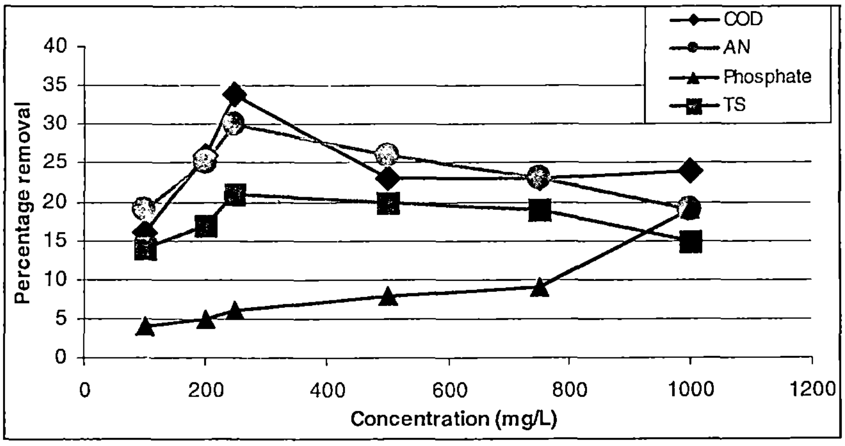


Fig. 3.A.8. Effect of concentration of aluminium sulphate as coagulant

Effect of concentration of aluminium sulphate was studied by adding varying concentration of $Al_2(SO_4)_3.14H_2O$ viz: 100 mg/L, 200 mg/L, 250 mg/L, 500 mg/L, 750 mg/L, 1000 mg/L to the skim serum effluent and analysed the effect in terms of the removal of COD, AN, phosphate and total solids. From the Fig. 3.A. 8. it is clear that maximum removal of COD (34%), AN (30%) and total solids (20%) is obtained for a concentration of 250 mg/L of aluminium sulphate. Phosphate removal increased as the concentration of aluminium sulphate increased and maximum removal of 19 percent was obtained for 1000 mg/L. As the concentration of Al^{+3} ions increased it combined with phosphate to form sparingly soluble aluminium phosphate which was removed as precipitate or by adsorption on excess of metallic hydroxide. Since maximum removal of COD, AN and total solids was obtained for a concentration of 250 mg/L of aluminium sulphate, this could be taken as optimum concentration.

(d) Aluminium chloride

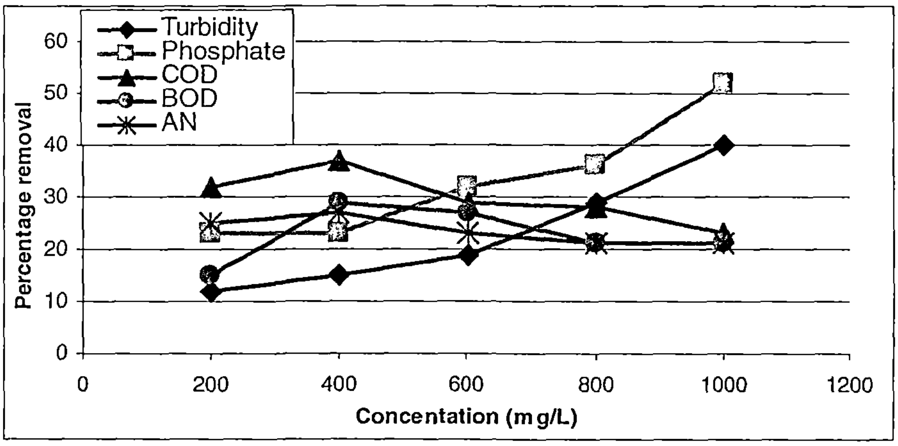


Fig. 3.A. 9. Effect of concentration of aluminium chloride as coagulant

Percentage removal of turbidity, phosphate, COD, BOD and AN were estimated for varying concentrations of aluminium chloride and the results are given in Fig. 3.A. 9. Turbidity and phosphate removal efficiency increased as the concentration of aluminium chloride increased and reached 40 % and 52% respectively for aluminium chloride concentration of 1000 mg/L, whereas the maximum percentage removal of COD, BOD and AN are 37, 29 and 27 respectively for a concentration of 400 mg/L. Aluminium chloride exists as a dimer $Al_2Cl_6.12\,H_2O$ in the solid state. When the halides dissolve in water, the high enthalpy of hydration is sufficient to break the covalent dimer into $[M(H_2O)_6]^{3+}$ and $3X^-$ ions. When the solution of $AlCl_3$ is added to the effluent, the coordinated water molecules in the $[M(H_2O)_6]^{3+}$ could be replaced by ammonia molecules or phosphate molecules. Chemical changes in the structure of hydrated metal, metal-OH-metal linkages and concurrent change in the floc sorbability made AlCl3 a good coagulant[14]. Maximum percentage removal of COD, BOD and AN were for a concentration of 400 mg/L of $AlCl_3$ and hence this could be taken as the optimum concentration.

(e) Ferrous sulphate

Effect of concentration of ferrous sulphate was assessed by adding ferrous sulphate in the range of 200 to 1000 mg/L to the skim serum effluent at pH 9. Analysis of the supernatant liquid showed that the highest percentage removal of turbidity, phosphate, BOD and AN were 75, 67, 33 and 26 respectively for a concentration of 600 mg/L (Fig. 3.A. 10). Phosphate removal efficiency was comparatively high since iron combines with phosphate to form a precipitate of iron hydroxophosphate which is insoluble and settle down.

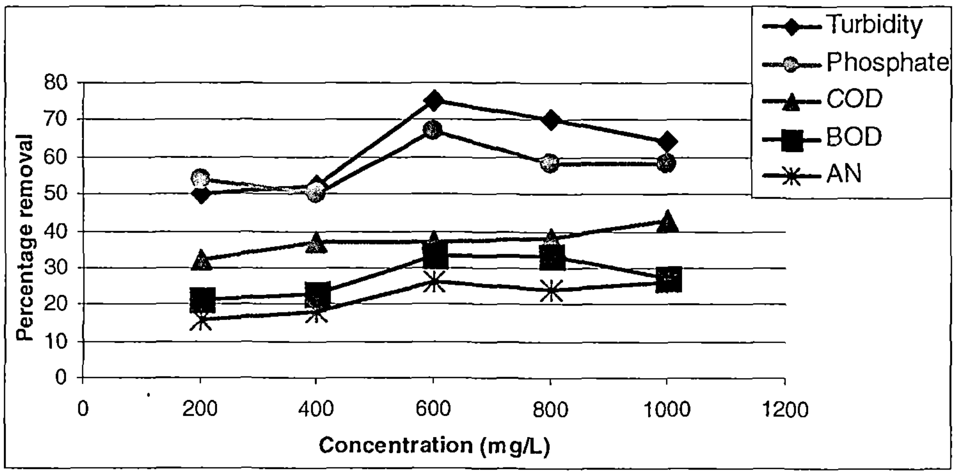


Fig.3.A.10. Effect of concentration of ferrous sulphate as coagulant

COD removal efficiency increased as the concentration of ferrous sulphate increased and reached a maximum value of 43 percent. The highest percentage removal of turbidity, phosphate, BOD and AN was observed for a concentration of 600 mg/L and hence this could be taken as the optimum concentration.

The addition of electrolytes have the effect of reducing the thickness of the diffuse electric layer and thereby, reduce the zeta potential and coagulate colloidal suspension. Increased concentration of the electrolyte will cause a decrease in zeta potential and a corresponding decrease in repulsive forces. The concentration of an electrolyte that is needed to destabilise a colloidal suspension is known as the critical coagulation concentration. A change in particle size will occur when chemicals are added to adjust the pH of the effluent to optimise the performance of hydrolysed metal ions used as coagulants[6].

3.A.4.3. Sludge settling characteristics

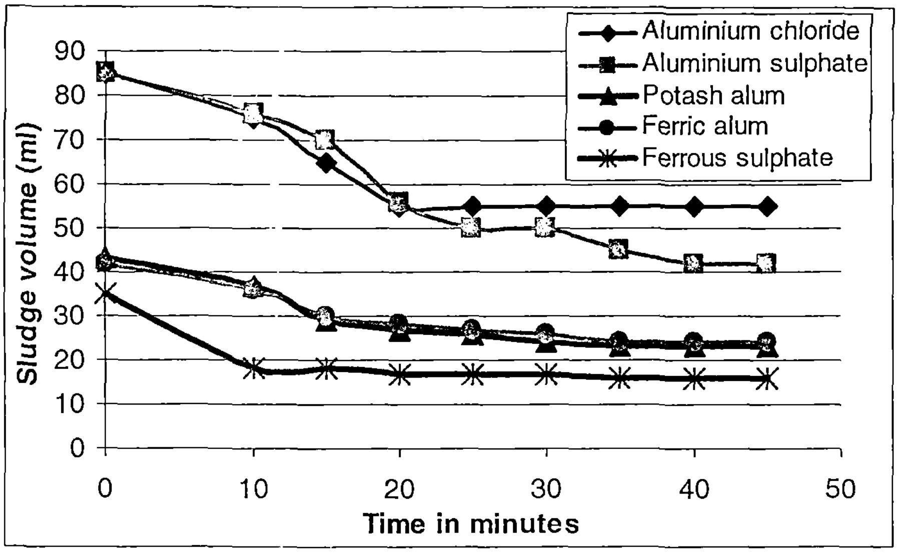


Fig. 3.A. 11. Sludge settling characteristics of coagulants

Imhoff cones of one litre capacity were used for measuring the settling rates and sludge volume.

In order to study the rate of sludge settling of different coagulants, 400 mg/L each of potash alum, ferric alum, ferrous sulphate, aluminium chloride and aluminium sulphate were added to 250 mL of effluent taken in separate imhoff cones. These were stirred well and allowed to settle and the sludge height measured at every five minutes interval and the results are shown in the Fig. 3.A. 11. Aluminium chloride and aluminium sulphate showed poor characteristics of the sludge in terms of settling rate and compactness. The highest sludge height was observed for aluminium chloride and aluminium sulphate. This could be due to the gelatinous nature of the aluminium precipitate formed. The sludge settling characteristics of potash alum and ferric alum were almost similar and settling became constant within 35 minutes. Settling of the precipitate formed by potash alum and ferric alum showed moderate rate. On the other hand with ferrous sulphate, floc formation was rapid and settling rate was also fast. Sludge settling became almost constant within 20 minutes.

3.A.5. Conclusion

Optimum pH values for the effective action of five coagulants, viz. potash alum, ferric alum, aluminium sulphate, aluminium chloride and ferrous sulphate were determined. The optimum pH value is 9 for potash alum, aluminium sulphate and ferrous sulphate. For ferric alum and aluminium chloride the optimum pH is 7.

Among the five coagulants studied, COD removal efficiency is in the range of 34 to 38 percent for all coagulants except ferric alum.

Sludge settling characteristics of potash alum and ferric alum show moderate rate, whereas with ferrous sulphate floc formation is rapid and settling rate is also fast. Sludge settling becomes almost constant within 20 minutes. The compressibility of the sludge is comparatively low for aluminium chloride and aluminium sulphate.

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PART B:

NATURAL AND SYNTHETIC POLYELECTROLYTES

3.B.1. Introduction

Polyelectrolytes are polymers with high molecular weight having electrical charges and ionisable groups. The term polyelectrolyte was first coined by Fuoss[1] for those polymers which, by action of an acid or alkali, can be converted into charged ions. Polyelectrolytes are divided into natural and synthetic, based on their origin. Naturally occurring polyelectrolytes are termed as biocolloids[2]. The common feature of natural and synthetic polyelectrolytes is the presence of recurring units containing ionisable groups in the molecule. Polyelectrolytes are classified into cationic, anionic and non-ionic depending on the charge of the recurring units. The choice of a proper polyelectrolyte for a given application is of paramount importance. The bacteria and colloidal matter present in natural waters generally carry negative charge. Hence, cationic polyelectrolyte which produce positively charged functional group on dissolution in water, are most suitable. If water contains only mineral suspended solids, cationic polyelectrolytes are found to be very effective as primary coagulants[3] and if both mineral and organic substances are present, a combination of inorganic coagulants such as alum or ferrous sulphate with cationic polyelectrolytes gives the best results[4]. Even for waters with mineral solids, a combination of alum with a cationic polyelectrolyte may prove to be more economical than any single coagulant[5] because the polyelectrolytes do not have great capacity for charge neutralization due to their large molecules and small doses used. In non-ionic polyelectrolytes, charge neutralization has to be brought about solely by inorganic coagulants. The non-ionic polyelectrolyte serves as a bridge between neutralized particles[6]. The combination generally results in excellent clarification. But the doses of non-ionic polymers required are generally ten times that of cationic ones[7]. Anionic polymers are found to be inefficient as primary coagulants for natural waters. This is due to the electrostatic repulsion between the colloids and the functional group obtained on dissolution of the polymer in water having similar charges. The higher the molecular weight of a polyelectrolyte, the more effective it is. The bridging mechanism basically depends on the molecular weight which determines the size and segments of the polymer formed on dissolution in water. The effectiveness of polyelectrolytes for various uses can be found out by suitable experimental procedures. However, jar test can give valuable information about the effectiveness of polyelectrolytes not only for coagulation but also for filtration and sludge conditioning. It is used to indicate the relative efficiency of various flocculants and possible range of effective doses[8].

Natural and synthetic polyelectrolytes are used in water and wastewater treatment either alone or along with metal coagulants to improve clarification. These are long chain compounds and in water they act as polyvalent ions[9]. Charge neutralization of colloids and agglomeration of smaller particles into bigger particles are the processes involved in the coagulation using polyelectrolytes. Large number of ionic sites are present in the polymer chain. Coagulation takes place by the neutralization of charge on the electronegative colloid particles by these ionized centers or by hydrogen bonding among the charged surfaces. Electrostatic cross-linking also binds the linear chains together. Effective removal of colloids is obtained when zeta potential of particles (15-25 mV) is reduced to zero plus or minus 5 mV. Even if a polyelectrolyte is not very efficient in charge neutralization, they may be effective in formation of floc by what is known as “bridging”. The polymer is adsorbed on the surface of the particle and bridges between them with the aid of free segments of the adsorbed polymer[9]. The bridging results in the formation of large, strong, three dimensional and quickly settleable flocs.

3.B.2. Natural polyelectrolytes

Natural organic polyelectrolytes are highly active materials that are practically present in all natural water systems. They interact with both organic and inorganic pollutants and nutrients, influencing and in many instances controlling the toxicity, rate of movement, persistence and rate of degradation of the pollutants and nutrients in aquatic environment. Detailed knowledge of the interaction and chemistry of natural polyelectrolytes is, therefore, of primary importance. Vanerkar studied herbal pharmaceutical wastewater treatment using conventional coagulants coupled with synthetic polyelectrolytes[10]. A mixture of aluminium salts and natural polyelectrolytes extracted from the cactus *Opuntia ficus indica*, has been used for the cleaning of wastewater from slaughterhouse[11]. Ganjidoust[12] investigated the effect of synthetic polymer and the natural polymer chitosan and the coagulant alum on the removal of lignin from pulp and paper industrial wastewater. Kawamura[13] conducted bench and pilot scale tests and confirmed the effectiveness of the natural polyelectrolytes, chitosan, and sodium alginate, in water treatment. Chitosan performed better than synthetic polyelectrolytes, both as a coagulant aid and as a filtration aid. A paste from the seed of *Strychnos potatorum Linn* commonly known as clearing nut or nirmali seed precipitates suspended siliceous impurities in raw water[14]. Electrophoretic measurements of the seed paste showed a weak negative charge and attributed its coagulating properties to its strychnine and albumin content[15]. Jamuna River water was treated using nirmali seed alone and in conjunction with metal coagulants[16]. Bulusu and Sharma[17] have confirmed these findings on a pilot plant using Jamuna River water having high turbidity. Rao and Sastry[18] found that 2 mg/L of nirmali seed extract reduce the alum dose by more than 50%. Bulusu and Pathak[19] identified the seeds of *Red Sorella* to be very effective as primary coagulant.

Natural polyelectrolytes have been used as auxiliary materials for flocculation and coagulation in wastewater treatment and water cleaning process[20]. Floccotan, extracted from quebracho and chemically modified, is largely used as a water clarifier in South America. *Opuntia ficus indica* a cactus that grows naturally in South America is also a source of viscous natural polyelectrolytes bearing negative surface charges[21]. The flocculation process induced by anionic polyelectrolytes such as the natural polyelectrolytes extracted from *Opuntia ficus indica* is greatly facilitated by the presence of low concentration of a metal ion (Al^{+3}) capable of binding with the functional groups on the polyelectrolytes. The positive metal ion serves to form a bridge among the anionic polyelectrolytes and negatively charged functional groups on the colloidal particle surface. Due to its hydrophilic character, several hydrogen bonds are formed among polyelectrolytes and water molecules. This association tends to occupy larger surface area causing very high viscosity[22]. Sadgir tested amla as coagulant and coagulant aid in the water purification system and confirmed its effectiveness[23].

Tripathi et al[24] established the anionic nature of nirmali seed extract by I.R. spectral studies and found that even though it is very efficient in the coagulation-flocculation of hydrophobic colloids, it is a poor flocculant in the case of hydrophilic colloids such as bacteria. Prasad and Jivendra[25] investigated the performance of *Tamarindus indica* seed extract as coagulant aid in water clarification and reported that it can reduce the alum dose by 50 percent. Vaidya and Bulusu[26] studied the potential of chitosan as a coagulant and coagulant aid in water clarification and Karim[27] investigated the use of inorganic salts and polymers in the treatment of palm oil effluent.

Table 3. B. 1. Indian herbs useful for coagulation in water purification[28]

No	Local name	Botanical Name	Family	Part used
1	Khas	Veteveria zizanioide, staff	*Gramineae*	Root
2	Amla	Phyllanthus emblica	*Euphorbicease*	Fruit
3	Anantvel	Hemidesmus indicus, R.Br.	*Asclepiadacease*	Root
4	Singhada	Trap bispinosa. Roxb	*Trapa*	Fruit
5	Shewaga	Moringo oleifera Lam. Gaertn	*Moringaceae*	Seed
6	Hirda	Terminalia chebula, Retz.	*Combretaceae*	Fruit
7	Elaichi	Elattaria cardamomum	*Scitamineae*	Seed
8	Nirmali	Strychnos potatorum Linn.	*Loganiaceae*	Fruit

Different types of herbs have been used as coagulant and coagulant aid for water purification[28]. Natural polyelectrolytes were used since 2000 B.C. where *Strychno potatorium Linn* extracted from seeds was used for water clarification[29]. Plant based substitute to treat water is economical and a number of herbs are useful for purification of water. In ancient India many Acharyas like Dhanvantari,Charaka, Sushruta and Varaharnihir made serious efforts to purify water by using herbal extracts[28]. Sushruta advocated the use of natural herbs like nirmali seed, roots of kamal, rhizomes of Algae and three stones namely gomedmani, moti, sphatik and he recommended the disinfection of contaminated water by exposing it to the sun or immersing red hot iron and hot sand in it[30]. Table 3. B. 1 gives a description of various herbs traditionally used for purification of water[28].

Table 3.B.2. Products and uses of the *Moringa oleifera* tree

Parts of tree	Products and uses
Seed	Crushed whole seed or press cake remaining after oil extraction as a coagulant for water and wastewater treatment
Vegetable	Green pods as fresh or canned vegetable, leaves and flowers used as a relish
Oil	Seeds contain 40 % oil by weight used for cooking, soap manufacture, as a cosmetics base and to provide illumination
Other uses	All plant organs as constituents in traditional medicines, leaves and oil press cakes as cattle fodder

Drum stick *(Moringa oleifera)*

Seeds of a few plants like *Moringa oleifera* and *Strychnos potatorum* make effective coagulant in the clarification of turbid water[31-37]. Sudanese villagers used powdered *Moringa oleifera* seeds to clarify river water from Nile[38]. Alum and broad beans were used for the traditional clarification of Nile water[39]. Raveendra Babu and Malay Chaudhuri[40] used a coagulation-filtration test to examine quality improvement of surface water treatment by direct filtration with *S. potatorum seed* or *Moringa oleifera* seed as the coagulant. Branches of *Moringa oleifera* tree are lopped and thrown into turbid and contaminated wells that turn clear[41]. Table 3. B.2 give a description of the products and uses of the *Moringa oleifera* tree.

Drum stick, *Moringa oleifera* tree [Drumstick (describing the shape of the pods) or horseradish (describing the taste of its roots)] though a native species of India, is now grown extensively throughout the tropics and is found in many countries of Africa, Asia and South America. The seed pods are allowed to dry naturally on the tree prior to harvesting. The seeds are crushed and sieved. The seed contains 40 percent by weight of oil, with the remaining press cake containing the active ingredients for natural coagulation. The crushed seed powder, when added to water, yields water soluble proteins that possesses a net positive charge. The solution, therefore, acts as a natural cationic polyelectrolyte during treatment[32]. Using *Moringa oleifera* as a replacement for proprietary coagulants meets the need for water and wastewater technology in developing countries which is simple to use, robust and cheap.

Goose berry [Amla or Indian Goose berry]

The emblic tree is native to tropical South Eastern Asia, particularly in central and southern India, Pakistan, Bangladesh, Sri Lanka, Malaysia, and China. Its botanical name is *Phyllanthus Embilica linn* and the family is Euphorbiaceae. The stem less fruit is round and its skin is thin and translucent, light green at first and becomes greenish- yellow as it matures. Ripe fruits are astringent, extremely acidic, and some are distinctly bitter.

Ascorbic acid in the emblic is highly stable, apparently protected by tannins which retard oxidation. Biochemical studies show the presence of 13 tannins plus 3 or 4 colloidal complexes. The dry powdered fruit contains 6. 3% phyllemblinic acid, 6% fatty matter, 5% gallic acid, ellagic acid, emblicol (a phenolic product) and other constituents like carbohydrate, sucrose, fiber etc[23].

Tamarind seed powder

The seeds of *Tamarindus indica* exhibit coagulating properties. The Seeds of the tamarind collected was dehusked in mills and the kernel of the seed is powdered. The ground seeds are sieved through 200 ASTM mesh and the solution was prepared by boiling. It gives a gelatin material that is used like other creaming agents. This powder is not soluble in water. The powder is first allowed to swell in water for some time before making it into slurry. It holds large quantity of water in a loosely bound condition and gives a viscous solution in water. The slurry is then boiled. It is a hydrophilic colloid.

3. B. 3. Synthetic polyelectrolytes

Synthetic polyelectrolytes are acrylamide and acrylic acid polymers and copolymers having a very high molecular weight. Molecules of polyelectrolytes are tightly bound and compact and they carry ionic charges. Unwinding and opening of the polymer molecules take place in aqueous media and ionic charges present in the polymer attract suspended fine particles in the media which get agglomerated forming macroflocs. As flocculation proceeds these macroflocs settle to the bottom of the container giving a very clear supernatant solution. Prasad and Belsare[42] studied the effectiveness of polyacrylamide as a coagulant aid in water treatment and found that a relatively small concentration (0.1 ppm) of polyacrylamide could remove high turbidity to the acceptable level and reduce half of the original amount of alum without any difference in the achievement of steady state. Sihorwala and Reddy[43] studied the use of synthetic polyelectrolytes and metal salts in the treatment of cotton textile waste. Madhu[44] and Ponnamma[45] studied the effect of a few natural and synthetic polyelectrolytes to treat composite effluent from latex centrifuge units.

No systematic study has been reported in literature on the use of natural and synthetic polyelectrolytes as coagulants and coagulant aids in the treatment of skim serum effluent from natural rubber latex centrifuging units. The aim of the present study is to evaluate the effectiveness of three natural polyelectrolytes viz: dried goose-berry powder, dried drum stick seed powder and tamarind seed powder and two synthetic polyelectrolytes as coagulant and coagulant aid to treat skim serum effluent from natural rubber centrifuging factory.

3. B. 4. Experimental

Skim serum effluent from natural rubber centrifuging factory was collected and analyzed as per standard methods[46]. It was then subjected to treatment with three natural coagulants and two synthetic coagulants using jar test apparatus. Stock solution of the coagulants were prepared and used for the studies. After mixing, the samples were allowed to settle for half an hour. The supernatant liquid was withdrawn and analyzed for various parameters. To find out the optimum pH needed for effective coagulation, the pH of the samples were adjusted to optimum levels using 2N sodium hydroxide and sulphuric acid.

Polyelectrolytes used for the study were

a) Natural polyelectrolytes

1. Drumstick seed powder [*Moringa oleifera*]

2. Goose-berry powder [*Phyllanthus embilica linn*]
3. Tamarind seed powder [*Tamarindus indica*]

The natural polyelectrolytes was prepared in the following way-*Moringa oleifera* seeds were dried and thoroughly powdered in a mortar to produce a pasty powder, 0.1% solution was prepared by boiling and the solution was filtered to remove suspended particles.

Phyllanthus embilica linn fruits were collected from the market, dried in the sunlight, removed the seeds, powdered the fleshy part and sieved through 200 ASTM mesh. 0.1% solution was prepared by boiling the powder. It was filtered to remove suspended particles.

Tamarindus indica. Tamarind seed powder was collected from the Rubber Research Institute of India. A 0.1% solution was prepared by boiling.

b) Synthetic polyelectrolytes

1. Cationic polyelectrolyte, Maxflock –504. Polyacrylamide based polyelectrolyte. Trade name-Sonimer. Supplier-Roop Rasayan Ind.Pvt.Ltd., Mumbai, India. It is a viscous liquid. A 0.1 percent solution was prepared by dissolving 100 mg of the polyelectrolyte in 100 mL of water.
2. Anionic polyelectrolyte, Maxflock-107. Polyamine based polyelectrolyte. Trade name-Sonimer. Supplier-Roop Rasayan Ind.Pvt.Ltd., Mumbai, India. It is a crystalline solid and 0.1% solution was prepared by dissolving 100 mg of the polyelectrolyte in100 mL of water.

Turbidity, a measure of the light-transmitting properties of water, is used to indicate the quality of wastewaters and natural waters with respect to colloidal matter. Turbidity is measured to evaluate the performance of water treatment. Higher the intensity of scattered light, the higher the turbidity. Turbidity was measured using digital Nephelo Turbidity Meter (Range 1-1000, No.24). pH was determined using digital pH meter 335-Systronics.

All polyelectrolytes, both natural and synthetic, were assessed for their effectiveness, both as a coagulant and coagulant aid in the treatment of serum effluent of natural rubber centrifuging factory.

3.B.5. Results and discussion

Polyelectrolytes have many advantages as coagulant and coagulant aid. The relationship between polymer molecular weight, charge density, polymer type, mixing condition and flocculated particle size distribution are the main factors in the selection of polyelectrolytes in water and wastewater treatment [47]. Presence of polyelectrolytes in low doses enhances settling properties.

3.B.5.A Optimisation of pH

To determine the optimum pH for the coagulation of polyelectrolytes, the pH of effluent samples was varied between 3 and 11.5. The coagulation efficiency was estimated in terms of turbidity removal achieved for the polyelectrolyte dosage of 4 mg/L.

(i) Drumstick seed powder

Effect of pH on the coagulation efficiency of drumstick seed powder is shown in Table 3. B. 3.

Table 3.B.3. Effect of pH on the coagulation efficiency of drumstick seed powder

pH	Turbidity before treatment (NTU)	Turbidity after treatment (NTU)
3	164	154
5	67	62
7	63	183
9	112	194
10	30	10
11.5	183	201

From the table it is clear that as the pH increased from 3 to 5, the turbidity decreased. But for pH 7 and 9, turbidity increased when drumstick seed powder was added to the effluent. For pH 10, maximum removal of turbidity was observed and the solution became very clear at this pH. On increasing the pH to 11.5, a negative effect was again observed. From the study it was clear that pH 10 could be taken as the optimum for drumstick seed powder when used as coagulant. Drumstick seed powder is a natural cationic polyelectrolyte and act as a coagulant that neutralises or lower the charge of the wastewater particles. Since the wastewater particles are normally negatively charged, cationic polyelectrolytes are found to be highly effective for coagulation. When chemicals are added to adjust the pH of the wastewater, a change in the particle charge will occur. Depending on the pH, the formation of mono and polynuclear and polymer hydroxide species occurs in fraction of a second[49].

(ii) Goose-berry powder

The pH dependence on the coagulation of goose-berry is shown in Table 3. B. 4.

Table 3. B. 4. Effect of pH on the coagulation efficiency of goose-berry powder

pH	Turbidity before treatment (NTU)	Turbidity after treatment (NTU)
3	164	148
5	67	45
7	63	161
9	112	170
10	30	5
11.5	183	181

For goose-berry, maximum removal of turbidity was observed at pH 10. At this pH, the turbidity came down from 30 to 5 NTU when goose-berry was added as coagulant. On further increase of the pH to 11.5, again a negative removal of turbidity was observed.

(iii) Tamarind seed powder

Table 3. B. 5. Effect of pH on the coagulation efficiency of tamarind seed powder

pH	Turbidity before (NTU)	Turbidity after (NTU)
3	164	131
5	67	60
7	63	165
9	112	181
10	30	3
11.5	183	192

When pH increased from 3 to 5 turbidity removal efficiency decreased and for pH 7 and 9, turbidity increased when tamarind seed powder was added to the effluent. Maximum removal of turbidity was observed at pH 10 (Table 3. B. 5). At this pH, the turbidity came down from 30 to 3 when tamarind seed powder was added giving 93 percent removal.

(iv) Cationic polyelectrolyte

Table 3. B. 6. Effect of pH on the coagulation efficiency of cationic polyelectrolyte

pH	Turbidity before (NTU)	Turbidity after (NTU)
3	164	131
5	67	60
7	63	165
9	112	181
9.5	70	15
10	30	10
11.5	183	192

Table 3. B. 6 shows that at pH 10, 67 % turbidity was removed. This was the maximum percentage removal efficiency obtained for a change in pH from 3 to 11.5. Therefore, pH 10 could be taken as the optimum pH when cationic polyelectrolyte was used as coagulant.

(v) Anionic polyelectrolyte

Table 3. B. 7. Effect of pH on the coagulation efficiency of anionic polyelectrolyte

pH	Turbidity before (NTU)	Turbidity after (NTU)
3	127	97
5	23	18
7	88	127
9	123	148
9.5	70	25
10	21	6
11.5	126	115

At pH 10, 71 % turbidity was removed (Table 3. B. 7). On increasing the pH to 11.5, a negative removal of turbidity was observed. Therefore pH 10 could be taken as the optimum pH.

3.B.5.B Effect of concentration of polyelectrolyte

To study the effect of coagulant dosage, the concentration of polyelectrolytes was varied between 2 mg/L to 12 mg/L at pH 10 and its effect was estimated in terms of turbidity removal.

(i) Concentration of drumstick seed powder.

Table 3.B.8. Effects of concentration of drumstick seed powder on turbidity removal

Concentration of drumstick seed powder mg/L	Initial turbidity (NTU)	Final turbidity (NTU)
2	30	14
4	30	0
6	30	14
8	30	14
10	30	12
12	30	12

From the Table 3.B.8 it is clear that 4 mg/L could be taken as the optimum dosage for drumstick seed powder since at this concentration complete removal of turbidity was observed.

(ii) Concentration of goose-berry powder

Table 3. B. 9. Effect of concentration of goose-berry powder on turbidity removal

Concentration of goose-berry powder (mg/L)	Initial turbidity (NTU)	Final turbidity (NTU)
2	30	6
4	30	4
6	30	6
8	30	7
10	30	8
12	30	10

Table 3. B. 9 shows that minimum turbidity was observed for a dosage of 4 mg/L and this could be taken as the optimum dosage of gooseberry when used as coagulant.

(iii) Concentration of tamarind seed powder

Minimum turbidity of 3 NTU was observed for a dosage of 4 mg/L (Table 3. B. 10) and this could be taken as the optimum dosage of tamarind seed powder when used as coagulant.

Table 3. B. 10. Effect of concentration of tamarind seed powder on turbidity removal

Concentration of tamarind seed powder (mg/L)	Initial turbidity (NTU)	Final turbidity (NTU)
2	30	5
4	30	3
6	30	4
8	30	5
10	30	10
12	30	10

(iv) Concentration of cationic polyelectrolyte.

Table 3. B. 11. Effect of concentration of cationic polyelectrolyte on turbidity removal

Concentration of cationic polyelectrolyte (mg/L)	Initial turbidity (NTU)	Final turbidity (NTU)
1	30	14
2	30	10
4	30	15
6	30	15
8	30	15
10	30	12
12	30	12

For cationic polyelectrolyte the concentration was varied between 1 mg/L to 12 mg/L at pH 10 and minimum turbidity of 10 NTU was observed for a concentration of 2 mg/L and this could be taken as the optimum concentration to treat serum effluent

(v) Concentration of anionic polyelectrolyte.

Table 3.B.12. Effect of concentration of anionic polyelectrolyte on turbidity removal

Concentration of anionic polyelectrolyte (mg/L)	Initial turbidity (NTU)	Final turbidity (NTU)
2	21	7
4	21	5
6	21	8
8	21	8
10	21	10
12	21	10

From Table 3. B. 12 it is clear that minimum turbidity of 5 NTU was observed for a concentration of 4 mg/L of the anionic polyelectrolyte and this could be taken as the optimum concentration of anionic polyelectrolyte to treat serum effluent.

3.B.5.C. Polyelectrolytes as coagulant aid

Four different metal coagulants viz. aluminium chloride ($AlCl_3$), potash alum ($KAl(SO_4)_2.12\ H_2O$), ferrous sulphate ($FeSO_4.7\ H_2O$) and ferric alum ($NH_4Fe(SO_4)_2.\ 12\ H_2O$) were selected for the present study. 200 mg/L of metal coagulant was added to the effluent having pH 10 along with 4 mg/L of polyelectrolyte as coagulant aid and its effect was observed in terms of turbidity removal.

(i) Drumstick seed powder

Table 3. B. 13. Effect of drumstick seed powder as a coagulant aid

200 mg/L metal coagulant + 4mg/L drumstick seed powder	Initial turbidity (NTU)	Final turbidity (NTU)
$AlCl_3$	30	6
$KAl(SO_4)_2.12\ H_2O$	30	0
$FeSO_4.7\ H_2O$	30	18
$NH_4Fe(SO_4)_2.\ 12\ H_2O$	30	19

The Table 3. B. 13 shows that among the four coagulants studied, $KAl(SO_4)_2.12\ H_2O$ along with drum stick seed powder removed the turbidity completely.

(ii) Goose-berry

Table 3. B. 14. Effect of goose-berry powder as a coagulant aid

200 mg/L metal coagulant + 4mg/L goose-berry	Initial turbidity (NTU)	Final turbidity (NTU)
$AlCl_3$	30	2
$KAl(SO_4)_2.12\ H_2O$	30	0
$FeSO_4.7\ H_2O$	30	13
$NH_4Fe(SO_4)_2.\ 12\ H_2O$	30	23

Complete removal of turbidity was observed when $KAl(SO_4)_2.12\ H_2O$ along with goose-berry was used as coagulant (Table 3. B. 14).

(iii) Tamarind seed powder

Table 3. B. 15. Effect of tamarind seed powder as a coagulant aid

200 mg/L metal coagulant + 4mg/L tamarind seed powder	Initial turbidity NTU	Final turbidity NTU
$AlCl_3$	30	3
$KAl(SO_4)_2.12\ H_2O$	30	0
$FeSO_4.7\ H_2O$	30	19
$NH_4Fe(SO_4)_2.\ 12\ H_2O$	30	19

Turbidity was completely removed when potash alum along with tamarind seed powder was used as coagulant (Table 3. B. 15).

(iv) Cationic poly electrolyte

When cationic polyelectrolyte was used as coagulant aid, 2 mg/L of polyelectrolyte was added to the effluent at pH 10 since the optimum concentration determined was 2 mg/L. Its effect was observed in terms of turbidity removal and the results are given in the Table 3. B. 16.

Turbidity was completely removed when aluminium chloride along with the cationic polyelectrolyte was used as coagulant.

Table 3. B. 16. Effect of cationic polyelectrolyte as a coagulant aid

200 mg/L metal coagulant + 2mg/L cationic polyelectrolyte	Initial turbidity (NTU)	Final turbidity (NTU)
$AlCl_3$	30	0
$KAl(SO_4)_2.12\ H_2O$	30	2
$FeSO_4.7\ H_2O$	30	12
$NH_4Fe(SO_4)_2.\ 12\ H_2O$	30	18

(v) Anionic polyelectrolyte

Table 3. B. 17. Effect of anionic polyelectrolyte as a coagulant aid

200 mg/L metal coagulant + 4mg/L anionic polyelectrolyte	Initial turbidity (NTU)	Final turbidity (NTU)
$AlCl_3$	21	15
$KAl(SO_4)_2.12\ H_2O$	21	9
$FeSO_4.7\ H_2O$	21	10
$NH_4Fe(SO_4)_2.\ 12\ H_2O$	21	22

Table 3. B. 17 shows that minimum turbidity was observed when potash alum along with anionic polyelectrolyte was used as coagulant.

3.B.5.D. Effect of polyelectrolyte as coagulant and coagulant aid to remove COD, phosphate and TKN.

A study on the effectiveness of potash alum and polyelectrolyte individually and in combination to remove COD, phosphate and TKN or AN were carried out at pH 10. 200 mg/L of potash alum and 4 mg/L of polyelectrolyte were used for the study.

(i) Drumstick seed powder

Percentage removal of COD and TKN increased when moved from drumstick to potash alum and in combination of both (Table 3. B.18). But high phosphate removal was observed for drumstick than for potash alum. Drumstick seed in combination with potash alum showed maximum percentage removal of COD, phosphate and TKN and the values are 37, 38 and 32 respectively.

Table 3. B. 18. Drumstick seed powder as a coagulant and coagulant aid

Coagulant	Percentage removal of		
	COD	PO_4	TKN
Drumstick seed	18	32	20
Potash alum	35	27	24
Potash alum + Drum stick seed	37	38	32

Water soluble proteins present in the seed causes a net positive charge and the solution therefore acts as a natural cationic polyelectrolyte during treatment[32]. Coagulation may take place by the neutralization of charge on the electronegative colloid particles by these ionized centers or by hydrogen bonding among the charged surfaces.

(ii) Goose-berry powder

Table 3. B.19. Goose-berry powder as a coagulant and coagulant aid

Coagulant	Percentage removal of		
	COD	PO_4	TKN
Goose-berry powder	23	14	15
Potash alum	35	27	24
Potash alum + Goose-berry powder	40	29	20

The effect of goose-berry as a coagulant and coagulant aid in the presence of potash alum to treat serum effluent was studied at pH 10 (Table 3. B.19). 40 % COD could be removed when it was used as a coagulant aid. Goose-berry could be used as a good coagulant since it removed 23 % of COD. Percentage removal of phosphate and TKN were 29% and 20% respectively when goose-berry was used as a coagulant aid.

Since the powdered dry fruit contains acid groups like phyllemblinic acid, gallic acid, ellagic acid[23], it may produce $-COO^-$ and H^+ ions during ionization and can neutralize the charge on the colloidal particles causing coagulation. The presence of low concentration of metal ion (Al^{+3}) is capable of binding with the functional groups on the polyelectrolytes facilitating rapid coagulation.

(iii) Tamarind seed powder

Table 3. B. 20. Tamarind seed powder as a coagulant and coagulant aid

Coagulant	Percentage removal of		
	COD	PO_4	TKN
Tamarind seed powder	27	44	16
Potash alum	35	27	24
Potash alum + Tamarind seed powder	42	46	28

The coagulation efficiency of tamarind seed powder to treat serum effluent was carried out in the presence of potash alum at pH 10 (Table 3. B.20). 200 mg/L of potash alum and 4 mg/L of tamarind seed powder were used for the study. Tamarind seed powder was good in removing phosphate. 44 percent phosphate was removed when tamarind seed powder was used individually and 46% phosphate was removed when used in combination with potash alum.

Maximum percentage removals of COD and TKN were 42 and 28 respectively when tamarind seed and potash alum were used in combination.

The activity of tamarind seed powder as a polyelectrolyte may be attributed to the presence of carboxylic group (–COOH) in the molecule which dissociates into $-COO^-$ at higher pH[24]. Hence, tamarind seed acts as anionic polyelectrolyte and effective formation of floc may be due to "bridging" mechanism.

(iv) Cationic polyelectrolyte

The effectiveness of cationic polyelectrolyte as a coagulant and coagulant aid to remove COD, phosphate and ammoniacal nitrogen from serum effluent was assessed in the presence of 200 mg/L aluminium chloride and 4 mg/L of cationic polyelectrolyte at pH 9.5 (Table 3. B.21).

Table 3. B. 21 Cationic polyelectrolyte as a coagulant and coagulant aid

Coagulant	Percentage removal of		
	COD	PO_4	AN
Cationic polyelectrolyte	15	05	16
Aluminium chloride	32	23	25
Aluminium chloride + Cationic polyelectrolyte	35	28	27

The coagulation efficiency was assessed in terms of COD, phosphate and AN removal. Cationic polyelectrolyte in the presence of aluminium chloride could remove 35 % of COD, 28 % phosphate and 27 % AN. The activity of cationic polyelectrolyte as a polyelectrolyte might be attributed to the charge neutralization and bridging action. Polyacrylamide is a non-ionic polyelectrolyte which is made cationic by special processes[48] and gives best results when molecular weight is high. Percentage removal of COD, phosphate and AN were comparatively less when cationic polyelectrolyte was used individually. This may be due to the inadequate positive charge imparted to the polyacrylamide molecule by the special process. The action of high molecular weight cationic polyelectrolytes is due to the charge neutralisation and bridging phenomenon. Besides lowering the surface charge on the particle, these polyelectrolytes also form particle bridges[49].

(v) Anionic polyelectrolyte

Table 3. B. 22. Anionic polyelectrolyte as a coagulant and coagulant aid

Coagulant	Percentage removal of		
	COD	PO_4	AN
Anionic polyelectrolyte	13	04	18
Potash alum	32	23	27
Potash alum + Anionic polyelectrolyte	34	26	27

Efficiency of anionic polyelectrolyte as a coagulant and coagulant aid was determined in terms of the percentage removal of COD, phosphate and ammoniacal nitrogen in the presence of 200 mg/L of potash alum and 4 mg/L of the anionic polyelectrolyte at pH 10 (Table 3. B.22). The anionic polyelectrolyte in the presence of potash alum could remove 34 % of COD, 26 % phosphate and 27 % AN. The coagulation action of the anionic polyelectrolytes could be attributed to the interparticle bridging mechanism. Anionic polymers become attached to a number of adsorption sites to the surface of the particles found in the wastewater. A bridge is formed when two or more particles become adsorbed along the length of the polymer. Bridged particles become more intertwined with other bridged particles during the flocculation process. The size of the resulting three dimensional particles grows until they can be removed easily by sedimentation[49].

The flocculation process induced by anionic polyelectrolytes is facilitated by the presence of low concentration of a metal ion (Al^{+3}) capable of binding with the functional groups on the polyelectrolytes. The positive metal ion serves to form a bridge among the anionic polyelectrolytes and negatively charged functional groups on the colloidal particle surface. Due to its hydrophilic character, several hydrogen bonds are formed among polyelectrolytes and water molecules. This association tends to occupy larger surface area causing very high viscosity[22].

The efficiency of the five polyelectrolytes as primary coagulant and coagulant aid were assessed in terms of the percentage removal of COD. The COD removal efficiency is in the order of tamarind seed powder followed by goose berry, drumstick seed powder, cationic polyelectrolyte and anionic polyelectrolyte. The study showed that natural polyelectrolytes perform better than the synthetic polyelectrolytes to remove COD from the serum effluent. The efficiency to remove phosphate from the effluent follows the order of tamarind seed powder followed by drumstick seed powder, goose berry, cationic polyelectrolyte and anionic polyelectrolyte. The removal efficiency in terms of TKN follows the order of tamarind seed powder followed by drumstick seed powder and goose berry. In the case of synthetic polyelectrolytes, AN removal is compared instead of TKN and both polyelectrolytes showed the same efficiency in removing AN when used as a coagulant aid.

3. B. 6. Conclusion

The optimum pH values for the effective coagulation of the five polyelectrolytes, viz., tamarind seed powder, goose berry powder, drumstick seed powder, cationic polyelectrolyte and anionic polyelectrolyte were determined. The optimum pH value was 10 for all polyelectrolytes.

The natural polyelectrolytes showed high potential as coagulant aid in the treatment of skim serum effluent from natural rubber centrifuging unit. A small quantity of polyelectrolyte, 4 mg/L, along with very small amount of metal coagulant could reduce considerable amount of COD and phosphate.

Among the five polyelectrolytes tamarind seed powder was found to be the most effective primary coagulant and coagulant aid to remove COD.

Potash alum was found to be the most effective among the metal coagulants studied in most of the cases. In the case of cationic polyelectrolyte the combination with aluminium chloride was more effective.

Among the synthetic polyelectrolytes, the cationic polyelectrolyte was found to be more effective to treat serum effluent than the anionic polyelectrolyte.

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CHAPTER 4

ANAEROBIC TREATMENT
PART A: UASB REACTOR

4. A. 1. Introduction

Bioremediation is defined as the recuperation of a contaminated site through the activity of living organisms, usually microorganisms, that breakdown the pollutant[1]. Bioremediation offers the potential to protect human health and the environment, maintains the protection over time and maximize waste treatment by exploiting naturally occurring biodegradable processes[2]. When oxygen is used as the electron acceptor, the reaction is termed aerobic and reactions involving other electron acceptors are considered anaerobic. The term anoxic is used to distinguish the use of nitrite or nitrate as electron acceptors from others under anaerobic conditions. Under anoxic conditions, reduction of nitrite or nitrate to gaseous nitrogen occurs, and this reaction is referred to as biological denitrification[3]. Anaerobic digestion is a biological treatment process for the degradation of simple organics in air free environment.

Anaerobic fermentation and oxidation processes are used primarily for the treatment of sludge and high strength organic wastes. The main advantages of anaerobic fermentation process are lower yield of biomass and energy formation from methane during the biological conversion of organic substrates. It is a cost effective method to treat high strength organic wastes in comparison to aerobic treatment with savings in energy, nutrient addition and reactor volume[3]. Anaerobic organisms utilize part of the carbon substrate for cell growth, and convert the other part to methane and carbon dioxide. Since anaerobic decomposition results in less efficient utilization of organic substrate for cell growth than aerobic decomposition the process has the advantage of low waste solids generation. Anaerobic treatment is economically more viable than the aerobic process[4]. The sludge production in anaerobic process is less than in the aerobic process. Aerobic process metabolises up to 40% of the BOD into sludge, whereas, in anaerobic process 5 to 10% of BOD metabolises to produce sludge[5]. High strength organic industrial wastes have been treated by this method with a minimum of land usage and energy, using filters, fluidized beds and UASB systems. Anderson et al[6] stated that anaerobic treatment is more economical for industrial effluents containing CODs greater than 4000 mg/L, and showed that if the COD of the effluent is above 20,000 mg/L then anaerobic treatment could save 75% of the capital and operating costs compared to an aerobic system. The process does not need energy intensive oxygen transfer for oxidation of organic pollutants in the wastewater. Rather, energy rich gaseous fuel methane is the by-product of this process that can supplement the energy needs of the industry.

Anaerobic digestion is applicable only to simple organic waste streams–primarily carbohydrates, lipids, proteins, alcohols, and organic acids[7]. If total suspended solid concentration is greater than 6 g TSS/L, anaerobic digestion or anaerobic contact process may be more appropriate[3]. Essentially no degradation of long-chain or cyclic hydrocarbons occurs. Metal concentrations are tolerated in the system as long as they are insoluble. Only soluble metal species are toxic to microbial activity and generally the heavier the metal ion, the greater the inhibition[7].

4. A. 2. Reactions in anaerobic process

The major steps involved in the anaerobic process are hydrolysis, acidogenesis and methanogenesis. In hydrolysis, materials like polysaccharides, proteins, lipids and nucleic acids are converted to simple and soluble monomers which is the first step in anaerobic process. Polysaccharides are converted to monosaccharides, proteins to amino acids, lipids to fatty acids and nucleic acids to purines and pyrimidines. In acidogenesis these are degraded further to acetate, hydrogen, CO_2, propionate and butyrate. Propionate and butyrate are also further degraded to acetate, hydrogen and CO_2. Organic substrates serve as both electron donors and acceptors. The precursors of methanogenesis are acetate, hydrogen and CO_2.[3]

In methanogenesis, the third step in anaerobic oxidation, two groups of methanogenic organisms are involved. One group is *aceticlastic methanogens* which split acetate into methane and carbon dioxide. The second group is hydrogen utilizing methanogens, use hydrogen as the electron donor and CO_2 as the electron acceptor to produce methane. Acetogens, the bacteria present in anaerobic process, use CO_2 to oxidize hydrogen and form acetic acid. This again gets converted to methane and therefore the impact of this reaction is minor. In anaerobic process about 72% of the methane is produced from acetate formation[3]. Based on the genetic traits, cellular life is divided into three basic domains, two composed of prokaryotic cells (Archaea and bacteria) and the third group is eukarya cells. Among the nonmethanogenic bacteria that are commonly found in anaerobic sludge digesters are *Clostridium* spp., *Peptococcus anaerobus.*, *Bifidobacterium* spp., *Desulphovibrio* spp., *Corynebacterium* spp., *Lactobacillus*, *Actinomyces*, *Staphylococcus* and *Escherichia coli* [3,8].

Many of the anaerobic bacteria isolated from anaerobic digesters are similar to those found in the stomach of ruminant animals and in organic sediments taken from lakes and rivers[3, 9]. Microorganisms responsible for methane production are archaea, which are strict obligate anaerobes. *Methanosarcina* and *Methanothrix* (spheres) are the only organisms which are able to use acetate to produce methane and carbon dioxide[3]. The methanogens and the acidogens form a syntrophic (mutually beneficial) relationship in which the methanogens convert fermentation end products such as hydrogen, formate, and acetate to methane and carbon dioxide. Methanogens reduces partial pressure of hydrogen; the equilibrium of fermentation reaction is shifted towards the formation of more oxidized end products like formate and acetate thus serving as a sink for hydrogen that allows the fermentation reactions to proceed. If methanogens are not able to utilize hydrogen due to process upset, the propionate and butyrate fermentation will be slowed down with the accumulation of volatile fatty acids in the anaerobic reactor and a reduction in the pH.

Madigan et al[10] showed that methanogens use limited number of substrates as shown below.

$$4\,H_2 + CO_2 \rightarrow CH_4 + 2H_2O$$

$$4\,HCOO^- + 4H^+ \rightarrow CH_4 + 3CO_2 + 2H_2O$$

$$4\,CO + 2H_2O \rightarrow CH_4 + 3CO_2$$

$$4\,CH_3\,OH \rightarrow 3\,CH_4 + CO_2 + 2H_2O$$

$$4\,(CH_3)_3N + H_2O \rightarrow 9\,CH_4 + 3CO_2 + 6H_2O + 4NH_3$$

$$CH_3COOH \rightarrow CH_4 + CO_2$$

The above reactions involve the oxidation of hydrogen, formic acid, carbon monoxide, methanol, methylamine and acetate.

Rate determining steps in the anaerobic process are hydrolysis conversion and the soluble substrate utilization rate for fermentation and methanogenesis. Minimum level of VFA concentration can be taken as an indication of sufficient methanogenic population. The rate determining step is the conversion of VFAs by methanogenic organisms and not the fermentation of soluble substrates by fermentating bacteria. 30 - 35 % of CO_2 is present in the gas developed in the anaerobic process and hence a high alkalinity is needed to ensure neutrality. Environmental factors such as pH and temperature have important effect on the selection, survival and growth of microorganisms. The key factor in the growth of microorganism is the pH as most bacteria cannot tolerate pH levels above 9.5. A pH value near neutral is preferred and below 6.8 the methanogenic activity is inhibited. The optimum pH for bacterial growth lies between 6.5 and 7.5. Table 4.A.1 shows the reactions of anaerobic bacteria and the products formed during anaerobic reaction.

Table 4.A. 1. Reactions of anaerobic bacteria showing carbon source, electron donor, electron acceptor and end products[3].

Common reaction names	Carbon source	Electron donor	Electron acceptor	Products
Acid fermentation	Organic compounds	Organic compounds	Organic compounds	VFAs such as acetate, propionate, butrate
Iron reduction	Organic compounds	Organic compounds	Fe(III)	Fe(II), CO_2, H_2O
Sulphate reduction	Organic compounds	Organic compounds	SO_4	H_2S, CO_2, H_2O
Methanogenesis	Organic compounds	VFAs	CO_2	Methane

According to the operating temperature range bacteria can be classified as psychrophilic (10-30^{0}C), mesophilic (20-50^{0}C) and thermophilic (35-75^{0}C)[3].

Solid retention time (SRT) greater than 20 days is needed for anaerobic process at 30^{0}C for effective treatment performance with higher SRT values at low temperatures[11].

Anaerobic processes are widely used through out the world for wastewater treatment due to the continuing rise in the cost of energy and the emergence of more stringent legislation concerning the discharge of industrial effluents. Anaerobic processes have been found to provide a feasible alternative to aerobic and chemical methods. Effect of particulate COD on anaerobic degradability was evaluated by Prashanth[12] using synthetically prepared wastewater at different food to microorganism ratios. An anaerobic-facultative lagoon system could remove 96% BOD, 89% COD, and 66% total nitrogen from latex concentrate effluent[13]. Saravanane[14] investigated the viability of biogas generation by integrating pressmud, a waste product of sugar industry with municipal sewage using cow dung and water as inoculum in the biomethanation process. The optimum total solid content was found to be 5% and maximum COD removal efficiency was 70% with biogas yield of 0.63 m^3/kg of COD. An innovative anaerobic digestion elutriated phase treatment (ADEPT) has been evaluated at mesophilic (M-ADEPT, 35^{0}C) and thermophlic (T-ADEPT, 55^{0}C) temperatures in which the organic loading rate was increased until the reactor fails[15].

4. A.3. Anaerobic treatment process and some important parameters

The following are some of the parameters that influence the anaerobic treatment process.

4.A.3.1. Types of anaerobic treatment process

(a) General

Anaerobic processes include anaerobic suspended growth, upflow and down flow anaerobic attached growth, fluidized-bed attached growth, upflow anaerobic sludge blanket (UASB), anaerobic lagoons and membrane separation anaerobic process[3]. A disadvantage of the anaerobic process is the low bacterial growth rate which may result in the washout of biomass. Upflow anaerobic sludge blanket (UASB) reactor developed in the late 1970s in the Netherlands by Lettinga and coworkers[16-18] is now the most widely used anaerobic treatment system for the biodegradation of industrial wastewaters[19]. The modifications of the original design of UASB process are horizontal flow filter, expanded granular sludge bed, upflow anaerobic fixed biofilm reactor etc. [20-25]. Other principal types of anaerobic sludge blanket process include the anaerobic baffled reactor (ABR) developed in early 1980s[26] and anaerobic migrating blanket reactor (AMBR) developed by Angenent et al[27] in 2000. UASB reactor has high loading capacity and simple design. This process is a combination of physical and biological process. No separate settler with sludge return pump is required as in anaerobic contact process.

(b) UASB reactor

UASB reactor consists of a vertical column containing an active population of anaerobic bacteria, which settle under gravity to form a thick fluid sludge bed in the lower part of the reactor. UASB reactor has the following major components; influent distribution system, sludge bed, sludge blanket, and a three phase separator consisting of gas-solid separator, and the effluent withdrawal design[16,17] (Fig. 4.A.1.). In the UASB reactor the wastewater is distributed at the bottom and travels in an upflow mode through the sludge blanket. The layer of biomass settled at the bottom of the reactor is called sludge bed. In this zone, the organic compounds in the influent are converted to the end products, such as biogas and cellular biomass, as flow passes upward through a highly active biomass. Bulk of stabilization occurs in this zone. Very high sludge concentration is maintained in this zone. The sludge blanket zone is on the top of the sludge bed. The sludge blanket zone is a suspension of sludge particles mixed with gases produced in the process. Degradation of residual organic matter from the sludge bed takes place in the sludge blanket zone. The biomass in a UASB reactor aggregates to form compact granules or pellets that may be up to 5 mm in diameter.

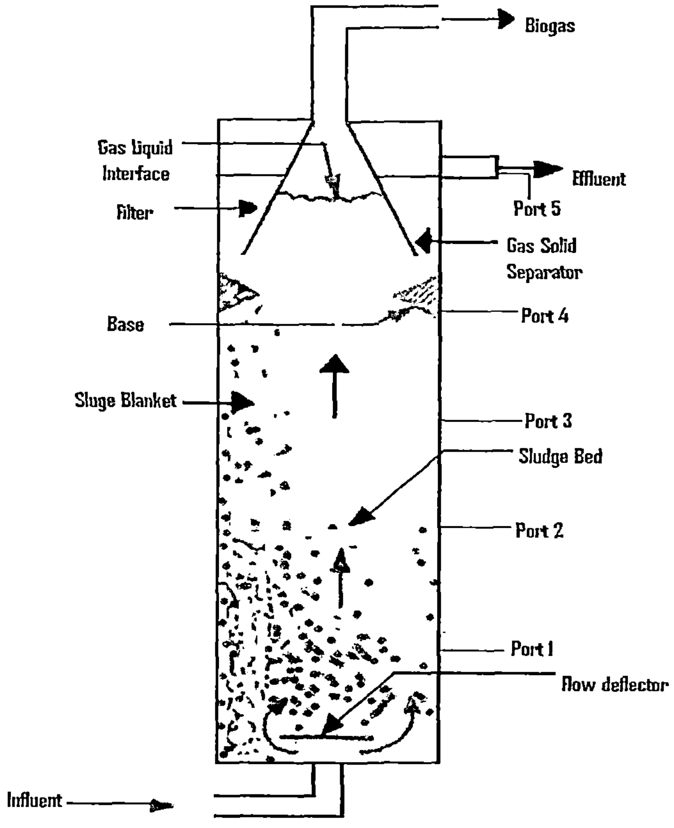

Fig. 4.A. 1. The schematic diagram of UASB reactor (After Lettinga et al.,[16])

The conditions suitable for the granular sludge formation were established by Speece[28]. A neutral pH, a plug- flow hydraulic regime, a zone of high hydrogen partial pressure, a non-limiting supply of ammoniacal nitrogen and a limited amount of the amino acid cysteine favour the formation of dense granulated sludge floc particles. In presence of sufficient amount of ammoniacal nitrogen and hydrogen, the bacteria responsible for granulation may produce amino acids, but their synthesis is limited by cysteine supply. The extracellular polypeptides which bind organisms together to form dense pellets or floc granules are some of the excess amino acids secreted[3]. At high rate of hydraulic loading, excessive agitation can lead to wash out of the sludge and system failure, if poorly flocculated sludge is formed. UASB reactor concept is based on the formation of well settable flocs or granules that are retained in the reactor by an efficient gas /liquid solid separator (GLSS) device located at the top of the reactor[5, 19]. Mixing in the reactor is caused by the evolved gases.

The input arrangement of the UASB reactor has the following advantages:

Slopping bottom of the settler serves as gas separator. No additional space is required for settling. The sludge separated in the settler can flow back directly into the reactor without mechanical means and thus prevents the washout of solids. The sludge is not exposed to the alien environment as it remains within the system[5]. Inoculums generally used are anaerobic digested sewage sludge or cow dung manure. The amount of inoculums required in UASB reactor has been found to be 10 to 20 kg VSS/m^3 of the reactor volume[29]. Development of dense granular sludge having a particle size of 1 to 3 mm helps the use of high volumetric COD loadings.

The UASB reactor is usually operated on long hydraulic retention time (HRTs) initially providing very low organic loading rate (OLR). This is necessary in the beginning so as to acclimatize the microorganisms especially the methanogens, to avoid shock loading and to increase methanogens' population. This steady state of operation indicated sufficient population of microorganisms[20]. During start-up period, COD removal gradually increases in the UASB as sludge accumulation occurs. Once proper startup of the reactor is achieved with generation of good quality of granular sludge, having good settling properties and activity, very high organic loading rates, greater than 30 kg COD/ m^3 can be applied[5].

UASB based plants have proved to be effective in the treatment of distillery wastewater, brewery wastewater, maize starch processing effluent and paper mill wastewater[30]. A laboratory study established the potential of anaerobic upflow filter for treating rubber effluents at an organic loading of 8 kg COD per day[31]. Anaerobic process with two UASB reactors was proposed as the best alternative to treat the wastewater from citric production by big manufacturing units[32, 33]. Batstone[34] examined specific aspects in the treatment of complex wastewater in high rate anaerobic treatment plants and the influence of different conversion processes and microbial characteristics on design and operation. The macroscopic conversion processes were examined by investigating and modelling a two stage high rate anaerobic reactor in Australia. He found that degradation of soluble proteins was rapid and all proteins were fully acidified in the acidogenic stage even at low retention times. Anaerobic treatment of alcoholic wastewaters in UASB reactors were investigated by Florencio[35].

The saving in operating power requirements makes the UASB cheaper in terms of overall cost than many other systems in warm countries (with reactor temperatures of more than 20^{0}C). The method is useful for meeting land irrigation requirements economically in a country like India[36]. In terms of simplicity and compactness, the most attractive approach is where micro-organisms are kept in the reactor either through their natural characteristics or through mechanical means and no external separation is needed. The best example of this approach is the UASB reactor, where the micro-organisms grow naturally in a dense, granular form. The anaerobic unit does not need to be filled with any stones or other media; the upflowing sewage itself forms millions of small 'granules' or particles which are held in suspension and provide a large surface area on which organic matter can attach and undergo biodegradation. A high solid retention time (SRT) of 30-50 days or more occurs within the unit[36]. Particular attractive features of the UASB reactor design include its independence from mechanical mixing of the digester contents, recycling of sludge biomass[37] and its ability to cope with perturbances due to high loading rates and temperature fluctuations[38]. No mixtures or aerators are required, thus conserving energy and resulting in very low operating cost.

4. A. 3. 2. Nutrients

To support microbial growth in biological systems, appropriate nutrients must be provided[3]. Nutrients other than carbon or energy sources may at times be the limiting material for microbial cell synthesis and growth. The principal inorganic nutrients needed by microorganisms are nitrogen, phosphorus, sulphur, potassium, magnesium, calcium, iron, sodium, and chlorine. Nitrogen and phosphorus are considered as macro nutrients because they are required in large amounts. They also require trace amounts of metallic ions or micro nutrients such as zinc, manganese, copper, molybdenum, selenium, nickel and cobalt[39]. The three major growth requirements are amino acids, nitrogen bases and vitamins. Using the formula $C_{12}H_{87}O_{23}N_{12}P$ for the composition of cell biomass, about 12.2 g of nitrogen and 2.3g of phosphorus are needed per 100 g of cell biomass[3]. The recommended COD:N:P during start up is 300:5:1 and during steady state process the ratio is 600:5:1[3].

4. A.3. 3. Methane gas production

The amount of CH_4 produced per unit of COD converted under anaerobic conditions at 35^0C is equal to 0.48 L[3]. Buswell and Boruff [40] suggested a relationship to estimate the products of anaerobic reaction. If the constitution of the waste is known, and neglecting the amount of the constituent used for cell synthesis, the following relationship, can be used to estimate the amount of methane (CH_4), carbon dioxide (CO_2), ammonia (NH_3) and hydrogen sulphide (H_2S) that will be produced under anaerobic conditions[3].

$$C_vH_wO_xN_YS_z+\left(v-\frac{w}{4}+\frac{x}{2}+\frac{3y}{4}+\frac{z}{2}\right)H_2O\rightarrow\left(\frac{v}{2}+\frac{w}{8}+\frac{x}{4}+\frac{3y}{8}+\frac{z}{4}\right)CH_4+$$
$$\left(\frac{v}{2}-\frac{w}{8}+\frac{x}{4}+\frac{3y}{8}+\frac{z}{4}\right)CO_2+yNH_3+zH_2S \qquad \text{Eq. 1}$$

The gaseous ammonia (NH_3) that is formed will react with the carbon dioxide to form ammonium ion and bicarbonate according to the following relationship.

$$NH_3+H_2O+CO_2\rightarrow NH_4^++HCO_3^- \qquad \text{Eq. 2}$$

Eq. 2 is the representation of the formation of alkalinity under anaerobic conditions, due to the conversion of organic compounds containing proteins (i.e., nitrogen). Percentage of carbon dioxide can be as high as 50 percent.

However since COD is removed in various ways, the effective gas production may be 0.10-0.3 m^3 per kilogram of COD removed. 1 m^3 of biogas with 75 percent of methane is equivalent to 1.4 kWh electricity[36]. Recovery of gas is optional and if collected but not used, a flare may be installed to burn the biogas. The burning of biogas avoids not only odour nuisance from the H_2S present in the gas[36] but also to prevent emission of CH_4 (a powerful green house gas compared CO_2).

4. A.3.4. Sulphide production

A group of bacteria (mainly obligate anaerobes) reduce sulphate in excess than their metabolic requirements and produce hydrogen sulphide in large amounts[41]. This type of sulphate reduction is known as dissimilatory sulphate reduction. Sulphate functions as the terminal electron acceptor in the anaerobic respiration by these organisms and the amount of sulphide produced is proportional to the amount of hydrogen or organic material dissimilated[41, 42].

Sulphate reducing bacteria can become a nuisance when the wastewater contains significant concentration of sulphate. These bacteria reduce sulphate to sulphide, and can become toxic to methanogenic bacteria. One solution to this problem is to add iron at controlled amounts to form iron sulphide precipitate. *Desulphovibrio* are group I sulphate reducers which use organic compound as their donor, oxidizing them to acetate and reducing sulphate to sulphide. Group II sulphate reducers are *Desulphobacter* which reduce fatty acids particularly acetate to carbon dioxide[3]. In addition, facultative anaerobic bacteria such as *Pseudomonas* reduce nitrate to nitrogen gas (denitrification). Reduction of sulphate to H_2S using methanol as the electron donor is as follows[3]

$0.119\ SO_4^{2-} + 0.167\ CH_3OH + 0.010\ CO_2 + 0.003\ NH_4^+ + 0.003\ HCO_3^- + 0.178$
$H^+ = 0.003\ C_5H_7NO_2$ (new cells) $+ 0.060\ H_2S + 0.060\ HS^- + 0.331\ H_2O$

Production of ferrous sulphide leads to black colour in the reactor. Blackening of the medium is a diagnostic characteristic of *Desulhoovibrio desulphuricans* growth. *Desulphovibrio desulphuricans* can cause significant removal of heavy metal ions by precipitation[43].

H_2S is malodorous and corrosive to metals and highly soluble in water, with a solubility of 2650 mg/L at 35^0C. Sulphate reducing bacteria compete with the methanogenic bacteria for COD and thus can decrease the amount of methane gas production. While low concentrations of sulphide (less than 20 mg/L) are needed for optimal methanogenic activity, higher concentrations can be toxic[29]. Methanogenic activity has been decreased by 50 percent or more at H_2S concentration ranging from 50 – 250 mg/L[44]. Unionised H_2S is more toxic than ionized H_2S, and therefore the pH is important in determining its toxicity. Type of anaerobic biomass, type of methanogenic population and the feed COD/SO_4 ratio determines the H_2S toxicity. At a pH value of 7 and 30^0C, about 60 percent of the total H_2S is present as gaseous H_2S.

4. A. 3. 5. Ammonia toxicity

Ammonia has been found to be toxic to anaerobic treatment of wastewater. High concentration of proteins and amino acids present in wastewater may get degraded to produce ammonium ions. High concentrations of free ammonia are also toxic to methanogenic bacteria. Since ammonia is a weak acid, the amount of free ammonia is a function of temperature and pH 2 to 4 percent of ammonium ion is in the form of free ammonia at a pH of 7.5 and a temperature of $30\text{-}35^0C$[29]. 100 mg/L is reported as the threshold toxicity for ammoniacal nitrogen[45]. Lay et al [46] found that 500 mg/L is the threshold toxicity level for methanogenic activity. McCarty[47] reported that 3000 mg/L of ammoniacal nitrogen is toxic at any pH. No inhibition for mesophilic and thermophilic digestion of municipal sludge was observed for NH_4-N concentration up to 2400 mg/L[48]. Parkin and Miller[49] and vanVelsen[50] have reported no effect of ammonia toxicity of ammoniacal nitrogen in the range of 5000 to 8000 mg/L.

4. A. 3. 6. Biological phosphorus removal

In biological phosphorus removal occurring in the anaerobic zone, acetate is produced by fermentation that can be assimilated by the biomass. Using energy available from stored polyphosphates, the phosphorus accumulating organisms (PAOs) assimilate acetate and produce intracellular polyhydroxybutrate (PHB). Some glycogen contained in the cell is also used. Concurrent with the acetate uptake is the release of orthophosphate (O-PO_4), as well as magnesium, potassium, and calcium cations. The PHB content in the PAOs increases while the polyphosphate decreases. Acetate uptake is critical in determining the amount of PAOs that can be produced and the amount of phosphorus that can be removed by this pathway. About 10 g of biodegradable soluble COD (bsCOD) will be required to remove 1g of phosphorus by biological storage mechanism. Periods of starvation or low bsCOD concentrations result in changes in the intracellular storage reserves of glycogen, PHB and polyphosphates and rapidly lead to decreased phosphorus removal efficiency[51]. In biological phosphorus removal, for an influent soluble phosphorus concentration of 10 mg/L, 5.6, 6.3 and 3.2 m/L of Mg, K, and Ca respectively would be required[3].

4. A. 3. 7. Advantages of UASB.

The daily operation of the UASB requires minimum attention. No special instrumentation is necessary for control, and the reactor may be emptied completely once in five years while the scum accumulated inside the gas collector channels may have to be removed every two years to ensure free flow of gas[52]. High loading rate, up to 30 kg/m^3 per reactor per day and low detention times and elimination of cost of packing material are the significant advantages of this method. It is a proven process in operation. Low land requirement, minimum sludge production, low need of nutrients, low operating power cost (even after neglecting the gas recovery and economic benefits of energy savings) are the major advantages of UASB over high capital costs for effluent treatment. Power supply is needed only for initial pumping of raw effluent and the whole plant can be kept operational at all times and this is a great advantage in many parts of India where regular power cuts occur[36]. Organic contaminants are transformed into green energy (biogas). The main advantages of UASB systems are the simplicity in design, ability to retain high biomass that leads to the efficient removal of organics at high loading rates and low energy demands [19].

UASB system is a well–proven technology for treatment of a wide range of effluents including toxic and inhibitory compounds. The knowledge on the importance of the role of nutrients and trace metals that affect the metabolic intermediates and end products facilitates process control.

4. A. 3. 8. Disadvantages

Disadvantages of anaerobic process are longer start-up time to develop necessary biomass intensity, alkalinity addition, upset due to toxic substances and the need for further treatment[3]. The presence of sulphates in the influent may lead to the production of highly corrosive and toxic form of sulphides. Certain heavy metals present in the industrial waste can either impede biological action in UASBs or get precipitated. Inflow fluctuations may affect the upflow velocities causing carry over some solids[36].

The aim of the present study is to evaluate the various aspects of treating highly polluted skim serum effluent from natural rubber latex centrifuging unit employing UASB technique.

4. A. 4. Experimental

A bench scale UASB reactor was fabricated using PVC pipe having internal diameter 10.5 cm and 460 cm height with a total volume of 36 liters. A flexible side tube of 20 mm diameter was provided to feed the effluent at the bottom. An exhaust was provided at the bottom to avoid clogging. Five sampling ports (Port 1from bottom to Port 5 at the top of the reactor) were provided at every 100 cm intervals. A space of 30 cm height was provided at the top. A hood having an outlet tube, which was free to move inside the outer tube, had been provided to collect the generated gas at the top. The wastewater was fed by gravity to the bottom of the reactor (Fig. 4.A 2.). To startup, the reactor was filled with water and filtered cow dung slurry.

pH of the serum was maintained above seven by adding lime and sodium hydroxide. After coagulating with potash alum, the clear solution was filtered and analysed as per standard methods[53]. Exogenous addition of bicarbonate is recommended for proper anaerobic conversion[35]. To the clear solution sodium bicarbonate was added to maintain the pH buffering capacity of the system. It was then fed in drops to the bottom of the reactor through the side tube at the rate of one litre per day. After 36 days of continuous acclimatization significant growth of bacteria was observed being evident from the volatile fatty acid and methane gas formation. When the reactor efficiency attained a steady state, the COD removal efficiency became almost constant (more than 85%) for a constant organic load. Continued the feeding with effluent samples at the rate of one litre per day (maintaining COD 0.75 kg/m^3 and 1.07 kg/m^3) and two litres per day for an HRT of 36 days and 18 days respectively. For HRTs of 12 days and 9 days, 2 litres of the effluent was diluted to 3 and 4 litres respectively to minimise the overloading of the reactor. Evaluated the performance of the reactor by drawing samples from the five different ports for different hydraulic retention times (HRTs) and various organic loadings for a period of fourteen months.

Samples were drawn every week from the sampling ports and analysed as per standard methods[53] for various parameters like pH, COD, BOD, sulphide, VFA, TKN, AN, TS, DS, phosphate and VSS. The biogas produced was also collected and measured by the downward displacement of water.

Estimated the presence of heavy metals using atomic absorption spectrophotometer (AAS) at the Rubber Research Institute of India (RRII), Kottayam, India. Instrument parameters are :- System type –Flame., Instrument mode –Abs.BC On., Measurement mode – Integration., Read Time- 3.0 s., Flame type- Air-Acetylene.

Biochemical analysis of raw as well as anaerobically treated effluent was done using a UV–visible recording spectrophotometer UV-240. Concentration of total sugars[54], reducing sugars[55], proteins[56], phenols[57] and free amino acids[58] were estimated as per standard analytical techniques, and as described in Chapter 2.

Fig. 4.A. 2. UASB reactor

4. A. 5. Results and discussion

4. A. 5. 1. COD removal

(a) Effect of organic loading rate and hydraulic retention time

An organic loading of 0.75 kg/m^3/d for 36 days was used for acclimatisation of the reactor. Feeding was continued for an organic loading of 0.75 and 1.07 kg/m^3/d for the same HRT. Effluent was withdrawn from the five sampling ports every week and analysed. The percentage COD removal for HRT of 36 days and 18 days at two different organic loadings are given in Fig.4.A. 3 to 6. The COD removal efficiency is the range of 41 to 72 percent at the bottom of the reactor (Port 1), 80 to 92 in Port 2, 80 to 93 in Port 3, 81 to 94 in Port 4 and 85 to 95 percent in Port 5, at the top of the reactor for HRT of 36 days and for an organic loading of 0.75 kg/m^3/d in different weeks of treatment. Gradual increases in values were observed up to the third week and the values become almost steady during the next two weeks. In the sixth week, again it increased and reached up to 95 percent which was the highest removal efficiency. This observation shows that an organic loading of 0.75 kg/m^3/d for an HRT of 36 days can be successfully used to treat the serum effluent. When organic loading was increased to 1.07 kg/m^3/d for the same HRT, the percentage removal of COD dropped to 60 and then it started increasing reaching a steady value of 87 (Fig. 4. A.4). In Port 1, for this HRT and OLR the percentage removal was in the range of 60 to 68 and in Port 5, it was 84 to 87 when moved from week 1 to week 5. COD values decreased gradually on moving from Port 1 to Port 5. The same trend was observed when HRT was reduced to 18 days and organic loading was increased to 3 and 4.3 kg/m^3/d (for 2 litres of the effluent per day) (Fig. 4.A. 5 & 6). Percentage COD removal, though decreased initially, it gradually increased and reached a value of 88 percent for an organic loading of 3 kg/m^3/d. But when organic loading increased further to 4.3 kg/m^3/d for the same HRT of 18 days, it dropped to 53 percent and only a maximum of 70 percent COD removal could be achieved at this HRT. For HRT of 12 days, the average COD removal efficiency dropped to 58 and 52 percent for the same organic loading of 3 and 4.3 kg/m^3/d respectively and for HRT of 9 days it dropped to 52 and 47 percent respectively for the same organic loading (Fig. 4.A. 7).

In most of the cases, the efficiency of treatment reached a steady state after a period of operation at a given organic loading. The initial decrease in percentage removal of COD for higher organic loadings might be due to initial substrate inhibition or insufficient, immobilized and balanced microbial communities[19].

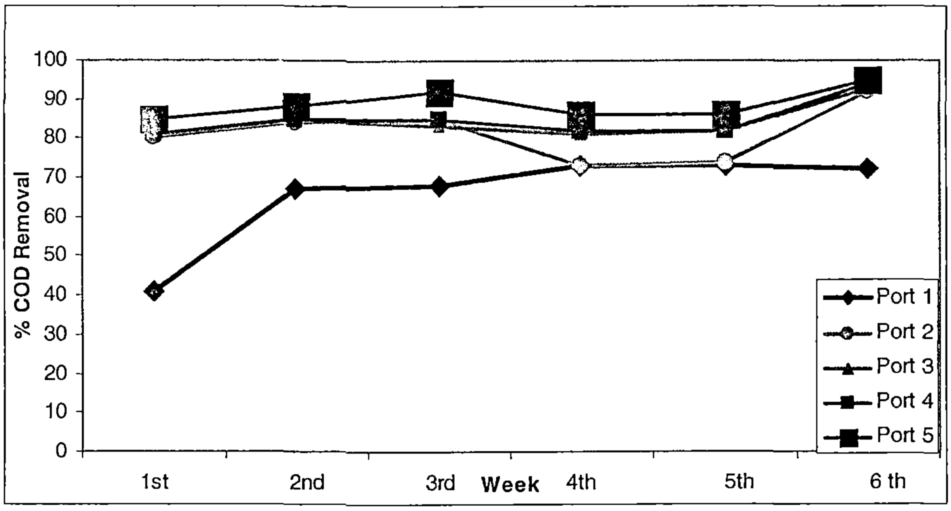

Fig. 4.A. 3. Percentage COD removal vs weeks of operation for HRT 36 days & OLR 0.75 kg/m^3/d

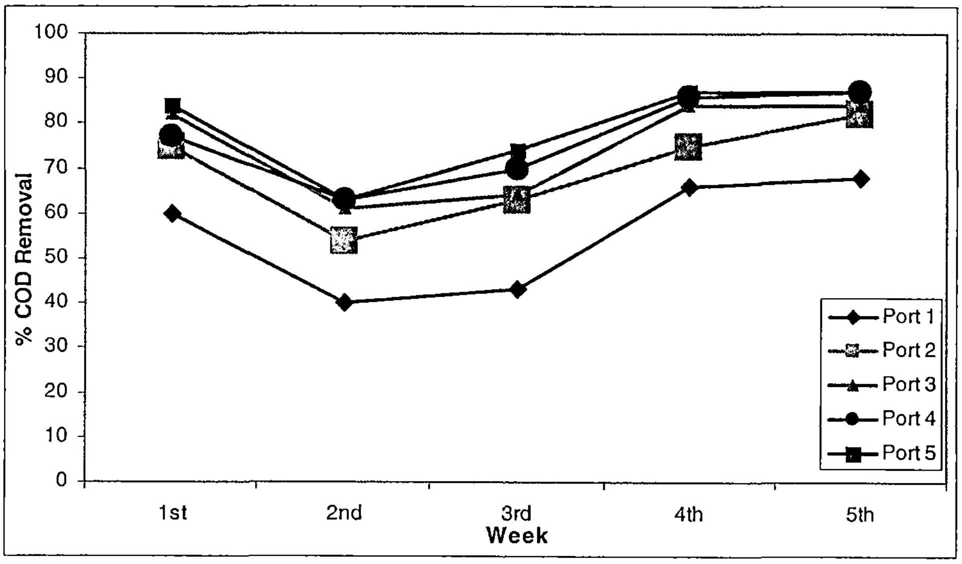

Fig. 4. A. 4. Percentage COD removal vs weeks of operation for HRT 36days & OLR 1.07 kg/m^3/d

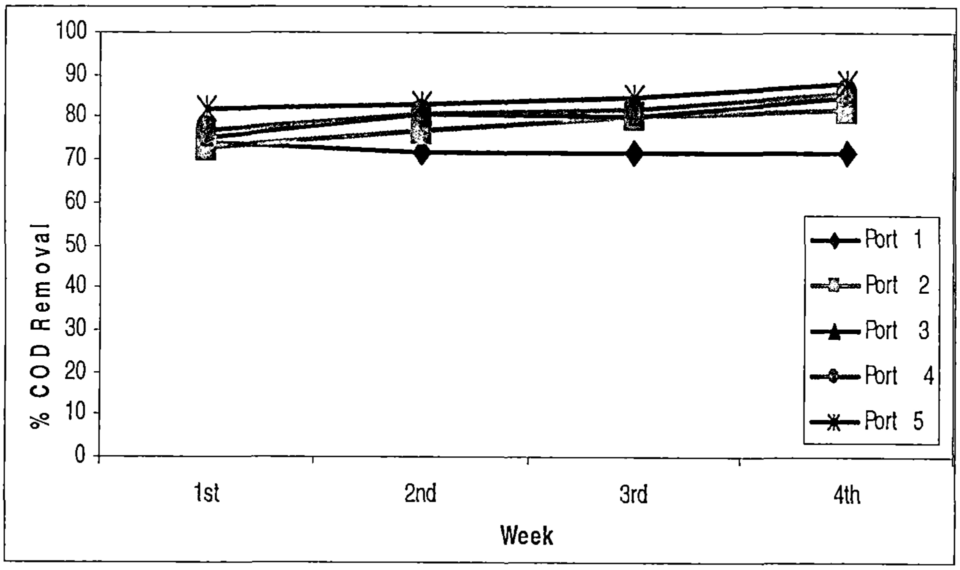

Fig. 4.A. 5. Percentage COD removal vs weeks of operation for HRT 18 days & OLR 3 kg/m^3/d

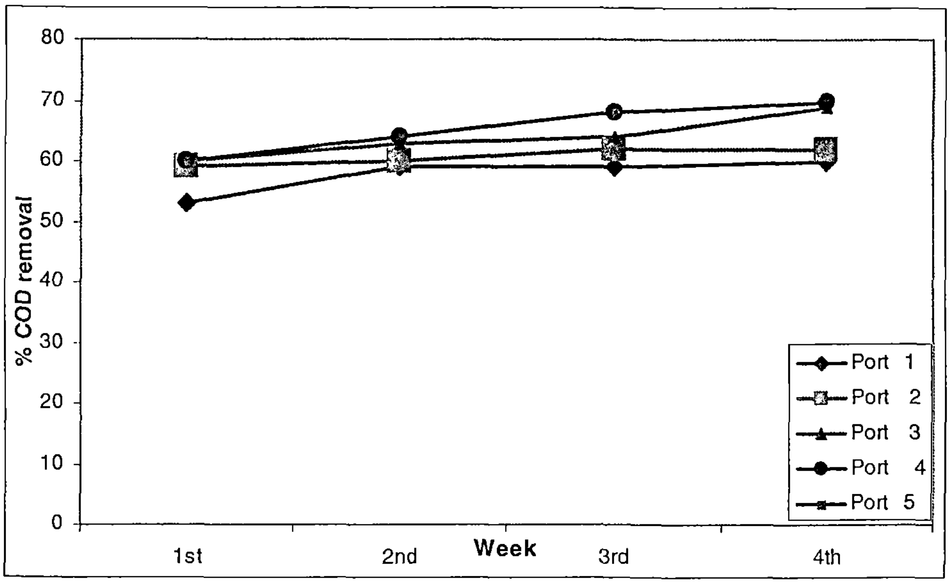

Fig. 4. A. 6. Percentage COD removal vs weeks of operation for HRT 18 days & OLR 4.3 kg/m^3/d

Influence of HRT on COD removal for the same organic loading was compared for HRTs of 18, 12 and 9 days since the same organic loading was used only for these HRTs. For 18 to 9 days of HRTs' and for an organic loading of 3 kg/m^3/d, COD removal efficiency dropped from 84.5 to 64 percent and for an organic loading of 4.3 kg/m^3/d the value dropped to 51 from 65.5 percent respectively (Fig. 4. A. 8).

As the HRT increased, COD reduction efficiencies of the system also increased and *vice versa.* At lower HRTs, higher liquid velocities caused shorter contact period between organism and organics, resulting in poor mass transfer rates, leading to decreased performance efficiency[20]. At lower HRTs, The possibility of washout of biomass is more prominent. This makes it difficult to maintain the effective number of useful micro organisms in the systems [59].

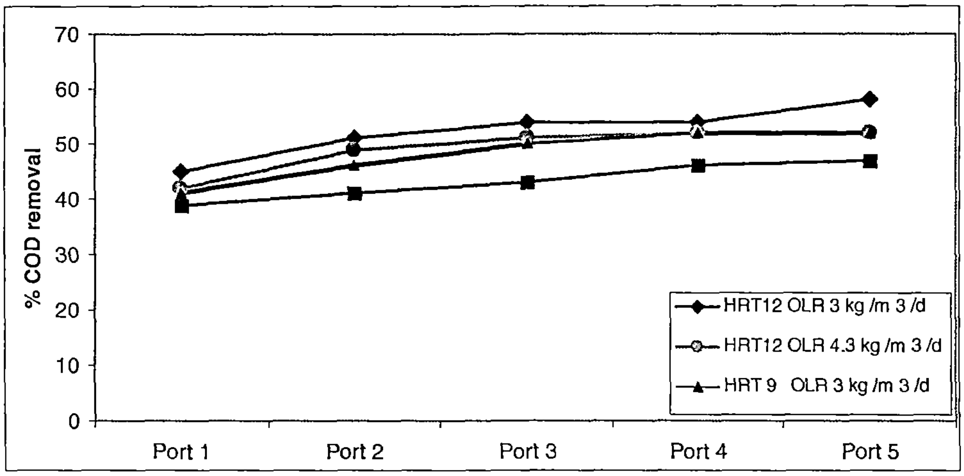

Fig. 4.A. 7. Percentage COD removal for HRT 12 & 9 days and for OLR 3 & 4.3 $kg/m^3/d$

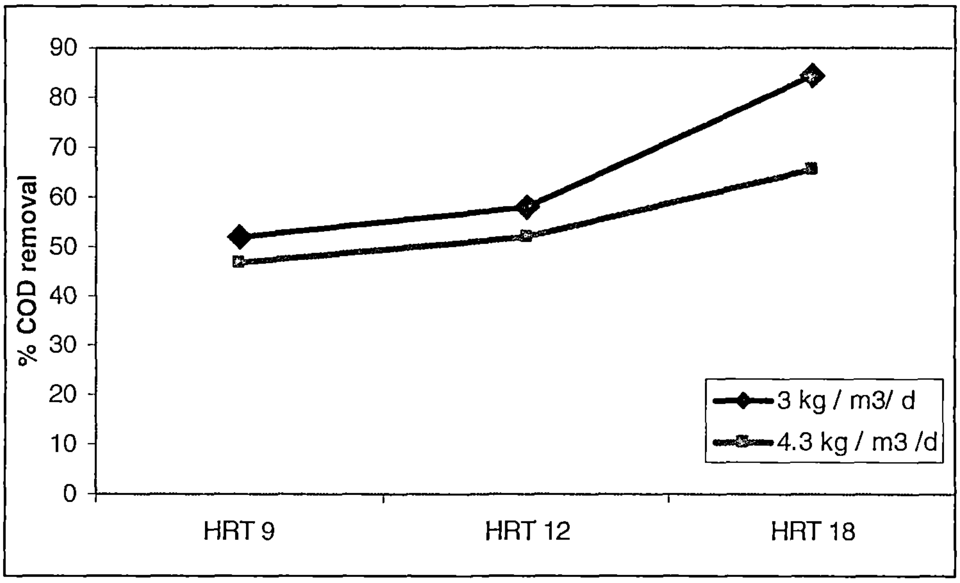

Fig. 4.A. 8. Percentage COD removal vs HRT for OLR 3 & 4.3 $kg/m^3/d$

(b) Influence of filter height

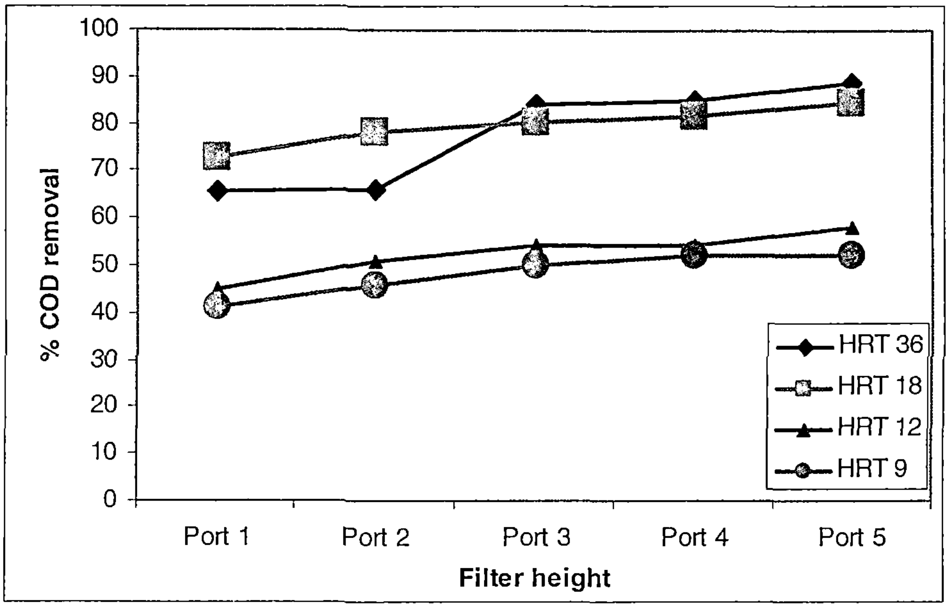

Fig. 4.A. 9. Percentage COD removal vs filter height for the effluent having COD 0.75 kg/m^3/d

From Fig. 4. A. 9, it is clear that percentage removal of COD increased as the effluent moved upwards and maximum percentage removal was on the top of reactor. The average percentage removal increased from 65.7 to 88.7 for HRT 36 days and OLR 0.75 kg/m^3/d. It increased from 73 to 85, 45 to 58 and 41 to 52% respectively for HRTs18, 12, 9 days and for an organic loading of 3 kg/m^3/d as the height of the reactor increased from Port 1 to Port 5. The influence of the reactor height was almost same for the two different organic loadings having the same HRT (Table 4. A. 2). A major portion of the COD was removed below 1.3 metre. The high percentage removal of COD at the lower part of the reactor was due to the presence of active population of anaerobic bacteria which settle under gravity to form a thick fluid sludge blanket in the lower part of the reactor[19]. The COD removal efficiencies achieved for different HRTs, OLRs and filter heights are summarized (as five weeks average) in Table 4.A. 2.

Table 4.A. 2. Summary of the effect of HRT, OLR and filter height on the percentage removal of COD

HRT(days) & OLR (kg/m^3/d)	Percentage removal of COD				
	Port 1 (bottom)	Port 2	Port 3	Port 4	Port 5 (Top)
HRT 36 days & OLR 0.75	65.7	66	84	84.8	88.7
HRT 36 days & OLR 1.07	55.4	69.8	75	76.6	79
HRT 18 days & OLR 3	73	78	80	82	85
HRT 18 days & OLR 4.3	58	61	64	66	66
HRT 12 days & OLR 3	45	51	54	54	58
HRT 12 days & OLR 4.3	42	49	51	52	52
HRT 9 days & OLR 3	41	46	50	52	52
HRT 9 days & OLR 4.3	39	41	43	46	47

4. A. 5. 2. BOD removal

Effluent samples having 27.5 kg/m^3 COD and 10.5 kg/m^3 BOD was used during the start up period. Then the reactor was fed with effluent having COD 27 kg/m^3 and BOD 14 kg/m^3. During the whole treatment period, the effluent used was having BOD less than COD and in most cases it was almost half of COD. As anaerobic treatment proceeds, an unusual increase in the value of BOD was observed for HRTs 36 and 18 days as is evident from Table 4.A. 3 to 6. The results are summarized in Table 4.A. 7.a and 7.

b. The values of BOD were found to exceed the value of COD during this experiment, though the estimation of COD shows 80 to 95 percent treatment efficiency for HRTs of 36 and 18 days. The same trend was observed even after repeating the process to confirm its efficiency. For HRTs of 12 and 9 days, the treatment efficiency is low and the BOD values did not exceed the COD values. For HRTs of 9 days the BOD/COD ratio was only 0.6. The higher value of BOD than COD does not mean that the treatment is not effective. For higher HRTs it showed some kind of interference in the measurement of BOD. This may be due to ions like nitrates, sulphides, sulphites etc.

Table 4.A. 3. The values of COD and BOD for an HRT of 36 days and OLR 0.75 kg/m^3/d

Week	COD					BOD				
	Port 1	Port 2	Port 3	Port 4	Port 5	Port 1	Port 2	Port 3	Port 4	Port 5
1	15927	5556	5345	5185	4400	18528	16320	15456	15648	15400
2	8968	4248	4115	4012	3900	20305	15540	16660	16576	16470
3	8779	3965	4531	4106	3823	19000	16000	14400	15600	15000
4	7439	7298	5235	4912	4070	20800	20000	17000	17600	17400
5	7415	7130	5035	4814	3972	18490	16792	16037	15849	15094
6	7579	2246	1825	1684	1403	18480	8310	7250	6260	4235

Table 4.A. 4. The values of COD and BOD for an HRT of 36 days and OLR 1.07 kg/m^3/d

Week	COD					BOD				
	Port 1	Port 2	Port 3	Port 4	Port 5	Port 1	Port 2	Port 3	Port 4	Port 5
1	14882	9354	6803	8504	8952	15810	15220	14586	9690	9384
2	22110	17008	14456	13606	13606	11016	9435	9282	8670	8313
3	21260	13606	13464	11120	9719	12240	11526	11200	11832	13056
4	12753	9285	5927	5029	5029	11760	12642	13328	14112	14504
5	12075	6792	6038	5014	4817	15484	16807	15827	14210	17493

Table 4.A. 5. The values of COD and BOD for an HRT of 18 days and OLR 3 kg/m^3/d

Week	COD					BOD				
	Port 1	Port 2	Port 3	Port 4	Port 5	Port 1	Port 2	Port 3	Port 4	Port 5
1	7177	7373	6902	6275	5333	18454	15292	14773	15622	16425
2	7683	5802	5174	5174	4860	17606	16223	16808	17606	15478
3	7526	5488	5331	5018	4233	17024	14044	13406	14258	14044
4	7526	5018	4077	3920	3293	14688	14112	12192	12000	9549

Table 4.A. 6. The values of COD and BOD for an HRT of 18 days and OLR 4.3 kg/m^3/d

Week	COD					BOD				
	Port 1	Port 2	Port 3	Port 4	Port 5	Port 1	Port 2	Port 3	Port 4	Port 5
1	18230	15840	15471	15460	15451	19320	17128	16760	15568	15254
2	15900	15390	14350	13900	13890	19004	17320	16320	17070	15254
3	15880	14750	13900	12410	12420	18560	17115	15760	15350	16750
4	15440	14730	12000	11640	11650	18032	16072	15680	14504	14798

Table 4.A 7a. Summary of the COD removal during anaerobic treatment

HRT (days) & OLR (kg/m^3/d)	COD during anaerobic treatment				
	Port 1 (bottom)	Port 2	Port 3	Port 4	Port 5 (Top)
HRT 36 days & OLR 0.75	9351	5074	4348	4119	3595
HRT 36 days & OLR 1.07	16616	11209	9338	8655	8425
HRT 18 days & OLR 3	7478	5920	5371	5489	4430
HRT 18 days & OLR 4.3	16262	15177	13930	13352	13352
HRT 12 days & OLR 3	14850	13230	12425	12390	11340
HRT 12 days & OLR 4.3	22564	19780	19000	18620	18620
HRT 9 days & OLR 3	15930	14580	13500	12960	12930
HRT 9 days & OLR 4.3	23660	22830	22170	20900	20560

Table 4. A. 7b. Summary of the BOD removal during anaerobic treatment

HRT (days) & OLR (kg/m^3/d)	BOD during anaerobic treatment				
	Port 1 (bottom)	Port 2	Port 3	Port 4	Port 5 (Top)
HRT 36 days & OLR 0.75	19267	15493	14467	14588	13933
HRT 36 days & OLR 1.07	13262	13126	12844	11702	12550
HRT 18 days & OLR 3	16943	14917	14294	14871	13874
HRT 18 days & OLR 4.3	18032	16079	15680	15123	14798
HRT 12 days & OLR 3	13680	13250	13180	12052	11012
HRT 12 days & OLR 4.3	15960	15177	14550	14034	14000
HRT 9 days & OLR 3	12400	11320	10800	9974	8102
HRT 9 days & OLR 4.3	15300	14443	13840	13152	12690

Table 4. A. 8. COD & BOD relationship of raw & anaerobically treated effluent

Raw effluent			Anaerobically treated effluent			
COD	BOD	BOD/COD	HRT(days)	COD	BOD	BOD/COD
27500	10500	0.38	36	3595	13933	3.9
37000	22500	.60		8425	12550	1.5
27000	14000	.52	18	4430	13874	3.1
27500	13500	.49		13352	14798	1.1
38800	23280	.60	12	11340	11012	0.9
				18620	14000	0.8
			9	12930	8102	0.6
				20560	12690	0.6

Table 4. A. 8 shows the COD and BOD relationship of the raw effluent and anaerobically treated effluent. The table gives the average values of four to five weeks of anaerobic treatment of the effluent at various HRTs. The BOD/COD ratio of the raw effluent is in the range of 0.38 to 0.60 whereas for anaerobically treated effluent it is in between 0.6 to 3.9. For lower HRTs the ratio is almost the same as that of the raw effluent.

Biochemical analysis of the raw effluent (Table 4.A.18) showed that it contained 865 mg/L soluble protein, 16000 mg/L of amino acids and 3900 mg/L of ammonia. There is a possibility of non-carbonaceous matter such as ammonia being produced during the hydrolysis of proteins[3]. Free ammonia in the anaerobically treated effluent was estimated occasionally and the values were between 3500 mg/L and 4500 mg/L for higher HRTs. Dense white fumes of ammonium chloride was observed when HCl was added to the anaerobically treated effluent during the estimation of sulphide. A number of bacteria are capable of oxidizing ammonia to nitrite and subsequently to nitrate[3]. Conversion of ammonia to nitrite by *Nitrosomonas* is

$NH_3 + 3/2\ O_2 \rightarrow HNO_2 + H_2O$

Conversion of nitrite to nitrate by *Nitrobacter* is

$HNO_2 + 1/2\ O_2 \rightarrow HNO_3$

And overall conversion of ammonia to nitrate is

$NH_3 + 2\ O_2 \rightarrow HNO_3 + H_2O$

The oxygen demand associated with the oxidation of ammonia to nitrate is called the nitrogenous biochemical oxygen demand (NBOD). This may interfere with BOD values at higher HRTs leading to erroneous interpretations of treatment as reflected in the table 4.A.8. At lower HRT's the hydrolysis of proteins and amino acids will be slow because of the high upflow velocities. The more the hydrolysis of proteins and amino acids, the higher will be the production of ammonia. When nitrification occurs, the measured BOD values will be higher than the BOD exerted by the carbonaceous material[3]. According to Ramalho[60], the oxygen demand of wastewaters is exerted by three classes of materials: carbonaceous organic materials used as a source of food by aerobic organisms; oxidisable nitrogen derived from nitrite, ammonia and organic nitrogen compounds which serve as food for specific bacteria and chemical reducing compounds like ferrous ion, sulphites, and sulphides, which are oxidized by dissolved oxygen. All these have a bearing on the oxygen balance of the receiving water[60,53]. Young[61] suggested that various methods like pasteurization and chlorination could be used to suppress the nitrifying organism. To study the influence of pasteurization on BOD test to suppress the nitrifying organism, the test was done after pasteurization. An effluent sample (COD of 15158 mg/L) having a BOD of 15600 mg/L before pasteurization, gives a BOD of 15445 mg/L after pasteurization. This showed that pasteurization was not so effective to suppress the nitrifying organism in this effluent. This might be due to the presence of high amount of free ammonia in the treated effluent. The raw effluent contained around 17000 mg/L of sulphate. During anaerobic treatment, it was reduced to sulphide equivalent to one third of the sulphate removed stoichiometrically[3]. The high amount of sulphide produced during anaerobic treatment was oxidisable by molecular oxygen and might cause interference in BOD values. Therefore, it is concluded that BOD values have limited use in assessing the efficiency of anaerobic treatment of skim serum effluent at higher HRTs.

4. A. 5. 3. pH and VFA

Table 4. A. 9.a. Variation of pH at different stages of the reactor

HRT(days) & OLR(kg/m^3/d)	pH				
	Port 1	Port 2	Port 3	Port 4	Port 5
HRT 36 days & OLR 0.75	5.60	5.30	5.30	5.30	5.30
HRT 36 days & OLR 1.07	7.60	7.30	7.20	7.20	7.20
HRT 18 days & OLR 3	7.25	7.05	7.10	7.10	7.10
HRT 18 days & OLR 4.3	6.90	5.00	6.60	6.60	6.70
HRT 12 days & OLR 3	6.20	5.75	5.70	5.70	5.70
HRT 12 days & OLR 4.3	6.00	5.65	5.60	5.60	5.60
HRT 9 days & OLR 3	7.20	7.10	7.10	7.00	7.00
HRT 9 days & OLR 4.3	7.00	6.95	6.80	6.75	6.75

pH of the raw effluent was in the range of 3.6 to 4.7 and VFA of the raw effluent was in the range of 1200 to 2000. Before subjecting it for anaerobic treatment, pH was brought up to 7 by adding sodium hydroxide, lime and sodium bicarbonate. The average pH and VFA values for four to five weeks at different HRTs and OLRs are given in the Table 4.A.9. a and b. In the initial stage, pH decreased from 7 to 5.3 (HRT 36) and this may be due to the production of volatile fatty acid (23 to 15.3 g/L as acetic acid). It seems that low pH values are not influencing much on the COD removal efficiency (Table 4.A.2). Florencio[35] reported that methylotrophic methanogens are active over a broad pH range.

Table 4. A. 9.b. Variation of VFA at different stages of the reactor

HRT(days) & OLR(kg/m^3/d)	VFA g/L				
	Port 1	Port 2	Port 3	Port 4	Port 5
HRT 36 days & OLR 0.75	23.0	19.2	18.9	15.2	15.3
HRT 36 days & OLR 1.07	13.7	12.9	13.9	11.5	11.2
HRT 18 days & OLR 3	11.4	14.3	14.1	14.1	13.0
HRT 18 days & OLR 4.3	16.9	17.4	16.0	15.8	15.2
HRT 12 days & OLR 3	12.5	12.8	11.9	11.0	10.2
HRT 12 days & OLR 4.3	11.8	11.7	11.4	11.3	10.8
HRT 9 days & OLR 3	10.6	10.8	10.4	10.3	10.2
HRT 9 days & OLR 4.3	10.2	10.5	9.8	9.6	9.3

In order to avoid the fall of pH to a minimum level, the pH of the influent was kept around 7.5 to 8.5 by adding NaOH, $NaHCO_3$ (25 meq/L) and lime for HRT 36 days and OLR 1.07kg/m^3/d onwards. The basic steps in the anaerobic process are hydrolysis in which particulate material is converted to its monomers. The second step is fermentation or acidogenesis and the principle products are acetate, hydrogen and CO_2. The third step is methanogenesis which is carried out by two groups. One group is *aceticlastic methanogens* which split acetate into methane and CO_2. The second group, termed hydrogen–utilising methanogens, use hydrogen as the electron donor and CO_2 as the electron acceptor to produce methane. Bacteria with in anaerobic process, termed acetogens, are also able to use CO_2 to oxidise hydrogen and form acetic acid. About 72 % of the methane produced in anaerobic digestion is from acetate formation[3]. Undissociated VFA is toxic to methanogens. In order to prevent the accumulation of undissociated VFA, high levels of alkalinity should also be applied[35]. High concentration of VFA for HRT 36 and 18 days shows the formation of acetate. But it may be the buffer action exerted by the added $NaHCO_3$ to proceed to acetogenesis as evinced from the removal of more than 80 percent COD (Table 4A.2). Bicarbonate plays an important role in the anaerobic conversion as a weak acid in the pH buffering system and as a cosubstrate in acetogenesis[35]. Since 30 to 35 % of CO_2 is present in the gas developed in the anaerobic process, a high alkalinity is needed to ensure neutrality. For sludge digestion sufficient alkalinity is produced by the breakdown of protein and amino acids producing ammonia. The gaseous ammonia (NH_3) that is formed will react with the carbon dioxide to form ammonium ion and bicarbonate (Eq. 2). This equation is the representation of the formation of alkalinity under anaerobic conditions, due to the conversion of organic compounds containing proteins (i.e., nitrogen). Meagre endogenous resources of bicarbonate that are generated by methanogenesis cannot support significant acetogenesis. Exogenous addition of bicarbonate is recommended for proper anaerobic conversion[35].

When loading continued with 1.07 kg/m^3/d for the same HRT of the effluent having initial pH around 8, the pH of the treated sample decreased only up to 7.20 and volatile fatty acid (VFA) decreased to 11.2 g/L. This may be due to conversion of VFA to biogas.When HRT was reduced to 18 days and organic loading was increased to 3 kg/m^3/d, VFA increased to 13 g/L and pH decreased initially and later it became steady. On varying HRT to 12 and 9 days, pH was in the range of 5.70 and 7.00 respectively. In this treatment there may be the dominance of methylotrophic methanogens since they are active in a wide pH range. The rate determining step is the conversion of VFAs by methanogenic organisms and not the fermentation of soluble substrates by fermentating bacteria. Environmental factors such as pH and temperature have important effect on the selection, survival and growth of microorganisms. The key factor in the growth of microorganism is the pH and most bacteria cannot tolerate pH levels above 9.5.

It is clear from the Table 4.A.9.b that during the whole treatment process, VFA decreased as the effluent moves upwards in the reactor and the decrease was steadier after Port 2. At the bottom level (below Port 2) the VFA concentration was always higher and showed slight fluctuations in values between Port 1 and Port 2. After Port 2, the VFA value always decreased. The same trend was observed in pH values also. Change in pH was more prominent below Port 2, and beyond Port 2, the pH values remained almost steady. pH and VFA changes were more pronounced below 1.3 metre (i.e., below Port 2), indicating high rate of anaerobic reactions at the lower part of the reactor. As HRT decreased, gradual drop in VFA values was observed. VFA formation gradually reached 9.3 g/L for an HRT of 9 days. This might be due to the washout of methanogenic bacteria and proton reducing acetogenic bacteria for higher HRTs.

4. A. 5. 4. Removal of TS and DS

Effect of HRTs and organic loadings on the removal efficiencies of TS and DS was studied for four to five weeks. The lowering of HRT from 36 days to 9 days decreased the efficiency of removing TS and DS, whereas increasing organic loading, increased the percentage removal of TS and DS.

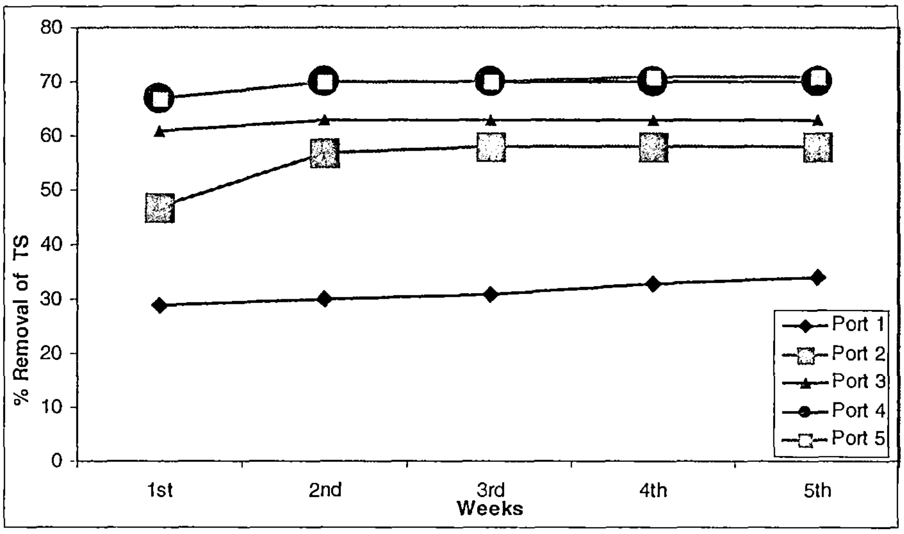

Fig.4. A. 10. Percentage TS removal vs weeks of operation for HRT 36 days & OLR 0.75 $kg/m^3/d$

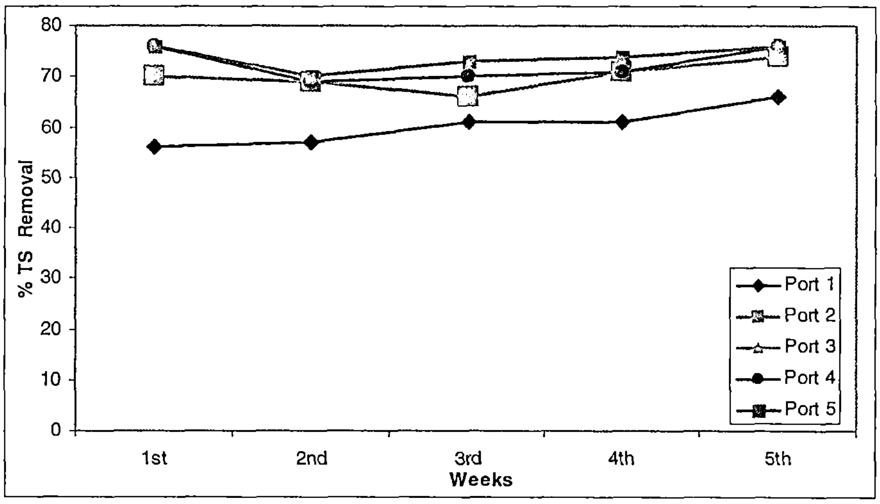

Fig. 4.A.11. Percentage TS removal vs weeks of operation for HRT 36 days & OLR 1.07 $kg/m^3/d$

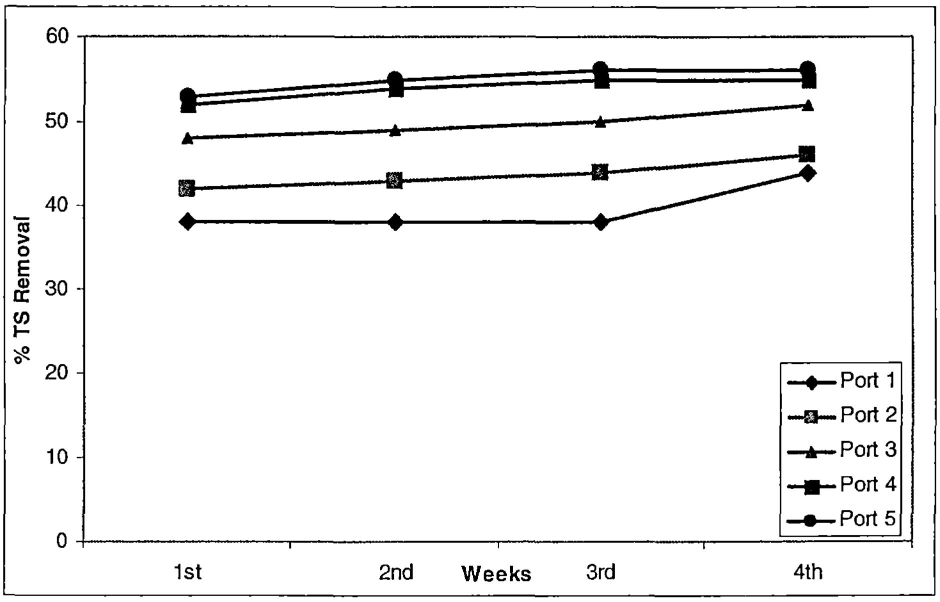

Fig. 4.A. 12. Percentage TS removal vs weeks of operation for HRT 18 days & OLR 3 $kg/m^3/d$

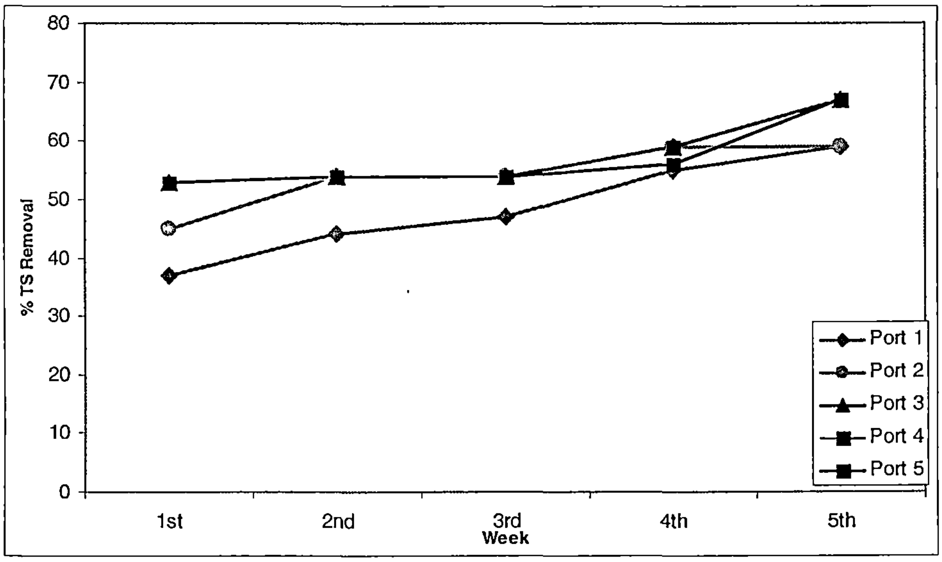

Fig. 4.A. 13. Percentage TS removal vs weeks of operation for HRT 18 days & OLR 4.3 $kg/m^3/d$

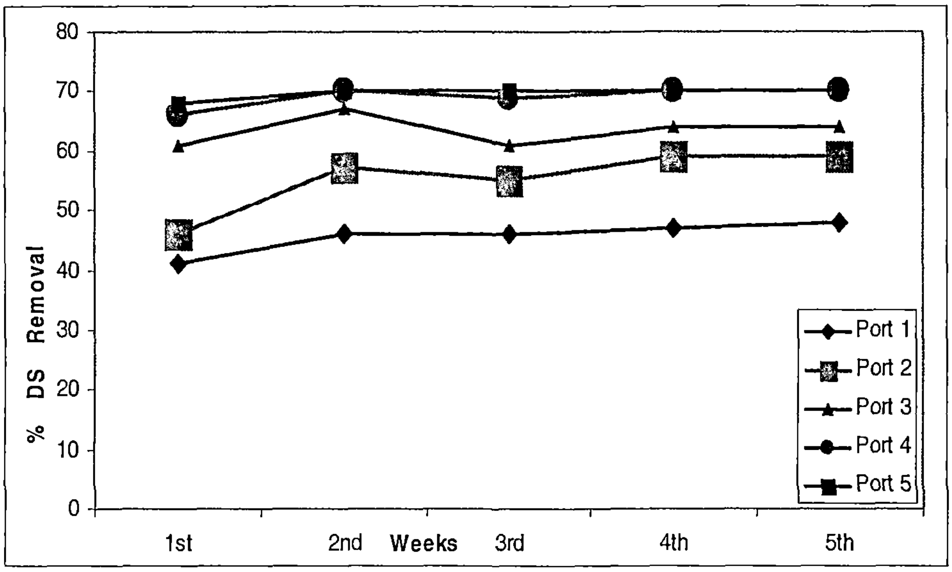

Fig. 4.A. 14. Percentage DS removal vs weeks of operation for HRT 36days & OLR 0.75 kg/m^3/d

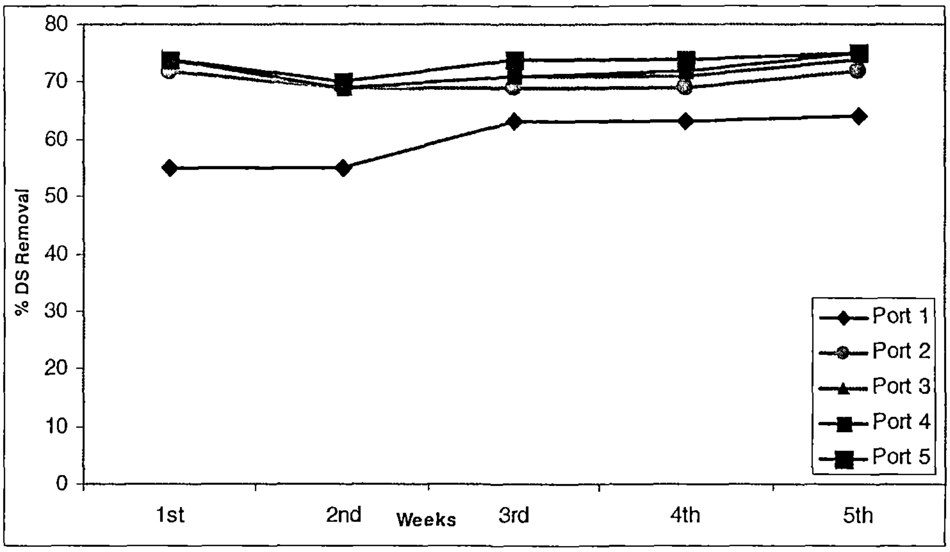

Fig. 4.A. 15. Percentage DS removal vs weeks of operation for HRT 36days & OLR 1.07 kg/m^3/d

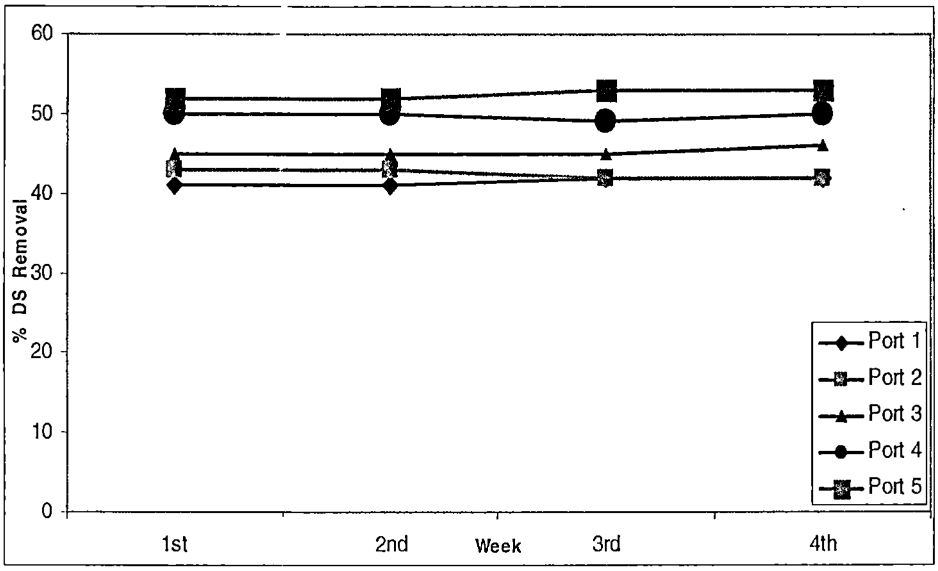

Fig. 4.A. 16. Percentage DS removal vs weeks of operation for HRT 18days & OLR 3 $kg/m^3/d$

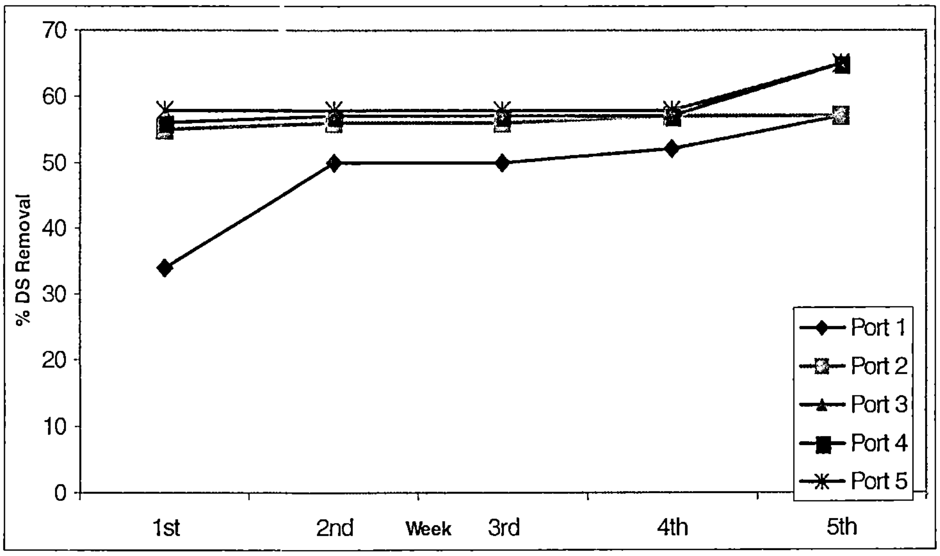

Fig. 4.A. 17. Percentage DS removal vs weeks of operation for HRT 18days & OLR 4.3 $kg/m^3/d$

Table 4. A. 10.a. Summary of the effect of HRT, OLR and filter height on percentage removal of TS

HRT(days) & OLR (kg/m^3/d)	Percentage removal of TS				
	Port 1 (bottom)	Port 2	Port 3	Port 4	Port 5 (Top)
HRT 36 days & OLR 0.75	31	56	63	69	70
HRT 36 days & OLR 1.07	60	70	73	73	74
HRT 18 days & OLR 3	40	44	49	54	55
HRT 18 days & OLR 4.3	48	54	57	60	61
HRT 12 days & OLR 3	40	45	45	47	53
HRT 12 days & OLR 4.3	42	46	49	53	55
HRT 9 days & OLR 3	23	24	24	25	26
HRT 9 days & OLR 4.3	27	30	32	33	33

Table 4. A. 10.b. Summary of the effect of HRT, OLR and filter height on percentage removal of DS

HRT(days) & OLR (kg/m^3/d)	Percentage removal of DS				
	Port 1 (bottom)	Port 2	Port 3	Port 4	Port 5 (Top)
HRT 36 days & OLR 0.75	46	54	63	69	70
HRT 36 days & OLR 1.07	60	70	71	73	74
HRT 18 days & OLR 3	42	43	45	50	53
HRT 18 days & OLR 4.3	49	56	57	59	60
HRT 12 days & OLR 3	45	46	50	52	53
HRT 12 days & OLR 4.3	46	48	52	55	56
HRT 9 days & OLR 3	20	22	23	25	26
HRT 9 days & OLR 4.3	23	25	28	32	33

Fig. 4.A. 10 to 13 show the percentage removal of TS and Fig. 4.A. 14 to 17 show the percentage removal of DS for various HRTs and OLRs. On decreasing HRT from 36 days to 9 days percentage removal efficiency decreased from 70 and 74 to 26 and 33 respectively for TS and DS. For the same HRT, higher organic loading showed more percentage removal of TS and DS. Since the effluent contained very high amount of TS and DS, longer period of retention time in the reactor removed appreciable amounts of TS and DS. Increasing the filter height increased the removal efficiency of TS and DS, but beyond the third port the effect was only marginal. Major portion of the TS and DS was removed below 1.3 meter height.

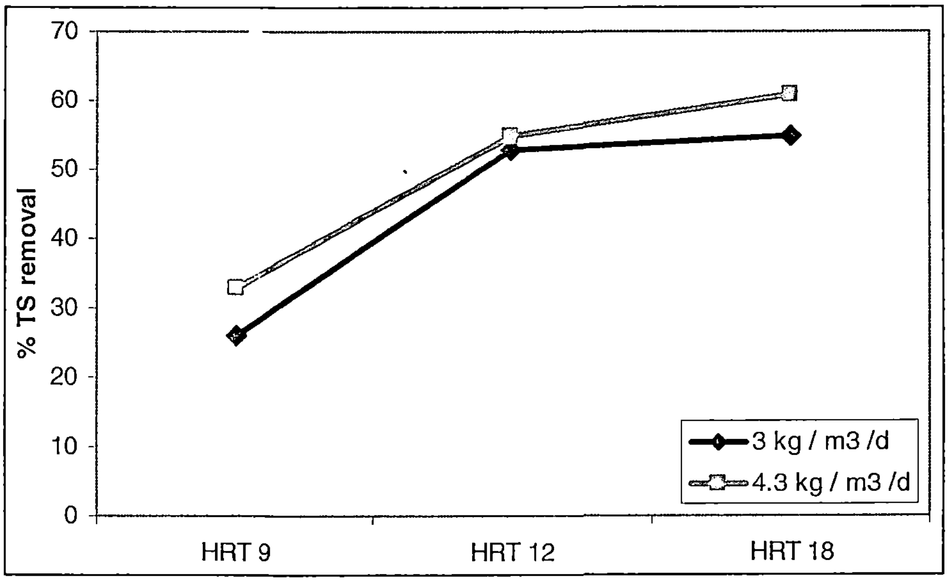

Fig. 4.A. 18. Percentage removal of TS vs HRT at different OLRs

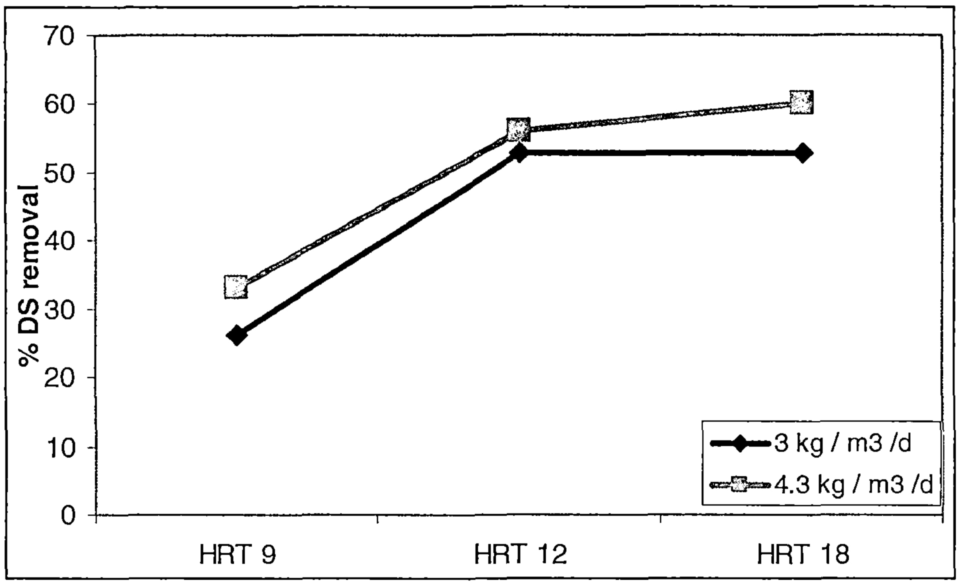

Fig. 4.A. 19. Percentage removal of DS vs HRT at different OLRs

Hulschoff Pol et al[62] have shown that three phases can be distinguished in the development of granular sludge. In phase I, expansion of sludge bed occurs due to gas production causing the development of less settleable sludge. In phase 2, the poorly settling sludge begins to washout and the heavier particles begin to concentrate at the bottom. Phase 3 occurs as the granular growth begins to exceed washout and a net accumulation of biomass occurs. Loading rates can be increased more rapidly in this stage and the increase should be small to avoid overloading of the biomass present. When this highly polluted effluent was run for an HRT of 36 days, the percentage reduction of TS and DS was in the range of 70 to 74 which showed that a high retention time was needed for granular sludge formation. In the second stage, HRT was reduced to half (18days) and organic loading was increased, it had a negative impact on the sludge formation due to the washout of biomass and overloading[19]. This resulted in the low percentage removal of TS and DS for lower HRTs.

Filter height has a profound influence on the removal of TS and DS which was more prominent in the lower parts of the reactor (Table 4.A.10 a & b). As the reactor height increased, the percentage removal of TS and DS also increased. Almost the same trend was observed for Port 4 and 5 which showed that good settling of the sludge took place at lower heights.

Fig. 4.A. 18 and 19 shows the influence of HRT on TS and DS removal. From the figure it is clear that as the HRT decreased from 18 to 9, the TS and DS removal efficiency also decreased. TS decreased from 55 to 26 and 61 to 33 when organic loading was 3 kg/m3/d and 4.3 kg/m3/d respectively for the change of HRT from 18 to 9. Similarly for the same HRT change and OLR, corresponding decrease in DS was 53 to 26 and 60 to 33.

4.A.5.5. Volatile suspended solid

The increase in biomass in an anaerobic system can be measured by VSS. Production of biomass indicates cell growth and in biological treatment process, cell growth occurs concurrent with the oxidation of organic or inorganic compounds. The ratio of the amount of biomass produced to the amount of substrate consumed is defined as the biomass yield[3].

VSS is the parameter most commonly used to follow biomass growth in full-scale biological wastewater treatment systems because its measurement is simple and requires minimum time for analysis. VSS measured also includes other particulate organic matter in addition to biomass. VSS measurement is used as an apparent indicator of biomass production and also provides a useful measurement of reactor solids in general[3].

Table 4.A. 11 shows the formation of VSS during anaerobic treatment. At the lower part of the reactor, high amount of VSS was formed compared to the top part of the reactor. This shows that formation of biomass is high at the lower part of the reactor. In the middle region of the reactor, concentration of VSS showed slight fluctuations though the general trend was a decreasing one. A slight increase in the concentration of VSS observed at the top of the reactor may be due to the accumulation of non settleable floating solids formed during the process of treatment. High concentration of VSS was observed for HRTs of 18 and 36 days. Lowering the HRT further to 12 and 9 days decreased the concentration of VSS. At higher upflow velocities (i.e., lower HRTs) granules are partially disintegrated and are washed out of the bed[63]. The fraction of granules with slow settling rate increases and thus influences the distribution of sludge granules.

Table 4.A.11. Summary of the effect of HRT, OLR and filter height on the formation of volatile suspended solid

HRT (days) & OLR ($kg/m^3/d$)	Concentration of VSS (mg/L)				
	Port 1 (bottom)	Port 2	Port 3	Port 4	Port 5 (Top)
HRT 36 days & OLR 0.75	6797	2875	2830	1250	2020
HRT 36 days & OLR 1.07	7820	4536	4472	2668	2762
HRT 18 days & OLR 3	7880	4890	4830	4850	4930
HRT 18 days & OLR 4.3	8510	3330	3810	3850	4900
HRT 12 days & OLR 3	4780	1500	1330	1400	1600
HRT 12 days & OLR 4.3	6110	2150	2100	2190	2210
HRT 9 days & OLR 3	3370	1470	1275	1360	1550
HRT 9 days & OLR 4.3	3150	1100	700	730	1030

4. A. 5. 6. TKN and AN removal

TKN and AN removal were rather low compared to COD removal. Fig.4.A. 20 to 23 shows percentage TKN removal and Table 4.A.13 and 14 shows percentage AN removal during different weeks of treatment at various HRTs and OLRs. The average values of the percentage removal of TKN and AN at different HRTs are given in Table 4.A. 14 a and 14 b. At an HRT of 36 days the maximum and minimum percentage removal of TKN were 56 and 25 respectively for an OLR 0.75 kg/m^3/d (Fig.4.A.20 and Table 4.A. 14 a). The average value was found to be 48 percent. But as the organic loading increased to 1.07 kg/m^3/d, the maximum percentage removal efficiency increased up to 71, giving an average value of 67 percent removal (Fig.4.A.21 and Table 4.A.14 a).

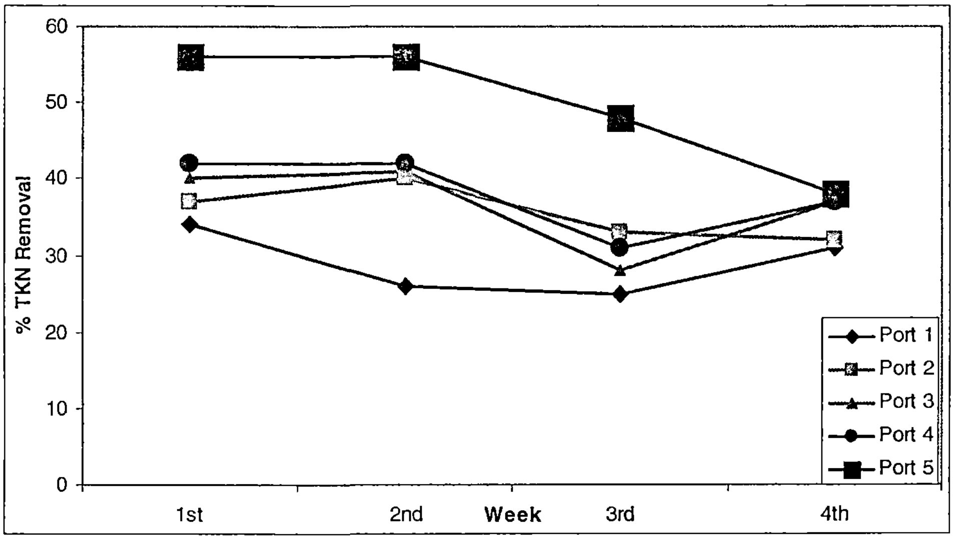

Fig. 4.A. 20. Percentage TKN removal vs weeks of operation for HRT 36days & OLR 0.75 kg/m^3/d.

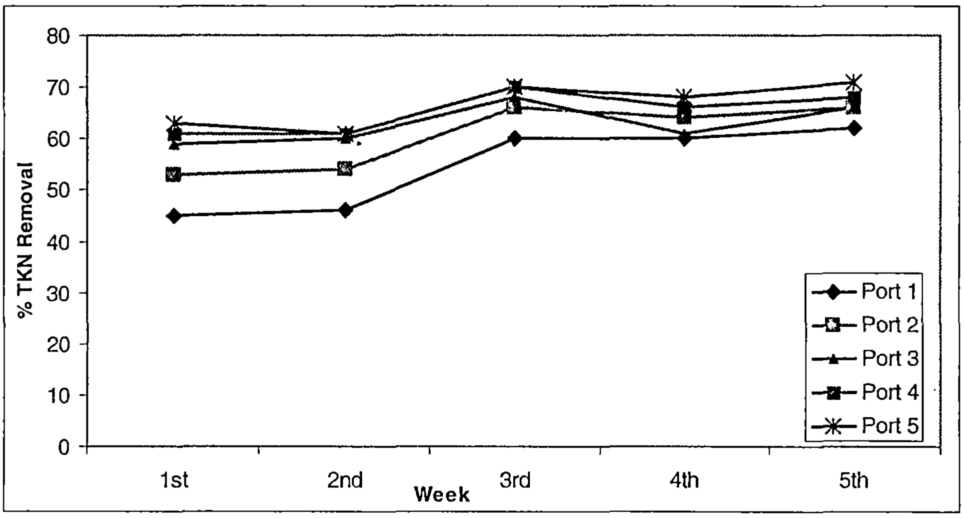

Fig. 4.A. 21. Percentage TKN removal vs weeks of operation for HRT 36 days & OLR 1.07 kg/m^3/d

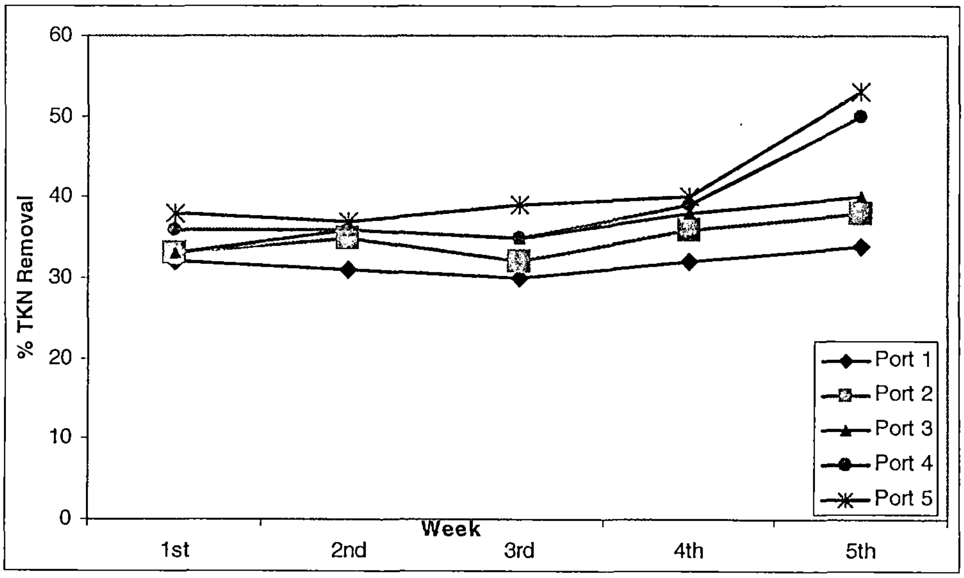

Fig. 4.A. 22. Percentage TKN removal vs weeks of operation for HRT 18 days & OLR 3 kg/m^3/d.

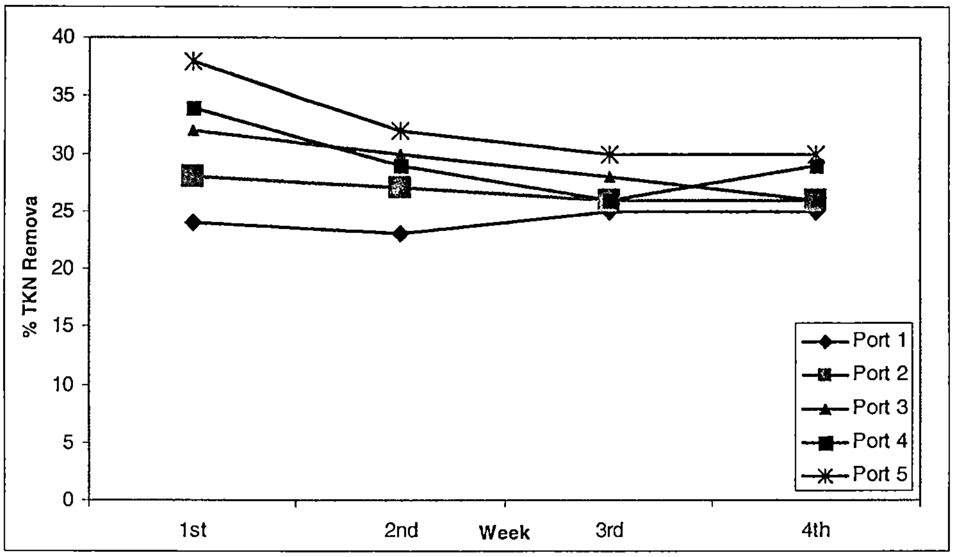

Fig. 4.A. 23. Percentage TKN removal vs weeks of operation for HRT 18 days & OLR 4.3 kg/m^3/d.

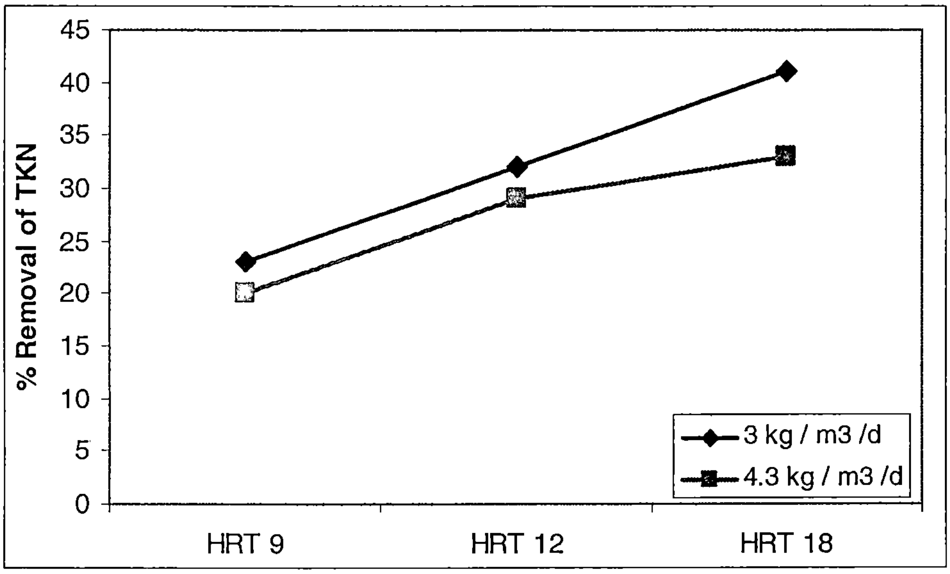

Fig. 4.A. 24. Percentage removal of TKN vs HRT at two OLRs

Table 4.A.12. Percentage AN removal for HRT 36 days & organic loading 0.75 & 1.07 kg/m^3/d

Week	% AN removal for HRT 36 OLR 0.75 kg/m^3/d					% AN removal for HRT 36 OLR 1.07 kg/m^3/d				
	Port 1	Port2	Port3	Port 4	Port 5	Port1	Port2	Port3	Port 4	Port 5
1	7	24	25	25	27	-11	-5	17	12	12
2	21	23	25	30	36	-15	-9	4	6	12
3	3	7	16	11	32	15	31	37	38	42
4	15	4	15	18	8	37	38	38	38	37
5	5	5	21	15	4	28	28	28	33	33

Table 4.A. 13. Percentage AN removal for HRT 18 days & organic loading 3 & 4.3 kg/m^3/d

Week	% AN removal for HRT 18 OLR 3 kg/m^3/d					%AN removal for HRT 18 OLR 4.3 kg/m^3/d				
	Port 1	Port2	Port3	Port 4	Port 5	Port1	Port2	Port3	Port 4	Port 5
1	6	10	10	15	17	19	28	30	35	39
2	6	10	8	6	15	20	29	35	48	52
3	3	3	6	8	10	3	5	6	9	12
4	10	15	15	15	15	0	3	9	12	17
5	6	10	18	23	23					

Table 4.A.14.a. Summary of the effect of HRT, OLR and filter height on percentage removal of TKN

HRT (days) & OLR (kg/m^3/d)	Percentage removal of TKN				
	Port 1 (bottom)	Port 2	Port 3	Port 4	Port 5 (Top)
HRT 36 days & OLR 0.75	31	36	37	39	48
HRT 36 days & OLR 1.07	55	61	63	65	67
HRT 18 days & OLR 3	32	35	36	39	41
HRT 18 days & OLR 4.3	24	27	29	30	33
HRT 12 days & OLR 3	29	22	26	30	32
HRT 12 days & OLR 4.3	16	19	22	28	29
HRT 9 days & OLR 3	15	17	20	22	23
HRT 9 days & OLR 4.3	10	14	17	20	20

Table 4.A. 14.b. Summary of the effect of HRT, OLR and filter height on percentage removal of AN

HRT (days) & OLR (kg/m^3/d)	Percentage removal of AN				
	Port 1 (bottom)	Port 2	Port 3	Port 4	Port 5 (Top)
HRT 36 days & OLR 0.75	10	13	20	20	21
HRT 36 days & OLR 1.07	11	17	25	25	27
HRT 18 days & OLR 3	6	10	11	13	16
HRT 18 days & OLR 4.3	11	16	20	26	30
HRT 12 days & OLR 3	4	7	10	15	19
HRT 12 days & OLR 4.3	9	10	20	25	26
HRT 9 days & OLR 3	4	7	7	16	31
HRT 9 days & OLR 4.3	5	6	8	19	33

When HRT was reduced to 18 and OLR was increased to 3 kg/m^3/d, the value dropped to 53 percent giving an average removal of 41 percent (Fig.4.A. 22 and Table 4.A. 14 a). On increasing OLR to 4.3 kg/m^3/d for the same HRT, the removal efficiency again dropped to 30 percent showing an average value of 33 percent (Fig.4.A. 23 and Table 4, 14 a). Percentage removal of TKN decreased further to 32 and 29 when HRT was reduced to 12 days and 23 and 20 for 9 days of HRT for the corresponding OLRs of 3 and 4.3 kg/m^3/d respectively. The relationship between percentage removal of TKN and HRT for two OLRs is given in Fig. 4.A.24. It shows that as HRT decreases the percentage removal of TKN also decreases. Anaerobic treatment is unable to remove total Kjeldahl nitrogen by means of oxidation and very low amount of nitrogen is removed in the UASB process[64].

Percentage removal of ammoniacal nitrogen shows wide variation ranging from negative to zero as shown in the Tables 4.A.12 and 13. Estimation of AN for various organic loading did not follow a regular pattern. The maximum (average percentage) removal of AN observed was 33 for a lower HRT of 9 days.

Anaerobic digestion process does not remove nitrogen effectively as expected in any anaerobic treatment process[65]. Effluent from anaerobic treatment will typically contain nitrogen in the form of ammonium ion ($NH4^+$). Some organic nitrogen is also reduced to AN. In the lower part of the reactor, the percentage removal of AN was low compared to the top. This might be due to the formation of ammonia from the degradation of proteins and amino acids at the lower part of the reactor. As the height of the reactor increased, a part of the ammonia formed escaped along with the other gases. In fact the negative percentage removal of ammoniacal nitrogen showed that ammoniacal nitrogen level increased after anaerobic digestion. The same observation was also reported by Zaid Isa [65] during the anaerobic treatment of diluted skim serum.

Though the presence of ammonia is considered to be toxic in anaerobic treatment as reported by McCarty[47] and Moen[48], no toxicity was found during this process of anaerobic treatment as reported by Parkin and Miller[49] and van Velsen[50]. For irrigation purpose use this is not a problem. However, for surface water discharge TKN should be limited to 100 mg/L in India. Therefore, post treatment like nitrification or denitrification is required after UASB[36].

4.A.5.7. Soluble organic nitrogen

Soluble organic nitrogen influences the total nitrogen concentration in the effluent. Raw effluent contains 6000 to 2900 mg/L of soluble organic nitrogen. After anaerobic treatment, the value dropped from 1690 to 178 mg/L (Table 4.A. 15). For higher HRTs, 97 percent removal of soluble organic nitrogen content was achieved during anaerobic treatment. High percentage removal of soluble organic nitrogen indicated the degradation of nitrogen containing compounds like proteins and amino acids during anaerobic treatment.

Table 4.A. 15. Effect of anaerobic treatment on soluble organic nitrogen

Anaerobically treated effluent		Raw effluent
HRT days	Soluble organic nitrogen (mg/L)	Soluble organic nitrogen (mg/L)
36	402	4500
36	178	6000
18	686	4500
18	947	3500
12	1400	2900
12	1513	
9	1614	
9	1690	

4.A.5.8. Phosphate removal

Microbial growth in biological systems needs nutrients like nitrogen, phosphorus and sulphur. Nutrients other than carbon may at times be the limiting factor for microbial cell synthesis and growth. Phosphorus is considered as a macro nutrient because it is required in large amounts. About 2.3 g of phosphorus are needed per 100 g of cell biomass and the recommended COD: N: P ratio during start up is 300:5:1 and during the steady state process the ratio is 600:5:1[3]. About 10 g of biodegradable soluble COD (bsCOD) will be required to remove 1g of phosphorus by biological storage mechanism. Phosphorus removal efficiency decreases due to starvation or low bsCOD[51]. Raw effluent contains 1500 to 4000 mg/L phosphate and therefore, addition of phosphate was needed only during the start up period. High rate removal of phosphate was observed during anaerobic treatment process since it was used for the biomass cell formation. In the anaerobically treated effluent, only trace amount of phosphate was found for an HRT of 36 days though before treatment it contained 3000 mg/L of phosphate. For an HRT of 18 days and 12 days, the concentration of phosphate was 525 and 680 mg/L respectively. This showed that biological phosphorus removal was higher for longer retention times in the UASB reactor.

4.A.5.9. Sulphide formation

One of the main disadvantages of anaerobic process is the production of foul smelling hydrogen sulphide if sulphates are present in the effluent. During processing of natural rubber latex, the skim latex (4% DRC) from the centrifuge machine is deammoniated and concentrated sulphuric acid is added as a coagulant. The serum effluent contains 16000 m/L to17000 mg/L of sulphate. Sulphate reducing bacteria can become a nuisance when the wastewater contains significant concentration of sulphate. These bacteria reduce sulphate to sulphide, which is toxic to methanogenic bacteria. Table 4.A. 16 shows the average concentration of sulphide present in the effluent drawn every week from the reactor for analysis. From the table it is clear that large amount of sulphide (up to140 mg/L) was formed during this process, but its presence did not seriously affect the treatment efficiency since high amount of COD was removed during this treatment process. Tadesse[69] reported that there is a competition between sulphate reducing bacteria and methanogenic archaea in the anaerobic treatment of tannery effluent and the relative electron flow towards sulphate reduction was higher than towards methanogenesis, although the COD recovery within the reactor varied between 15 and 90 percent. Sulphidogenesis also depends on the COD/SO_4^{-2} ratio. Increase of sulphate concentration and decrease of the COD/SO_4^{-2} ratio increases the flow of electron towards sulphate reducing bacteria[69]. Increase of OLR increased the formation of sulphide and the decrease of HRT decreased the sulphide formation with very few exceptions (Table 4.A. 16).

Table 4.A.16. Summary of the effect of HRT, OLR and filter height on the formation of sulphide

HRT(days) & OLR (kg/m^3/d)	Concentration of sulphide formed (mg/L)				
	Port 1 (bottom)	Port 2	Port 3	Port 4	Port 5 (Top)
HRT 36 days & OLR 0.75	43.4	20.9	23.92	22.5	22.0
HRT 36 days & OLR 1.07	87.4	83.0	94.8	86.9	86.9
HRT 18 days & OLR 3	118.5	134.5	138.8	135.4	140.0
HRT 18 days & OLR 4.3	101.8	102.0	111.0	107.6	100.4
HRT 12 days & OLR 3	75.3	72.0	73.5	71.3	69.4
HRT 12 days & OLR 4.3	83.4	80.6	75.2	77.7	75.02
HRT 9 days & OLR 3	51.0	54.2	53.7	49.5	48.7
HRT 9 days & OLR 4.3	56.3	58.0	54.5	51.0	49.0

As anaerobic reaction proceeded, formation of iron sulphide was evident from the black colour of the effluent. Blackening of the medium is a diagnostic characteristic of *Desulphovibrio desulphuricans* growth. *Desulphovibrio desulphuricans* can cause significant removal of heavy metal ions by precipitation[44]. Analysis of metal ion concentration showed that high concentrations of metal ions were removed from the effluent after anaerobic treatment. Malodorous and corrosive hydrogen sulphide has to be removed from the effluent. The discharge standards in India allow sulphides up to 2 mg/L in effluents to be discharged to surface waters and 5 mg/L to marine coastal waters[36]. This demands further treatment after anaerobic digestion.

4. A. 5. 10. Biogas formation.

Production of methane gas is the slowest and the most sensitive step of the anaerobic digestion process. The gas produced was collected by the downward displacement of water. For an HRT of 36 days, production of gas on an average was 2 to 3 litres per day and it increased to 3.5 litres when OLR increased for an HRT of 18 days. But when HRT was reduced to 12 and 9 days the production of gas decreased to one litre and 0.75 litre per day respectively. Past studies indicated that the presence of 1650 mg/L of sulphate could reduce the biogas production by 50 percent[66]. Since the skim serum effluent used contains 16000 to 17000 mg/L of sulphate, production of biogas is comparatively low and the gas collected was having foul smell due to the presence of hydrogen sulphide, and other gases like ammonia, mercaptans etc. Hence, the recovery of gas is optional. Odour nuisance is of great concern during the anaerobic treatment of wastewaters with high sulphate content in the wastewater. It is difficult to control foul smell from the reactor as long as sulphuric acid is used in skim processing. It is better not to use the gas collected. A flare may be installed to burn the biogas since burning avoid odour nuisance from the H_2S present in the gas[36].

4. A. 5. 11. Removal of heavy metals

In the anaerobic treatment process heavy metals were removed by adsorption and complexation of metals with the microorganisms. Transformation and precipitation of metals occur in the biological treatment process. Adsorption and combination of metal ions take place due to the interaction of metal ions and negatively charged microbial surfaces. Metal ions complex with carbonyl groups found in microbial polysaccharides and other polymers adsorbed by protein materials in the biological cells[3]. Concentration of heavy metal ions in the raw effluent and anaerobically treated effluent was estimated using atomic absorption spectrophotometer (AAS) and the results are given in the Table 4. A. 17.

Table 4.A. 17. Estimation of metal ions using AAS.

Element	Lamp current mA	Wavelength nm	Concentration in ppm		Percentage removal
			RE	ATE	
Fe	7.0	248.3	7.099	1.725	75.7
Cu	3.0	324.7	0.025	0.014	44.00
Zn	8.0	213.9	204.500	1.750	99.144
Mn	8.0	279.5	0.037	0.198	increased
Ca	5.0	422.7	3.440	871.900	increased
Mg	4.0	285.2	1.610	13.100	increased

(RE: Raw effluent., ATE: Anaerobically treated effluent)

All living organisms require varying amounts of (macro and micro) metallic elements. Presence of adequate amounts of phosphate, magnesium and calcium are required for satisfactory reactor performance and sludge granulation[19]. Although metals are required for growth of microorganisms, their higher concentration will lead to toxicity. Macro nutrients like Ca, Fe, Mg, K and Na and micro nutrients like Cr, Co, Cu, Pb, Mg, Mn, Mo, Ni, Se, W, V and Zn are needed for biological growth [3, 39].

Table 4. A. 17. reveals that the anaerobic process removes 75.7% Fe, 44% Cu and 99.144% Zn for an HRT of 18 days. 204.5 ppm of Zn was present in the raw effluent and after anaerobic treatment it was only 1.75 ppm. High removal of Zn, Fe and Cu may be either due to formation of sulphides or adsorption by microorganism during anaerobic reaction. *Desulphovibrio desulphuricans* can cause significant removal of heavy metal ions by precipitation[44]. In anaerobic process the reduction of sulphate to hydrogen sulphide can promote precipitation of metal sulphides[3]. Metals like iron, copper and zinc are removed as their sulphides during anaerobic treatment process. Increase in the concentration of Ca, Mg and Mn could be due to the addition of lime (commercial grade) to adjust pH of the raw effluent.

4.A.5.12. Biochemical analysis

Table 4.A. 18. Results of biochemical analysis

Sample details	Soluble protein	Phenol	Total sugar	Reducing sugar	Non reducing sugar	Free amino acids
RE	865	586	1250	1095	155	15952
ATE (HRT 12)	450	434	107	83	24	7932
ATE (HRT 18)	310	370	96	42	54	5719
ATE (HRT 36)	280	45	65	56	9	5779

(RE: Raw effluent., ATE: Anaerobically treated effluent., All values are expressed in mg/L)

Biochemical studies of the serum effluent show that it contained 865 mg/L soluble protein, 586 mg/L phenol, 1250 mg/L total sugar, 1095 reducing sugar, 155 mg/L non reducing sugar, 15952 mg/L of free amino acids. Anaerobic treatment by UASB method could reduce all these constituents (Table 4. A. 18.), but when HRT was reduced to 18 and 12 days, the percentage removal of all these were low. From the table it is clear that anaerobic treatment could remove soluble protein, sugar and even phenol. To degrade phenol by anaerobic treatment, longer periods of retention was required and organic loadings should be low. Toxic and recalcitrant organic compounds like phenols were degraded under anaerobic conditions, with the compound serving as a growth substrate with fermentation and ultimate methane production[3]. These toxic compounds were transformed into non-toxic entities by specialized microbes. A long-term exposure to the organic compound may be needed to induce and sustain the enzymes and bacteria required for degradation[68]. Removal of amino acids by UASB was comparatively low. In the presence of sufficient amount of ammoniacal nitrogen and hydrogen concentration, the bacteria responsible for granulation may produce amino acids, but their synthesis was limited by cysteine supply. The extracellular polypeptides which bind organisms together to form dense pellets or floc granules were some of the excess amino acids secreted during UASB treatment[3, 29].

Biochemical analysis also revealed that anaerobic treatment is an effective method to treat highly polluted skim serum effluent.

4. A. 6. Conclusion

1. UASB reactor could be successfully used to treat skim serum effluent, a high-strength organic waste (COD 38800 mg/L). For an HRT of 36 days, the average COD removal efficiency achieved was 89 and 79 percent at OLRs of 0.75 and 1.07 kg/m^3/d respectively. In most cases, there was an initial decrease in the efficiency of COD removal for higher organic loadings but it reached a steady state after a period of operation at a given organic loading.

2. As the HRT increased COD reduction efficiencies of the system also increased and *vice-versa*. On varying the HRT from 18 to 9 days, for an organic loading of 3 kg/m^3/d, COD removal efficiency dropped from 84.5 to 64 percent.

3. The influence of the reactor height was almost the same for the two different organic loadings having the same HRT. Percentage removal of COD increased as the effluent moved upwards and the maximum percentage removal was obtained at the top of the reactor.

4. Efficiency of treatment could not be assessed in terms of BOD removal since BOD values were unusually high during anaerobic treatment for longer HRTs.

5. During the whole treatment process, VFA decreased as the effluent moved upwards in the reactor and the decrease was steadier beyond Port 2. At the bottom level (below Port 2) the VFA concentration was always higher. The same trend was observed in pH values also. Change in pH was more prominent below 1.3 metre, and above 1.3 metre, the pH remained almost steady. pH and VFA changes were

more pronounced below 1.3 metre, indicating high rate of anaerobic reactions at the lower part of the reactor.

6. Lowering HRT from 36 days to 9 days decreased the efficiency of removing TS and DS, whereas increasing organic loading increased the percentage removal of TS and DS.
7. Increasing the filter height increased the removal efficiency of TS and DS, but beyond the third port the effect was only marginal. Major portion of the TS and DS were removed below 1.3 meter height which showed that good settling of the sludge took place below this height.
8. High amount of VSS was formed at the lower part of the reactor, compared to the top part of it, showing the formation of high biomass in the lower part of the reactor. High concentration of VSS was found for HRTs of 18 and 36 days and decrease of HRT resulted in a drop in the concentration of VSS.
9. Total Kjeldahl nitrogen and ammoniacal nitrogen removal was rather low compared to COD removal. Highest percentage removal of TKN achieved was 67 for 36 days of HRT having organic loading of 1.07 kg/m^3/d.
10. Estimation of AN for various organic loadings did not follow a regular pattern. Anaerobic digestion process did not remove nitrogen effectively. Wide fluctuations in the values of AN were observed showing negative to zero percentage removal for higher OLRs.
11. For higher HRTs, 97 percent removal of soluble organic nitrogen content took place during anaerobic treatment. High percentage removal of soluble organic nitrogen indicated the degradation of

nitrogen containing compounds like proteins and amino acids during anaerobic treatment.

12. Biological phosphorus removal was higher for longer retention times in the UASB reactor and *vice-versa.*

13. Anaerobic treatment of serum effluent using UASB reactor produces hydrogen sulphide. Large amount of sulphide (up to 140 mg/L) formed during this process of treatment did not seriously affect the treatment efficiency of the UASB reactor as it was evident from 85 to 95 percent COD removal.

14. Production of biogas was found to decrease when HRT was reduced from 36 days to 9 days. The gas collected was having foul smell owing to the presence of hydrogen sulphide.

15. Changes in the concentration of heavy metals were estimated using AAS and the anaerobic process was found to remove 75.7% Fe, 44% Cu and 99.144% Zn for an HRT of 18 days. Addition of lime to adjust pH of the effluent was the reason for the increase in the concentration of Ca, Mg and Mn.

16. Biochemical studies of the serum effluent showed that anaerobic treatment by UASB method could remove protein, sugar and even phenol. To degrade phenol by anaerobic treatment, longer periods of retention was required and organic loadings should be low. Biochemical analysis also proved that anaerobic treatment is an effective method to treat highly polluted skim serum effluent.

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PART B:

TRACE METAL EFFECT IN ANAEROBIC TREATMENT

4. B. 1. Introduction

Growth factors differ from one organism to another. Major growth factors are amino acids, nitrogen bases and vitamins[1]. Nutrients like iron (Fe), cobalt (Co), nickel (Ni), molybdenum (Mo), zinc (Zn), manganese (Mn), magnesium (Mg) and copper (Cu) are the trace metal elements required for methanogens for growth and activity[1-4]. Addition of the missing elements accelerates anaerobic degradation.

Speece[5] studied the importance of trace metals to stimulate methanogenic activity and the recommended requirements for iron, cobalt, nickel and zinc as 0.02, 0.004, 0.003 and 0.02 mg/g acetate produced respectively. Increased anaerobic activity was noted after trace additions of iron, nickel and cobalt, but their concentrations vary for different wastewaters with high VFA concentrations. A recommended dose of trace metals per liter of reactor volume is 1.0 mg $FeCl_2$, 0.1 mg $CoCl_2$, 0.1 mg $NiCl_2$ and 0.1 mg $ZnCl_2$.

Metabolic capability of microorganisms in wastewater treatment is determined by cell enzymes. It consists of protein and a cofactor such as a metal ion (Zn, Fe, Cu, Mn and Ni). Enzymes are large organic molecules that catalyse biological reactions like cell synthesis, hydrolysis and oxidation-reduction. Prokaryotes require trace amounts of metallic ions Zn, Fe, Cu, Mo, Co, Mn and Ni and all these elements are derived from the environment. Shortage of these metals can inhibit or alter growth[1]. It has been reported that metal deficiencies can limit the performance of anaerobic digestion systems and that metal supplementation may substantially improve anaerobic treatment of different types of waste streams[6-12]. Sharma et al[2] reported that micronutrients (Ni, Fe, and Co) improve both sludge volume index and methanogenic activities of the sludge. Addition of salts of Ni, Fe, and Co at 0.10 and 0.5 mg/L to distillery effluent improved its anaerobic digestion and sedimentability of sludge[13]. Increased concentration of Ni and Co accelerated the initial exponential and final arithmetic increases in the methane production rate and reduced the temporary decrease in rate due to micronutrient limitation[14]. Florencio studied the effect of cobalt in the anaerobic degradation of methanol and found that addition of cobalt stimulated only those trophic groups which directly utilized methanol, while other trophic groups utilizing down stream intermediates, H_2/CO_2 or acetate, were largely unaffected[15, 16].

4. B. 2. Aim

Natural rubber serum contains sulphur, potassium, phosphorus, chlorine, zinc, magnesium, sodium, calcium, iron, aluminium and rubidium whereas presence of Co, Ni and Mo were not reported in the natural rubber serum[17]. Therefore, whether the addition of these trace metals will stimulate the anaerobic degradation of serum effluent like methanol was a pertinent question and this study is focusing on it.

The present study is an investigation on the effect of Co, Ni and Mo individually and in combination on the anaerobic treatment of skim serum effluent, using a batch type upflow anaerobic sludge blanket reactor and comparing it with another set without any added metal (control).

Fig. 4. B. 1. Batch type reactor

Commercial grade metal salts like cobalt chloride, nickel chloride and ammonium hepta molybdate tetra hydrate (all from Merck) were used for the addition of Co, Ni and Mo. 100 mL of the effluent was added drop by drop every day. In the first phase of the experiment 100 µg/L (i.e.10 µg/100 mL) and in the second phase 10 µg/L (i.e.1 µg/100 mL) of metals were added to the effluent. Over flowed effluent was collected every week and analysed for various parameters like COD, BOD, pH, turbidity, sulphide, TKN and AN.

Characteristic of the serum effluent are given in the Table 4. B. 1.

4. B. 3. Experimental

Five polyethylene containers of 2.5 litre capacity each were filled with water and diluted and filtered cow dung slurry, one litre each. It was closed with a lid having two holes. Five sets of bottles with tubes were collected, washed and sterilized and placed on a tall supporting stand. One end of the tube from the bottle was passed through a hole on the lid to reach the bottom of the container. The effluent from the bottle passed through this tube, and then reaching the bottom of the container and rising gradually. Another small tube was fixed above the level of water through the other hole on the lid for gases to escape. An outlet tube was fixed on the polyethylene containers exactly at the position where the volume of container reached 2 litre. The pH of the effluent was adjusted to 7.5 by adding lime and sodium bicarbonate. It was allowed to settle, filtered and analysed as per standard methods[18]. 100 mL of effluent was taken in each bottle. The five bottles were marked as E, Co, Ni, Mo, and Co+Ni+Mo. In bottle E, effluent alone was taken. In other bottles marked as Co, Ni, Mo, and Co+Ni+Mo, 100 mL effluent and Co, 100 mL of effluent and Ni, 100 mL effluent and Mo, 100 mL effluent and equal amounts of Co, Ni, and Mo together respectively were taken, mixed well and allowed to flow drop wise. During feeding, an equal volume of treated effluent overflowed through the outlet tube and collected for analysis (Fig. 4. B.1). A period of 30 days was allowed for a proper start up.

Table 4. B. 1. Characteristics of effluent

No	Parameters	Values (mg/L) (Except for pH)
1	pH	7.5
2	COD	24627
3	BOD	9649
4	Sulphates	16000
5	TS	59000
6	DS	57100
7	TSS	1900
8	TKN	5740
9	AN	3750
10	Sulphide	24

4. B. 4. Results and discussion

(a) pH

Fig. 4. B. 2 shows the average variation of pH resulting from the addition of 100 μg/L of different metal ions to the effluent. The average pH of the effluent was found to be above 7.57. This reveals that VFA formation did not influence much on the pH of the serum effluent during the anaerobic treatment in the presence of 100 μg/L of trace metals. Minimum level of volatile fatty acid (VFA) concentration can be taken as an indication of sufficient methanogenic population. The low pH due to undissociated VFA produced by acetogens is toxic to methanogens[19, 20]. The pH around 7 indicated the conversion of VFAs by methanogenic organisms which is the predominant reaction and not the fermentation of soluble substrates by fermentating bacteria (Table 4. B. 2 & 3).

Table 4. B. 2. Variation of pH by the addition of 100 µg/L of trace metal ions

Type of metal added and pH (Conc. of metal-100 µg/L)					
Week	E	Co	Ni	Mo	Co+Ni+Mo
1	7.75	7.7	7.7	7.7	7.65
2	7.8	8.05	7.7	7.7	8.4
3	7.65	7.5	7.4	7.35	7.35
4	7.95	7.9	7.45	7.45	7.5
5	8.1	8.1	7.85	7.7	7.8
6	7.9	7.95	7.85	7.65	7.75
7	7.5	7.55	7.65	7.45	7.5

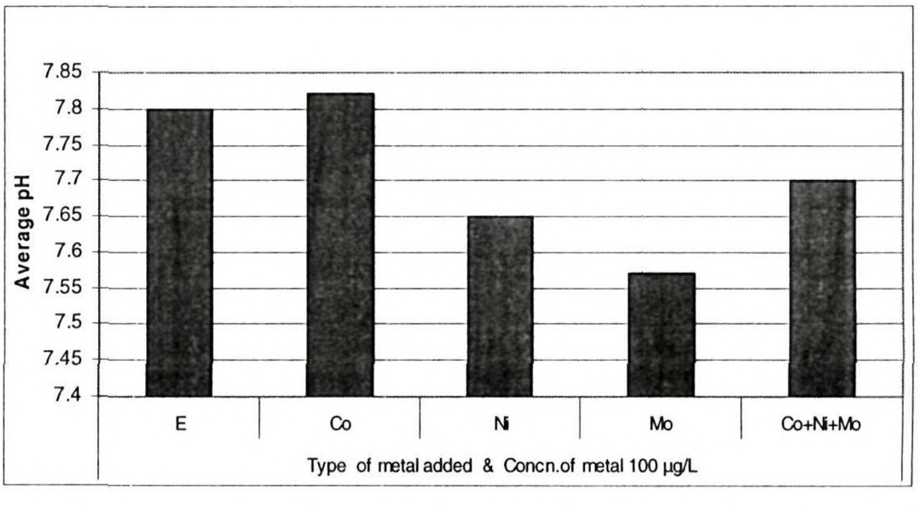

Fig.4. B. 2. Average variation of pH caused by the addition of 100 µg/L of trace metal ions

If sufficient buffering capacity is present VFA produced will not decrease the pH and consequently the undissociated VFA fraction will be too small to significantly disturb the methanogens[15]. To provide a buffering medium sodium bicarbonate was added along with lime to adjust the pH of the raw effluent.

Table 4. B. 3. Variation of pH by the addition of 10 μg/L of trace metal ions

Type of metal added and pH (Conc. of metal; 10 μg/L)					
Week	E	Co	Ni	Mo	Co+Ni+Mo
1	7.60	7.80	7.75	7.70	7.50
2	7.55	7.70	7.25	7.45	7.05
3	7.75	7.90	7.55	7.55	7.75
4	7.65	7.70	7.55	7.45	7.65
5	7.90	7.95	7.85	7.80	7.90

Bicarbonate is used not only as a weak acid in the pH buffering system but also as a cosubstrate in the acetogenic breakdown. When undissociated VFA remains high for prolonged period, methanogens are slowly wiped out and acetogens predominate in the bioreactors[15]. The presence of Mo and Ni lower the pH of the effluent whereas Co increases the pH. This may be due to the production of bicarbonate (in presence of Co) during the formation of methane[15].

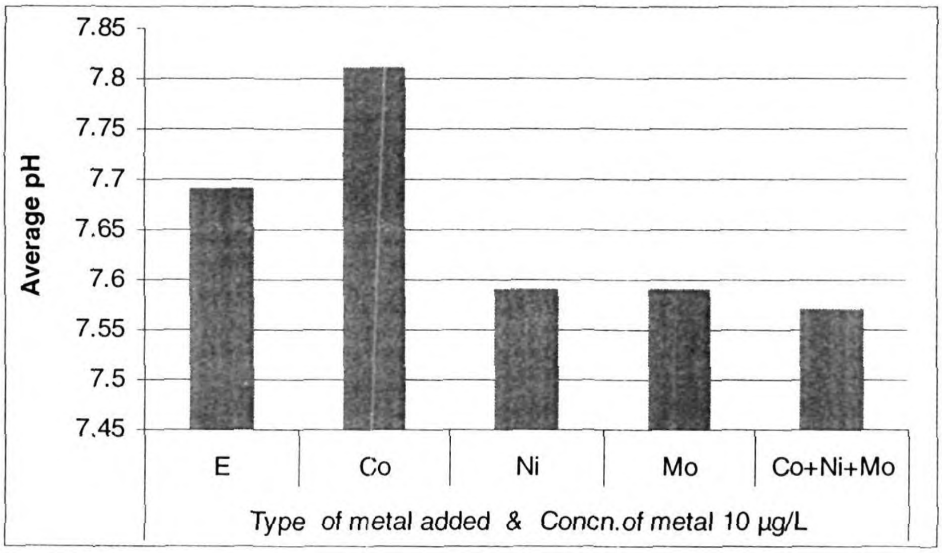

Fig. 4. B. 3. Average variation of pH by the addition of 10 µg/L of trace metal ions

In the second phase of the experiment, addition of 10 µg/L of trace metal also has almost the same effect on pH as that of 100 µg/L. Addition of Ni and Mo and combination of all the metals lower the pH of the effluent compared to that of the control without any metal. In the presence of Co (Fig. 4. B. 3) pH showed a high value than that of the control. Here also the presence of bicarbonate acts as a buffering system and therefore, pH is not lowered much.

(b) Sulphide formation

Sulphide formation was found to be the maximum in the presence of 100 µg/L cobalt when compared to the other trace metals. When three metals are added in combination, the amount of sulphide formed was also high, but not as high as that of cobalt (Fig. 4. B. 4). Formation of sulphide was almost constant in the control where heavy metals were absent (Table 4.B.4). The continuous addition of most metals resulted in a gradual increase in the formation of sulphide up to a certain level and then gradually declining. This indicates that as anaerobic reaction proceeds with increasing concentration of metals, growth of *Desulphovibrio desulphuricans* also increases, and after a particular concentration it becomes inhibitory. Thick black colour of the effluent is a diagnostic characteristic of *Desulphovibrio desulphuricans* growth and formation of metal sulphides. *Desulphovibrio desulphuricans* can cause significant removal of metal ions by precipitation[21]. Since the raw effluent contains 16000 mg/L of sulphate, formation of high amount of sulphide is expected. Sulphide formation is comparatively less in the presence of Ni and Mo.

Table 4. B. 4. Formation of sulphide in the presence 100 μg/L of trace metal ions

Type of metal added and sulphide formed (mg/L) (Conc.of metal; 100 μg/L)					
Week	E	Co	Ni	Mo	Co + Ni + Mo
1	9	19	27	33	24
2	151	69	36	120	27
3	82	302	148	159	77
4	82	297	165	77	234
5	82	173	34	15	155
6	82	128	35	42	170
7	102	38	18	23	30

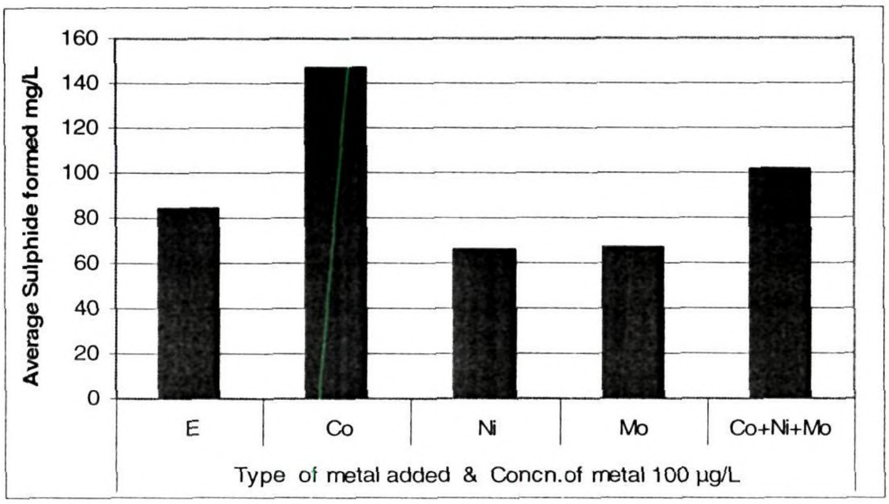

Fig. 4. B. 4. Average sulphide formation in the presence of 100 µg/L of trace metal ions

In the second phase of experiment with 10 µg/L of metal concentration, formation of sulphide (average) is high (Table 4. B. 5 & Fig. 4. B. 5). Effluent containing Mo shows low value for sulphide. Ni containing effluent shows the next higher value.

Table 4. B. 5. Sulphide formation in the presence of 10 µg/L of trace metals

Type of metal added and sulphide formed (mg/L) (Conc. of metal; 10 µg/L)					
Week	E	Co	Ni	Mo	Co + Ni + Mo
1	232	212	100	96	216
2	162	298	227	94	310
3	197	246	187	94	134
4	161	265	26	44	65
5	117	352	9	43	270

Like the first phase of the experiment with 100 µg/L of metal, the effluent containing 10 µg/L of Co shows the highest value for sulphide formation. This shows that the presence of cobalt enhances the growth of *Desulphovibrio desulphuricans.*

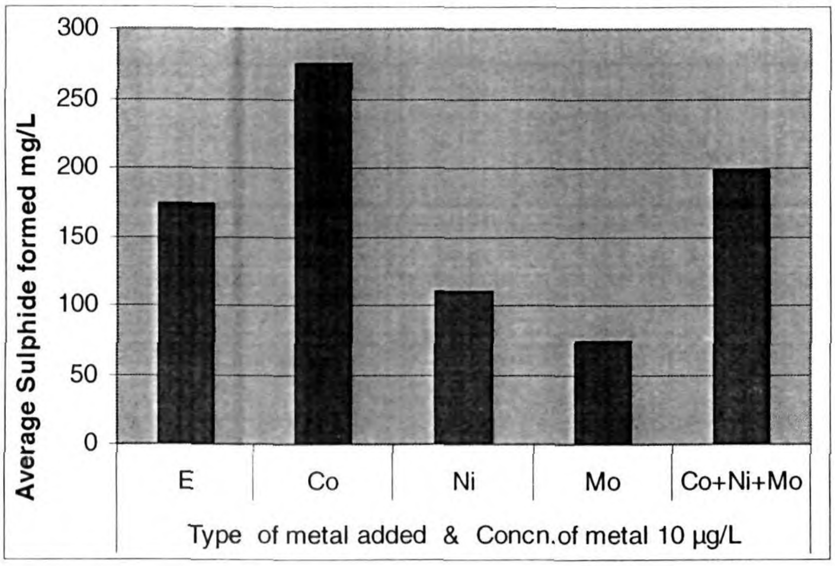

Fig. 4. B. 5. Average sulphide formation in the presence of 10 µg/L of trace metal ions

(c) Turbidity

Turbidity values in the presence of 100 µg/L of different metal ions showed that metal ions influenced the formation of turbidity. The highest value for turbidity was in the presence of Co (Table 4. B. 6. & Fig. 4. B. 6.). In the presence of cobalt, turbidity gradually increased, reached a maximum and then decreased. The decreasing trend was observed during the sixth and seventh week. The average value of turbidity for seven weeks showed that presence of Co in the effluent enhanced turbidity. The next higher values were observed for the addition of Mo.

Table 4. B. 6. Formation of turbidity in the presence of 100 μg/L of trace metals

Type of metal added and turbidity (NTU) (Conc. of metal ;100 μg/L)					
Week	E	Co	Ni	Mo	Co+Ni+Mo
1	135	152	124	193	47
2	144	194	136	234	54
3	245	234	229	257	211
4	134	248	80	157	111
5	91	288	84	207	226
6	94	227	191	252	230
7	278	279	193	220	237

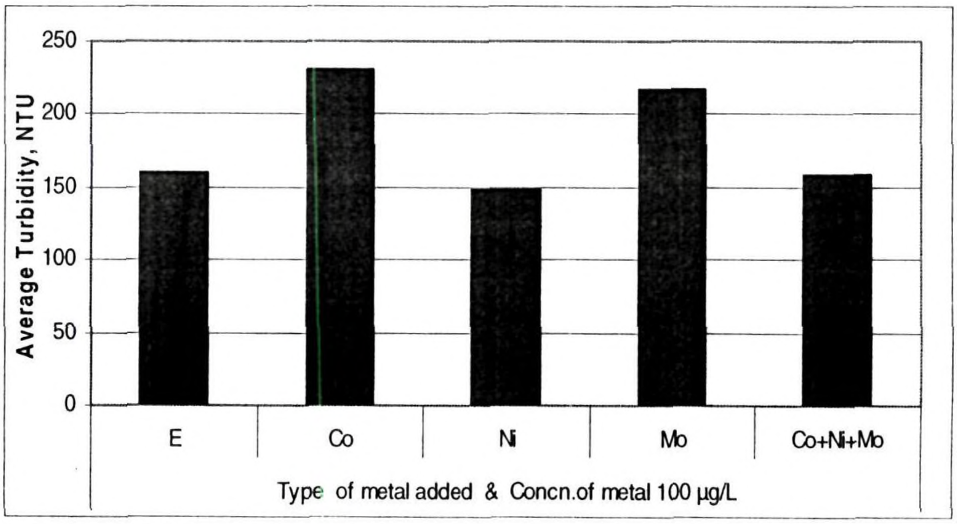

Fig. 4. B. 6. Average turbidity in the presence of 100 μg/L of trace metals

Turbidity measurements may be used to provide a rapid and simple means of observing cell growth[1]. It was observed that the presence of cobalt favoured cell growth compared to other metals. Presence of Co also increases the COD removal efficiency which further confirmed the influence of Co on biomass formation.

Table 4. B. 7. Formation of turbidity in the presence of 10 µg/L of trace metals

Type of metal added and turbidity(NTU) (Conc. of metal;10 µg/L)					
Week	E	Co	Ni	Mo	Co+Ni+Mo
1	141	148	104	145	117
2	145	152	143	165	130
3	170	144	130	185	128
4	164	145	113	169	137
5	171	153	153	174	157

The turbidity values of the anaerobically treated effluent after adding 10 µg/L of trace metals showed that Mo containing effluent had high value of turbidity (Table 4. B. 7. & Fig. 4. B. 7). This is an indicator of greater biomass growth in this reactor and the presence of high biomass content is helpful to treat the effluent anaerobically. But the average value of turbidity is higher for effluent treated with 100 µg/L of metal than with 10 µg/L of metal. Similarly COD removal efficiency is also higher for the former which confirms the relation of turbidity and biomass concentration.

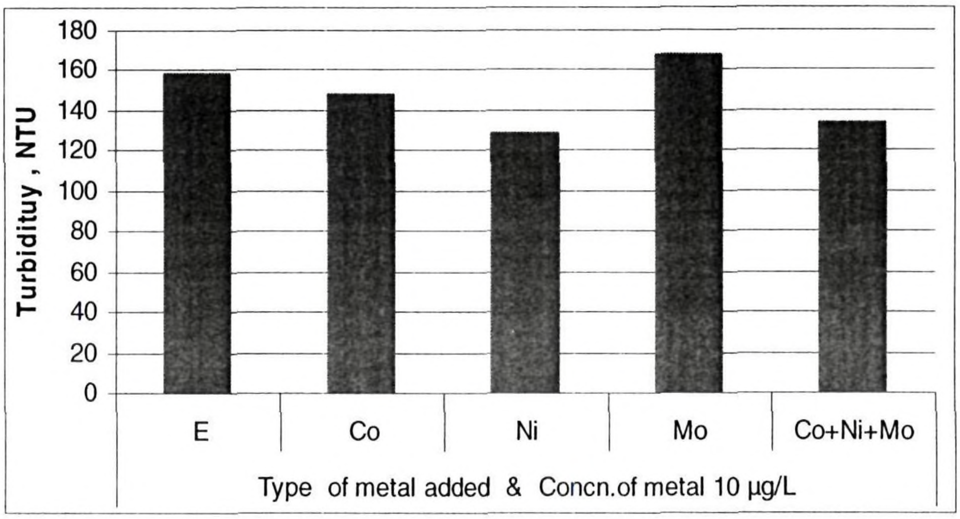

Fig. 4. B. 7. Average turbidity in the presence of 10 µg/L of trace metals

(d) COD removal

Addition of 100 µg/L of trace metals on COD removal efficiency was estimated for seven weeks and the results are given in Table 4. B. 8.

Table 4. B. 8. Effect of 100 µg/L of trace metal ions on COD removal

Type of metal added (Conc. of metal; 100 µg/L) and % COD removal					
Week	E	Co	Ni	Mo	Co+Ni+Mo
1	64	52	67	82	62
2	60	67	46	45	46
3	51	55	53	38	40
4	43	49	41	55	53
5	46	48	42	44	43
6	46	23	13	21	13
7	42	22	13	21	11

During the first week, addition of Mo and Ni resulted in a high percentage removal of COD, pointing to their initial high influence. Influence of these metals was not so prominent in the later weeks. Though the influence of cobalt was not so prominent in the first week, its continuous addition increased the percentage removal of COD to 67% in the second week. After the fifth week, a sudden decrease in the COD removal efficiency was observed in all metal ion concentrations. An abrupt drop in percentage removal of COD from 42 to 13 was observed when Ni was added continuously (sixth and seventh week). The same effect was also observed during the continuous addition of a combination of all the three metals. The sudden decrease in COD removal could be attributed to the high concentration of Ni added. Addition of Ni became inhibitory during anaerobic treatment. The same effect was observed by Gonzalez-Gil et al[14] during the methanogenic conversion of methanol and they reported that 400 μM of Ni was inhibitory. For effluent without any metal, the decrease in the percentage removal of COD was gradual. This gradual decrease might be due to the wash out of biomass since the rector was of low height. The drop in the COD removal efficiency was also observed during the addition of Co and Mo after the fifth week but not as high as that of Ni. Addition of metals enhances the treatment of effluents, but excess of nutrients can lead to inhibitory effects on the biomass and/or to metal accumulation in the sludge[14]. For anaerobic treatment, Ni and Mo were needed only initially and its continuous addition did not show any positive influence on the treatment of effluent. Influence of cobalt in treating serum effluent was evident from the high percentage removal of COD in the presence of this metal, when compared to the treatment without using any metal (Table 4. B. 8, second to fifth week). But high concentration showed inhibitory effect which was evident from the sudden fall of COD removal from 48 to 23. According to Florencio[15] methanogens are better scavengers for cobalt than acetogens and cobalt levels can be used to prevent acetate formation from methanol. For growth and activity, both trophic groups had similar cobalt optima with the values around 0.05 mg/L. With methylotrophic substrates, cobalt is an important nutrient during anaerobic treatment and this high requirement for cobalt is presumably due to the production of corrinoids (i.e., vitamin B12) where cobalt is the central ion[22].

In the second phase of the experiment 10 μg/L of these metals were added daily and its effect on COD removal is given in Table 4. B. 9. From the table it is clear that as the concentration of metal goes on increasing, percentage removal also increases for Co and Mo. Influence of Mo was higher than Co for 10 μg/L concentration, whereas for 100 μg/L reverse effect was observed. As concentration of Mo increased, percentage COD removal also increased, indicating the need for traces of Mo in anaerobic treatment. Increase in Co concentration had a corresponding impact in the percentage removal of COD and it was more prominent when 100 μg/L was added.

Table 4. B. 9. Effect of 10 μg/L of trace metal ions on COD removal

Type of metal added (Conc. of metal; 10 μg/L) and % COD removal					
Week	E	Co	Ni	Mo	Co+Ni+Mo
1	31	36	22	52	43
2	62	54	30	52	36
3	65	46	41	58	45
4	49	55	29	61	41
5	49	55	35	64	46

(e) BOD removal

Percentage BOD removal efficiency decreased on moving from the first week to the third week and then it started showing negative percentage removal. As anaerobic treatment continued further, BOD values were found to increase above the values of the raw effluent as shown in Fig. 4. B. 8. The same type of negative percentage BOD removal was observed in the UASB reactor also. When anaerobic treatment proceeds, an unusual increase in the value of BOD was observed. The value of BOD exceeds the value of COD from the fourth week onwards which indicated a constant interference in the measurement of BOD. Metcalf & Eddy[1] reported the interference due to nitrogenous biochemical oxygen demand (NBOD) in BOD values leading to erroneous interpretations of the treatment.

The measured BOD values were higher than the true value due to oxidation of nitrogenous compounds along with carbonaceous materials. BOD test measures the oxygen demand produced by carbonaceous and nitrogenous compounds. Sulphite, sulphide and ferrous iron are oxdisable by molecular oxygen and these also have a bearing on the oxygen balance of the receiving water[18, 23].

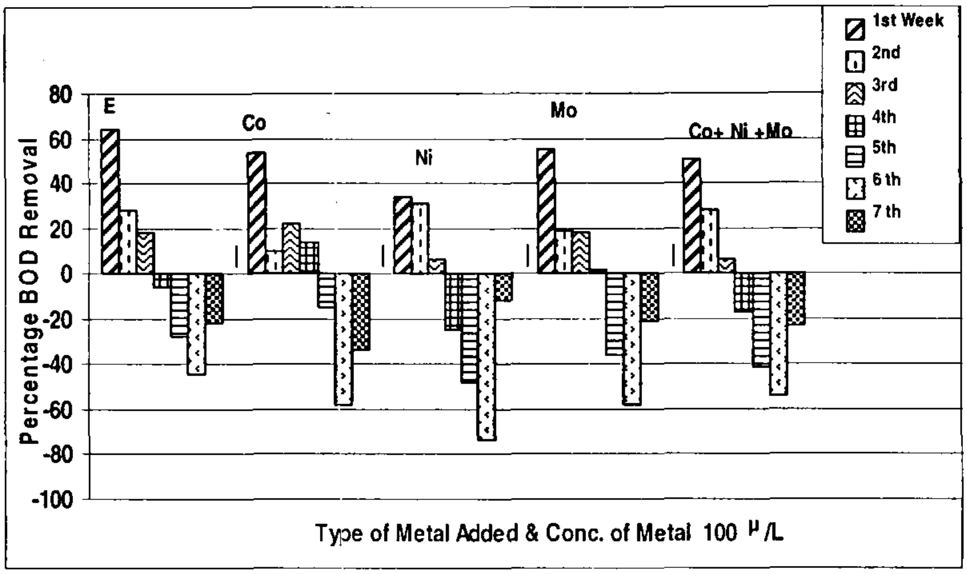

Fig. 4. B. 8. Effect of trace metal (100μg/L) on percentage BOD removal

The same effect was also observed when 10 μg/L of trace metal was added to the effluent daily (Table 4.B.10.). Since some kind of constant interference was observed in the BOD values, the efficiency of treatment cannot be assessed in terms of BOD alone.

Table 4. B. 10. Effect of trace metals (10μg/L) on BOD removal

Type of metal added and % BOD removal (Conc. of metal 10 μg/L)					
Week	E	Co	Ni	Mo	Co+Ni+Mo
1	29	10	9	31	18
2	4	-5	-5	0	-7
3	-16	-6	-34	-27	-36
4	-18	-14	-10	-25	-36
5	-17	-19	-19	-17	-13

(f) TKN and AN removal

Trace metals did not have much influence in the removal of TKN and AN. Removal of TKN decreased as anaerobic reaction progressed (Table 4.B.11). During the initial stage, TKN removal was comparatively high in the presence of 100 μg/L Co, Ni and Mo. Anaerobic treatment is not considered as an efficient method to remove nitrogen since it is unable to remove TKN by means of oxidation[24] and very low amount of nitrogen was removed in the UASB process also.

Ammoniacal nitrogen (AN) concentration increased after the first week (Table 4. B.11). Anaerobic digestion process does not remove nitrogen effectively and this can be expected in anaerobic treatment[25]. Anaerobically treated effluent contains nitrogen in the form of ammonium ($NH4^+$). Organic nitrogen present in the proteins and amino acids is converted to AN during anaerobic treatment. Removal of ammoniacal nitrogen was negative during anaerobic treatment, due to formation of AN. No significant influence of trace metals was observed in the anaerobic treatment of serum effluent to remove total Kjeldahl nitrogen and ammoniacal nitrogen.

In the second phase of experiment when 10 μg/L of metals were added daily, TKN removal increased for Co, Ni and for the combined addition of metals. When Mo was added, no regular pattern for the removal of TKN was observed. Removal of ammoniacal nitrogen also did not follow any regular pattern. During the first three weeks ammoniacal nitrogen concentration increased which showed a negative removal and then its concentration decreased showing a positive removal.

Table 4. B. 11. Percentage removal of TKN & AN in the presence of 100 µg/L of trace metals

Type of metal added (Conc. of metal 100 µg/L)										
Percentage **TKN** removal						Percentage **AN** removal				
Week	E	Co	Ni	Mo	Co+ Ni+Mo	E	Co	Ni	Mo	Co+ Ni+Mo
1	31	52	49	39	43	30	26	22	25	25
2	20	38	36	32	37	-5	-12	-12	-12	6
3	4	10	10	8	22	-25	-23	-25	-21	-7
4	3	2	3	0	5	-12	-19	-23	-19	-19
5	0	0	3	0	3	-18	-30	-34	-34	-23
6	17	12	12	12	17	-34	-34	-34	-34	-27
7	5	4	8	3	3	-23	-26	-30	-23	-27

Table 4. B. 12. Percentage removal of TKN & AN in the presence of 10 µg/L of trace metals

Type of metal added (Conc. of metal 10 µg/L)										
Percentage **TKN** removal						Percentage **AN** removal				
Week	E	Co	Ni	Mo	CO+Ni+Mo	E	Co	Ni	Mo	Co+ Ni+Mo
1	15	10	15	15	19	-12	-15	-14	-8	-1
2	16	10	17	15	15	-12	-12	-15	-13	-5
3	18	25	20	25	15	-14	-1	-5	-8	-1
4	29	29	29	32	24	7	14	3	5	14
5	25	46	32	25	34	7	1	7	8	10

Low percentage removal of total nitrogen and increase in the level of ammoniacal nitrogen were due to the conversion of organically bound nitrogen to ammonium by anaerobic bacteria[25].

4. B. 5. Conclusion

- Addition of trace metals like Mo and Ni individually and in combination (Mo, Ni and Co) at 100 and 10 µg/L concentration reduced the pH of the serum effluent, whereas individual Co metal addition increased the pH at both level of concentrations.
- During the addition of 100 µg/L of metals consecutively at weekly intervals, sulphide formation increased gradually and then decreased. Average sulphide formation is higher for the metal concentration of 10 µg/L than for 100 µg/L and formation of sulphide is comparatively low in the presence of Ni and Mo

In the presence of cobalt (100 µg/L) turbidity gradually increased, reached a maximum and then decreased showing that the presence of cobalt favoured cell growth than any other metals. Addition of 10 µg/L of trace metals to the effluent showed that Mo containing effluent had high value of turbidity. But the average value of turbidity was higher for effluent with 100 µg/L of metals compared to effluent containing 10 µg/L.

Continuous addition of 100 µg/L of Co increased the rate of removal of COD, reached a maximum and then decreased. An abrupt drop in percentage removal of COD was observed during the addition of Ni alone and all the three metals together, showing the inhibitory effect of metals. In the removal of COD, influence of Mo was higher than Co when 10 µg/L of the trace metals were added indicating the need for traces of Mo in anaerobic treatment.

- Increase in BOD was observed during anaerobic treatment and the value of BOD exceeded the value of COD, which indicates a constant interference in the measurement of BOD.
- Trace metals did not have much influence on the removal of TKN and AN. Percentage removal of TKN decreased as anaerobic reaction progressed. Percentage removal of ammoniacal nitrogen was negative, which confirmed the formation of ammonia during anaerobic treatment. No significant influence of trace meals was observed in the anaerobic treatment of serum effluent to remove total Kjeldahl nitrogen and ammoniacal nitrogen.
- The study confirms the significant role of microgram quantities of trace metals like Co and Mo in the anaerobic treatment of serum effluent.

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CHAPTER 5

ELECTROCHEMICAL TREATMENT

5. 1. Introduction

Electrochemical treatment enables protection of the environment reducing substantially the polluting effects of wastes and toxic materials in effluents. Electrolysis is a non-biological, chemical free and environment friendly process that ensures a better quality of treated water. It is a noiseless and odourless operation and purifies water, which is getting scarce day by day. Biological processes are used to remove biodegradable organics[1], and if part of the organic compound present in the effluent is persistent, microorganisms are not able to degrade it. Further treatment, following biological process, is inevitable and electrochemical treatment is an effective method for the purification of this kind of wastewater. Electrochemical treatment method is widely accepted and applied because of its versatility, energy efficiency, amenability to automation and environmental compatibility[2]. In fact the main reagent in the electrochemical treatment is the electron which is a "clean reagent"[3]. Application of electrolytic dissociation at the anode for wastewater treatment is more than a century old process. The earliest reported use of electrolytic treatment was in the year 1880 in England[4]. Electrolysis has been used since then for treatment for various wastes and wastewaters, including wastewaters from a number of industries, such as textile[5-8], pulp[4], food processing[9], petrochemical[10], defence[11], metal working[12], tannery[13 14], slaughter house[4, 15], diary products[12], domestic wastewater[16], and land fill leachate[17].

Electrochemical oxidation (ECO) or electro coagulation has been used in North and South America and Europe to treat industrial wastewaters containing metal ions[18]. Destruction of naphthol[19], 4-chlorophenol[20], lignin, EDTA and chlorotetracycline[1] in simulated wastewaters can be achieved through electrochemical oxidation. Real wastewaters can also be treated by this method[21-25]. Electrochemical oxidation using cast iron electrode for the effective treatment of pharmaceutical wastewaters[26] and cigarette industry wastewaters[27], colour removal of distillery effluent using catalytic anodes[28] and dye wastewaters with graphite anode and steel cathode[29] were also reported. Fenton's reagent was used for the treatment of olive oil mill and textile dyeing wastewater[30, 31]. Electrochemical treatment has shown to be an effective alternative way for the treatment of these different types of industrial effluents.

Electrocoagulation (EC) is based on scientific principles involving the responses of water-based contaminants to strong electric fields and electrically induced oxidation and reduction reactions. The process removes over 99% of some heavy metal cations and electrocutes microorganisms in water. The EC technology offers an alternative to the use of metal salts or polymers and ployelectrolytes for breaking stable emulsions and suspensions. This technology removes metals, colloidal solids and particulates and soluble inorganic pollutants from aqueous media by introducing highly charged polymeric metal hydroxide species. These species neutralise the electrostatic charges on suspended solids and oil droplets to facilitate agglomeration or coagulation and the resultant separation from aqueous solutions.

5. 2. Advantages of electrochemical treatment

Electrochemical treatment is a simple and easy method and at the same time ensures rapid processing. It removes very fine colloidal particles through coagulation resulting from the faster motion of the colloidal particles in the applied electric field. Electrochemical treatment as such does not need any chemicals unless oxidants are used. The use of oxidants will result in additional cost, additional sludge and salt production since they are based on the addition of supplementary chemical reactants. In EC treatment the pollutants are carried to the top of the solution through the gas bubbles formed during electrolysis which can be removed. The quantity of the sludge generated and water bound to the sludge is less and therefore easy for dewatering. The desired efficiency can be achieved at very low concentration of ions. Another advantage of EC is emulsion breaking resulting from the oxygen and hydrogen ions that bond to the water receptor sites of oil molecules. Metal ions complex with chlorine in a chlorinated hydrocarbon molecule resulting in large insoluble complex materials. Bleaching by oxygen ions produced in the reaction chamber oxidises dyes, cyanides, bacteria, viruses, biohazards etc[57]. One of the main attractions of electrochemical treatment is the use of solar energy for electrolysis with the help of photovoltaic cells and therefore this method can be applied in rural areas too. Since electricity is expensive, use of solar energy reduces the running cost of the treatment processes. Replacement of the electrodes as a result of oxidation is the main disadvantage of this process.

5. 3. Theory of electrochemical treatment.

The term electrolysis or electrolyte reaction is applied to those reactions, oxidation or reduction, which take place at the surface of conductive electrodes immersed in a chemical medium, under the influence of an applied potential. At the negative electrode, or cathode, the reactions may include the reduction of a metal ion.

$$M^+ + e^- \rightarrow M \qquad (1)$$

or, in aqueous media, the reduction of hydrogen ion or water to hydrogen gas

$$2H^+ + 2e^- \rightarrow H_2 \qquad (2)$$

or

$$2H_2O + 2e^- \rightarrow H_2 + 2OH^- \qquad (3)$$

The potential at which such reactions take place at 25^0C and with all reactants and products at unit activity is termed E^0 of the reaction. For conditions in which all reactants and products are not at unit activity, the required potential is given by the Nernst equation[32];

$$E = E^0 - \frac{0.0592}{n} \log Q \qquad \mathbf{(4)}$$

where 'n' is the number of electrons required in the reduction (or oxidation) step and 'Q' is the product of activities of product materials divided by the activities of the reactants.

Following are the oxidation reactions[32].

$$2Cl^- \rightarrow Cl_2 + 2e^- \qquad (5)$$

or

$$2H_2O \rightarrow O_2 + 4H^+ + 4e^- \qquad (6)$$

or

$$4OH^- \rightarrow 2H_2O + 4e^- + O_2 \qquad (7)$$

When DC power is turned on, electrons flow to the metal anode. They readily combine with hydrogen ions dissociated from water molecules to form hydrogen gas which escapes as rising bubbles. The remaining hydroxyl ions combine with the metal ions to form coagulants in the cell. Oxygen and ozone are also generated. Chloride ions are oxidised to chlorine gas; the electrons that are formed, go through the graphite cathode back to the power source to complete the current flow cycle. Chlorine formation takes place only in the top layer and is therefore, not excessive. Heyrovsky[33] showed that the migration current can be completely eliminated by adding an indifferent electrolyte in a concentration so large that its ions carry essentially all the current. An indifferent electrolyte is one which conducts the current but does not react with the material under investigation, nor at the electrodes within the potential range studied. This means that the concentration of the added electrolyte ('supporting electrolyte') must be at least 100-fold that of the electro active material[33]. Sodium chloride added to the electrolyte results in the production of hypochlorite upon electrolysis. The hypochlorite then reacts with the organic constituents to yield ultimately carbon dioxide, nitrogen, and sodium hydroxide and it is an effective disinfectant[34].

In electro coagulation a metal coagulant is provided by electrolysing an electrode of an electrolytic cell which then reacts to form metal-hydroxide and settle down. One advantage of electro coagulation over chemical coagulation is that, in chemical coagulation, anions such as sulphate or chloride are introduced to the solution, and these anions can cause problems with reuse of the water. In addition, for electro coagulation, the coagulant is introduced into the water in direct relationship with the amperage applied to the electrolytic cell and therefore can be controlled by controlling the applied current[35]. Therefore, it helps the process of decantation, coagulation and flocculation and if applied before the conventional biological treatment it increases the efficiency of the aerobic and anaerobic lagoons. Also it avoids the use of chemical products for neutralisation and the reacted waste may be reused in the process; thus projected as a treatment method compatible with the conservation of the environment[36]. In electro coagulation the released ions remove undesirable contaminants either by chemical reactions causing precipitation or by causing the colloidal particles to coalesce, which can be removed by flotation. In addition, water contaminants move through the applied electric field, and there may be ionisation, electrolysis, hydrolysis and free-radical formation which can alter the physical and chemical properties of water and contaminants[57].

Anodic oxidation schemes generally involve the destruction of specific organic chemicals. It reduces the toxicity through the transformation of resistant groups (aromatic rings etc) to biodegradable substances. This can be carried out through the electro oxidation of organic substance, either through the electrode or through the potential of oxide-reduction[37].

Fenton's reagent generates ferrous ions to react with hydrogen peroxide, producing hydroxyl radicals, which are strong oxidising agents. They can react with organics and degrade them[38, 39].

$$H_2O_2 + Fe^{+2} \rightarrow {}^{\cdot}OH + OH^{-} + Fe^{+3} \qquad k = 70\ M^{-1}s^{-1} \qquad (8)$$

The hydroxyl radical propagate the reaction by reacting with the organics to produce further radicals, which can then react in many ways.

$${}^{\cdot}OH + RH \rightarrow H_2O + R^{\cdot} \quad k = 10^{9} - 10^{10}\ M^{-1}s^{-1} \qquad (9)$$

$R^{\cdot} + H_2O_2 \rightarrow ROH + HO^{\cdot}$ **(10)**

Additionally many other reactions are also possible, which include the radical-radical interaction or the reaction of the OH radical with H_2O_2

$^{\cdot}OH + ^{\cdot}OH \rightarrow H_2O_2$ (11)

$^{\cdot}OH + H_2O_2 \rightarrow H_2O + HO_2^{\cdot}$ $k = 3.3 \times 10^7 M^{-1}s^{-1}$ (12)

The peroxide radical ($HO_2^{\cdot}$) produced in the above case can further oxidise other species present in the solution.

$HO_2^{\cdot} + Fe^{+3} \rightarrow O_2 + Fe^{+2} + H^+$ $k = 1.26 \times 10^6 M^{-1}s^{-1}$ (13)

The rapid consumption of H_2O_2 that is often observed with Fenton's reagents is probably due to combined effect of reactions (8) to (11). However, since reaction 9 has the highest rate constant, it is mainly responsible for the degradation of the organic compounds. Furthermore, it is possible for Fe^{+2} to be auto-regenerated in this system, and act as catalyst[40].

$Fe^{+3} + H_2O_2 \rightarrow Fe^{+2} + H^+ + HO_2^{\cdot}$ (14)

Two processes namely passivity and mass transport, control the electrolytic process[41]. For some systems, increase in current does not lead to corresponding increase in electrolysis of the electrode. Instead, a decrease in electrolysis is observed; this phenomenon is called passivity. Passivity is caused by the accumulation of a coating of insoluble salts, oxides, organic materials, and biological films on the surface of the electrodes, which increases the résistance to the electrolytic process. Because of the limitations on the transport of ions to and from the electrodes, for every system there is a limiting current density, at which mass transport controls the rate of the reaction instead of the current[35].

Electrochemical cells generally adopt some form of stirring to reduce concentration changes in the neighbourhood of the electrode and narrow electrode spacing minimises resistance. Electroflotation serves as an interesting example of the indirect use of electrolysis to achieve useful end in water treatment. Electrolytically generated bubbles are used for separation of oil-water mixtures[32].

5. 3. 1. **Theory of chemical oxidant** -Fenton's reagent (Hydrogen peroxide and Ferrous sulphate)

Fenton's reagent is used to treat different types of industrial wastes containing toxic organic compounds (phenols, formaldehyde and complex wastes derived from dyestuffs, pesticides, wood preservatives, plastics additives, and rubber chemicals)[42]. Many metals have special oxygen transfer properties especially iron when used in the prescribed manner, results in the generation of highly reactive hydroxyl radicals ($^{\cdot}OH$). The reactivity of this system was first observed in 1894 by its inventor H.J.H. Fenton, but its utility was not recognized until the 1930's once the mechanisms were identified. This can be applied to treat wastewaters and sludge since it has the ability to remove odour, colour, BOD/COD and organic pollutants. It reduces toxicity and improves biodegradability[42].

The reaction can be represented as

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + {}^{\cdot}OH$$

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + {}^{\cdot}OOH + H^+$$

To attain maximum efficiency of the reagent the procedure needs to satisfy the following conditions. The pH of the water should be in the range of 3 to 5. Iron catalyst should be added and the addition of H_2O_2 should be slow. If the pH is too high, the iron precipitates as $Fe(OH)_3$ and catalytically decomposes the H_2O_2 to oxygen, potentially creating a hazardous situation[42].

Reaction rates with Fenton's reagent are generally limited by the rate of $^{\cdot}OH$ generation (i.e., concentration of iron catalyst) and less so by the specific wastewater being treated. Fe: H_2O_2 ratios are 1:5-10 wt/wt, though iron levels < 25-50 mg/L can require excessive reaction times (10-24 hours). This is particularly true where the oxidation products (organic acids) sequester the iron and remove it from the catalytic cycle[42]. Fenton's reagent is most effective as a pre-treatment tool, where COD's are > 500 mg/L. This is due to the loss in selectivity as pollutant levels decrease.

$$^{\cdot}OH + H_2O_2 \rightarrow {}^{\cdot}O_2H + H_2O$$

$$^{\cdot}OH + Fe^{3+} \rightarrow Fe^{2+} + OH^-$$

In addition to free radical scavengers, the process is inhibited by (iron) chelants such as phosphates, EDTA, formaldehyde, and citric/oxalic acids. Fenton's Reagent is sensitive to different wastewaters[42]. The hydroxyl radical is one of the most reactive chemical species known, second only to elemental fluorine in its reactivity. The chemical reactions of the hydroxyl radical in water are of four types:

Addition: $^{\cdot}OH + C_6H_6 \rightarrow (OH)C_6H_6^{\cdot}$

where the hydroxyl radical adds to an unsaturated compound, aliphatic or aromatic, to form a free radical product (cyclohexadienyl radical shown above).

Hydrogen abstraction: $^{\cdot}OH + CH_3OH \rightarrow {}^{\cdot}CH_2OH + H_2O$

where an organic free radical and water are formed.

Electron transfer: $^{\cdot}OH + [Fe(CN)_6]^{4-} \rightarrow [Fe(CN)_6]^{3-} + OH^-$

where ions of a higher valence state are formed, or an atom or free radical, if a mononegative ion is oxidized.

Radical interaction: $^{\cdot}OH + {}^{\cdot}OH \rightarrow H_2O_2$

where the hydroxyl radical reacts with another hydroxyl radical, or with an unlike radical, to combine or to disproportionate to form a stable product.

Fenton's reagent when applied for industrial waste treatment, the conditions of the reaction are adjusted so that the first two mechanisms (hydrogen abstraction and oxygen addition) predominate. Typical rates of reaction between the hydroxyl radical and organic materials are 10^9 - 10^{10} k $(M^{-1}\ s^{-1})$[42].

Concentration of iron and type of iron used (Ferrous or Ferric), H_2O_2 and its concentration, temperature, pH and reaction time are the factors which affect the reaction rate in presence of Fenton's reagent. Hydroxyl radical formation is not observed in the absence of iron. A minimal threshold concentration of 3-15 mg/L Fe allows the reaction to proceed within a reasonable period of time regardless of the concentration of organic material. A constant ratio of Fe: substrate above the minimal threshold, typically 1 part Fe per 10-50 parts substrate, which produces the desired end products. Though it does not matter whether Fe^{2+} or Fe^{3+} salts, if low doses of Fenton's reagent are being used, ferrous iron may be preferred. As the H_2O_2 dose is increased, a steady reduction in COD may occur with little or no change in toxicity until a threshold is attained, whereupon further addition of H_2O_2 results in a rapid decrease in wastewater toxicity. Most commercial applications of Fenton's reagent occur at temperatures in between 20-40^{0}C. The optimal pH for Fenton's reagent occurs between pH 3 and 6. In alkaline pH hydrated ferrous iron is transformed into colloidal ferric species which decompose H_2O_2 to oxygen and water. The time needed to complete a Fenton reaction will depend on catalyst dose and wastewater strength.

5. 4. Aim

The objective of the study is to investigate the effect of anodic oxidation of raw and anaerobically treated skim serum effluent using different electrodes like aluminium, stainless steel, mild steel, and cast iron. Photovoltaic cells harnessing solar energy were used to treat these effluents. Their efficiency was compared with that of DC power. Sodium chloride was used as the supporting electrolyte. In order to study the efficiency of treatment using advanced oxidation process Fenton's reagent was used as the chemical oxidant. The effect of operating factors such as supporting electrolyte, duration of electrolysis, solar radiation, direct current, pH, concentration of effluent and the presence of chemical oxidant on the removal of COD, BOD, TKN, AN, turbidity, sulphides and phosphates were studied. The influence of these factors on the biochemical constituents and population of total bacteria was also investigated.

5. 5. Experimental

Natural rubber skim serum effluent was collected according to the methods described in Chapter 2. To obtain anaerobically treated sample for the study, the effluent was subjected to anaerobic treatment in a bench scale an upflow anaerobic sludge blanket (UASB) reactor as explained in Chapter 4. Raw and treated samples were analysed for various parameters as per standard methods [43] and the results of analysis are given in Table 5. 1.

Table 5. 1. Characteristics of effluent used for electrochemical treatment

No	Parameters	RE	ATE (1)	ATE (2)
1	pH	**3.6**	**8.2**	8.5
2	COD	38800	10900	4508
3	BOD	27650	7890	1015
4	TKN	7000	2380	4480
5	AN	5000	1680	3500
6	Phosphate	2583	680	525
7	Sulphide	**14**	**45**	**235**
8	Turbidity (NTU)	350	110	107

(All values are in mg/L except pH & turbidity; RE- Raw effluent; ATE- anaerobically treated effluent,(1) Sample 1- HRT-18 days, (2) Sample 2- HRT- 36 days)

A glass reactor with a capacity of 500 cm^3 was used for all the electro oxidation experiments. Plates made of cast iron, aluminium, mild steel and stainless steel (7cmx5cm) were used as anodes. Graphite of similar geometric dimensions was used as the cathode. The area of the electrode exposed to electrolysis was fixed at 25 cm^2 and the remaining area was prevented from exposure. The cathode and anode were placed vertically and parallel to each other at a fixed distance of 2 cm apart using a nonconducting material in the electrolytic cell (Fig.5.1).

To compare the efficiency of different electrodes in removing pollutants, galvanostatic conditions were maintained using D.C power supply (Systronics dual channel power supply 615D) and a current of 1.3A was applied. 10 g/L of NaCl solution was added to the effluent prior to electrolysis as the supporting electrolyte. The cell was equipped with a magnetic stirrer in order to keep the electrolyte well mixed during electrolysis and voltage and current were recorded at every five minute intervals. Fenton's reagent was prepared from 35% hydrogen peroxide and $FeSO_4$ (obtained from Merck). 200 mg/L of Fe and 2 mL/L of H_2O_2 were added during electrolysis.

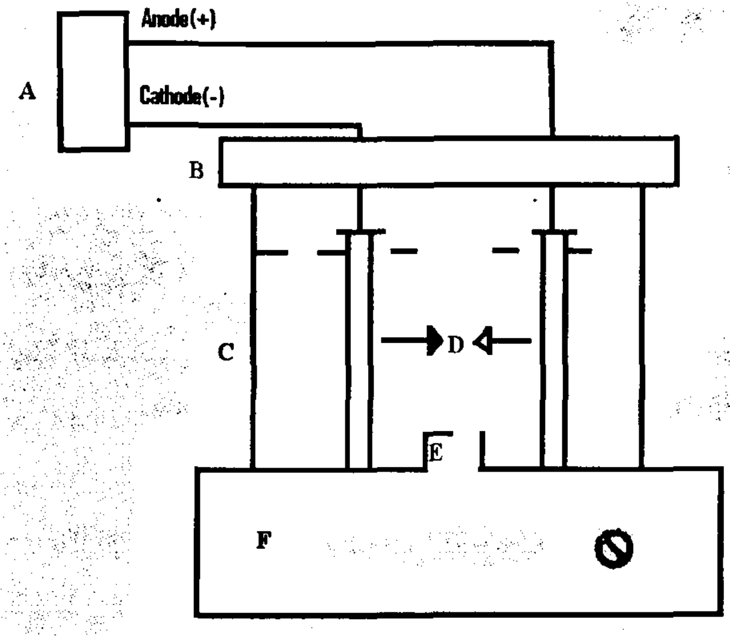

Fig 5. 1 Schematic diagram of the electrochemical reactor[26]

A:- Regulated D.C power supply B:- Head plate C:-Glass reactor

D:-Pair of electrodes E:-Magnetic bar stirrer F:-Magnetic stirrer unit

A photovoltaic cell (of 12volt) was employed to convert solar energy to electrical energy and electrolysis was carried out using aluminium as anode and graphite as cathode (Fig. 5. 2)

Fig. 5.2. Electrochemical oxidation using photovoltaic cell

Raw and anaerobically treated samples were subjected to electrochemical treatment for different intervals. During electrochemical treatment, samples were drawn at regular intervals and parameters like pH, turbidity, COD, BOD, TKN, AN, sulphides and phosphates were determined as per standard methods[43].

Biochemical analysis of the different types of effluent was done using a UV-visible recording spectrophotometer, UV-240. Concentration of total sugars[44], reducing sugars[45], soluble proteins[46], phenols[47] and free amino acids[48]were estimated as per standard analytical techniques.

The population of total bacteria in the raw and treated effluent was enumerated using appropriate media. The standard serial dilution plate technique of Pramer and Schmidt[49] was employed for the enumeration of microbiological population.

5. 6. Results and discussion

5. 6. 1. Effect of supporting electrolyte in electrochemical treatment

The effect of NaCl (supporting electrolyte) in electrochemical treatment was ascertained by electrolysing the raw effluent with steel as anode and graphite as cathode for three hours in the presence as well as absence of NaCl. The variation of cell voltage and current with time for the two cases are shown in Fig. 5. 3 and Fig.5. 4

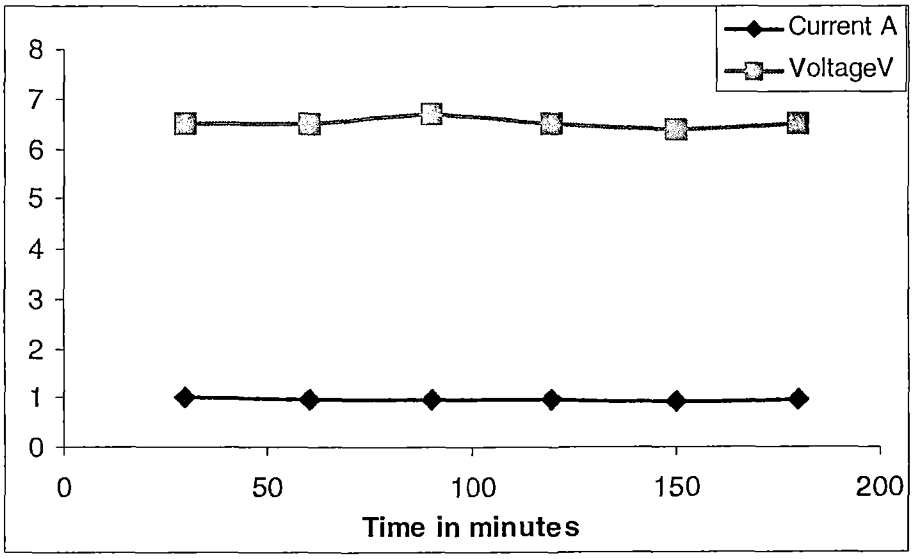

Fig. 5. 3. Variations of current and voltage with time (without of NaCl)

Fig. 5.3. shows that current and cell voltage remained almost constant when electrolysed without NaCl. When repeated in the presence of (10 g/L) NaCl current and voltage dropped in the beginning and then

maintained a steady value as shown in Fig. 5.4. The cell voltage and current were unchanged during electrolysis in the absence of NaCl, suggesting the absence of electrode degradation. The higher values of cell voltage in Fig.5.3 might be due to the absence of sodium chloride, the supporting electrolyte. The presence of the supporting electrolyte decreased the cell voltage, which lead to a decrease in power consumption.

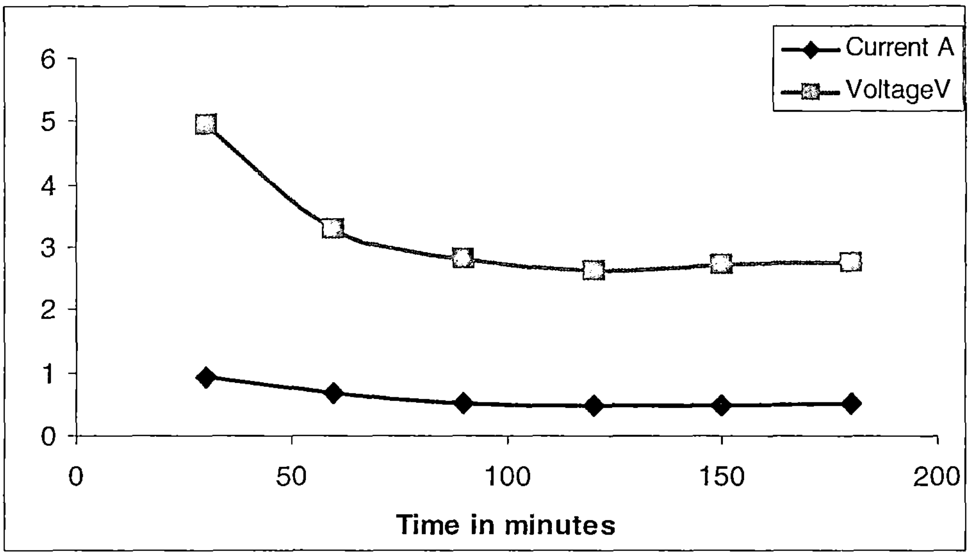

Fig. 5. 4. Variations of current and voltage with time in the presence of NaCl

Table 5. 2. Effect of supporting electrolyte on effluent parameters

Supporting electrolyte	pH before	pH after	Percentage removal			
			COD	BOD	TKN	AN
Without NaCl	3.45	3.5	17.3	40	13	23
With NaCl	3.8	5.65	31.5	58	16	30

In the absence of supporting electrolyte slight variation in pH was observed during electrolysis and pH was found to increase from 3.8 to 5.65 in the presence of NaCl. The increase in pH of the treated effluent might be due to the formation of hydroxyl radical during electrolysis. Better performance was observed when electrolysis was carried out in the presence of sodium chloride. When electrolysed in the presence of supporting electrolyte percentage removal of COD, BOD, TKN and AN was found to increase (Table 5. 2). The reason for this trend can be attributed to the different oxidation mechanisms prevailing in the presence of NaCl[28] and absence of it. At higher concentration of NaCl, the destruction of organics might be due to the presence of chlorine and hypochlorites. The off-gases from the cell were collected and tested. When the gas was passed through lime water, the latter turned out milky indicating the formation of carbon dioxide during electrolysis. The gas emanated from the anode formed a white curdy precipitate when treated with dilute nitric acid and silver nitrate. Presence of chlorine gas caused this change. Presence of hypochlorite was also evident from its smell during electrolysis. The removal of organics through electro generated oxidising agents like chlorine / hypochlorite has already been established[54,55]. In general the following reactions took place during electrolysis of the effluent. At the anode :

$$2Cl^- \rightarrow Cl_2 + 2e^-$$

$$4OH^- \rightarrow O_2 + 2H_2O + 4e^-$$

At the cathode:

$$2H_2O + 2e^- \rightarrow H_2 + 2OH^-$$

In the undivided cell, chlorine formed at the anode and hydroxides formed at the cathode reacted to form chlorine and hypochlorites. Both the hypochlorites and free chlorine were chemically reactive and oxidised organics in the effluent to carbon dioxide and water.

5. 6. 2. Comparison of different electrodes

Electrolysis of raw effluent (RE) was carried out for 20 minutes using electrodes made of aluminium, cast iron, mild steel (MS), and stainless steel (SS) in the presence of 10 g/L of NaCl as supporting electrolyte. After electrolysis samples were analysed as per standard methods[43]. Analysis of the result proved that aluminium electrode has greater efficiency in removing COD, BOD, TKN and AN as shown in Fig.5.5.

But in phosphate (PO_4) removal the reverse order was observed. Steel electrode showed the maximum phosphate removal of 74 percent followed by MS, cast iron and aluminium. When aluminium electrode was used as anode, the current variation was in the range of 1.38A to 1.34A and voltage 5.9V to 5.7V. For cast iron the current variation was in the range of 1.29A to 1.23A and voltage 10.3V to 7.9V. For MS the current variation was from 1.34A to 1.32A and voltage was 6V to 5.8V. For steel electrode the current variation was in the range of 1.35 A to 1.33 A and voltage variation was from 5.5V to 4.3V. Aluminium electrode produced a very clear solution after settling the coagulated particles. This might be due to the greater charge neutralization of colloidal particles by positively charged metal ions generated *in situ* by electrolytic oxidation.

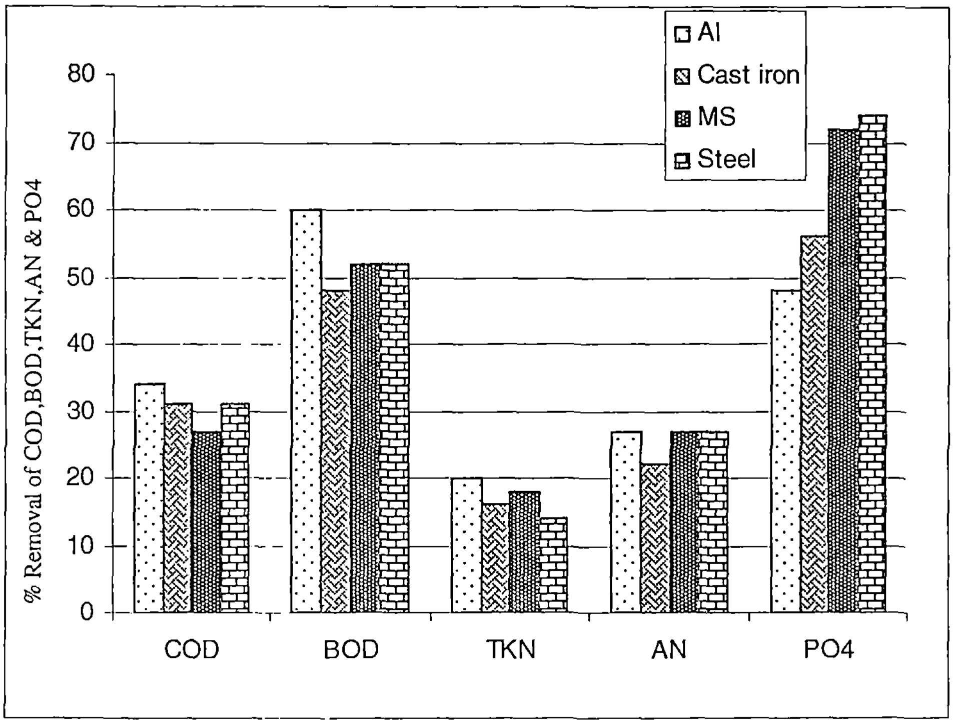

Fig. 5. 5. Comparative plots of COD, BOD, TKN, AN and PO_4 reduction using different electrodes.

Aluminium is the best anode material. Also it is the most affordable material that provides trivalent cations and can be used in almost all kinds of wastewater treatment applications. The trivalent metal ions have a higher charge density, which allows for the superior adsorption capabilities[56].

The dominant reactions at the anode and cathode with aluminium electrodes are:

$2Al_{(s)} \rightarrow 2Al^{3+} + 6e^-$ (Anode)

$6H_2O + 6e^- \rightarrow 3H_{2(g)} + 6OH^-$ (cathode)

The dissolution of anode can be represented by the equation as:

$2Al_{(s)} + 6H_2O \rightarrow 2Al(OH)_3 + 3H_{2(g)}$

The aluminium ions released from the sacrificial electrodes neutralise the electrostatic charges on the colloidal/suspended solids, enabling the formation of flocs. It has been reported that electrolytically added aluminium ions are much more active than chemically added aluminium ions and cause higher degree of destabilisation and flocculation in relatively small dosages[56]. The electro coagulated floc differs significantly from the floc formed during chemical coagulation process. The former contain less bound water and has more resistance to shear. Hence, they exhibit better dewatering characteristics[56]. Aluminium electrode has proven to be very effective when compared to iron and combination of iron/aluminium electrodes and aluminium electrode has the ability to handle a wide variety of wastewater compositions and flow rates[5, 50].

5. 6. 3. Pollutant removal as a function of time

Raw effluent was subjected to electrolysis at pH 3 for 150 minutes to study the effect of duration of electrolysis on pollutant removal using aluminium anode and graphite cathode. 10 g/L of NaCl was added as supporting electrolyte. After every 15 minutes a minimum quantity of effluent was drawn from the cell for analysis.

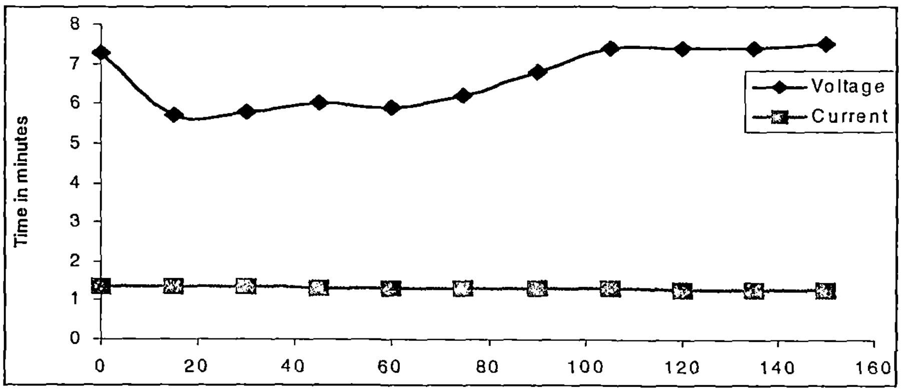

Fig. 5. 6. Variation of voltage and current as a function of time

Table 5. 3. Pollutant removal as a function of time using Al electrode

Time min	pH	Percentage removal			
		COD	BOD	TKN	AN
0	3.00	-	-	-	-
15	3.60	23	69	18	14
30	4.30	32	83	20	16
45	4.55	35	83	21	16
60	4.70	35	82	20	16
75	4.90	35	80	20	16
90	5.10	34	73	20	16
105	5.20	32	69	21	16
120	5.35	32	71	20	16
135	5.45	34	70	21	16
150	5.65	34	62	21	16

Current and voltage were noted at every 5 min. interval. Current was in the range of 1.29 A to 1.36 A and cell voltage was in the range of 5.7 V to 7.3 V. (Fig. 5. 6.). Current remained almost constant during electrolysis. This demonstrates that the electrochemical cell constituents are unaffected during the entire period of electrolysis. Though voltage dropped as electrolysis proceeds, it remained constant at the end of the electrolysis, indicating the absence of adsorption or passivation of the electrode[28]. Maximum removal of BOD within 30 minutes and COD took place within 45 minutes (Table 5. 3). After this, electrolysis did not show much effect on the pollutant removal. Therefore 45 minutes could be taken as the optimum time for electrolysis. The long duration of electrolysis leads to the dissolution of electrode which makes the effluent more viscous. The pH of the solution was found to be increasing from 3 to 5.65 at the end of the electrolysis.

5. 6. 4. Determination of instantaneous current efficiency

Current efficiency decreases during the electrochemical treatment of wastewater containing organic pollutants due to the side reaction of oxygen evolution[51]. Instantaneous current efficiency (ICE) is the current efficiency measured at a particular time or constant time intervals during the electrochemical treatment of wastewater. Calculation of ICE gives information about the formation of polymeric products at the anode during treatment. Two methods have been used for the determination of the ICE during electrochemical oxidation: the oxygen flow rate (OFR) method and the chemical oxygen demand method[51]. The choice of the method depends on the solubility of the electrolysis product. The COD method is used only if the electrolytic products are soluble in the electrolyte. The OFR method is used where electrolysis products are soluble or insoluble. In the OFR method, the ICE is calculated by the oxygen production measured during the electrochemical oxidation of organic pollutants.

In the present study, ICE values were calculated by the COD method using the following relation

$$\mathrm{ICE} = \frac{[(\mathrm{COD})_t - (\mathrm{COD})_{t+\Delta t}]\,FV}{8\,I\Delta t}$$

where [(COD)$_t$ and (COD)$_{t+\Delta t}$ are the chemical oxygen demands at times t and t + Δt (in mg O_2/dm^3) respectively, 'I' is the current (A), 'F' the Faraday constant (96487 C/mol) and 'V' the volume of the electrolyte (dm^3). The calculated ICE values were plotted with respect to time when aluminium was used as anode (Fig. 5. 7.).

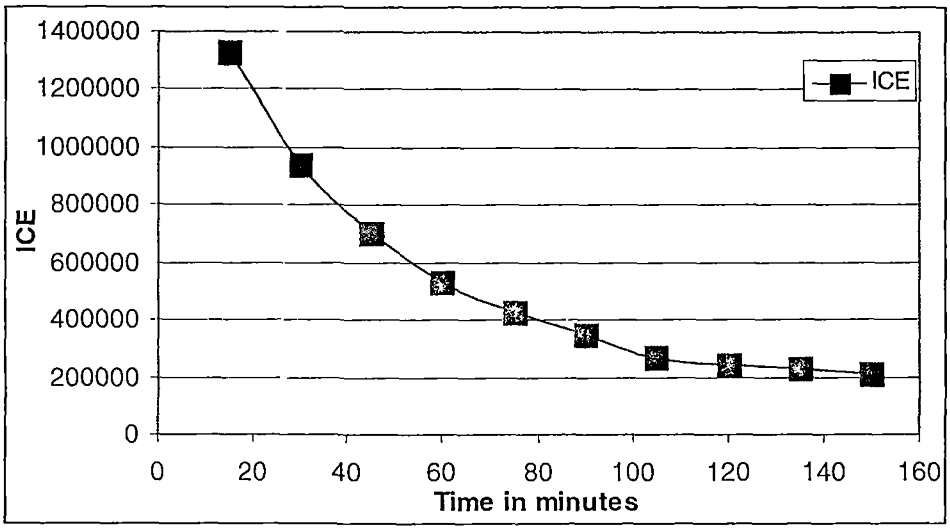

Fig. 5. 7. Plots of ICE values with respect to time

The ICE was found to decrease with time during electrolysis and gradually reached a constant value after 120 minutes. The initial decrease might be due to partial coverage of the active electrode surface by bulky molecules in the medium. Because of continuous oxidation, surface coverage was not increased further. At longer duration, not much change in ICE was observed. This indicated that the electrode surface reactions were not affected much during electrolysis and the constancy in ICE values was due to the steady state obtained between the mass and charge transfers. This was in agreement with the results of earlier observations[28].

5. 6. 5. Effect of cast iron electrode

To study the effect of Fenton's reagent in the presence of cast iron electrode at different pH, raw effluent was subjected to electrolysis at pH 3 and 6.5 using cast iron as anode and graphite as cathode and 10 g/L of NaCl as supporting electrolyte[27]. Current (1.32 - 1.29 A) remained almost constant for the variation of cell voltage range of 11- 6.9 V. Another set of effluents under the same conditions but with 20 mg/L Fe and 2 mL/L H_2O_2 (Fenton's reagent) was also electrolysed for a current of 1.32 to 1.26 A having a potential of 8.5 - 9 V.

Table 5. 4. Pollutant removal in the presence of Fenton's reagent using cast iron electrode

pH	Reagent	Percentage removal			
		COD	BOD	TKN	AN
3	Nil	24.9	41.5	17.5	15.1
6.5	Nil	26.1	42.8	21.6	21.2
3	FR	32.5	75.8	18.3	6
6.5	FR	32.5	76.1	18.3	6

(FR: Fenton's reagent)

The results are given in Table 5. 4 showed that the change of pH from 3 to 6.5 increased the percentage removal efficiency of COD from 24.9 to 26.1, BOD from 41.5 to 42.8, TKN from 17.5 to 21.6 and AN 15.1 to 21.2. Addition of Fenton's reagent increased the COD removal efficiency to 32.5% and BOD removal efficiency to 76.1%. But TKN and AN removal efficiency dropped to 18.3 and 6% respectively. The results show that cast iron electrode in the presence of Fenton's reagent is effective in removing high percentage of BOD.

5. 6. 6. Effect of pollutant concentration on electro chemical treatment

Raw effluent and two different forms of anaerobically treated effluents viz:-ATE (1) & ATE (2) were electrolysed using aluminium anode and graphite cathode for 45 minutes by adding Fenton's reagent and 10 g/L of NaCl as supporting electrolyte. It was found that for the RE, current varied from 1.37 to 1.29A for a cell voltage of 5.7 to 9.4V, for ATE (1) the current was 1.38 to 1.34A for a constant potential of 5.8V and for ATE(2)the current was in the range of 1.32 to 1.29A having a constant potential of 6.7V.

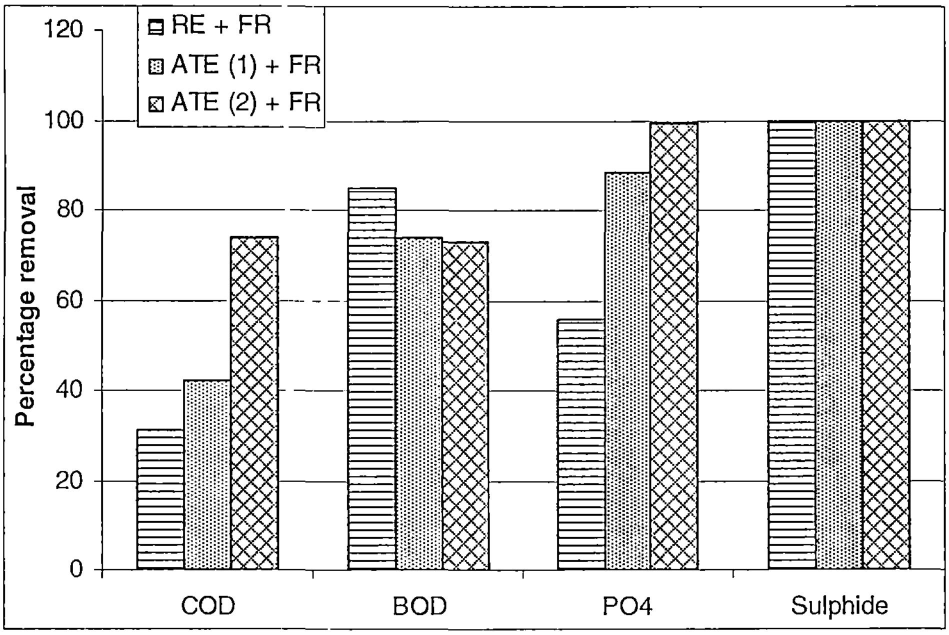

Fig. 5. 8. Effect of pollutant concentration of effluent on electrolysis

Fig. 5. 8. shows that the percentage COD removal efficiencies we 31, 42 and 74 corresponding to the initial COD concentrations of 388 mg/L, 10900 mg/L and 4508 mg/L, respectively. The lower the COD of t effluent used for electrolysis, the higher the rate of removal. But in the ca of BOD, the removal efficiency was 83 percent for RE and it decreased to 7 and finally to 73 percent for anaerobically treated effluents. This may be d to the removal of biodegradable substrates during anaerobic treatment. Aft electrolysis phosphate removal efficiency was 56 percent for raw effluent a

it increased to 99.5 percent for anaerobically treated effluent which showed that when the concentration of phosphate dropped from 2583 to 525 mg/L by anaerobic treatment, its removal efficiency also increased. This might be due to the ability of metal ions (Al^{3+}) to combine with phosphates to form $AlPO_4$. Metal ions formed from the anode become new centres for large, stable and insoluble complexes[56]. Complete removal of sulphide was observed in all the treatment trials. From this it is clear that electrochemical method is more effective for the treatment of anaerobically treated effluent having lower organic load.

5. 6. 7. Influence of concentration of iron in Fenton's reagent using aluminium electrode

To study the effect of concentration of iron in Fenton's reagent, anaerobically treated effluent was subjected to electrolysis for 30 minutes after adding 20, 100, 200 and 300 mg/L of Fe (as Fe^{+2}) respectively in four different sets of effluent (250 mL each) along with 2 mL/L of H_2O_2 in each. A control cell without any Fenton's reagent was also subjected to electrolysis. A current in the range of 1.26A to 1.32A and cell voltage in the range of 5.9V to 7.4V was used in all set of experiments. Current and voltage were recorded at every 5 minute intervals. 10 g/L of NaCl was added as supporting electrolyte in each set. The results are given in Table 5. 5. Turbidity was completely removed after electrolysis using Fenton's reagent. Percentage removal of COD, BOD, TKN and AN were maximum for 200 mg/L of iron concentration in Fenton's reagent. But maximum removal of sulphide and phosphate were for 300 mg/L of iron. As the concentration of iron increased, more sulphides and phosphates combined to form corresponding iron salts. The increase in the removal of pollutants with the increase in the concentration of iron might be due to the formation of hydroxyl radical in presence of Fe^{+2} and H_2O_2. A constant ratio of Fe: substrate above the minimal threshold level produced the desired end product[42].

Table 5. 5. Effect of concentration of Fe in Fenton's reagent on ATE using Al electrode

Reagent	pH	Percentage Removal of						
		Turbidity	COD	BOD	TKN	AN	Sulphide	PO_4
Nil	8.5	75	57	20	22	17	57	52
20 mg/L Fe	8.5	100	57	20	28	15	65	60
100 mg/L Fe	8.5	100	60	22	21	15	72	68
200 mg/L Fe	8.5	100	67	54	30	20	78	83
300 mg/L Fe	8.5	100	66	53	28	16	81	86

5. 6. 8. Effect of pH on electrolysis of ATE using aluminium electrode in presence of Fenton's reagent

One of the parameters that possibly affect the extent of treatment is the pH of the wastewater. To study the influence of pH on pollutant removal, pH of the anaerobically treated effluent was adjusted from 8.5 to 3, 5, 7 and 9 using sulphuric acid and sodium hydroxide. 200 mg/L of Fe (as Fe^{+2}) and 2 mL/L of 30 % H_2O_2 were added to each set. It was then subjected to electrolysis for 20 minutes using aluminium anode and graphite cathode. 10 g/L of NaCl was added as supporting electrolyte. Current strength and voltage were noted at every five minutes interval. The values were plotted against time intervals. (Fig. 5. 9, 10, 11 and 12).

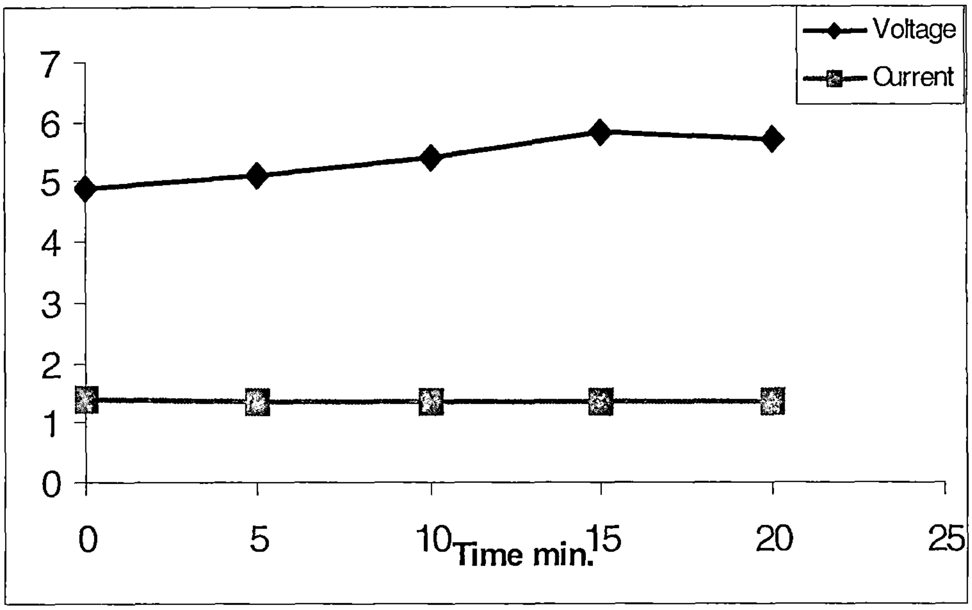

Fig. 5. 9. Voltage & current variation with time at pH 3

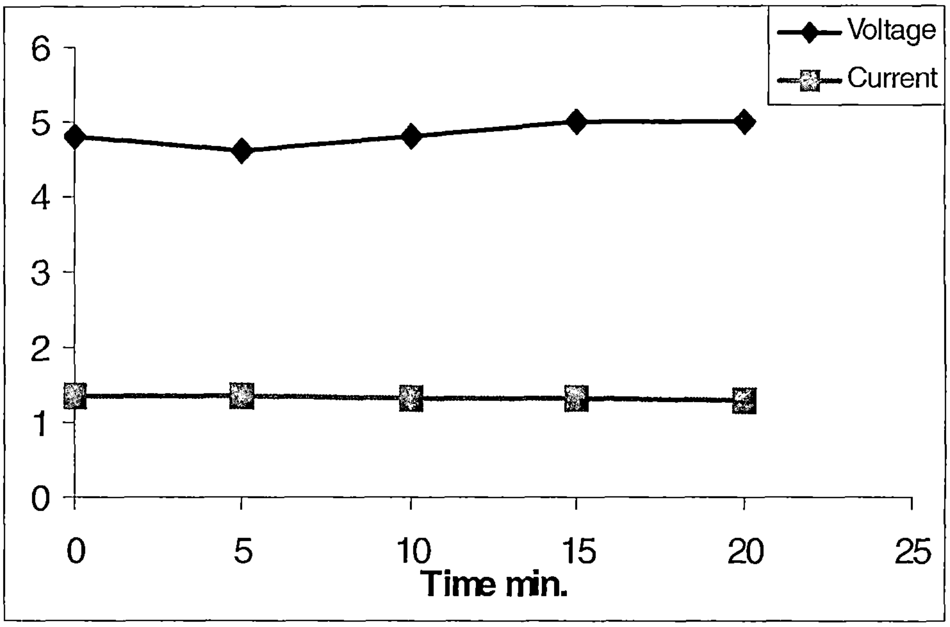

Fig. 5. 10. Voltage & current variation with time at pH 5

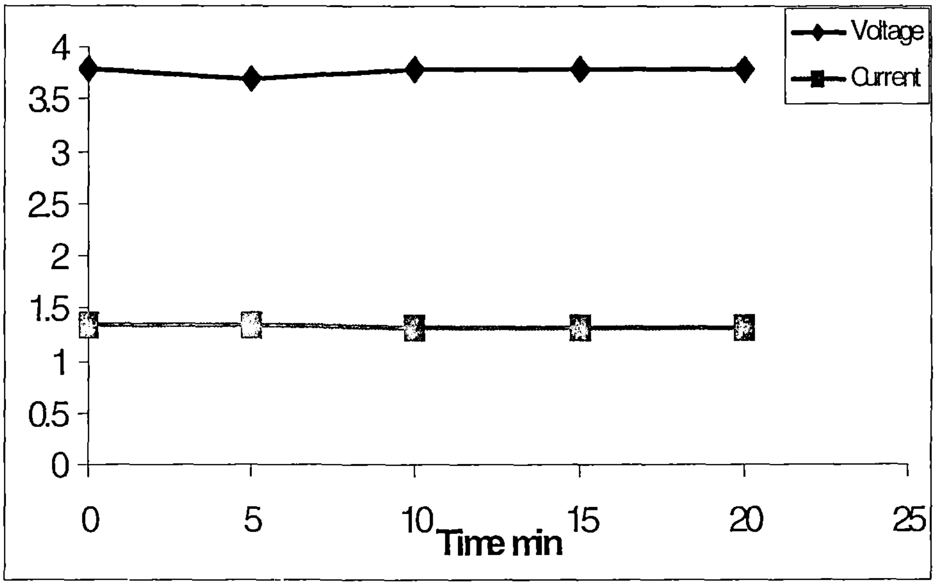

Fig. 5. 11. Voltage & current variation with time at pH 7

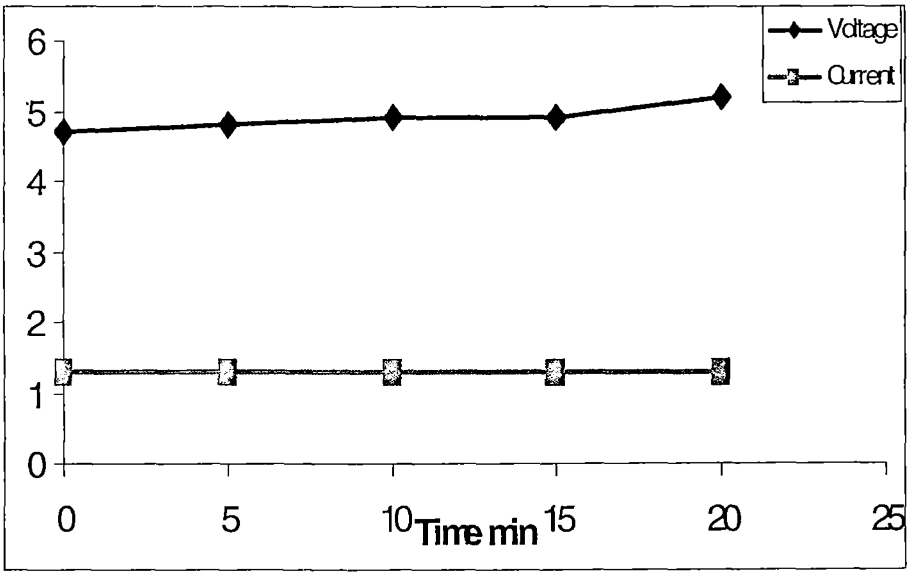

Fig. 5. 12. Voltage & current variation with time at pH 9

Table 5. 6. Effect of pH on pollutant removal during electrolysis

pH		Percentage Removal						
Before electrolysis	After electrolysis	Turbidity (NTU)	COD	BOD	TKN	AN	Sulphide	PO_4
3	4.9	100	65	50	31	20	100	89
5	6.8	100	70	79	30	20	85	81
7	7.6	100	62	59	28	20	40	96
9	8.7	100	55	48	29	20	29	85

When $FeSO_4$ was added to the anaerobically treated effluent the colour of the solution changed to black, due to the formation of iron sulphide, as anaerobically treated effluent contains sulphide. When pH changed from 3 to 9 the colour of the effluent changed from colourless to brown after electrolysis; the brown was due to the formation of ferric hydroxide in alkaline pH. From the figures (Fig.5.9 to 5.12) it is clear that current and cell voltage remained almost constant throughout the experiment. This demonstrates that the electrochemical cell constituents are unaffected during the entire period of electrolysis. It is an indication of the absence of adsorption, passivation and anode dissolution during electrolysis. Froth formation was observed as electrolysis progressed and more froth was on the cathode (graphite) side and the quantity of froth decreased as pH increased. Maximum percentage removal of COD and BOD were observed at pH 5, TKN and sulphide at pH 3 and phosphate at pH 7 (Table 5. 6). Removal of ammoniacal nitrogen was not affected by pH change. Complete removal of sulphide was possible at pH 3. As pH increases, the removal of sulphide decreases. Therefore pH around 5 can be taken as optimum pH for electrolysis in the presence of Fenton's reagent. In alkaline pH hydrated ferrous iron is transformed into colloidal ferric species which decompose H_2O_2 to oxygen and water[42].

5. 6. 9. Electrolysis using solar cell and its comparison with DC power supply

Electrolysis of raw as well as anaerobically treated effluent was carried out with aluminium electrode using solar energy and DC power supply at the optimum pH of 5 and compared their efficiency. It was observed that solar radiation was effective in treating RE and anaerobically treated effluent (ATE).

Table 5. 7. Comparison of the effect of solar energy and DC power supply

Type of Electrolysis	Percentage Removal					
	Turbidity	COD	BOD	TKN	AN	PO_4
RE+SE	75	29	87	12	13	68
RE + DC	99	21	78	20	16	71
RE +FR+SE	89	35	89	14	22	56
RE +FR+DC	99	27	83	20	24	74
ATE+SE	25	40	24	20	23	89
ATE+DC	78	46	20	22	17	65
ATE+F+SE	74	62	74	20	23	58
ATE+F+DC	99	67	54	23	20	94

(Abbreviations used: RE; Raw Effluent, SE: Solar Energy, ATE: Anaerobically Treated Effluent DC: Direct Current, FR: Fenton's Reagent).

From the Table 5. 7 it is clear that electrolysis of RE using solar energy removes more COD (29 %) and BOD (87 %) than the steady DC power supply. But during electrolysis of ATE using solar energy BOD removal efficiency of 24 % and COD removal efficiency of 40 % were observed compared 20 % BOD removal efficiency and 46 % COD removal efficiency achieved using DC power supply. When anaerobically treated effluent was electrolysed in the presence of Fenton's reagent, 62% and 67% of COD removal was observed in solar radiation and DC power supply respectively compared to 35% and 27% in the case of RE. In the anaerobic reaction organic molecules are degraded by hydrolysis, fermentation, and methanogenesis into simple monomers, acetate, hydrogen, methane and carbon dioxide[52], presumably facilitating the easy decomposition by electrolysis. Percentage removal of BOD was very high compared to COD and this effect was more pronounced in the case of RE. Low percentage removal of BOD in the case of ATE may be due to the removal of biologically degradable substrate by methanogenic bacteria into methane and carbon dioxide during anaerobic treatment. Addition of Fenton's reagent increased the removal efficiency of COD as well as BOD. Fenton's reagent plays an important role in the photolytic oxidation of organic pollutants. Photolysis of H_2O_2 produced hydroxyl radical in a pH rage of 3 to 5. Many metals have special oxygen transfer properties which improve the utility of hydrogen peroxide. The most common of these is iron which results in the generation of highly reactive hydroxyl radicals (.OH). Iron acts as catalyst and if the pH is too high, the iron precipitates as Fe $(OH)_3$ and catalytically decomposes the H_2O_2 to oxygen[42]. Presence of Fenton's reagent enhanced the COD and BOD removal efficiency in both types of effluents. Since there is a possibility of sulphide in the anaerobically treated effluent forming iron sulphide (visualized by the formation of black colour) and phosphates present in the skim serum chelating with iron, there is a need for supplemental aliquot of Fe which saturates the chelating properties in the effluent thereby availing unsequestered iron to catalyze the formation of hydroxyl radicals in Fenton's reagent. 200 mg/L of Fe and 2 mL/L of H_2O_2 was used to prepare Fenton's reagent. By electrolysis 56 to 94 percent of phosphate was removed since metal ions formed or added may chelate with the phosphate. Chlorides have been widely used in the treatment of different types of effluents as supporting electrolyte[22-24,27]. Chloride ion in the supporting electrolyte not only promotes current flow for direct oxidation but reacts to form oxidized chlorine species responsible for indirect oxidation of the pollutants. TKN and AN removal were in the range of 20 to 24 percent in the case of anaerobically treated effluent in both types of electrolysis. Ammonia was decomposed by electrolysis with the production of hydrogen gas[53]. The gases produced when passed through lime water turned milky, indicating the formation of CO_2. Formation of chlorine gas was detected by the formation of white precipitate when the gas was passed through silver nitrate solution.

Colloidal particles in the effluent were invisible and assumed to be hydrophilic, since the turbidity values are high with an average value of 350 NTU for RE and 120 NTU for ATE. 99 percent of turbidity was removed when raw and anaerobically treated effluent was electrolysed using DC current in the presence of Fenton's reagent. Electron flooding of the water eliminates the polar effect of the water complex, allowing colloidal materials to precipitate[57]. When solar radiation was used, percentage turbidity removal dropped to 89 and 74 in the case of RE and ATE respectively. The positively charged metal ions formed during electrolysis and hydroxyl free radicals produced by addition of H_2O_2 may neutralise the charge which tends to destabilize colloidal particles. It was observed that significant amount of floc generated settle at the bottom of the reactor causing electro coagulation and contributing to COD removal. Presence of sulphide in the ATE was quite high (235 & 45 mg/L), since H_2SO_4 was used as a coagulant for skim latex. After anodic oxidation H_2S was completely removed in the presence of Fenton's reagent.

5. 6. 10. Biochemical and microbiological analysis of the electrolysed Effluent

Electrolysis using solar and direct current was equally good in removing soluble protein, phenol and total sugar from raw effluent. Electrolysis was more effective in treating anaerobically treated effluent. Soluble protein and total sugar were completely removed from the anaerobically treated effluent when electrolysed using solar or DC power even in the absence of Fenton's reagent. Results of biochemical analysis of raw and treated effluent are given in Table 5. 8. Electrocoagulation can be successfully employed for the removal of protein and fat present in wastewater[56].

Comparison of biochemical constituents of anaerobically treated effluents having 10900 and 4508 mg/L COD (No. 7 & 13 in Table 5. 8) showed that as COD decreases, biochemical constituents also decrease. As time of electrolysis of ATE increases from 30 minutes to 45 minutes (No.14 & 15 in Table 5. 8), a reduction in the biochemical parameters were observed. Complete removal of free amino acids by electrolysis was not possible. This may be due to the generation of individual amino acids by the degradation of proteins.

Table 5.8. Biochemical analysis of raw and treated effluent

No	Sample details	Soluble protein	Phenol	Total sugar	Reducing sugar	Non reducing sugar	Free amino acid
1	RE	870	586	1250	1095	155	16120
2	RE+SE	305	195	335	264	71	15060
3	RE + DC	285	182	390	295	95	14724
4	RE +FR	351	335	639	450	189	20082
5	RE +FR+SE	48	153	285	195	90	14280
6	RE+FR+DC	65	165	270	156	114	16611
7	ATE(1,COD 10900)	425	434	107	83	24	7932
8	ATE+SE	nil	89	nil	nil	nil	7100
9	ATE +DC	nil	59	nil	nil	nil	6831
10	ATE+FR	114	224	42	25	17	6417
11	ATE+FR+SE	nil	20	nil	nil	nil	5980
12	ATE+ FR+DC	nil	25	nil	nil	nil	6157
13	ATE(2,COD 4508)	289	45	65	56	9	5779
14	ATE+FR+DC(30min)	17	17	nil	nil	nil	4322
15	ATE+FR+DC (45min)	nil	7	nil	nil	nil	4290

(Abbreviations used: RE; Raw effluent., SE: Solar energy, ATE: anaerobically treated effluent, DC: direct current, FR: Fenton's reagent. All values are expressed in mg/L)

The population of total bacteria present in raw, anaerobically treated and electrolysed effluent was enumerated using appropriate media and found that RE and ATE contains 25 x 10^{4} and 30x 10^{5} total bacteria respectively. After electrolysis using Fenton's reagent for 20 minutes no such colony forming units were observed which shows that electrolysis in the presence of Fenton's reagent could remove total bacterial population completely (Table 5. 9). The photographs of some of the cultured samples (1 to 8) are given in Fig 5.13. The same type of observation was reported by Mohanasundaram that electrocoagulation process destroys fecal coliform by 99.999 percent[56]. Without Fenton's reagent a little more time for electrolysis (45min) was needed to remove total bacterial count. The increase of electrons creates an osmotic pressure that ruptures bacteria, cysts and viruses and thus electrocute microorganism in the water[57].

Table 5.9. Results of microbiological analysis

No	Sample	cfu per mL	Serial No. of Photograph given in the figure 5.13
1	RE	25x 10^4	1
2	RE+EL	5 x 10	2
3	RE+FR	8 x 10^3	3
4	RE+FR+EL	nil	4
5	ATE	30 x 10^5	5
6	ATE+FR	4 x 10^2	6
7	ATE+EL 15'	3 x 10^2	
8	ATE+EL 20'	1 x 10^2	
9	ATE+EL 25'	23	7
10	ATE+EL 30'	3	
11	ATE+EL 45'	nil	
12	ATE+FR + EL 20'	nil	8
13	ATE+FR + EL 45'	nil	

(RE: raw effluent; EL: Electrolysis; FR: Fenton's reagent)

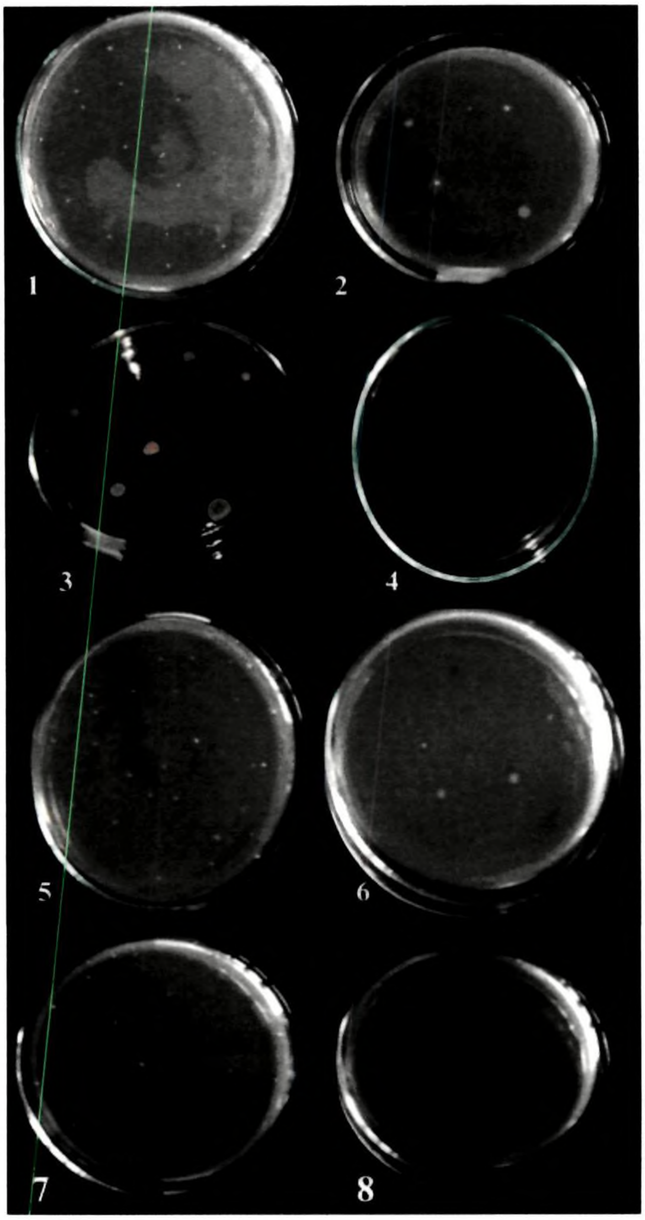

Fig. 5.13. Photographs showing the effect of electrolysis on the population of total bacteria

1-RE, 2-RE+EL, 3-RE+FR, 4-RE +FR+EL, 5-ATE, 6-ATE+FR, 7- ATE+ EL for 25min. 8-ATE+FR+EL (RE = raw effluent, EL = Electrolysed, FR = Fenton's Reagent, ATE = Anaerobically treated effluent)

5.7. Conclusion

Application of electrochemical oxidation to treat raw and anaerobically treated serum effluent was the focus of the study. It was found that electrochemical method could be used for pre-treatment as well as for post-treatment of this effluent, but the latter being more effective.

Various metal electrodes like aluminium, cast iron, steel, and mild steel were compared for their efficiency in removing COD, BOD,TKN, AN and phosphate and found that aluminium anode was more effective to remove pollutants compared to others. Role of Fenton's reagent in electrolysis was also studied and found that its addition during electrolysis was very effective in removing pollutants.

Electrochemical oxidation in the absence of supporting electrolyte does not cause much change in the pH of the solution, but by adding supporting electrolyte the pH increased from 3.8 to 5.65. Presence of supporting electrolyte increased the percentage removal of COD, BOD, TKN and AN.

The maximum removal of COD took place within 45 minutes and BOD within 30 minutes of electrolysis. After this, electrolysis did not show any significant effect on pollutant removal.

The ICE decreases with time during electrolysis and gradually reaches a constant value. Effect of pollutant concentration of effluent on electrochemical oxidation showed that the lower the COD of the effluent used for electrolysis, the higher the rate of removal. After electrochemical oxidation phosphate removal efficiency was 56 percent for raw effluent and it increased to 99.5 percent for the anaerobically treated effluent. Complete removal of sulphide was observed in all trials. It is concluded that electrochemical oxidation is very effective for the treatment of anaerobically treated effluent having low organic load.

Percentage removal of COD, BOD, TKN and AN were maximum for 200 mg/L of iron concentrations in Fenton's reagent. But maximum removal of sulphide and phosphate were for 300 mg/L of iron.

Electrochemical oxidation of ATE using aluminium electrode at various pH showed that H_2S was completely removed at pH 3. Highest percentage removal of COD and BOD could be attained at pH 5. Therefore, pH around 5 can be taken as optimum pH for electrolysis in the presence of Fenton's reagent.

The present work showed the potential of solar cell as an alternate source of power for the electrochemical treatment of effluent. Electrolysis was found to be more effective for anaerobically treated effluent.

Biochemical analysis revealed that electrolysis in the presence of Fenton's reagent is effective in removing soluble protein, phenol and sugars especially from anaerobically treated effluent. Microbiological analysis showed the complete removal of total bacteria by 20 minute electrolysis in the presence of Fenton's reagent. Thus it is concluded that anodic oxidation could be effectively used for the post-treatment of anaerobically treated effluents from latex centrifuging unit.

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CHAPTER 6

PURIFICATION BY ADSORPTION

6.1 Introduction

Adsorption is an effective purification and separation technology for wastewater treatment. Among the physico-chemical treatment processes adsorption is found to be highly effective, cheap and easy to adopt. Adsorption is the process by which a solid adsorbent can attach a component dissolved in wastewater via physical or chemical bonds, thus removing the component from the fluid[1]. When adsorption takes place, molecular species is accumulated more at the surface than in the bulk of a solid or liquid. The molecular species or substance, which concentrates or accumulates at the surface, is termed adsorbate and the material (solid, liquid or gas) on the surface of which adsorption takes place is called adsorbent. Adsorption is a surface phenomenon. It is the process of accumulating substances that are in solution on a suitable interface. During adsorption molecules adhere to the internal walls of the pores of adsorbent particles. It is a mass transfer phenomenon in which a constituent in the liquid phase is transferred to the solid phase. Mass transfer zone (MTZ) is defined as the zone where the adsorption process takes place. In the MTZ, the solute particles are transferred from the bulk of the solution onto the solid adsorbent. Adsorption is being extensively used for the treatment of wastewater containing organics, where better quality of treated wastewater, including toxicity reduction, is in demand. Adsorption is used to attach relatively small quantities of soluble organic and inorganic compounds such as nitrogen, sulphides and heavy metals remaining in the wastewater already subjected to biological or physico–chemical treatment. The efficacy of this method to reduce contaminants from water and to improve its taste, colour and odour has been known for a long time. The ancient Egyptians used charcoal lined vessels to store water for drinking purposes[2]. It is used in industry for product separation and waste treatment. The concentration of adsorbents in the effluent should be less than 1% when recovery is not needed; concentrations up to 5% are acceptable when recovery is essential.

The most widely accepted adsorbent in wastewater treatment is activated carbon[3-5]. The major benefits of carbon treatment include the removal of a wide variety of organics and inorganics, insensitivity to toxic materials and minimum land requirement[5]. The adsorption of phenol from aqueous solution onto carbon is widely known, and an exhaustive review has been published by Radovie et al[6]. Granular activated carbon[7,8], bituminous coals and coal based adsorbents[9-12], petroleum coke[13] are some of the adsorbents that have been used to remove pollutants. High cost and low rate of regeneration of activated carbon induced the search for other carbonaceous materials from active biomass. Farm waste is generated in ample quantities annually in our country and their use in the treatment of wastewater is not only economical but also environment friendly. These are lignocellulosic materials, which have an inherent ability to adsorb waste chemicals such as dyes, organics, metals and non-metals. The process of treatment is environment friendly and the materials are biodegradable. These materials include rice husk,[14-20] saw dust[21-24], palm seed coat carbon[25] and used tea leaves[26,27]. Carbonised coir pith has been examined for the removal of dyes[28], phenols and chlorophenols[29,30] and tobacco stems for the removal of chromium (VI)[31]. Metal adsorption from acidic synthetic solutions were carried out using oyster shells, cedar bark, vermiculite, coco shells and pea nut shells and found that they are effective natural adsorbents for the recovery of lead and chromium[32]. Coconut shell carbon and its nitrated and sulphonated forms were used for the adsorption of chromium (VI) [33].

Wastewater can be treated using natural adsorbents like quartz, bentonite and kaolinite for the adsorption of hexachlorobenzene[34]. Large number of minerals like red mud[35, 36] and montmorillonite[37] have been utilised as adsorbents for wastewater treatment. Srihari studied the adsorption capacity of three different types of untreated natural clays namely laterite, kaolinite and montmorillonite for the sugar industry effluents[38]. Adsorption of the pesticide dazomet and copper ion on bentonite and clinoptilolite[39,40], zinc on fuller's earth[41] heavy toxic metals on usar soil[42] and ammonium ion on zeolite (contains 50% clinoptilolite) deposit[43] are some of the adsorption studies carried out using natural clay type adsorbents.

6.2.1 Theory

Adsorption is a mechanism in which the forces of interaction between the surface atoms and the adsorbate molecules are similar to van der Waals forces that exist between adjacent molecules. There are both attractive forces and repulsive forces with the net force depending on the distance between the surface of the adsorbent and the adsorbate molecule. In general, adsorption is the process by which a component moves from one phase to another phase while crossing some boundary. Experiments by several scientists including Brunauer, Emmet and Teller, McBain and Langmuir[44] focused on the manner in which adsorbents removed adsorbates from both gases and liquids. The observed effect of adsorption was achieved within porous solids and that adsorption was the result of interactive forces of physical attraction between the surface of porous solids and component molecules being removed from the bulk phase.

Adsorption can be classified as either physical or chemical. Physical adsorption involves weak forces and is therefore reversible. Physical adsorption occurs at low temperatures and is very similar to condensation process and thus is exothermic with heat of adsorption similar to that of the latent heat of condensation. Chemical adsorption or chemisorption is important in gas phase catalysis, but is not generally relevant to liquid – solid adsorption at ordinary temperatures.

There are several factors that influence physical adsorption. The major factors which affect physical adsorption include the surface area of the adsorbent, pore structure of the adsorbent, surface chemistry of the adsorbent, nature of the adsorbate, pH of the solution and the presence of competing adsorbates. Due to these factors, physical adsorption is considered to be a complex phenomenon.

The ability of carbon to remove desired constituents such as COD, BOD, nitrogen, phosphate, colour and phenol, etc. is known as its adsorption capacity. The 'phenol number' states the carbon's ability to remove taste and odour of compounds. The capacity of carbon to adsorb low-molecular weight substances is given by its 'iodine number'. The carbon's ability to adsorb high-molecular-weight substances is given by its 'molasses number'[45].

6.2.2 Process of adsorption

The four different processes that occur in different phases of adsorption are (1) bulk solution transport, (2) film diffusion transport, (3) pore transport and (4) adsorption (or sorption)[46]. The process in bulk solution transport is the movement of the organic material to be adsorbed through the bulk liquid by advection and dispersion in carbon contactors to the boundary layer of fixed film of liquid surrounding the adsorbent. The process in film diffusion transport involves the transport by diffusion of the organic material through the stagnant liquid film to the entrance of the pores of the adsorbent. Pore transport is the process in which the transport of the material to be adsorbed through pores by a combination of molecular diffusion through the pore liquid and /or by diffusion along the surface of the adsorbent. Adsorption involves the attachment of the material to be adsorbed to the adsorbent at an available adsorption site[47]. Adsorption can occur on the outer surface of the adsorbent and in the macropores, mesopores, micropores, and submicropores, but the surface area of the macro and mesopores is small compared with the surface area of the micropores and submicropores and the amount of material adsorbed there is usually considered negligible. Adsorption forces include coulombic-unlike charges, point charge and a dipole, dipole–dipole interactions, point charge neutral species, London or van der Waals forces, covalent bonding with reaction and hydrogen bonding[48]. After initially contacting an adsorbent with a solution, an equilibrium will be reached such that the rates of solute adsorption and desorption are equal. General rules of adsorption are: - higher surface area will give greater adsorption, larger pore size will give greater adsorption capacity for large molecules, adsorptivity will increase as the solubility of the solute decrease, adsorption capacity will decrease with increase in temperature and for ionisable groups, maximum adsorption at a pH corresponding to minimum ionisation[2].

6.2.3 Adsorption isotherms

Adsorption is a function of both the characteristic and concentration of adsorbate and temperature. The amount of material adsorbed is determined as a function of the concentration at a constant temperature, and the resulting function is called an adsorption isotherm. Adsorption isotherms can be developed by exposing a given amount of adsorbate in a fixed volume of the liquid in varying amounts of activated carbon. The adsorbent phase concentration after equilibrium is computed using the equation

$$q_e = \frac{(C_0 - C_e)V}{m}$$

where q_e = adsorbent phase concentration after equilibrium, mg adsorbate/g adsorbent

C_0 = initial concentration of the adsorbate, mg/L

C_e = final equilibrium concentration of the adsorbate after adsorption has occurred, mg/L

V = volume of liquid in the reactor, L

m = mass of adsorbent, g

6.2.4 Freundlich isotherm

The basic ideas behind this equilibrium model are: it is a multilayer sorption and the sorption process does not obey Henry's law at lower concentrations. Freundlich isotherm is used to describe the adsorption characteristics of the activated carbon used in water and wastewater treatment. Derived empirically in1992, the Freundlich isotherm is defined as follows[46]:

$$x/m = K_f C_e^{1/n}$$

where x/m = mass of adsorbate adsorbed per unit mass of adsorbent, mg adsorbate /g activated carbon

K_f = Freundlich capacity factor, (mg adsorbate /g activated carbon) (L water/mg adsorbate) $^{1/n}$

C_e = equilibrium concentration of adsorbate in solution after adsorption, mg/L

1/n = Freundlich intensity parameter and the constants in the Freundlich isotherm can be determined by plotting log (x/m) versus log C_e and the above equation can be rewritten as

$$\text{Log}\,(x/m) = \log K_f + 1/n \log C_e$$

6.2.5 Langmuir isotherm

The basic ideas behind this equilibrium model are that it is a monolayer sorption and the adsorption process obeys Henry's law at lower initial concentration of adsorbate. At higher concentrations, it predicts a monolayer adsorption

Langmuir isotherm can be represented as [46]

$$x/m = \frac{abC_e}{1 + b\,C_e}$$

where x/m = mass of adsorbate adsorbed per unit mass of adsorbent, mg adsorbent/g activated carbon, a and b are empirical constants.

C_e = equilibrium concentration of adsorbate in solution after adsorption, mg/L

Assumptions in Langmuir isotherm are: a fixed number of accessible sites are available on the adsorbent surface, all of which have the same energy and adsorption is reversible. Equilibrium is reached when the rate of adsorption of molecules onto the surface is the same as the rate of desorption of molecules from the surface. The constants in the Langmuir isotherm can be determined by plotting $C_e/(x/m)$ versus C_e and the equation can be rewritten as

$$\frac{C_e}{(x/m)} = \frac{1}{ab} + \frac{1}{a} \times C_e$$

6.2.6 Types of adsorbents.

A wide variety of adsorbents are used for the purification and separation of organics and wastewaters. They include activated carbon, synthetic polymer, and silica based adsorbents, although the cost of the synthetic polymeric and silica based adsorbents are high. Activated carbon treatment of wastewater is usually thought of as a polishing process for wastewater that has already received normal biological treatment. The carbon in this case is used to remove a portion of the remaining organic matter. A suitable adsorbent has the following properties: - high affinity and high adsorption capacity for the adsorbate, safe and economically viable treatment, tolerance for a wide range of wastewater parameters, easy regeneration and should not produce secondary pollutants.

Activated carbon

The term activated carbon applies to any amorphous form of carbon that has been specially treated to give high adsorption capacities. Activated carbon is prepared by carbonisation of the organic material such as peat, wood, coconut shell, saw dust, rice husk, and lignite (a soft brown coal) by heating to red heat below 600^0C in a muffle furnace to drive off the hydrocarbons but with an insufficient supply of oxygen to sustain combustion. The carbonisation process is essentially a pyrolysis process. The char particle is then activated by exposure to oxidising gases such as steam and carbon dioxide at high temperature in the range from 800 to 900^0C. These gases develop a porous structure in the char and thus create a large internal surface area, 1400 square metres per gram which makes it an excellent absorbent. The resulting pore sizes are defined as follows[46]

Macropores > 25nm.
Mesopores > 1nm and < 25 nm
Micropores < 1nm.

Many variations in surface properties are possible depending on the type of material used and the preparation procedure. Most of the available surface area is non-polar in nature, but the interaction with oxygen (in production) does produce specific active sites which give the surface a slightly polar nature. The type of base material from which the activated carbon is derived may also affect the process distribution and the regeneration characteristics. After activation, carbon can be separated into or prepared in different sizes with different adsorption capacity. The two size classifications are powdered activated carbon (PAC), which typically has a diameter less than 0.074 mm (200 sieve), and granular activated carbon, (GAC) which has a diameter greater than 0.1mm (140 sieve)[46].

6.2.7 Carbon black

Carbon black is essentially elemental carbon and is composed of particles which are partially graphitic in structure. The carbon atoms in the particles are in layer planes, parallel alignment and overlapping, which give the particles their semi graphitic nature. The outer layers are more graphitic than those in the centre. It is produced by converting either liquid or gaseous hydrocarbons to elemental carbon and hydrogen by partial combustion or thermal decomposition. Depending on the process adopted for the preparation, carbon blacks are classified as furnace blacks, thermal blacks, channel blacks and lamp blacks. Furnace blacks are produced by incomplete combustion of natural gas or heavy aromatic residue oils from the petroleum industries. Thermal blacks are produced by the decomposition of natural gas or oil at 1300^0C in the absence of air. Channel blacks are produced by feeding the natural gas or oil into thousands of small burner tips where the small flames impinge onto a large rotating drum. Lamp blacks are made by burning oil and allowing the black formed to settle out by gravity in a series of chambers[49].

The carbon black particles are not discrete but are clusters of individual particles. Electron microscope is used to determine the particle size and it ranges from 10 nm to 400 nm in diameter, the smaller ones being less graphitic. Carbon particles are joined together to form long chains and tangled three dimensional aggregates. The oil furnace process using highly aromatic raw materials gives blacks of high structure. In a particle the carbon atoms are present in layer plane. Carbon blacks consist of 90-99 percent of elemental carbon, small amounts of hydrogen and oxygen and traces of sulphur depending on the hydrocarbon used in the manufacture. Due to the reaction of oxidising gases surface of the carbon black are not smooth. Oxidation takes place at the non-graphitic atoms and can progress into the particle to give pores. The types of carbon black selected for the present study and its particle size are given in the Table 6. 1.

Table 6. 1. Particle size of carbon black

No	Type of carbon black	Particle size (nm)
1	SAF (Super abrasion furnace black)	20-25
2	ISAF (Intermediate super abrasion furnace black)	24-33
3	HAF (High abrasion furnace black)	28-36
4	SRF (Semi reinforcing furnace black)	70-96

6.2.8 Clay-based adsorbents

Natural adsorbents like clay and nano clays like montmorillonite and bentonite (layered silicates), are used for the purification of wastewater through adsorption. Requirements needed to use this method of adsorption are; it should be active, stable, accessible, easy to regenerate and most important is that the exchange ions should be harmless and should not provoke secondary water pollution. The natural minerals respond to these requirements.

6.2.9 Structure and properties of layered silicates

The term layered silicates also known as phyllosilicates include natural clays and synthesised layered silicates such as mica and laponite. Montmorillonite (MMT) is the most abundant and widely used naturally occurring clay. It is the determinate components in bentonite. The colloidal state of the MMT particles in the bentonite dispersion is decisive in many practical applications. An outstanding property of dispersed MMT is delamination into single silicate layers or thin packets of them when the counter ions are alkali cations, and the salt concentration is sufficiently small[50]. It has an average size of 0.5μm and the particles are of irregular in shape. They can be compact but, mostly, they are foliated and look like paper sheets torn into smaller pieces[50] (**Fig.6.1**).

The frame work of layered silicates consists of a two dimensional layer of two fused SiO_4 tetrahedral sheets sandwiching and edge shared octahedral sheet of metal atoms, such as Al or Mg. The neighbouring layers are separated by a van der Waals gap, called a gallery or interstatum. These galleries are usually occupied by cations that counterbalance the excess of negative charges generated by the isomorphous substitutions of Si^{4+} for Al^{3+} in the tetrahedral lattice and Al^{3+} for Mg^{2+} in the octahedral sheet. Due to the partial positive charge formed within the gallery, layered silicates are highly hydrophilic. The model structure of layered silicates proposed by Hoffmann *et al*[51] is presented in Fig.6.2. The thickness of single layer of silicates is 0.96 nm. The nano regime is normally considered to encompass the range between 1 nm and 100 nm- the region intermediate between micron scale and molecular scale.

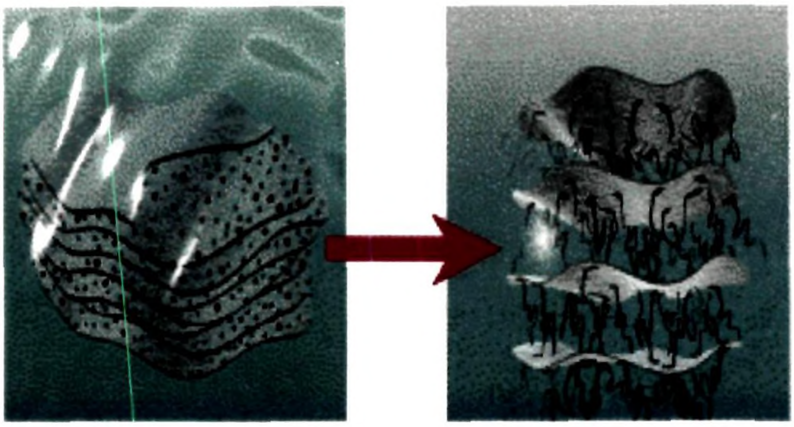

Fig.6.1. Delamination of layered silicates in water

G. Lagaly, S. Ziesmer, Adv. Colloi. Interf. Sci, **100**, 105, 2003[50].)

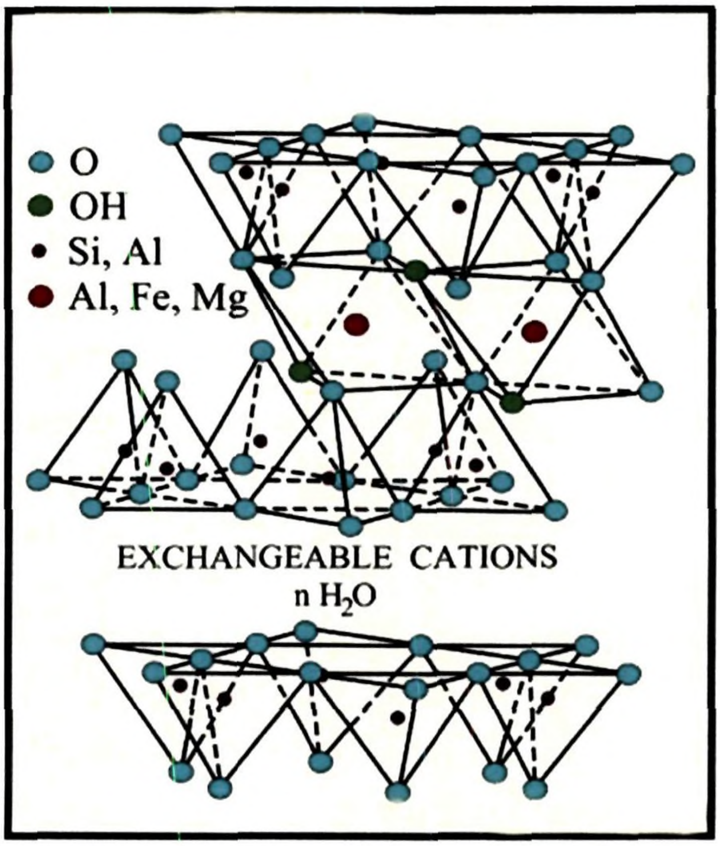

Fig.6.2. Structure of montmorillonite.

U. Hoffmann, K., Endell, D. Wilm , Krist, **86**, 340(1933 [51].)

6.3. Aim

This study was to assess the feasibility and efficiency of different adsorbents and to select a best adsorbent to purify anaerobically and electrolytically treated skim serum effluent. Adsorbents like activated carbon prepared from different natural sources and silica based adsorbents including nanoclays were used. The efficiency of treatment was evaluated in terms of the removal of COD, BOD, TKN, AN, and phosphate along with the biochemical and microbiological parameters. Since the present study concentrates mainly on the efficiency of different adsorbents to remove pollutants, not much attention was given to the kinetic and thermodynamic aspects of the adsorption system.

6.4. Experimental

Skim serum effluent after anaerobic treatment and electrolytic oxidation was used for adsorption studies.

6.4. 1. Raw materials: - activated carbon (AC)

Commercially available activated carbon was shaken with anaerobically and elctrolytically treated effluent in a rotary shaking machine at room temperature.

6.4. 2. Rice husk (RH)

Atmospheric dried and fresh golden brown rice husk was collected from a rice mill in central Kerala and used as raw material to produce activated carbon. The rice husk was subjected to pyrolysis at 205^0C and 550^0C in a muffle furnace. The activation agents used were sodium hydroxide and ortho phosphoric acid (Merck).

6.4. 2. (a). Activation with sodium hydroxide

20 g of dried rice husk was treated with 200 mL of 10% sodium hydroxide at 100^0C for one hour. Sodium hydroxide removed the silica present in the rice husk. It was then filtered and washed with distilled water to remove excess of sodium hydroxide. Washings were continued till the filtrate gave neutral pH. The treated husk was then dried in an air oven at 100^0C for 1hour. Half of it was subjected to pyrolysis at 205^0C in a muffle furnace and sieved through 200 mesh size and coded as NRH.

6.4. 2. (b). Activation with phosphoric acid

20 g of dried rice husk was treated with 100 mL of 40% phosphoric acid and kept overnight. It was filtered and washed with distilled water and dried over night in hot air oven at 100^0C and half of it was subjected to pyrolysis at 205^0C in a muffle furnace and sieved through 200 mesh size and coded as PRH.

6.4. 3. Coconut shell (CS)

Coconut shell which is available in plenty in Kerala was dried in the sun. The outside and inside of the shell was cleaned by removing the fibres. It was broken into small pieces and kept in a muffle furnace for 550^0C, ground and sieved to 170-200 mesh size. The powdered carbon was treated overnight with 0.75 N sulphuric acid. The carbon was washed repeatedly till the washings give a pH 7. It was then dried overnight in hot air oven at 100^0C and coded as CS

6.4. 4. (a). Saw dust (SD)

Saw dust was collected from a saw mill and dried. One part of it was subjected to pyrolysis in a muffle furnace at 550^0C and another part for

205^0C for 3 hours. One part of saw dust after pyrolysis at 205^0C was treated with concentrated sulphuric acid, filtered, washed with distilled water to remove sulphuric acid and finally dried in hot air oven at 100^0C. It was ground and sieved to 170-200 mesh size, and coded as SD.

6.4. 4. (b). Teak wood saw dust (TSD)

Saw dust from teak wood alone was collected and soaked in 20% sodium hydroxide and autoclaved for 3 hours. This converts fibres to micro fibres. Reagents for treating saw dust was prepared as follows. Solution (a): - 28 g of sodium hydroxide was dissolved in 75 mL of glacial acetic acid and diluted to 1 litre. Solution (b):- Sodium hypochlorite and water were mixed in the ratio 1:3 by volume. Equal volumes of solution (a) and (b) were mixed and teak wood saw dust was soaked in this reagent till the reagent covers the whole saw dust, heated to 80 ^0C till decolouration was observed, washed and dried. This treatment removes all other components leaving behind only cellulose. It was kept in muffle furnace for 3 hours at 205 ^0C, ground and sieved to 170-200 mesh size and coded as TSD

6.4. 5. Furnace carbon black

Furnace carbon blacks namely SAF, ISAF, HAF, SRF were collected from Rubber Research Institute of India, Kottayam, Kerala. These were of rubber compounding grade.

6.4. 6. Clay-based adsorbents

6.4. 6. (a). Clay

English Indian clay (commercial clay, amorphous). Grade and supplier- English Indian Clays, Thiruvnanthapuram, India.

The composition of clay is 45% SiO_2, 38% Al_2O_3, 0.5% Fe_2O_3, 0.55% TiO_2, 0.06% CaO, 0.07% MgO, 0.25% Na_2O, 0.1% K_2O, loss on ignition 14.5%.

6.4. 6. (b). Nano clay

(1) Bentonite (layered silicate)

Bentonite (EXM 757) is purified natural clay obtained from Sud Chemie, Germany. The characteristics of bentonite are: Trade name- EXM 757, chemical name- Na-bentonite, chemical formula-$(Al_{3.2}Mg_{0.8})\ Si_8O_{20}(OH)_4\ Na_{0.8}$, ion exchange capacity- 80 mequiv/100 g, layer distance- 1.24 nm

(2) Montmorillonite (layered silicate)

MMT- ODA. Grade and supplier- Nanomer 1.30P, Nanocor Inc, USA. Characteristics: Octadecylamine (ODA) modified montmorillonite, specific gravity 1.9 g/cm^3, particle size 16-20μm, inter layer distance 2.10 nm

(3) Montmorillonite (layered silicate) MMT-TMDA. Grade and supplier- Cloisite 30B, Southern Clay Products, USA. Characteristics: Methyltallow bis-2-hydroxyethyl quaternary ammonium (TMDA), modified montmorillonite, specific gravity 1.5-1.7 g/cm^3, interlayer distance 1.85nm

6.4. 7. Batch studies

Batch adsorption studies were carried out by shaking 50 mL of the anaerobically and electrolytically treated effluent samples with appropriate concentration of the adsorbent in 100 mL glass stoppered conical flasks using a temperature controlled heavy rotary shaking machine (Heavy Rotary Shaker, Kemi, model No.KRS-1). Adsorption tests were performed at room temperature. The progress of adsorption during the experiments was determined by removing the flasks from the shaking machine after the desired contact time, filtering and analysing the extent of treatment in terms of pH, COD, BOD, TKN, AN, and phosphate by using standard methods[52]. Different proportions of pre weighed adsorbents were also shaken with 50 mL of effluent samples and analysed to study the effect of adsorbent's concentration.

6.5. Results and discussion

6.5. 1. Adsorption using activated carbon (AC)

Adsorption experiments were conducted in batch mode with 50 mL of the anaerobically and electrolytically pre-treated effluent, having different concentrations with 1g of AC adsorbent for 30 minute in separate flasks at pH 8.

(a) Effect of pollutant concentration on AC adsorption

Fig.6. 3 shows the removal efficiency of COD, BOD and TKN with respect to the concentration of pollutants in the effluent when adsorbed on activated carbon. The percentage removal of BOD and TKN gradually decreased when the initial COD of the effluent used for adsorption increased. Percentage removal of COD remained almost constant when the initial COD of the effluent used for adsorption increased from 816 to 1449 mg/L (Fig.6. 3). But a sudden drop in the percentage removal of COD, BOD and TKN was observed for the initial COD change from 1449 to 1613 mg/L. From the figure it is clear that the lower the initial COD of the effluent used for adsorption, the higher the removal of pollutants. This is in agreement with the observations reported in literature[1].

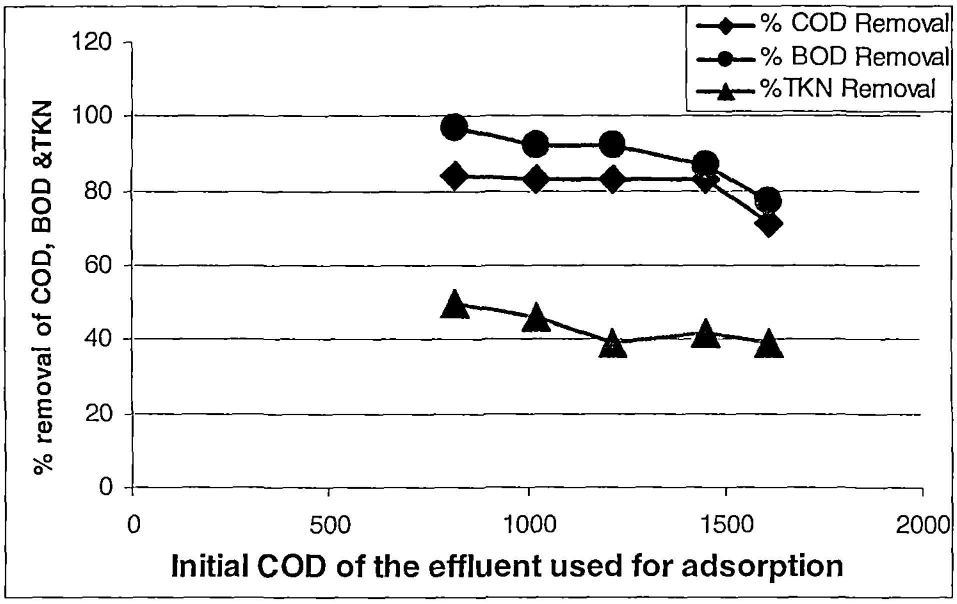

Fig.6. 3. Effect of initial COD of the effluent on the removal of COD, BOD & TKN by activated carbon adsorption

(b) Effect of pre-treatment of the effluent on AC adsorption

Another observation found in adsorption by activated carbon was that the effluent with high degree of pre-treatment by anaerobic and electrolytic method was more effectively purified by adsorption than with low level of pre-treatment.

Table 6. 2. Effect of pre-treatment on AC adsorption

	COD before adsorption	COD after adsorption	% removal of COD
Sample-1 (without dilution)	1185	180	83
Sample-2 (diluted)	366	224	39

Sample-1 in the Table 6. 2 is highly pre-treated by anaerobic (HRT 18 days) and electrolytic method (without dilution). Sample-2 has low percentage of pre-treatment by anaerobic (HRT 9 days) and electrolytic method (COD of 2481mg/L), but diluted to give a low COD. Though sample-2 had a low COD by dilution, adsorption was not so effective on activated carbon compared to sample-1. This observation showed that it is not the COD, but the efficiency of pre-treatment before adsorption that influenced adsorption. By anaerobic treatment highly polymeric organic molecules were converted to its monomers through hydrolysis, fermentation and methanogenesis[46]. Therefore, it can be concluded that small organic molecules are more susceptible to adsorption than large polymers and the size of the pore on activated carbon may be more suitable for the small molecules.

(c) Freundlich adsorption isotherm

The adsorption data were analysed at different initial concentration of effluent in the light of Freundlich equation

$$\text{Log}\,(x/m) = \log K_f + 1/n \log C_e$$

were K_f and 1/n are Freundlich constants related to adsorption capacity and adsorption intensity respectively (Fig.6. 4.).

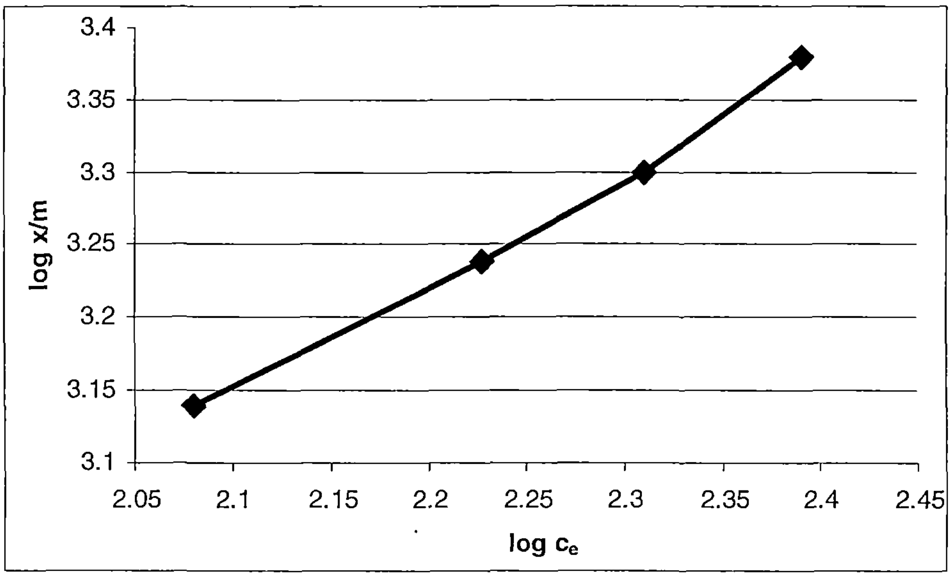

Fig.6. 4. Freundlich adsorption isotherm

The linear plots between log (x/m) and log C_e confirms the applicability of the model.

(d) Langmuir adsorption isotherm

Adsorption data were analysed in the light of Langmuir adsorption model. The linear plots of 1/x/m versus $1/C_e$ suggest the applicability of Langmuir adsorption model which is in the form

$$\frac{C_e}{(x/m)} = \frac{1}{ab} + \frac{1}{a} \times C_e$$

$$\frac{1}{x/m} = \frac{1}{a} + \frac{1}{ab} \times \frac{1}{C_e}$$

where C_e is the equilibrium concentration (mg/L) and x/m is the amount of COD adsorbed at equilibrium (mg/g) a and b are Langmuir constants related to adsorption capacity and equilibrium constant respectively. The linear plots of 1/x/m versus 1/ C_e clearly indicate monolayer coverage of adsorbate at the outer surface of the adsorbent (Fig.6. 5).

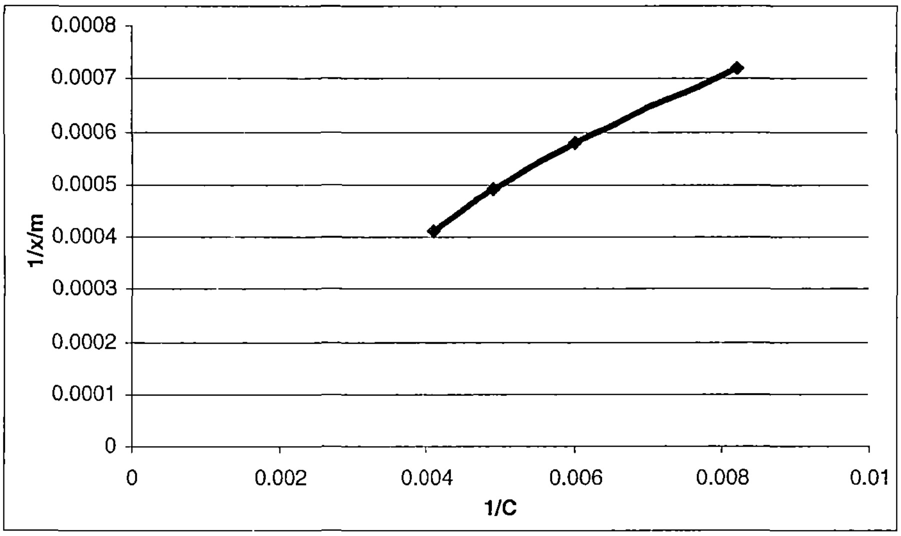

Fig.6. 5. Langmuir adsorption isotherm

6.5. 2. Rice husk (RH)

1 g of rice husk (different forms) was shaken with 50 mL of anaerobically and electrolytically pre-treated effluent in separate flasks at pH 8.1 and analysed for its efficiency to remove COD and BOD and found that rice husk muffled at 205^{0}C is a better adsorbent than rice husk muffled at 550^{0}C (Table 6. 3). Rice husk was activated with NaOH and H_3PO_4 and muffled at 205^{0}C. Batch adsorption studies were conducted with the activated form and muffled form of the rice husk. 29 percent COD and 75 percent BOD were removed by rice husk treated by NaOH and muffled whereas H_3PO_4 treated and muffled form removes only 21 percent COD and 69 percent BOD. Activation with sodium hydroxide along with carbonisation made it more efficient to treat the effluent. 10 percent NaOH can dissolve the silica present in the rice husk which provides a porous

structure for it[15]. Organic molecules can occupy these pores, resulting in greater efficiency of sodium hydroxide activated and carbonised rice husk.

Table 6. 3. Different forms of treated rice husk as adsorbents

No	Type of Treatment	% COD removal	% BOD removal
1	Rice Husk muffled at 550^{0}C	16	25
2	Rice Husk muffled at 205^{0}C	20	72
3	Rice Husk + NaOH (not muffled)	19	69
4	Rice Husk + H_3PO_4 (not muffled)	14	32
5	Rice Husk +NaOH muffled at 205^{0}C	29	75
6	Rice Husk + H_3PO_4 muffled at 205^{0}C	21	69

6.5. 3. Coconut shell (CS)

(a) Effect of pH

50 mL of the pre-treated effluent having COD 346 mg/L, BOD 90 mg/L, TKN 301 mg/L and AN 238 mg/L was shaken with 1 g of coconut shell carbon at pH 3, 5, 7, 9 for 30 minutes, filtered and analysed for their efficiency in removing COD, BOD, TKN and AN. Fig.6.6. shows the percentage removal of COD, BOD, TKN and AN as a function of pH. It is evident from the figure that maximum removal of COD and BOD were 30 and 78 respectively at pH 7 whereas maximum removal of TKN and AN were 21 percent and 30 percent respectively at pH 9.

It was observed that adsorption on CS is highly dependent on pH of the effluent, since pH influences the surface charges of CS as well as the degree of ionisation[53].

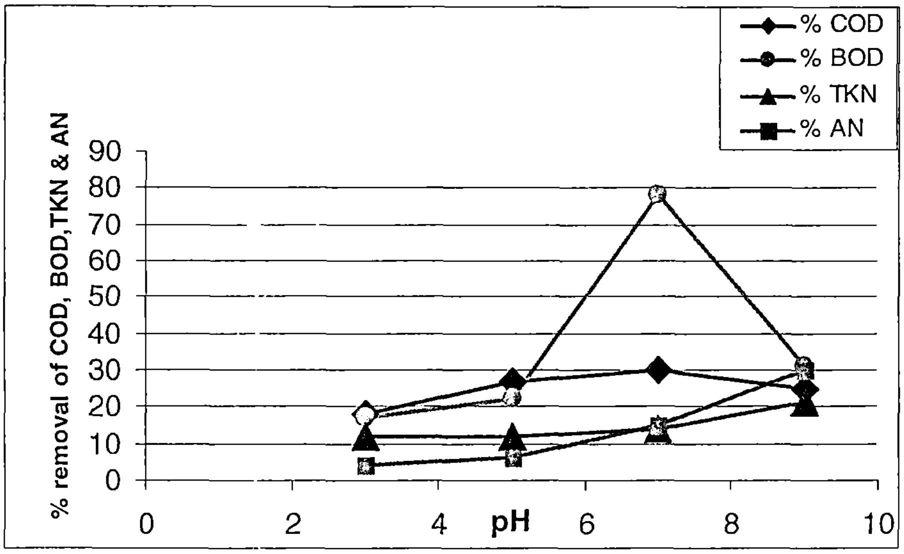

Fig.6. 6. Effect of pH on adsorption by coconut shell on the removal of COD, BOD, TKN & AN

(b) Effect of adsorbent (CS) concentration

50 mL of pre-treated effluent was shaken with different concentrations of CS for 45 minutes, filtered and analysed for various parameters. The results of treatment are given in the Table 6. 4.

Table 6. 4. Effect of adsorbent concentration (CS)

No	Concentration of CS g/L	Percentage removal			
		COD	BOD	TKN	AN
1	5	31	12	5	7
2	10	38	18	8	10
3	20	41	23	12	14
4	30	56	28	17	15
5	40	61	35	20	21

From the table it is evident that as the concentration of adsorbent increases, percentage removal of COD, BOD, TKN and AN also increases. This may be due to the availability of high surface area to adsorb pollutants in the effluent.

6.5. 4. Saw dust carbon (SD) and Teak wood saw dust carbon (TSD)

Adsorption experiments were conducted in batch mode with 50 mL of the anaerobically and electrolytically pre-treated effluent having pH 8 with 1g of adsorbent for 30 minutes. It was then filtered and analysed for its efficiency of treatment. The results are given in the Table 6. 5. From the table it is clear that SD pyrolysed at 205^{0}C is more efficient to treat this effluent than SD pyrolysed at 550^{0}C. Sulphuric acid treated SD is more efficient to adsorb organics since percentage removal of COD and BOD are 19 and 45 respectively (Table 6. 5.). But there is no change in the concentration of TKN and AN, though the removal efficiency of these were less in SD at 550^{0}C and 205^{0}C.

Table 6. 5. Effect adsorption on saw dust

No	Type of adsorbent	Percentage Removal			
		COD	BOD	TKN	AN
1	Saw dust (SD) 550^{0}C	11	14	3	5
2	Saw dust (SD) 205^{0}C	15	18	5	8
3	SD 205^{0}C +H_2SO_4 (SD)	19	45	0	0
4	Teak SD 205^{0}C (TSD)	22	80	54	31

Saw dust of teak wood was treated as described and pyrolysed at 205^{0}C and adsorption experiments were conducted with 50 mL of the anaerobically and electrolytically pre-treated effluent having pH 8 with 1g of adsorbent for 30 minutes. The results showed that percentage removal of COD, BOD, TKN and AN were 22, 80, 54 and 31 respectively. BOD, TKN and AN removals were much higher than that of other SD carbons. The higher efficiency of this treatment can be attributed to the removal of organic and inorganic components during the process of treatment, leaving behind micro fibrous particles having highly porous surface enhancing the process of adsorption.

6.5. 5. Furnace carbon black

(a) Comparison of different types of carbon black

50 mL of anaerobically and electrolytically pre-treated effluent having pH 8.15 was adsorbed on 1 gm of furnace carbon black using batch mode adsorption for 30 minutes. It was filtered and the pH of the resulting solution was measured and the results obtained are given in the Table 6.6. Adsorption on SAF, ISAF, HAF and SRF reduced the pH from 8.15 to 5.50, 6.15, 6.30 and 7 respectively.

Table 6. 6. Comparison of the efficacy of different types of carbon black

No	Type	pH before adsorption	pH after adsorption	Percentage removal				
				COD	BOD	PO4	TKN	AN
1	SAF	8.15	5.50	59	85	100	26	55
2	ISAF	8.15	6.15	48	63	100	10	14
3	HAF	8.15	6.30	44	80	100	57	65
4	SRF	8.15	7.00	50	84	100	24	39

When SAF was applied the COD and BOD removal were 59% and 85% respectively. This was the maximum removal achieved during adsorption using furnace carbon black. Due to the small size and large surface area, SAF could adsorb large amount of pollutants and this resulted in the high rate of removal of pollutants. Adsorption on ISAF removed 48 % COD and 63 % BOD, on HAF 44% COD and 80 % BOD, and on SRF 50 % COD and 84 % BOD when used as adsorbents. Maximum TKN (57%) and AN(65%) were removed when HAF was used as adsorbent. The pore size was appropriate to accommodate nitrogenous compounds and inorganic ammonium ions. All these adsorbents could remove the dirty white colour of the solution and gave very clear solution after adsorption. Adsorbents settled down at the bottom of the flasks after adsorption except for SAF. In SAF treated solution not much sedimentation was observed, instead it became fluffy, but filtration gave very clear solution.

(b) Effect of pH

Table 6. 7. Influence of pH on adsorption on ISAF

No	pH	Percentage removal			
		COD	BOD	TKN	AN
1	3	54	63	12	11
2	5	50	37	10	12
3	7	45	60	10	14
4	9	19	9	35	28

To study the effect of pH on furnace carbon black, 50 mL of the effluent at pH 3, 5, 7 and 9 were shaken with 0.5 gm of ISAF in separate flasks for 30 minutes, filtered and analysed. At pH 3, 54 % COD and 63 % BOD were removed (Table 6. 7) and this was the highest percentage of removal observed during this treatment. But maximum efficiency for the percentage removal of TKN and AN were 35 and 28 respectively at pH 9. High rate of removal of ammonia at higher pH, is inferred to the decomposition of ammonium ion to ammonia.

6.5. 6.1. Nano clays as adsorbents

(a) Influence of pH on montmorillonite

Table 6.8 shows the effect of pH onto the adsorption of montmorillonite. pH plays an important role in adsorption. After adsorption at pH 9 and 11, it dropped to 8.20 and 10.1. But for pH 3, 5 and 7 the post-treatment values increased to 3.15, 5.20, and 7.45 respectively. There was consistent increase in percentage reduction of COD, BOD, TKN and AN corresponding with the increase in pH up to 9 and thereafter the values stagnated. Percentage removal of COD, BOD, TKN and AN at pH 9 were 45, 19, 45 and 30. The physico-chemical characteristics of the adsorbent played an important role in the adsorption. The clays are aluminosilicates, having layered structure and bears charge on the surface and edges and stable bonds might be formed at alkaline pH.

Table 6. 8. Influence of pH on montmorillonite

No	pH before treatment	pH after treatment	Percentage removal			
			COD	BOD	TKN	AN
1	3	3.15	5	5	12	9
2	5	5.20	20	9	16	15
3	7	7.45	23	12	19	20
4	9	8.20	45	19	45	30
5	11	10.1	44	19	45	31

(b) Adsorption on montmorillonite as a function of time

Adsorption studies were carried out with 2g of adsorbent with 100 mL of pre-treated effluent having COD 983 mg/L in batch adsorption method. Fig.6.7 shows that a slight variation of pH from 8 to 7.5 as time of adsorption increased to 240 minutes due to the exchange of ions in solution with the adsorbent. Rate of removal of COD, TKN and AN increased as time of contact increased and reached a steady value after attaining the equilibrium in 180 minutes. But BOD reached a steady value after 210 minutes. The increase in removal efficiency with contact time was due to the transport of the material to be adsorbed through layers and hence availability of more active sites for adsorption.

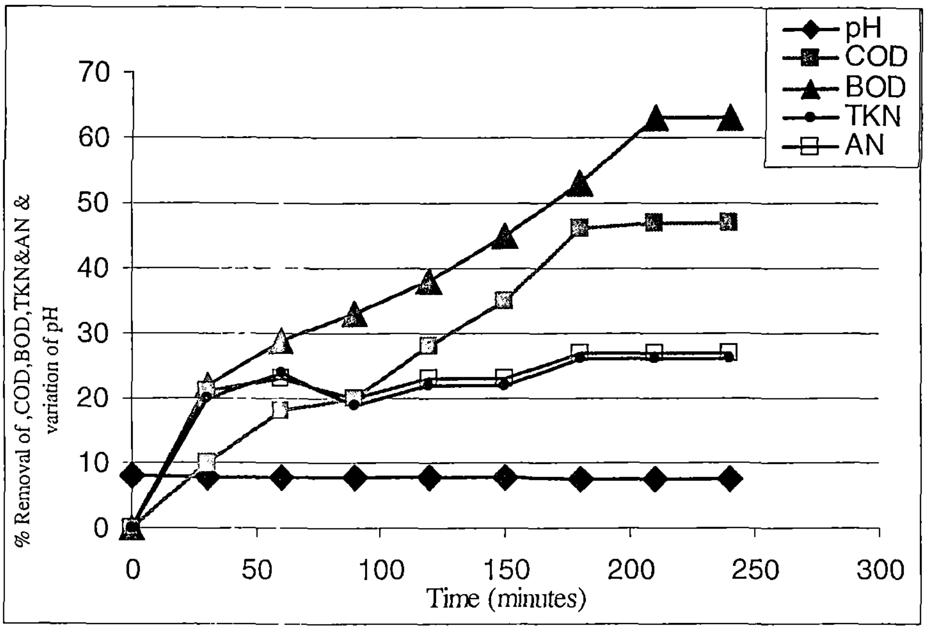

Fig.6. 7. Adsorption on montmorillonite as a function of time

(c) Effect of adsorbent dosage (montmorillonite)

Anaerobically and electrolytically pre-treated effluent having COD 369 mg/L was adsorbed on montmorillonite varying the dosage level from 5 g/L to 40 g/L. Fig.6. 8 shows the effect of dosage on the percentage removal of COD, BOD, TKN and AN. As the adsorbent dosage increased percentage removal of all parameters increased and became almost constant at 30 to 40 g/L. The increase in percentage reduction with increase in dosage is attributable to the surface area availability[20].

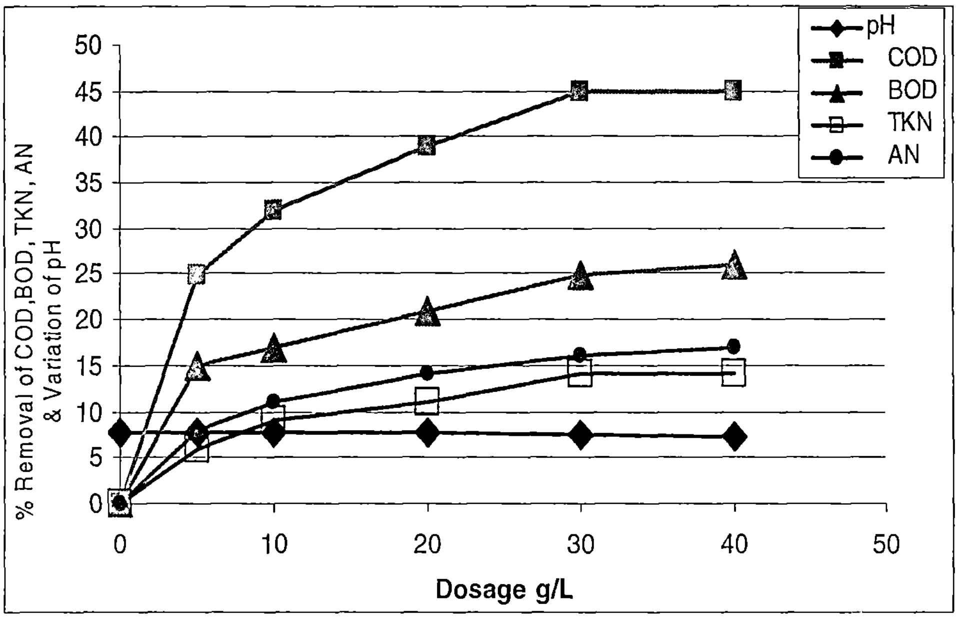

Fig.6. 8. Effect of adsorbent dosage (montmorillonite)

6.5. 6.2. Comparison of clays and nano clays

Clay (amorphous) was compared with modified nano clays montmorillonite (MMT–TMDA), montmorillonite (MMT-ODA) and bentonite (an unmodified natural layered silicate clay) with respect their efficiency in treating the pre-treated effluent. 50 mL of the effluent was shaken with 1gm of the adsorbent for 30 minutes. The efficiency of treatment was compared in terms of the percentage removal of COD, BOD, TKN and AN (Table 6. 9). pH of the effluent decreased from its original value after adsorption. Efficiency of treatment was in the order of clay < MMT-TMDA< MMT- ODA < bentonite. MMT-ODA and bentonite are of the same magnitude of treatment except in the removal of BOD and AN. But in the case of bentonite, though the removal efficiency was good compared to the other nano clays, it swells and was difficult to filter when treated with the effluent. Though MMT-ODA was difficult to wet, pollutant removal efficiency was better than MMT-TMDA. This is due to the difference in modifications adopted to expand the inter layer distance[49].

Table 6. 9. Comparison of clays and nano clays

Type of nano clay	pH before treatment	pH after treatment	Percentage removal			
			COD	BOD	TKN	AN
Clay	7.90	7.9	7	25	6	12
MMT -TMDA	7.90	7.8	25	20	18	23
MMT- ODA	7.90	7.5	29	35	23	43
Bentonite	7.90	7.6	29	40	23	50

Adsorption using nano clays to purify the effluent depends largely on the type of effluent used. Highly pre-treated effluents by anaerobic and electrolytic method gave better result. Though the probabilities of leaching of the modifiers were negligible a further careful analysis was needed to confirm that secondary pollutants were not produced during the process of treatment. Srihari[38] suggested the potential use of clay-blend and modified clays with pre-determined adsorption capacity for wastewater treatment. Environmental impact of using nano clays in wastewater treatment is another aspect that needs further research. High cost of nano clays was an impediment to use it as an adsorbent to treat wastewater, but in future, development of new technologies to produce modified and eco-friendly nano clays would help to tackle the problem and provide a very clean technology to treat wastewater.

6.5. 7. Comparison of different types of adsorbents

Adsorption on activated carbon purchased and prepared from different sources, clays and nano clays were compared for their efficiency to purify the biologically and electrolytically pre-treated effluent. The results of analysis are given in the Table 6. 10.

Table 6. 10. Comparison of different types of adsorbents

No	Type of adsorbent	Percentage removal			
		COD	BOD	TKN	AN
1	Activated carbon (commercial)	85	97	50	33
2	Furnace carbon black	59	85	26	55
3	Coconut shell carbon	41	78	14	15
4	Nano clay	29	40	23	50
5	Rice husk carbon	29	75	10	11
6	Teak wood saw dust carbon	22	80	54	31
7	Saw dust carbon	19	45	0	0
8	Clay	7	25	6	12

From the table it is clear that commercially available activated carbon is the best for treating biologically and electrolytically pre-treated effluent from rubber processing factories. It removed 85 percent of COD, 97 percent of BOD, 50 percent of TKN and 33 percent of AN. High removal efficiency of these parameters is attributed to the high surface area and large pore volume of activated carbon. The second best adsorbent was furnace carbon black which removed 59 percent of COD. Coconut shell stood the next with 41% COD removal. Treated teak wood carbon could remove TKN (54%) and AN (31%). Removal of total nitrogen and ammoniacal nitrogen were low compared to organic pollutant removal for most of the adsorbents.

Using nano clays as an adsorbent is a new approach and found that it is good in removing COD and BOD and ammoniacal nitrogen. Since nano clays are layered silicates, pollutants like ammonia, amino acids and phenols can be easily entrapped in their layers and thus increases their removal efficiency. Treated rice husk carbon removed 29% COD and 75% BOD and it can be suggested for adsorption since the raw material husk is cheap and easily available in Kerala. Saw dust and clay are poor adsorbents for this type of wastewater, since the percentage removal of pollutants was comparatively low.

6.5. 8. Estimation of metal ions

After adsorption on activated carbon the concentration of metals were estimated using AAS. And the results are given in Table 6. 11. Copper was completely removed after treatment. 97.6 percent of magnesium and 95.8 percent of calcium were removed after electrolysis and purification by adsorption. Percentage removal of iron and zinc were 75.77 and 2.17 respectively. An increase in the concentration of manganese was due to the impurities associated with the electrode used for electrolysis. Though Fenton's reagent (Fe^{+2} and H_2O_2) was added during electrolysis, corresponding increase in the concentration of iron was not observed after treatment. Iron precipitated along with the impurities present in effluent. The result shows that anaerobic treatment supplemented by electrolysis and adsorption on activated carbon removes most of the metal ions present in the effluent.

Table 6. 11. Effect of treatment on metal ion concentration

Element	Lamp current mA	Wavelength nm	After ATE ppm	After EL & Adsorption ppm	% Removal from ATE
Fe	7.0	248.3	1.725	0.418	75.77
Cu	3.0	324.7	0.014	nil	100
Zn	8.0	213.9	8.75	8.56	2.17
Mn	8.0	279.5	0.198	9.223	increased
Ca	5.0	422.7	8719	364	95.8
Mg	4.0	285.2	1310	31.5	97.6

(ATE: Anaerobically treated effluent., EL: Electrolytic treatment.)

6.5. 9. Biochemical and microbiological analysis

Table 6. 12. Results of biochemical analysis

Sample details	Soluble protein	Phenol	Total sugar	Reducing sugar	Non reducing sugar	Free amino acid
Adsorbed on AC	nil	6	nil	nil	nil	4235

(All values are expressed in mg/L)

Results of biochemical analysis of effluent adsorbed on activated carbon after anaerobic and electrolytic treatment are given in Table 6. 12. After adsorption on activated carbon, COD and BOD values are 122 and 9 respectively. Concentration of phenol and free amino acids are 6 and 4235 mg/L. Sugar and soluble proteins were completely removed by electrolysis itself. Comparatively less removal of phenol and amino acids took place by adsorption.

Presence of total bacteria was not detected after adsorption since total bacteria present in the anaerobically treated effluent was completely removed by electrolytic treatment.

6.6. Conclusion

The endeavour was to find out the efficiency of different carbon forms and nano clays to purify the pre-treated natural rubber skim serum effluent from centrifuge latex factory. This study shows that commercially available activated carbon and furnace carbon black are good adsorbents to purify this effluent.

Activated carbons prepared from coconut shell, rice husk, saw dust and teak wood saw dust can also be used as adsorbents since these natural substances are cheap and easily available in Kerala. Out of these, carbon prepared from coconut shell is the best adsorbent in terms of COD removal. The order of their efficiency in terms of COD and BOD removal is, coconut shell > rice husk > teak wood saw dust > saw dust. Nano clays are also good to remove COD, total nitrogen and ammoniacal nitrogen.

Table 6.13 shows characteristics of the effluent after electrochemical treatment and adsorption. After electrochemical treatment the COD and BOD values reached to 816 and 290 mg/L respectively.

Table 6. 13. Characteristics of effluent after treatment

Type of treatment	COD	BOD	TKN	AN	PO_4	Soluble protein	Total sugar	Amino acids	Phenol
After **electrolytic** Treatment	816	290	3480	2580	7	nil	nil	4322	7
After **adsorption** on activated carbon	122	9	1740	1728	6	nil	nil	4235	6

(All values are expressed in mg/L)

After adsorption on activated carbon the values of COD, BOD and phosphate are 122, 9 and 6 mg/L respectively which are below the limits prescribed by the Pollution Control Board. But further treatment is needed for the complete removal of TKN, AN and amino acids. Total sugar and soluble protein were completely removed though trace of phenol was detected in the purified effluent.

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CHAPTER 7

GAMMA RADIATION TREATMENT

7. 1. Introduction

Radiation treatment is emerging as an environment friendly technology for recycling wastewater. To overcome the problem of pollutants which pose a serious threat to public health, it is essential to develop cost effective and environment-friendly technologies. Radiation systems like gamma ray, electron beam, ultraviolet and x- ray are currently being used to treat waste and wastewater.

Gamma radiation has been widely accepted as a method of wastewater treatment and disinfection. Unlike UV radiation, gamma rays are highly penetrating. Gamma radiation has the ability to alter organic and inorganic molecules and this effect have application on tertiary treatment process[1]. Radiation technology is an environment–friendly approach for cleaning contaminated ground water and industrial wastewater. Radiation technologies treat multi-component waste streams and do not require any hazardous chemical additives, nor does it create any secondary wastes. Advanced oxidation process using gamma rays is used for treating industrial effluents that are very hard to be treated by conventional methods[2]. The mechanism of chain reactions which involves hydroxyl and hydroperoxy radicals (due to their high reactivity and low selectivity), enables the application of the process to a large number of compounds present in the wastewater, especially for decolouration. Advantages of the method mainly are (i) it does not create by-products, (that may cause secondary pollution of the environment), (ii) elimination of the hazard of overdosing of oxidizing agents; and (iii) high process rate and efficiency. Through primary and secondary products of water radiolysis, the ionizing radiation causes the decomposition of organic compounds contained in water. It was found that the presence of oxygen in the solution had a positive effect on the rate of destruction process due its contribution in the chain process of oxidation. The mechanism of the process is complex because of various agents that initiate the decomposition and a number of consecutive reactions that follow. As a result, there is a variety of intermediate and final products[3].

The development of new technologies which are able to minimize discharges are essential since unpolluted water is necessary for human beings. The chemical processes that are triggered by radiation of water are known. Therefore, radiation procedures could be advantageous in environmental applications compared to other techniques. Radiation is a flexible process in comparison to chemical process because of its safety and unpolluting mechanism[4].

7.2. Forms of radioisotopes and their applications

Radioisotopes are used for nucleonic control systems, radiation processing, non-destructive testing (NDT) and in radiotracer studies. Sealed neutron sources of Cs-137, Co-60, Am-241, Kr-85, Pm-147, Sr-90/Y-90, Tl-204, Cf-252, Ni- 63, Fe-55, Cd-109, Co-57 and Am-Be-241 are used in nucleonic control systems. Cobalt-60 in the form of high intensity sources is the main isotope used for radiation processing. Ir-192 sources are used in more than 90% of the gamma radiation devices. There is considerable interest in therapy using radiopharmaceuticals. The range of isotopes and their products in regular use is expected to expand significantly. A large number of isotopes in various chemical forms are used as tracers in industry. Identification and conservation of art objects and artifacts by neutron activation analysis, cargo inspection, sterilisation of medical devices, food irradiation, non-destructive testing (NDT), and modification of polymers are the major areas of application[5-7]. Cobalt-60 is a deliberately produced radio active isotope and the same is used in the treatment of cancer patients in hospitals. Cesium-137 is the major component of nuclear waste material, which is a by-product of nuclear power plants and is therefore available as a source of gamma rays for water purification, and thereby diminishes the amount of chlorine required for disinfection[1].

Gamma rays, electron beam or ion beam are used to reduce harmful industrial emissions like SOx and NOx and to reprocess waste streams. The other possible application of the technology is in the treatment of volatile organic compound (VOC) and polycyclic hydrocarbons e.g. in the gas purification units of municipal waste incinerator plants[5]. The electron beam flue gas treatment plants are operating in the coal–fired power plants in China and Poland. Radiation technology being a clean and environment friendly process helps to curb pollutants' emission[8]. Sterilisation of waste streams and sludge hygenisation are other applications of radiation[6].

Use of gamma irradiators in the disinfections of sewage sludge has been demonstrated on a full scale basis at a plant near Munich, Germany and at a biomedical waste steriliser in Arkansas, USA for the treatment of hospital wastes. Its use for degradation of toxics in soils is currently under investigation. Similarly electron beam has been proven effective in the disinfections of drinking water and wastewater. More recently, it has been used in pilot scale studies to break down contaminants in soils and industrial waste slurries. Irradiation facilities for treatment of water have been constructed in many countries of the world[7].

In pilot plant and industrial installations, the advanced oxidation process has been used in the treatment of drinking and industrial water. At present, such installations are used for wastewater treatments in several countries, including Germany, France, Canada and United States[3,9]. The first large-scale plant was the Geiselbullach gamma sludge irradiator, constructed in Germany in 1973. Another commercial application, also in Germany, is the use of irradiation to reduce biological fouling of drinking water wells[7].

Research and development on the use of electron accelerators for environmental conservation in Korea have covered water and wastewater treatment and air polluting waste treatment. Investigation was in progress in the application of radiation technique for the treatment of dyes in wastewater[3,10-14] and a pilot plant for dye factory wastewater treatment equipped with an electron accelerator has been constructed in South Korea[15].

Though, employment of radiation techniques in wastewater treatment on an industrial scale is rather non-existent; of late, much interest is evinced on the potential of using gamma radiation in the treatment of municipal and industrial wastewater and drinking water[3,16]. Radiation combined with biological processes was used in the treatment of wastewater originating from production of antibiotics and metallurgy where large quantities of detergents and fats are used in the production process[3,10,17]. Pikaev[18] conducted electron-beam purification of wastewater from carboxylic acids and removal of petroleum products (diesel fuel, motor oil and residual fuel oil) from water by gamma irradiation.

Investigations on the effect of gamma radiation on poliovirus infectivity seeded in sludge samples, elimination of the endocrine-disrupting activity ('feminization') of 17 beta-estradiol using reactive species, reduction in the population of coliphage, total coliforms and total flora present in raw sewage and secondary effluent, comparison of the toxicity responses of irradiated wastewater effluent samples to chlorinated municipal wastewater effluent samples and treatment of several animal viruses found in effluent from an animal disease laboratory have been reported[1,19-24].

Jung et al investigated radiation treatment using gamma rays in order to reclaim the secondary effluent from a sewage treatment plant at a dose of 15 kGy[25]. Gamma rays effectively disinfected microorganisms and completely removed them at a dose of 0.3 kGy. Radiation treatment reduced BOD by 85% irrespective of the absorbed dose, and the removal of COD, TOC and colour were up to 64%, 34%, and 88%, respectively, at a dose of 15 kGy.

The effect of gamma-ray radiolysis of aqueous phenol solutions dispersing TiO_2 and Al_2O_3 nanoparticles was studied and found that organic compounds, including phenol and aromatic intermediates induced by radiolysis, were effectively removed from the aqueous phase in the presence of the nanoparticles[26].

The application of a 5000 Ci cobalt-60 gamma radiation source as a surrogate for electron beam irradiation is potentially an emerging technology for wastewater treatment. A preliminary study to treat paper mill bleach effluent showed that for an absorbed dose of 800 krads, COD was reduced by 13.55 %[27]. Meeroff et al conducted laboratory and pilot test studies to investigate the use of ionizing radiation at an activated sludge wastewater treatment plant[28]. A dose of 2-3 kGy would be successful for bulking control and radiation assisted anaerobic digestion.

Pikaev studied radiation purification of wastewater from surfactant and petroleum products, dyeing complex and paper mill[29]. He investigated the removal of heavy metals (Cd, Pb, Cr, & Hg) from water and wastewater, decomposition of some dyes, hydrogen peroxide, chlorine-containing organic compounds and formic acid using electron-beam treatment in combination with ordinary methods (biological, coagulation, adsorption, flotation etc). Gamma-irradiation showed effective organic contaminant decomposition and resulted in the reduction of BOD, colour, COD and total organic carbon during the treatment of secondary effluent from a sewage treatment plant[30].

A study was conducted by Basfar et al[31] to determine the effectiveness of gamma irradiation in the disinfection of wastewater, and the improvement of the water quality by determining the changes in organic matter as indicated by the measurements of BOD, COD and TOC. The studies were conducted using a laboratory scale Co-60 gamma source.

An industrial plant for wastewater treatment is under development in Korea and a pilot plant for sewage sludge irradiation has been in operation in India for many years. There are over 160 gamma industrial irradiators and 1300 electron industrial accelerators in operation world wide[8]. India's sludge hygienization research radiator (SHRI) in Vadodara, furnished with Co-60 gamma source has been operating since 1992 and is the second such plant in the world.[7] A radiation dose of about 3 kGy delivered to the sewage sludge ensures removal of more than 99.9 % of disease causing bacteria. The irradiated sludge is "pathogen free" which can be beneficially used as manure in the agricultural fields as it is rich in nutrients required for the soil. At present this is the only operational plant in the world for demonstrating this technology[32].

Radiation treatment of sewage sludge offers an efficient, simple reliable method to produce pathogen free sludge. The wastewater sludge generated by a sewage plant is very odorous and contains a high level of pathogens which limits its reuse, which is otherwise a rich source of nutrients. Its disposal in the present form is an economic loss to the country. Further processing of the sludge to reduce odour and pathogens is therefore necessary, before beneficial utilization. Thus there exists a need to further extend the treatment processes to include a step that ensures removal of the pathogenic bacteria with high degree of reliability. The main advantage of the gamma radiation treatment is the ability to inactivate pathogens with a high degree of reliability associated with the process in a clean and efficient manner and easy operation and maintenance of the plant. India's Ministry of Environment and Forests has expressed interest in radiation technology for large scale treatment of municipal sewage effluent in cities along the Ganga River in North India[7, 32].

Since there is a growing interest in advanced oxidation processes like radiation technology to treat wastewater, a study was initiated to apply this method in combination with chemical oxidants (Fenton's reagent) to treat skim serum effluent from centrifuging rubber latex factory.

7.3. Theory of gamma radiation treatment

The action of ionizing radiation on water results in the formation of ions and excited state electrons close to its paths or track. The electrons so formed have sufficient energy to ionize a few other molecules of water. The clusters of ions thus produced are called ‘spurs’. A few other molecules of water, located a little further away, become excited.

The secondary electron, after losing its energy is solvated some distance away, forming a hydrated electron. The mother ion also reacts with water molecule producing an OH radical. The excited water molecule either loses its energy or decomposes into H and OH radicals which subsequently recombine to give water as they are formed close together.

The H and OH radicals formed, react with each other in those regions where their concentration is high. When linear energy transfer of radiation increases, the recombination of radicals among themselves becomes more, leading to decrease in radicals and an increase in H_2 and H_2O_2 production[33]. The major reactions in the radiolysis of water are given below[34].

$$H_2O \xrightarrow{\gamma} H_2O^+,\ e^- \text{ and } H_2O^*$$

$$H_2O^+ + H_2O \longrightarrow H_3O^+ + OH$$

$$H_2O^* \longrightarrow H_2O$$

$$e^- + nH_2O \longrightarrow e^-_{aq} \text{ (hydrated electron)}$$

$$e^-_{aq} + H_2O \longrightarrow H + OH^-$$

$$2\,e^-_{aq} \longrightarrow H_2 + 2OH^-$$

$$e^{-}_{aq} + H \longrightarrow H_2 + OH$$

$$e^{-}_{aq} + OH \longrightarrow OH^{-}$$

$$e^{-}_{aq} + H_3O^{+} \longrightarrow H + OH$$

$$2H \longrightarrow H_2$$

$$H + OH \longrightarrow H_2O$$

$$2OH \longrightarrow H_2O_2$$

$$OH + H_2O_2 \longrightarrow H_2O + HO_2$$

$$2HO_2 \longrightarrow H_2O_2 + O_2$$

The reactions dependent on pH, impurities, temperature, OH radicals; H radicals and hydrated electrons (e^{-}_{aq}) are formed as a result of radiolysis of water[34]. Based on this, the overall equation for radiolysis of water may be written as

$$H_2O \longrightarrow e^{-}_{aq}, H, OH, H_2, H_2O_2$$

In acidic solutions the hydrated electrons are converted to hydrogen atoms. In alkaline solutions, hydrogen atoms are converted to hydrated electrons and there is ionization of OH radicals. When oxygen is present in the solution, H and e^{-}_{aq} react very fast with it. In neutral and alkaline solutions HO_2 also ionizes. The yield of primary radical and molecular products depends on pH of the solutions[33].

In aqueous solutions, the primary species either interact among themselves or with the dissolved solute as mentioned earlier. The reactions of primary species with solutes are (i) oxidation (ii) reduction (iii) addition (iv) abstraction (v) electron transfer. The reactions with the organic solutes are addition, abstraction and electron transfer, whereas reactions within organic solutes are generally oxidation-reduction. The initial products from the reaction of primary radiolytic species with dissolved solutes may further undergo various types of reactions depending upon the type of the system being irradiated. These reactions may be different in the presence and absence of oxygen[33].

The primary products of water radiolysis, which are formed as a result of ionizing radiation form peroxide ($HO_2^{\cdot}$) radicals in the reactions with oxygen dissolved in the system[35]

$$H_2O \longrightarrow H^{\cdot} + OH^{\cdot} + e_{aq}^{-} \qquad (1)$$

$$H^{\cdot} + O_2 \longrightarrow HO_2^{\cdot} \qquad (2)$$

$$e_{aq}^{-} + O_2 \longrightarrow O_2^{-} \qquad (3)$$

$$O_2^{-} + H^{+} \longrightarrow HO_2^{\cdot}\text{, pK} = 4.88 \qquad (4)$$

Decomposition of organic molecules will take place in water solutions exposed to ionizing radiation, by the hydroxyl $OH^{\cdot}$ radicals formed during water radiolysis[35].

$$RH \xrightarrow{\gamma} R^{\cdot} + H^{\cdot} \qquad (5)$$

$$RH + OH^{\cdot} \longrightarrow R^{\cdot} + H_2O \qquad (6)$$

$$R^{\cdot} + O_2 \longrightarrow RO_2^{\cdot} \qquad (7)$$

In advanced oxidation process $OH^{\cdot}$ has got a predominant function in the decomposition of organic molecule[35]. The mechanism of generating

OH' radicals in water and their restoration in chain reactions with ozone, hydrogen peroxide and UV radiation has been presented by Benitez[36]. Reactions of wastewater play an important role in the decomposition of impurities by radiation. The highest decomposition was obtained at neutral or slightly alkaline pH[35].

The radioisotope most commonly used in making of high intensity radiation sources is Co-60. It is an isotope formed by thermal neutron capture in a nuclear reactor from Co-59

$$^{59}\mathrm{Co} + \mathrm{n} \longrightarrow {}^{60}\mathrm{Co} + \gamma$$

$$^{60}\mathrm{Co} \xrightarrow{\beta} {}^{60}\mathrm{Ni} + 2\,\gamma$$

Co-60 is a beta emitter and the emission of two gamma rays from Ni-60 having energy of 1.33 and 1.17 MeV combined with a useful half-life of 5.3 years makes it a useful radioisotope. Caesium-137 is another useful radioisotope which is formed from the fission of U-235 in a nuclear reactor. Caesium-137 is also primarily beta emitter but its daughter product Ba-137 (half- life 2.6 min) is a source of 0.66 MeV gamma rays. Caesium-137 has a half life of 30 years. A Co-60 source is usually kept in the form of metal slug, pellet or rod. They are doubly encapsulated with stainless steel in the form of a pencil. A final source assembly contains multiple pencils in a case or plaque with planar, circular, or other geometric configuration[33]. In most Co-60 irradiators, the pencils of Co-60 are mounted vertically in a source case. The source cases are assembled into a source rack. The rack is put on the lifter system or suspended from a hoist mechanism on the roof of the cell.

Radiation doses are almost always quoted in terms of absorbed dose to water, the only significant exception being the semiconductor industry, which uses absorbed dose to silicon. Unless otherwise stated, the use of the word **dose** should be taken to mean **absorbed dose to water**[6]. The amount of radiation absorbed by a material is defined as the dose. Its unit is 'rad' (radiation absorbed dose). 1 rad = 100 erg/gm Therefore, 100 rads =1 Gy

The standard international (SI) unit of dose is the Gray (Gy), which represents J/kg. When 1 kg of material absorbs 1 J of energy, then this matter is said to have received a dose of 1 Gray.

When 1 kg of material absorbs 1000 J of energy, then this matter is said to have received a dose of 1 kGy (Kilo Gray)

In industry, the amount of radio isotope is indicated by the Ci unit. One Ci of a radioisotope disintegrates at 3.7 x 10^{10} per second. A γ- ray of 2.506 MeV is emitted by each disintegration[34].

The theory and reactions of Fenton's reagent and the effect of various factors to treat toxic organic compounds in the presence of Fenton's reagent were discussed in detail in Chapter 5 dealing with electrochemical treatment[37-39].

7. 4. Aim

The objective of the present study was to investigate the impact of gamma irradiation on the treatment of raw and anaerobically treated skim serum effluent. The combined effect of gamma radiation and Fenton's reagent on these effluents was also included in the study. The treatment efficiency was determined in terms of effective organic contaminant decomposition as indicated by the measurements of COD, BOD, TKN, AN, phosphate, TS and DS. The impact of radiation treatment on the biochemical constituents like soluble protein, free amino acids, phenol, total sugar, reducing and non reducing sugars and population of total bacteria were also studied. The irradiation of the samples was conducted using a laboratory scale Co-60 gamma source.

7. 5. Experimental

7. 5. 1. Treatment procedure

Table 7. 1. Characteristics of raw and anaerobically treated effluent

Parameter	Raw effluent	Anaerobically treated effluent
pH	3.6	8.5
Turbidity (NTU)	350	107
COD	36400	4508
BOD	27300	815
TKN	7000	4480
AN	5000	3500
TS	60000	32418
DS	58000	30554
Sulphide	14.00	235
Phosphate	2600	1525

(All units except pH and turbidity are expressed in mg/L)

Natural rubber skim serum effluent was collected according to the methods described in Chapter 2 and analysed for various parameters as per standard methods[40] and the values are given in Table 7.1. It was then subjected to gamma radiation and the effect of the following factors were investigated: the dose of radiation viz. 0.5 kGy to 100 kGy, pH of the effluent, addition of various chemicals like ferrous sulphate, Fenton's reagent and pollutant concentration of the effluent. To study the effect of gamma radiation by adding Fenton's reagent on anaerobically treated effluent, the sample was collected from the UASB reactor as explained in Chapter 4 (Table 7.1). After irradiation, samples were analysed to study the extent of treatment in terms of pH, turbidity, COD, BOD, total Kjeldahl nitrogen, ammoniacal nitrogen, total solids, dissolved solids and sulphide.

Gamma irradiation of effluent samples was conducted at the Radiation Vulcanization of Natural Rubber Latex (RVNRL) Plant of Rubber Research Institute of India (RRII), Kottayam, Kerala, India.

7. 5. 2. Description of the radiation source

Table 7. 2. Specifications of Gamma chamber 5000:

1	Maximum Co-60 source capacity	444 (12000 Ci)
2	Dose rate at maximum capacity	-9 kGy/hr (0.9 Mega Rad/hr) at the centre of the sample chamber
3	Dose rate uniformity	+25% or better radially; -25% or better axially
4	Irradiation volume	5000 cc approx.
5	Size of sample chamber	17.2 cm (dia) X 20.5 cm (ht)
6	Shielding material	Lead & stainless steel
7	Weight of the unit	5600 kg. approx.
8	Size of the unit	125 cm(l) X 106.5 cm (w) X 150 cm (ht)
9	Timer range	6 seconds onwards.

Gamma Chamber 5000 is a compact self shielded Co-60 gamma irradiator providing an irradiation volume of approximately 5000 cc. The material for irradiation is placed in an irradiation chamber located in the vertical drawer inside the lead flask. This drawer can be moved up and down with the help of a system of motorized drive which enables precise positioning of the irradiation chamber at the centre of the radiation field. Specifications of Gamma Chamber 5000 are given in Table 7. 2.

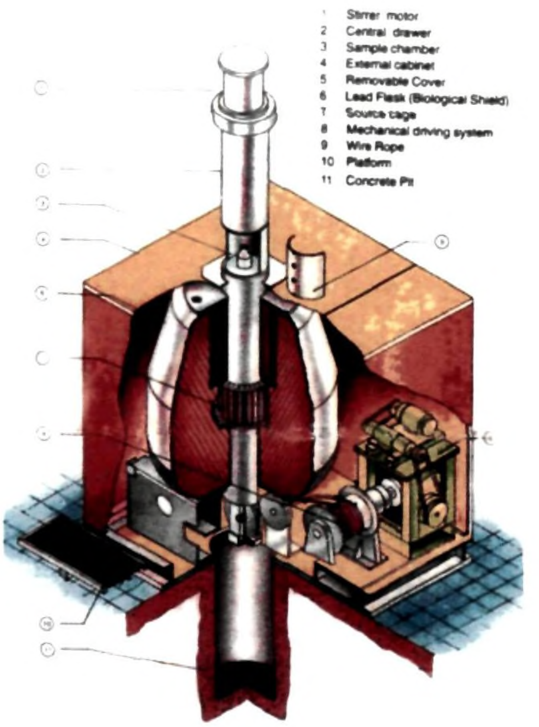

Fig.7. 1 Radiation plant

Radiation field is provided by a set of stationary Co-60 sources placed in a cylindrical cage. Details regarding Co-60 source are given Table 7. 3. The sources are doubly encapsulated in corrosion resistant stainless steel pencils and are tested in accordance with internal standard.

Two access holes of 8 mm diameter are provided in the vertical drawer for introduction of services sleeves for gases, thermocouple, etc. A mechanism for rotating /stirring samples during irradiation is also incorporated. The lead shield provided around the source is adequate to keep the external radiation field well within the permissible limits. The gamma chamber 5000 unit can be installed in a room measuring 4 meters x 4 meters x 4 meters

Table 7. 3. Details regarding Co-60 source

Name	Cobalt
Symbol	Co
Atomic number	27
Atomic weight	58
Mass number	59
Melting point	1495^0C
Boiling point	2900^0C
Crystal structure	Hexagonal
Density (g/mL)	8.9
Wave length of γ-ray of (1.25MeV) Co-60	10^{-2}-10^{-5} nm
Frequency of γ-rays	$3x10^{22}$ Hz

Irradiation time

Irradiation time is calculated using the formula

Irradiation time =Dose/Dose rate

The value of dose rate changes from day to day, which is obtained from the table. The dose rate decreases day by day due to the disintegration of Co-60

7. 5. 3. Biochemical and microbiological analysis

Biochemical analysis of the different types of effluent was done using a UV-visible Recording spectrophotometer UV-240. Concentration of total sugars[41], reducing sugars[42], total and soluble proteins[43], phenols[44], and free amino acids[45], were estimated as per standard analytical techniques.

The population of total bacteria of the raw and treated effluent was enumerated using appropriate media. The standard serial dilution plate technique of Pramer and Schmidt was employed for the enumeration of microbiological population[46].

7. 6. Results and discussion

7. 6. 1. Effect of irradiation dose on pollutant removal

(a) Effect on COD and BOD removal

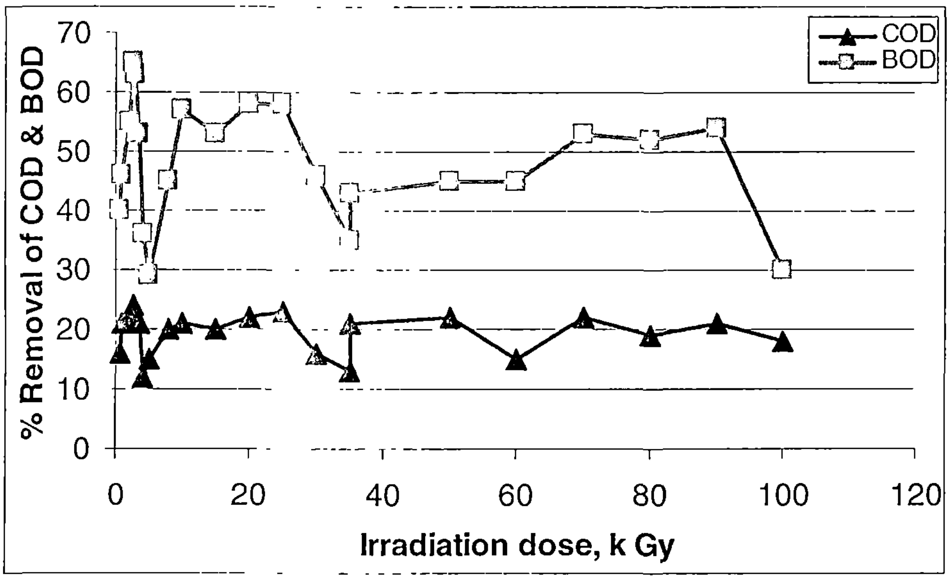

Fig. 7. 2. Percentage removal of COD & BOD vs radiation dose

The effect of exposure rate on the treatment of the raw effluent was investigated within the range of 0.5 kGy (0.05Mrad) to 100 kGy (10 Mrad).

The COD removal efficiency increases as the dose of gamma radiation increases from 0.5 to 2.5 kGy. The maximum COD removal efficiency was at 2.5 kGy which was 24 percent and thereafter it showed fluctuations as shown in the Fig. 7. 2. A further increase of the dose up to 100 kGy had practically little effect on COD removal.

In removing BOD almost the same trend was followed. From 2.5 to 3 kGy BOD removal efficiency was 60-65 percent which was the maximum observed during the treatment though the dose was increased up to 100 kGy

(b) Effect on pH

There was no significant change in pH after irradiating with gamma rays as it was evident from the Fig.7.3. Very slight increase in pH was observed for almost all irradiation doses and this may be due to the reactions of hydroxyl radicals generated during radiolysis.

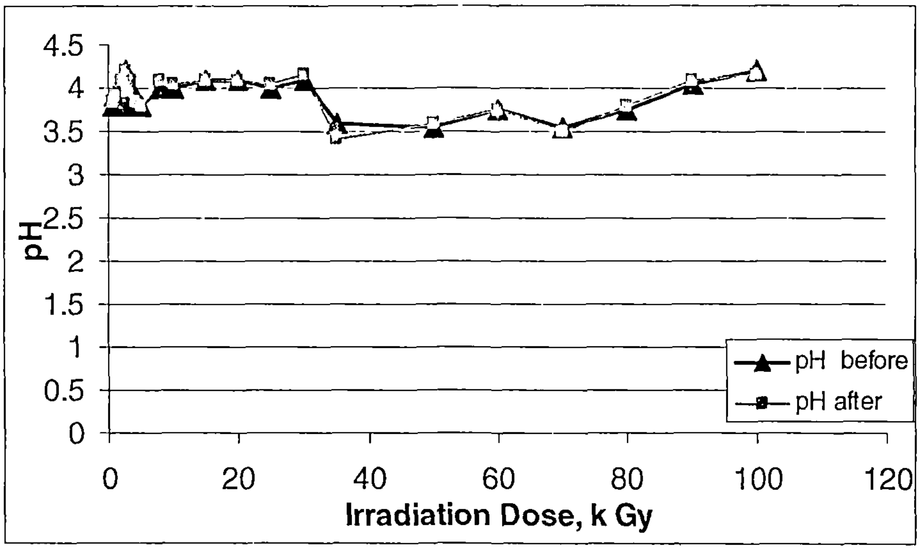

Fig.7. 3. Effect of irradiation on pH

(c) Effect on TKN, AN, TS, DS and turbidity removal

Gamma radiation showed only 23 percent and 28 percent removal of TKN and AN respectively in the range of 1 to 2.75 kGy (Fig.7. 4). Maximum removal of TS and DS were 16 percent and 14 percent respectively in between doses of 1 to 2.75 kGy. But the turbidity removal was in the range of 80 to 82 percent. Maximum turbidity removal was for an irradiation dose of 0.5 to 2.5 kGy and 70 to 100 kGy (Fig.7. 5). It was observed that after gamma irradiation the effluent became very clear and good settling was observed even when the dose was as low as 0.5 kGy.

From the percent removal of pollutants, 2.5 kGy was taken as optimum dose of irradiation to treat skim serum effluent.

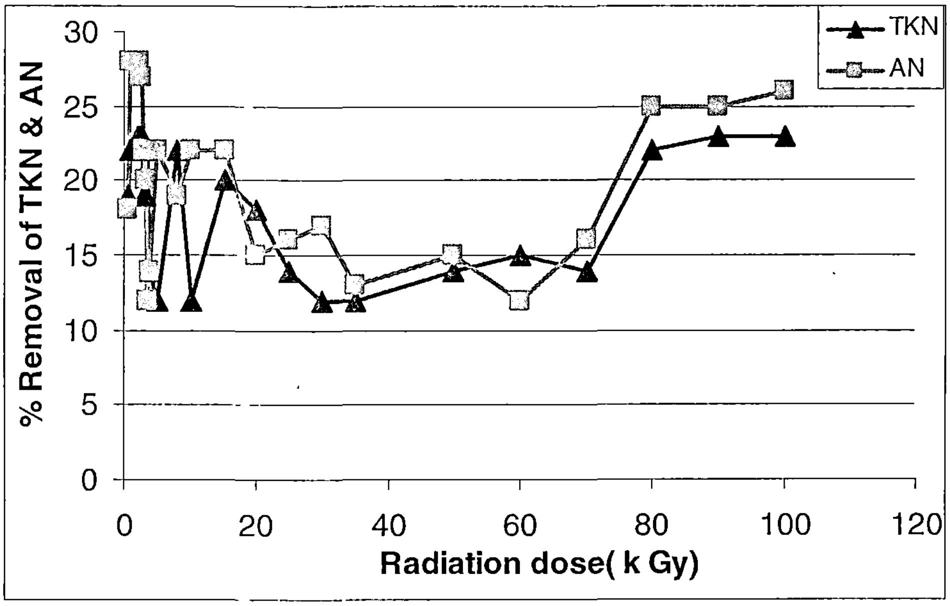

Fig. 7. 4. Effect of radiation dose on TKN & AN removal

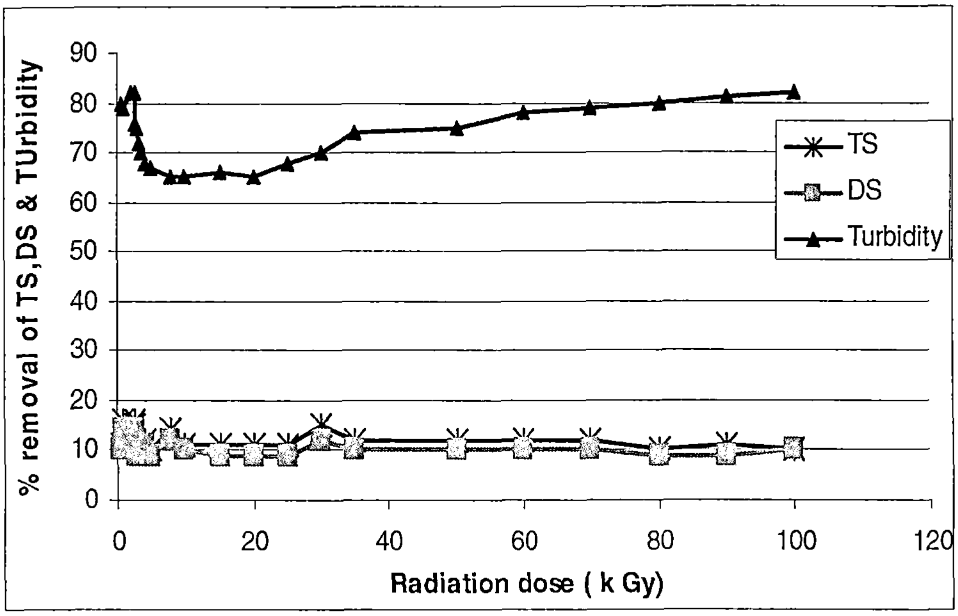

Fig. 7. 5. Effect of radiation dose on TS, DS & turbidity removal

7.6.2. Factors influencing the efficiency of radiation treatment

(1) Influence of pH and gamma radiation on COD and BOD removal

To study the effect of pH and gamma radiation on the removal of pollutants, the pH of the effluent was adjusted to 3, 4, 5, 6, 7, 8 and 9 before subjecting to gamma radiation at a dose of 2.5 kGy. The effect of pH on percentage reduction of COD and BOD is illustrated in Fig.7.6 . It was found that there was a decrease in the percentage removal of COD and BOD as the pH of the effluent was increased from 3 to 5 and then a steady increase when the pH was changed from 5 to 7. The values remained almost constant after pH 7 (Fig. 7.6). Therefore pH between 7 and 8 could be taken as the optimum.

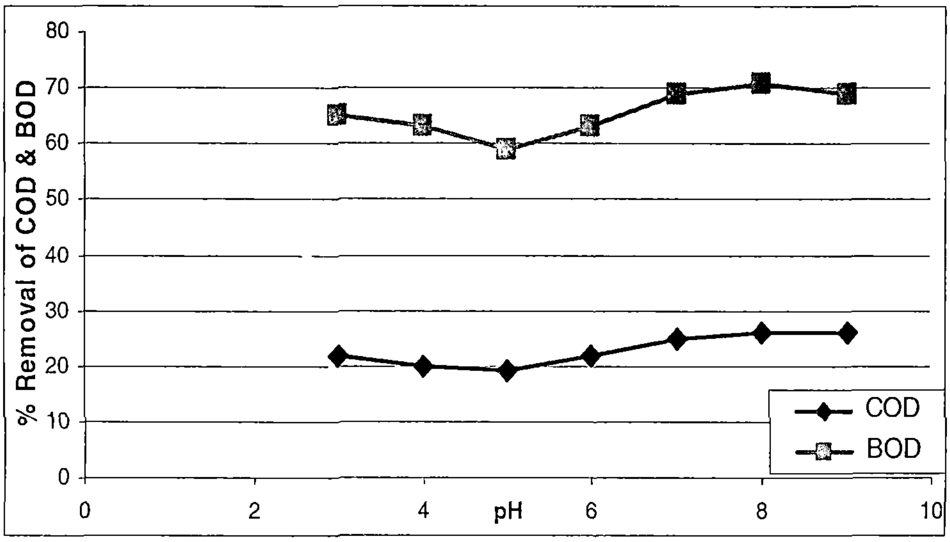

Fig. 7.6. Effect of pH on COD and BOD removal efficiency

In the advanced oxidation process OH' has got a predominant role in the decomposition of organic molecule. Perkowski[35] also observed the same effect that the highest decomposition was obtained at neutral or slightly alkaline pH in the decolouration of model dye house wastewater with advanced oxidation process.

(2) Influence of various reagents

Function of each reagent when separately added to the effluent and subjected to gamma radiation was the thrust of this study. The following reagents were added to the raw effluent (a) 200 mg/L of iron (b) 5 mL/L H_2O_2 (c) 200mg/L of iron and 5 mL/L H_2O_2 along with a control without any reagent, subjected to gamma radiation having a dose of 2.5 kGy and analysed for various parameters as per standard methods [40]. The results are given in the Table 7. 4.

A slight increase in pH occurs when the effluent was irradiated alone and a decrease in pH occurs when the effluent was irradiated either in the presence of iron or iron and hydrogen peroxide. When hydrogen peroxide alone was added there was no change in pH after irradiation.

Table 7. 4. Influence of gamma radiation in presence of various reagents to remove pollutants

RE and the following reagents	pH		Percentage removal		
	before	after	Turbidity (NTU)	COD	BOD
γ	4.15	4.20	45	23	64
γ + 200 mg/L of Fe	3.15	3.10	55	29	69
γ + 5 mL/L H_2O_2	4.15	4.15	-53	25	63
γ+ 200 mg/L iron + 5 mL/L H_2O_2	2.9	2.80	82	36	73

Maximum reduction of COD and BOD took place when the raw effluent was irradiated in the presence of 200 mg/L of iron + 5 mL/L of H_2O_2 than individual applications of the reagents. Radiation in the presence of H_2O_2 alone showed negative values in the removal of turbidity and the COD and BOD reduction were also comparatively low. In the absence of iron, formation of hydroxyl radical was negligible[37] and this negatively affected the treatment efficiency.

(3) Effect of gamma radiation at various concentration of iron in Fenton's reagent ($FeSO_4 + H_2O_2$)

Effect of gamma irradiation in the presence of Fenton's reagent to remove pollutant was studied. Oxidation by Fenton's reagent is catalysed by Fe^{+2}. Experiments were conducted to know the effect of Fe^{+2} and to find out the optimum dosage of Fe^{+2} on the degradation of pollutants in the wastewater. The effluent sample was subjected to gamma radiation having a dose of 2.5

kGy in the presence of Fenton's reagent having different concentrations of iron viz., 20 mg/L, 80 mg/L, 140 mg/L, 200 mg/L, 300 mg/L, 400 mg/L along with a fixed dose of H_2O_2 (5 mL/L of 30 percent H_2O_2). It was then analysed for various parameters as per standard methods[40].

By the addition of ferrous sulphate and hydrogen peroxide, pH decreased since the addition of $FeSO_4$ catalyst typically contains residual H_2SO_4. When the effluent was subjected to gamma radiation in the presence of Fenton's reagent, pH decreased again in all cases, which is evident from Table 7.5. This drop in pH could be attributed to the fragmenting of organic material into organic acids and the reaction is progressing as envisaged[37]. Optimum dose range for iron catalyst varies between wastewaters[37].

The effect of iron concentration in Fenton's reagent on the removal of turbidity showed that maximum turbidity removed was 95 percent when the concentration of iron was 200 mg/L to 400 mg/L. There was a steady increase in the removal of turbidity as the concentration of iron increased from 80 mg/L to 200 mg/L. Good sedimentation of coagulated particles were observed after irradiation and the effluent became very clear.

Optimum dose of iron concentration in Fenton's reagent was assessed in terms of its efficiency to remove COD and BOD. It was observed that on incremental increase of Fe^{+2} dosages, increase in the reduction of COD and BOD values were observed as is evident from Table 7. 5 and maximum percentage removal of COD and BOD took place at a concentration of 200 mg/L of iron.

Table 7.5. Effect of gamma radiation at various concentration of iron in Fenton's Reagent

$RE+H_2O_2$ (5mL/L+Fe (conc. given below)	pH		Percent Removal			
	before γ	after γ	Turbidity (NTU)	COD	BOD	Phosphate
RE+ γ(with out any reagents)	4.00	4.05	45	23	63	12
20 mg/L iron	3.90	3.80	87	29	70	17
80 mg/L iron	3.50	3.40	90	30	72	29
140 mg/L iron	3.25	3.15	92	31	74	35
200 mg/L iron	2.90	2.80	95	33	75	44
300 mg/L	2.75	2.70	95	33	74	46
400 mg/L iron	2.25	2.20	95	31	73	54

33 percent COD and 75 percent of BOD were removed at this concentration of iron. Further increase in the concentration of iron (200 mg/L to 400 mg/L) had only a marginal impact. The rate of ·OH generation (i.e., concentration of iron catalyst) limits the reaction rates with Fenton's reagent and less so by the specific wastewater being treated. A constant ratio of Fe: substrate above the minimal threshold level produced the desired end products. The ratio of Fe: substrate may affect the distribution of reaction products; and a supplemental aliquot of Fe which saturates the chelating properties in the wastewater, thereby availing unsequestered iron to catalyse the formation of hydroxyl radicals[37]. Since the pollution load is very high in this wastewater, a concentration of 200 mg/L of iron could be

taken as the optimum concentration in Fenton's reagent to treat this effluent.

In the case of phosphate, the removal efficiency increased as the concentration of iron increased from 20 mg/L to 400 mg/L. Without Fenton's reagent phosphate removal efficiency was only 12 percent and it increased from 17 to 54 percent when iron concentration in Fenton's reagent increased from 20 mg/L to 400 mg/L. As concentration of iron increased, it combined with the phosphate and removed as iron phosphate.

(4) Effect of gamma radiation at various concentration of H_2O_2 in Fenton's reagent

The effect of different concentrations of hydrogen peroxide on raw effluent in the presence of 200 mg/L of iron and gamma ray was investigated by adding 5 mL/L to 30 mL/L of 30% H_2O_2 to the effluent and irradiating it with a dose of 2.5 kGy. Optimum dose of H_2O_2 was 10 mL/L in the presence of 200 mg/L of iron since the highest removal of turbidity, COD, BOD and phosphate took place at this dose (Table 7. 6). A high ratio of Fe^{+2} and H_2O_2 was needed for chain initiation as shown in the Eq. 1[47].

$Fe^{+2} + H_2O_2 \rightarrow Fe^{+3} + OH^{*} + OH^{-}$ $K_I = 70\ m^{-1}s^{-1}$ (Chain initiation) Eq. 1

At low concentrations of H_2O_2 the radical chain reactions are quickly terminated since OH radicals produced mainly react with the ferrous iron and not with hydrogen peroxide. Eq. 2[48]

$OH^{*} + Fe^{+2} \rightarrow OH^{-} + Fe^{+3}$ $K_2 = 3.2\ x10^{y}\ m^{-1}s^{-1}$ (Chain termination) Eq. 2

Excess of H_2O_2 reacts with OH^{*} and thus scavenging hydroxyl radicals by H_2O_2 and consequently reducing the efficiency of treatment as shown in Eq. 3. The oxidation rate seems to be negatively affected by the increase of H_2O_2 concentration.

$OH^* + H_2O_2 \rightarrow H_2O + HO_2$ Eq. 3

The higher percentage degradation efficiency is that hydroxyl radicals can oxidise organic pollutants by abstraction of hydrogen producing organic radicals which are highly reactive and can be further oxidized [35, 49].

$RH + {}^*OH \rightarrow H_2O + R^*$ Eq. 4

Higher concentration of H_2O_2 reacts with the highly potent OH^* radicals to produce perhydroxyl radical OH_2 which has low oxidation potential than OH^* radical.

As concentration of H_2O_2 increased, a sudden fall of phosphate removal took place (from 46 to 5 and 2 for 10 to 20 and 30 mL/L respectively). This might be due to the interference caused by the excess perhydroxyl radical OH_2 produced by the addition of high amount of H_2O_2 during the formation of iron phosphate. Formation of large amount of froth was observed when concentration of hydrogen peroxide increased from 10 mL/L to 30 mL/L due to the evolution of gases like CO_2, H_2 and O_2.

Table 7. 6. Effect of gamma radiation at different concentrations of H_2O_2 in Fenton's reagent

RE + 200 mg/L of Fe + γ + H_2O_2 (Conc. given below)	pH		Percentage removal			
	Before γ	After γ	Turbidity (NTU)	COD	BOD	phosphate
5 mL/L	2.75	2.8	75	33	71	43
10 mL/L	2.70	2.8	82	35	77	46
20 mL/L	2.70	2.8	66	35	75	5
30 mL/L	2.25	2.3	40	30	75	2

From these studies it was concluded that optimum dose of iron and hydrogen peroxide needed in Fenton's reagent to treat skim serum effluent were 200 mg/L and 10 mL/L respectively.

(5) Effect of gamma radiation on different concentration levels of effluent

Raw effluent of different concentration like 100%, 75%, 50% and 25% were prepared by diluting raw effluent with distilled water and pH was adjusted to 4 and placed in gamma chamber for gamma radiation having a dose of 2.5 kGy. The effect of gamma radiation on different concentrations of effluent was then analysed in terms of COD, BOD and phosphate.

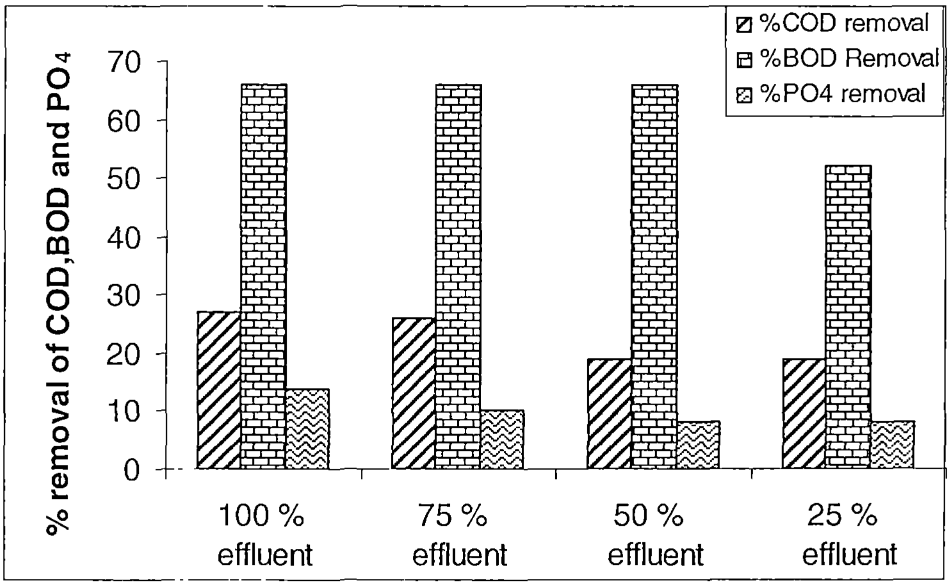

Fig. 7.7. Effect of effluent concentration and gamma radiation

Highest COD, BOD and phosphate removal were for undiluted effluent. When concentration of effluent decreased from 100 to 25 percent, COD removal decreased from 27 to 19 percent. But BOD removal was 66 percent for the dilution up to 50 percent effluent concentration, which declined to 52 percent for the 25 percent effluent concentration (Fig.7. 7).

In the case of phosphate, there was a steady decrease from 14 to 8 percent removal for the concentration change from 100 to 25 percent. As dilution increases the effective number of ions per mL that can be degraded by gamma radiation also decreases. This may be the reason for the low percentage removal of COD, BOD and phosphate when diluted.

7.6.3. Effect of gamma radiation and Fenton's reagent on anaerobically treated effluent (ATE).

7. 6. 3. A. Effect of gamma radiation and various reagents on ATE

When ATE effluent was subjected to gamma irradiation of 2.5 kGy without Fenton's reagent, 55% turbidity, 29 % COD, 45 % BOD and 27% sulphide were removed.

Table 7. 7. Effect of gamma radiation and various reagents on ATE

Reagent type ATE	pH		Percentage removal					
	before γ	after γ	Turbidity (NTU)	COD	BOD	sulphide	TKN	AN
ATE + γ (Without reagent)	7.8	8	55	29	45	27	8	6
ATE+ 200 mg/L Fe +5mL/L H_2O_2(without γ)	3.25	3	100	35	59	81	12	8
ATE+20 mg/L Fe + 5mL/L H_2O_2+ γ	3.75	3.5	100	41	57	52	16	8
ATE+80mg/LFe+5mL/L H_2O_2+ γ	3	2.9	100	54	80	64	18	9
ATE+140mg/L Fe + 5mL/L H_2O_2+ γ	3	2.9	100	66	81	83	19	9
ATE+200mg/L Fe +5mL/L H_2O_2 + γ	2.7	2.6	100	77	96	95	19	10
ATE+300mg/L Fe +5mL/L H_2O_2 + γ	2.6	2.5	100	76	95	94	19	10
ATE+400mg/L Fe +5mL/L H_2O_2 + γ	2.6	2.5	100	77	96	92	19	10

But when treated with Fenton's reagent alone (without gamma irradiation) 100% turbidity, 35 % COD, 59% BOD and 89 % sulphide were removed. This showed that Fenton's reagent is good to remove sulphide and turbidity. Removal of pollutants was high compared to raw effluent when anaerobically treated effluent was subjected to gamma radiation in the presence of Fenton's reagent (Table 7. 7). In the anaerobic reaction organic molecules are degraded by hydrolysis, fermentation and methanogenesis into simple monomers, acetate, hydrogen, methane and carbon dioxide[50] and this may be the reason for the high removal of COD and BOD compared to raw effluent.

As concentration of iron in Fenton's reagent increased from 20 mg/L to 200 mg/L, percentage removal efficiencies of COD increased from 41 to 77, BOD from 57 to 96, sulphide from 52 to 95 and TKN from 16 to 19 up to 200 mg/L. The values remained almost constant for further increase in the concentration of iron to 400 mg/L. But its effect on AN was not so prominent. Hundred percent removal of sulphide took place in all treatments where Fenton's reagent was used.

7. 6. 3. B. Effect of pH and Fenton's reagent on radiation treatment of anaerobically treated effluent

To study the effect of pH and gamma radiation in the presence of Fenton's reagent on ATE, pH of the ATE was adjusted to 2.5, 3, 5, 7 and 8. To these each set 5 mL/L of H_2O_2 and 200 mg/L of iron were added and irradiated with 2.5 kGy.

Table 7.8. Effect of pH and Fenton's reagent on radiation treatment of ATE

pH before	pH after	Percentage removal			
		Turbidity (NTU)	COD	BOD	Sulphide
2.5	2	97	70	86	100
3	2.3	97	72	88	100
5	4.5	63	68	76	99
7	7.4	98	33	74	95
8	8	98	23	75	77

From Table 7. 8 it is clear that 97% turbidity, 72% COD, 88% BOD and hundred percent sulphides were removed at pH 3. This was the maximum reduction of pollutants obtained when anaerobically treated effluent was subjected to gamma irradiation in the presence of Fenton's reagent at various pH. Previous studies using Fenton's reagent had shown that pH near 3 was optimum for Fenton oxidation[47]. At higher pH, the ferric ions form $Fe(OH)_3$ which has a low activity and does not react with hydrogen peroxide[47]

$$Fe^{+2} + H_2O_2 \rightarrow Fe^{+3} + OH^{*} + OH \qquad \text{Eq. 5}$$

$$2\,Fe^{+2} + H_2O_2 + 2\,H^{+} \rightarrow 2\,Fe^{+3} + 2\,H_2O \qquad \text{Eq. 6}$$

The pH value influenced the generation of *OH radicals and thus increased the oxidation efficiency. The precipitation of iron as its hydroxide reduced the availability of Fe^{+2} and oxidation transmission[51, 39]. The need of H^+ ion to decompose H_2O_2 is evident from Eq. 6 indicating the necessity for an acidic environment to produce the maximum amount of hydroxyl radicals.

7. 6. 4. Biochemical analysis of the radiated effluent

Biochemical analysis of raw and irradiated effluent were done by estimating change in the concentrations of soluble protein, phenols, total and reducing sugars and free amino acids.

Soluble protein concentration of raw effluent (RE) was 865 mg/L. Removal efficiency of it increased from 38.8 percent to 47 percent as the dose of radiation increased from 2.5 kGy to 50 kGy (Table 7. 9). Addition of Fenton's reagent alone removed 36.3 percent soluble protein. But gamma radiation of 2.5 kGy in the presence of Fenton's reagent removed 87 percent of soluble protein. In the case of phenol also maximum removal efficiency was for a radiation dose of 2.5 kGy which was 25 percent. But as the dose of radiation increased from 2.5 kGy to 50 kGy, an unusual increase in the concentration of phenol than the original one was observed. It is reported that the first step in the phenol decomposition by radiolysis and photo catalysis is oxidation of phenol forming various aromatic intermediates such as hydroquinone, catechol, pyrogallol and hydroxyhydroquinone[52]. The hydroxyl radicals produced during radiolysis might have combined with some of the organic molecules producing phenols. But in the presence of Fenton's reagent alone and combination of Fenton's reagent and gamma radiation, phenol removal efficiency was 43 and 48 percent respectively. Reports show that Fenton's reagent was used to treat a variety of industrial wastes containing a range of toxic organic compounds (phenols, formaldehyde and complex wastes derived from dyestuffs, pesticides, wood preservatives, plastics additives, and rubber chemicals)[37].

Table 7. 9. Results of biochemical analysis

No	Sample details (γ in kGy)	Soluble protein	Phenol	Total sugar	Reducing sugar	Non reducing sugar	Free amino acid
1	RE	865	586	1250	1095	155	15952
2	RE + 2.5 γ	529	440	793	666	127	11633
3	RE +50 γ	458	710	405	317	88	14929
4	RE +FR	551	335	456	267	189	20082
5	RE+FR+2.5 γ	110	307	1567	2912	203	17556
6	ATE	289	45	56	36	20	5779
7	ATE+ 2.5 γ	nil	52	nil	nil	nil	7726
8	ATE+ 3.5 γ	nil	60	nil	nil	nil	5145
9	ATE+ FR	nil	22	nil	nil	nil	5650
10	ATE+FR+2.5 γ	nil	27	nil	nil	nil	6442
11	ATE+FR+5 γ	nil	27	nil	nil	nil	6123

(Abbreviations used: RE; Raw effluent, ATE: anaerobically treated effluent, γ: Gamma radiation, FR: Fenton's reagent. All values are expressed in mg/L)

Total sugar concentration was 1250 mg/L for the raw effluent. Gamma radiation could remove 36.5 to 67.6 percent of total sugar when the dose of radiation increased from 2.5 kGy to 50. But the presence of Fenton's reagent and gamma radiation increased the concentration of total sugar from 1250 mg/L to 1567 mg /L. Estimation of reducing sugar and non reducing sugar also showed unusual values by the simultaneous effect of Fenton's reagent and gamma radiation. This may be due the decomposition of big organic molecules to small sugar molecules in the presence of Fenton's reagent and gamma radiation. The initial concentration of free amino acids was 15952 mg/L. For a radiation dose of 2.5 kGy its concentration decreased to 11633 mg/L which was 27 percent removal from the original. As the radiation dose increased further from 2.5 kGy to 50 kGy free amino acids removal decreased to 6.4 percent. But addition of Fenton's reagent alone and with gamma radiation, concentration of free amino acids increased to 20082 mg/L and 17556 mg/L. Increase of free amino acids may be due to the decomposition of proteins into individual amino acids in the presence of Fenton's reagent and gamma radiation.

Soluble protein was completely removed when anaerobically treated effluent was subjected to radiation at a dose of 2.5 kGy in the presence of Fenton's reagent. Phenol concentration was found to increase from 45 mg/L to 52 and 60 mg/L for a gamma radiation dose of 2.5 kGy and 3.5 kGy. But when treated with Fenton's reagent phenol concentration decreased by 51% and the combined effect of Fenton's reagent and gamma radiation removed only 40% phenol. Almost complete removal of total sugar as well as reducing and non-reducing sugars were possible when anaerobically treated effluent was subjected to irradiation. Estimation of free amino acid showed an increase from 5779 mg/L to 7726 mg/L when anaerobically treated effluent was subjected to a radiation dose of 2.5 kGy. This may be due to the complete decomposition of proteins leading to the formation amino acids. But when dose was increased to 3.5 kGy, amino acids content decreased from 5779 to 5145. Again its concentration increased to 6442 mg/L and 6123 mg/L when treated with Fenton's reagent and a combination of Fenton's reagent with gamma radiation. These changes may be due to the formation of radicals and the cleavage of carbon-carbon, carbon-hydrogen, carbon-oxygen and carbon-nitrogen bonds under high-energy radiation.

7. 6. 5. Microbiological analysis of the gamma irradiated effluent

Table 7. 10. Results of bacteriological analysis

No	Sample (γ in kGy)	Total bacteria (cfu/mL)	Serial No. of Photograph given in the figure 7.8
1	RE	27 x 10^4	1
2	RE+FR	2 x 10^3	
3	RE+FR+2.5 γ	nil	
4	RE+2.5 γ	nil	2
5	ATE	19 x 10^5	3
6	ATE + 0.5 γ	13 x 10^4	
7	ATE + 1 γ	6 x 10^4	
8	ATE + 1.5 γ	23 x 10^3	
9	ATE + 2 γ	60 x 10^2	
10	ATE+2.5 γ	46 x 10^2	
11	ATE + 3 γ	30 x 10^2	
12	ATE + 3.5 γ	18 x 10^2	
13	ATE+FR	4 x 10^4	4
14	ATE+FR+2.5 γ	2 x 10^2	5
15	ATE+FR+5 γ	1x 10	6
16	ATE+FR+10 γ	nil	7

(Abbreviations used: RE; Raw effluent., ATE: anaerobically treated effluent, γ: Gamma radiation., FR: Fenton's reagent.)

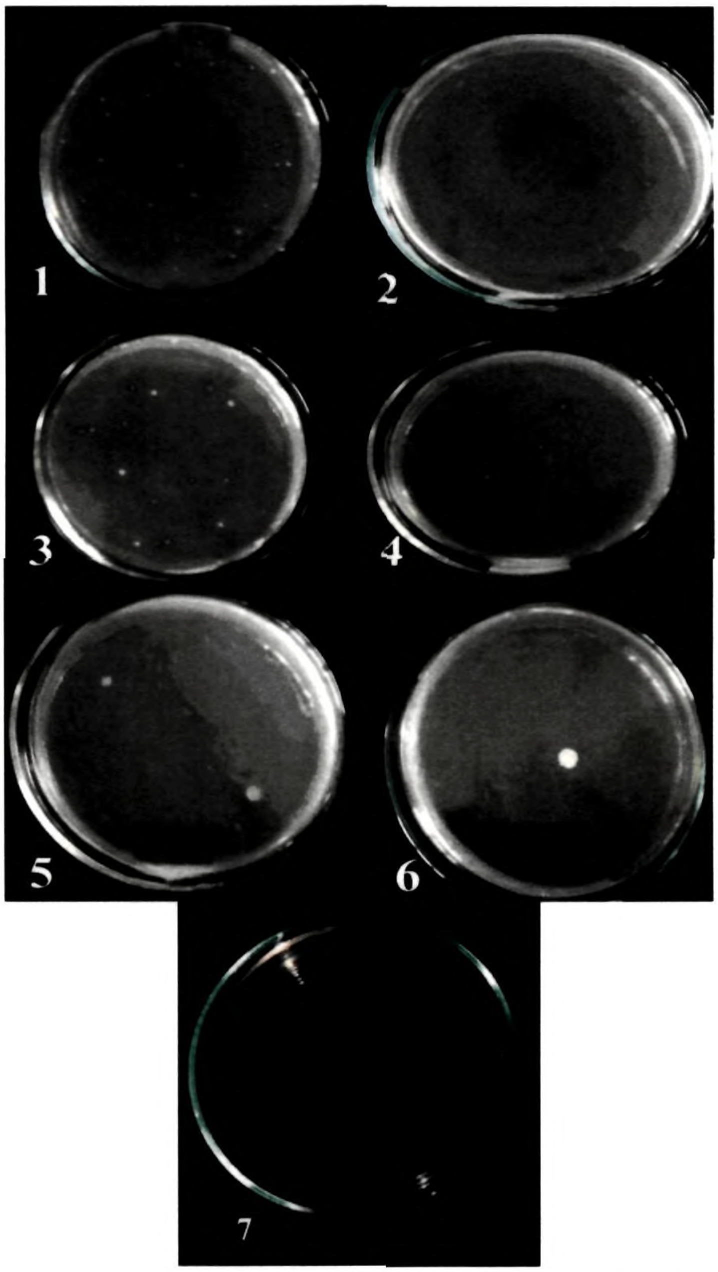

Fig.7.8. Effect of gamma radiation on total bacteria

(1 = RE., 2 = RE+2.5γ., 3 = ATE., 4 = ATE+FR., 5 = ATE +FR+2.5γ., 6 = ATE +FR+5γ ., 7 = ATE +FR+10γ)

(Abbreviations used: RE; Raw effluent, ATE: anaerobically treated effluent, γ: Gamma radiation., FR: Fenton's reagent.)

Total bacterial count of the raw as well as irradiated effluent was found out by culturing the samples. The results were given in the Table 7. 10 and photographs of some of the cultured samples (1 to 7) are given in Fig 7.8. Gamma irradiation having a dose of 2.5 kGy in the presence or absence of Fenton's reagent completely removed total bacterial population of raw effluent.

Anaerobically treated effluent when subjected to Gamma irradiation having a dose of 2.5 kGy in the presence or absence of Fenton's reagent did not remove total bacteria completely. Radiation dose of 5 to10 kGy in the presence of Fenton's reagent was needed to remove bacteria completely from the anaerobically treated effluent.

7. 7. Conclusion

When raw effluent was subjected to gamma radiation at a dose range of 0.5 to 100 kGy, maximum percentage removal of pollutants was observed for the dose range of 2.5 to 3 kGy. Therefore, 2.5 kGy could be taken as the optimum dose of radiation to treat raw serum effluent. Not much change in pH was observed after irradiating with gamma rays.

200 mg/L of iron and 10 mL/L of H_2O_2 could be taken as the optimum dose in Fenton's reagent since the highest removal of turbidity, COD and BOD took place at this dose. Phosphate removal efficiency increased as the iron concentration increased in Fenton's reagent. On comparison of the effect of concentration of effluent on gamma radiation, the highest COD, BOD and phosphate removal were for 100 percent effluent than for diluted effluent.

Effect of irradiation was more prominent in the presence of Fenton's reagent. pH around 7 could be taken as the optimum pH to treat raw effluent using gamma radiation but pH around 3 was effective for gamma irradiation along with Fenton's reagent.

Anaerobically treated effluent could be more effectively treated using gamma radiation and Fenton's reagent. Sulphides present in the anaerobically treated effluent could be completely removed when irradiated in the presence of Fenton's reagent.

Biochemical analysis of the radiated effluent (raw) showed that 87% soluble protein could be removed for a dose of 2.5 kGy in the presence of Fenton's reagent. 48 % of phenol was removed in the presence of Fenton's reagent and gamma radiation. Gamma radiation alone could remove 36.5 % of total sugar and 27 % of free amino acids for a dose of 2.5 kGy.

Anaerobically treated effluent when subjected to gamma radiation for a radiation dose of 2.5 kGy, soluble protein was completely removed. With Fenton's reagent phenol concentration decreased by 51% and almost complete removal of total sugar as well as reducing and non-reducing sugars was possible when anaerobically treated effluent was subjected to irradiation.

Gamma irradiation having a dose of 2.5 kGy, irrespective of Fenton's reagent added or not, completely removed total bacterial population of raw effluent, whereas in the case of anaerobically treated effluent, addition of Fenton's reagent in combination with a radiation dose of 5 to10 kGy was needed to remove bacteria completely.

Future research in this area is likely to pave the way for the application of radiation technology on a commercial basis to treat wastewater from rubber industries.

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CHAPTER 8

SUMMARY AND CONCLUSION

Pollution of water is a serious threat to public health and the situation calls for the development of cost effective and environmentally friendly technologies to ameliorate this problem. Skim serum effluent generated in latex centrifuging units is heavily polluted, requiring mandatory treatment so as to reduce the impact of pollution in land and water. This is more important in the context of high density of population and closely-knit dwellings in the state of Kerala. The water bodies which were once the life lines of human habitation have turned out to be the breeding grounds of disease causing microorganisms. Notwithstanding all kinds of pollution control measures imposed by the government and local bodies, untreated or partially treated effluents are discharged indiscriminately to the these water bodies. Consequently the water has turned out to be unfit for aquatic life and human needs.

The overall objective of the present work is to study the effectiveness of certain physico-chemical, electrochemical, biological and radiation methods for the treatment of the highly polluted acidic skim serum effluent on a laboratory scale and thus to develop a systematic treatment system. The treatment methods evaluated in this study had been effective proved to be effective for sewage and effluents from industries like pulp and paper mills, slaughterhouses, pharmaceutical units, citric acid units, distilleries, tanneries, textiles, dye industry and sugar industry.

It is necessary to know the composition of rubber latex to develop a treatment system and its processing. Therefore, a comprehensive description about natural rubber, composition of rubber latex, different methods of processing natural rubber latex and possible sources of effluent generation are given in the first chapter. The various physical, chemical and biological properties of the wastewater are discussed. The characteristics of effluent from natural rubber processing units, impact of effluent discharge on water bodies and the existing treatment practices are also discussed in brief. The objectives and scope of the work are also defined in this chapter.

In Chapter 2, the characteristics of skim serum effluent from latex centrifuging units are described. The average pH of the serum effluent is 4.11, indicating its acidic nature. COD and BOD of the serum effluent were estimated and the average values were 31603 mg/L and 16756 mg/L respectively. The average ratio between BOD/COD is 0.516 which indicates that the major pollutant is organic in nature. 95.5 per cent of the total solids is in the dissolved form indicating high concentration of organic matter. The major fraction of total suspended solids could be considered as organic matter, since 95.3 % of it is volatile in nature. The presence of total Kjeldahl nitrogen, ammoniacal nitrogen, phosphates, volatile fatty acids, sulphates, sulphide, oil and grease, turbidity and chlorides was also estimated. Concentration of metals as well as biochemical and bacteriological properties were estimated. Most of the characteristics studied are above the limits specified by the Kerala State Pollution Control Board. Therefore, it needs proper treatment before discharging.

Part A of the third chapter comprises the results of a comparative study of five commonly used metal coagulants to remove various pollutants from the serum effluent. The coagulants used were potash alum, ferric alum, aluminium sulphate, aluminium chloride and ferrous sulphates. The effectiveness of metal coagulants was evaluated in terms of the reduction of turbidity, COD, BOD, TKN, AN and phosphate. The effect of pH on coagulation was also investigated. The optimum pH value for potash alum, aluminium sulphate and ferrous sulphate is 9. For ferric alum and aluminium chloride the optimum pH is 7. Among the five coagulants studied, COD removal efficiency was above thirty percent for all coagulants except ferric alum. Sludge settling characteristics of potash alum and ferric alum were found to be moderate, whereas with ferrous sulphate, floc formation was rapid and settling rate was also fast. Settling of sludge became almost constant within 20 minutes. For aluminium chloride and aluminium sulphate sludge height was quite high showing low compressibility of the sludge.

Effectiveness of three natural polyelectrolytes viz: dried gooseberry powder, dried drum stick seed powder and tamarind seed powder and two synthetic polyelectrolytes (cationic and anionic) as coagulant and coagulant aid to treat skim serum effluent is described in Part B of the third chapter. The optimum pH value for the effective coagulation was 10 for all the polyelectrolytes. The natural polyelectrolytes show high potential as coagulant aid in the treatment process. Tamarind seed powder was found to be the most effective primary coagulant and coagulant aid among the five polyelectrolytes. A small quantity of polyelectrolyte, 4 mg/L, along with a very small amount of metal coagulant could reduce COD and phosphate considerably. When polyelectrolytes in conjunction with metal coagulants were studied, potash alum was found to be the most effective among the metal coagulants in all cases except cationic polyelectrolyte. In the case of cationic polyelectrolyte, aluminium chloride was more effective. Among the synthetic polyelectrolytes, cationic polyelectrolyte was more effective than anionic polyelectrolyte.

The topic of discussion in Part A of the fourth chapter is the treatment of skim serum effluent by UASB reactor. Performance of the reactor was evaluated in terms of the variations in pH, COD, BOD, sulphide, volatile fatty acids, total Kjeldahl nitrogen, ammoniacal nitrogen, total solids, dissolved solids, phosphate and volatile suspended solids. The volume of biogas produced was measured by the downward displacement of water. The studies revealed that UASB reactor could be successfully used to treat skim serum effluent. For an HRT of 36 days, average COD removal efficiency was 89 and 79 percent at an organic loading of 27 and 37 kg/day/m^3 respectively. Decreasing HRT and increasing organic loading decreased the efficiency of the treatment. The reactor height influenced the treatment efficiency and the percentage removal of COD increased as the effluent moves upwards and the maximum percentage removal was at the top of the reactor. Efficiency of treatment could not be assessed in terms of BOD since BOD values were unusually high during anaerobic treatment for longer HRTs. pH and VFA changes are more pronounced below 1.3 metre height, indicating high rate of anaerobic reactions at the lower part of the reactor. Lowering HRT from 36 days to 9 days decreased the efficiency of removing total solids and dissolved solids, whereas increasing organic loading increased the percentage removal of TS and DS showing the need for longer periods of retention for the removal of solids. Major portion of the TS and DS was removed below 1.3 meter height proving substantial settling of the sludge below this height.

High amount of VSS is formed at the lower part of the reactor. This could be a sign of high biomass formation at the lower part of the reactor. Total Kjeldahl nitrogen and ammoniacal nitrogen removal were rather low compared to COD. Percentage removal of ammoniacal nitrogen shows wide variation ranging from negative to zero. For higher HRTs, 97 percent removal of soluble organic nitrogen content was achieved during anaerobic treatment. High percentage removal of soluble organic nitrogen indicated the degradation of nitrogen containing compounds like proteins and amino acids during anaerobic treatment. Biological phosphorus removal was higher for longer retention times in the UASB reactor and *vice versa.* Large amount of sulphide was formed during this process of treatment but its presence did not seriously affect the treatment efficiency since 85 to 95% COD was removed. The biogas produced was measured and its quantity decreased when HRT decreased. Anaerobic process removed the concentration of heavy metals like iron, copper and zinc. Biochemical studies of the serum effluent showed that anaerobic treatment by UASB reactor is an effective method to remove protein, sugar and even phenol.

Part B of the fourth chapter explains the investigations into the effect of trace metals like Co, Ni and Mo individually and in combination at 100 and 10 μg/L concentrations on the anaerobic treatment of skim serum effluent using batch type upflow anaerobic sludge blanket in comparison with another set without any added metal. Average sulphide formation was higher when metal concentration was 10 μg/L than at 100 μg/L. But, formation of sulphide was comparatively low in the presence of Ni and Mo. Turbidity in the presence of cobalt was comparatively high, showing that the presence of cobalt favoured cell growth than any other metal. But for 10 μg/L concentration of trace metals Mo containing effluent had high value of turbidity. Measurement of turbidity could be used to assess the cell growth. Continuous addition of 100 μg/L of Co increased the percentage removal of COD to an optimum value and then declined. Continuous addition of Ni and all the three metals together caused an abrupt decline in the percentage removal of COD, showing the inhibitory effect of metals. In the removal of COD, influence of Mo was higher than Co when 10 μg/L of trace metal was added indicating the need for traces of Mo in the anaerobic treatment. Increase in BOD values during anaerobic treatment in the presence of trace metals indicated a constant interference in the measurement of BOD. Trace metals did not influence much in the removal of total Kjeldahl nitrogen and ammoniacal nitrogen. Percentage removal of ammoniacal nitrogen was negative, which confirmed the formation of ammonia during anaerobic treatment.

Chapter 5 discusses the effect of anodic oxidation of raw and anaerobically treated skim serum effluent. Electrochemical method could be used for pre-treatment as well as post-treatment of this effluent. But post-treatment was more effective. Sodium chloride was used as supporting electrolyte. Various metal electrodes like aluminium, cast iron, steel, and mild steel were compared for their efficiency in removing COD, BOD,TKN, AN and phosphate and found that aluminium anode was more effective to remove pollutants compared to others. Also studied the effect of Fenton's reagent in electrolysis and found that the presence of Fenton's reagent during electrolysis was very effective in removing pollutants. Maximum removal of COD took place within 45 minutes and BOD within 30 minutes. Therefore, 45 minutes could be taken as the optimum time for electrolysis. Instantaneous current efficiency decreased with time during electrolysis and finally reached a constant value after 120 minutes. Effect of concentration of effluent on electrolysis showed that the lower the COD of the effluent used for electrolysis, the higher the rate of removal and it was more prominent in the case of the anaerobically treated effluent. 99.5

percent phosphate could be removed from the anaerobically treated effluent by electrolysis. Electrolysis was more effective for the treatment of anaerobically treated effluent having low organic load. Complete removal of sulphide was observed during electrolysis. pH around 5 could be taken as optimum for electrolysis in the presence of Fenton's reagent since maximum percentage removal of COD and BOD took place at pH 5. With the help of a photovoltaic cell, solar radiation was used to treat these effluents and compared its efficiency with DC power and found that solar cell was very effective as an alternative source of power to treat serum effluent by electrochemical method. The use of photovoltaic cell is highly efficient and economical once it is installed. Biochemical analysis revealed that electrolysis in the presence of Fenton's reagent was good in removing soluble protein, phenol and sugars especially from the anaerobically treated effluent. Sugar and soluble proteins were completely removed by electrolysis. Microbiological analysis showed that population of total bacteria could be completely removed by 20 minute electrolysis in the presence of Fenton's reagent. It is concluded that anodic oxidation is one of the best methods for the treatment of anaerobically pre-treated effluent.

Chapter 6 discusses the feasibility and efficiency of different adsorbents and the selection of the best adsorbent to purify anaerobically and electrolytically pre-treated skim serum effluent. Different forms of activated carbon, carbon black and nano clays were used to purify the pre-treated natural serum effluent. This study showed that commercially available activated carbon and furnace carbon black were good adsorbents to purify this effluent. Activated carbons prepared from coconut shell, rice husk, saw dust and teak wood saw dust were also used as adsorbents, since these different natural substances are cheap and easily available in Kerala.

Out of these, activated carbon prepared from coconut shell was the best adsorbent for the removal of COD. The order of their efficiency in terms of COD and BOD removal is coconut shell > rice husk > teak wood saw dust > saw dust. Nano clays were good to remove COD, total nitrogen and ammoniacal nitrogen. Though nano clays were good adsorbents, further studies need be carried out to evaluate the biochemical as well as economical aspects.

Chapter 7 explains the impact of gamma irradiation from a Co-60 gamma source in the presence of Fenton's reagent on the treatment of raw and anaerobically treated skim serum effluent in terms of effective organic contaminant decomposition as indicated by the measurements of COD, BOD, total Kjeldahl nitrogen, ammoniacal nitrogen, total solids and dissolved solids. The impact of these factors on the biochemical constituents like soluble protein, free amino acids, phenol, total sugar, reducing and non-reducing sugars and population of total bacteria was also studied. Maximum removal of pollutants was observed at 2.5 to 3 kGy, when raw effluent was irradiated for a dose range of 0.5 kGy to 100 kGy. Effect of irradiation was more prominent in the presence of Fenton's reagent. Gamma radiation in the presence of Fenton's reagent was more effective on anaerobically treated effluent. pH around 7 was good to treat raw effluent using gamma radiation but pH around 3 was effective for gamma irradiation in the presence of Fenton's reagent. Sulphides present in the anaerobically treated effluent could be completely removed when irradiated in the presence of Fenton's reagent. Biochemical analysis of the raw effluent after irradiation in presence of Fenton's reagent showed that a dose of 2.5 kGy could remove appreciable amount of soluble protein, phenol and free amino acids. But the effect was more pronounced when anaerobically treated effluent was subjected to gamma radiation for the same radiation dose of 2.5 kGy. Complete removal of total sugar as well as reducing and non reducing sugar was possible when anaerobically treated effluent was subjected to irradiation in the presence of Fenton's reagent. Gamma irradiation having a dose of 2.5 kGy in the presence or absence of Fenton's reagent completely removed total bacterial population of raw effluent whereas in the case of anaerobically treated effluent the dose was in between 5 to 10 kGy.

Proposed treatment system for skim serum effluent

The study was undertaken with the objective of evolving a treatment system to mitigate the health hazards of the effluent, with special emphasis on the effluent discharged from the rubber processing units which are aplenty in the rubber growing belts of central Kerala, particularly Kottayam district.

Based on the studies undertaken and the observations made, the treatment system evolved in a laboratory scale is outlined below.

pH of the serum effluent collected from latex centrifuging unit was in the range of 3.6 to 4.7. This is raised to increase the pH to 8.5 to 9 by the addition of sodium hydroxide and lime. Sodium hydroxide was used along with lime to minimise the quantity of sludge formation. After adjusting the pH, potash alum was used as a coagulant to settle colloidal and suspended particles. Coagulation removed a part of the pollution load as evidenced by the decrease in the values of COD and BOD. Very low concentration of tamarind seed powder could also be added along with metal coagulant to enhance the coagulation process. COD of the raw effluent was in the rage of 27000 to 38800 and BOD was 10500 to 23280 mg/L respectively and by coagulation these parameters were reduced to 19000 to 27000 and 7350 to 15000 mg/L respectively. The clear solution after coagulation was filtered and fed into the UASB reactor for anaerobic treatment, where the organic pollutants were removed biologically. Microgram quantities of cobalt (100 μg/L) and molybdenum (10 μg/L) enhanced the treatment efficiency of the UASB reactor. Anaerobic treatment was essential for skim serum effluent due to its high pollution load. In the UASB reactor the effluent without dilution was used. Therefore, an HRT of 18 days was essential for proper treatment, especially for higher organic loadings. The main advantage of UASB reactor was the simplicity in design, ability to retain high biomass that lead to the efficient removal of organics at high loading rates and low energy demands. Only minimum power supply was needed and the whole plant could be kept operational at all times. COD of the effluent from the UASB reactor was in the range of 3595 to 4508. Most of the biochemical constituents including phenol were reduced. One of the disadvantages of the anaerobic treatment process was the formation of sulphide, since the effluent contained high concentration of sulphate.

The effluent from the UASB reactor was then subjected to electrochemical treatment in the presence of Fenton's reagent. Photovoltaic cell could be used as the source of power (DC power could also be conveniently used). Cost of treatment could be minimised since the source of power in photovoltaic cells is solar radiation. Electrochemical treatment in the presence of Fenton's reagent was an excellent method for the post-treatment of anaerobically treated effluent since it completely removed the sulphides from the anaerobic process. The COD of the effluent after electrochemical treatment was in between 816 to 1499 and BOD was 290 to 464. Biochemical constituents like soluble proteins and soluble sugars were completely removed by the electrochemical treatment.

Gamma radiation was effective to treat the anaerobically treated effluent in the presence of Fenton's reagent since it removed high percentage of COD, BOD and sulphides and inactivated pathogenic microorganisms from the effluent. But the non-availability of the radiation source limits its use as a common method of treatment. Electrochemical treatment can be replaced by gamma radiation treatment, if the radiation facility is not available.

UASB and electrochemical treatment when combined together removed the major part of the pollutants from the serum effluent. The disadvantages of the UASB were compensated by electrochemical treatment. The presence of electrons during electrochemical treatment electrocute microorganism in the biologically treated water and the microbial population present in the anaerobically treated effluent was completely removed by this method.

The effluent after electrochemical treatment was further purified by adsorption on activated carbon. This process gave a very clear solution and removed substantially the remaining pollutants, except TKN and AN for which further treatment was needed. The COD of the purified effluent was 122 to 240 and BOD was 9 to 14. These values were well below the standards prescribed by the Kerala State Pollution Control Board. A flow chart of the treatment system is given below.

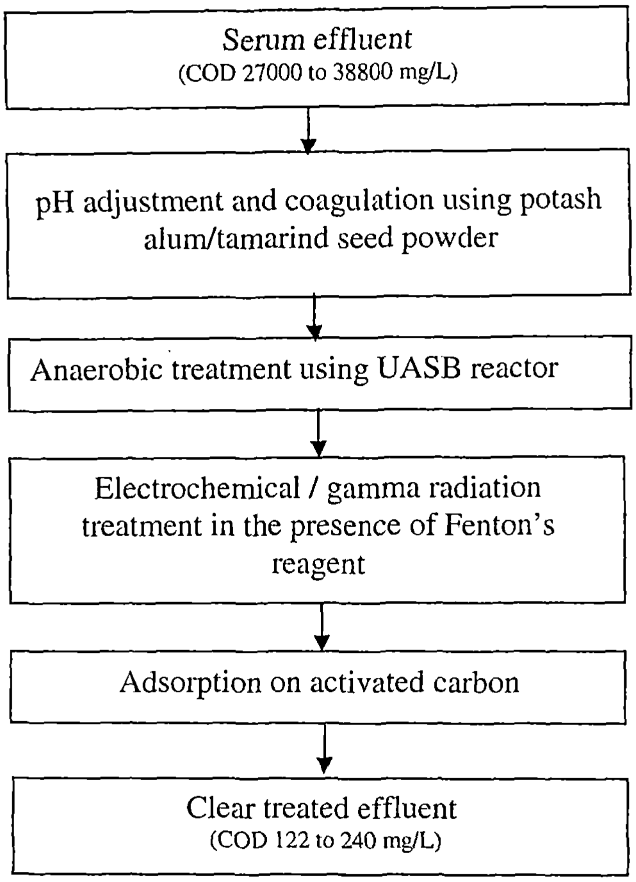

Fig. 8.1. Flow chart of the suggested treatment system

It has been finally concluded that the pollutant load of serum effluent can be brought down to below the tolerable levels-standards prescribed by the Pollution Control Board - through the following sequence of processes (i) coagulation of the serum effluent, (ii) anaerobic treatment using UASB reactor, (iii) electrochemical oxidation or gamma radiation process and (iv) adsorption on activated carbon.