MICROBIAL TREATMENT OF RUBBER LATEX CENTRIFUGATION EFFLUENT

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BY

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CERTIFICATE

This is to certify that the work presented in the thesis entitled **"MICROBIAL TREATMENT OF RUBBER LATEX CENTRIFUGATION EFFLUENT"** is based on the original research done by Mr. K. Jayachandran, under my guidance and supervision, at the Department of Biotechnology and no part of the work has been included in any other thesis for the award of any degree.

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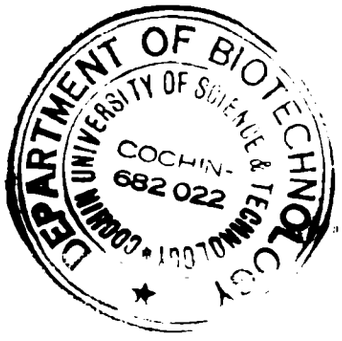

DECLARATION

I hereby declare that the work presented in the thesis entitled **"MICROBIAL TREATMENT OF RUBBER LATEX CENTRIFUGATION EFFLUENT"** is based on the original research carried out by me at the Department of Biotechnology, Cochin University of Science and Technology, under the guidance of Dr. M. Chandrasekaran, Reader and Head, Department of Biotechnology, and no part thereof has been presented for the award of any other degree.

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GENERAL INTRODUCTION

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GENERAL INTRODUCTION

1.1 PREFACE

The rapid increase in the population, accelerated exploitation of natural resources, urbanization, and industralization, along with the unchecked use of chemicals and pesticides have, consequently, led to an increase in the rate of pollution in the environment. Since destruction of the environment would ultimately result in the depletion of life on earth, in recent years there has been a growing concern for the pollution abatement, environmental protection and conservation of the environment which is necessitated towards a healthy life in the future.

Water is one of the most important requirements of man and there is dearth of pure water resources around the world owing to continuous pollution. The chemical reaction that occur in water and the chemical species found in it are strongly influenced by the environment in which the water is found (Sarkar and Gadgil, 1996). Hence there is an urgent need to protect our natural water resources from pollution.

According to Barness and Wilson (1978) three types of wastewater require treatment before disposal. They are (i) Domestic sewage (ii) Industrial wastewater (iii) Surface run-off.

The industrial effluents are generated by industrial and manufacturing enterprises from their production process. The amount and composition are therefore determined by the nature of the crude products and treatment process. They may be permitted to be discharged into the nearby water ways only after bringing down the pollution level as per the discharge regulations.

The wastewater treatment processes are generally grouped as the primary treatment, secondary treatment and the tertiary or the advanced waste treatment. Primary treatment removes identifiable suspended solids and floating matter. In the secondary treatment, also known as biological treatment, organic matter, that is soluble or in the colloidal form, is removed. Advanced wastewater treatment may involve the application of physical, chemical or biological processes or their combinations depending upon the impurities to be removed. Advanced waste water treatment processes are expensive and employed only when high quality water is required for direct use.

Available waste water treatment processes are broadly classified as physical, chemical or biological. Physical processes are based on the exploitation of the physical properties of the contaminants and are generally the simplest form of treatment. Physical methods comprise screening, sedimentation, floatation, and filtration. Chemical methods utilize the chemical properties of the effluent or of the added reagents. Commonly used chemical processes are precipitation, coagulation and disinfection. Other physical and chemical methods such as air stripping, carbon adsorption, oxidation and reduction, ion

exchange and membrane processes like reverse osmosis and electrodialysis are also important in certain cases (Gonazalez, 1996).

Biological treatment processes utilize biochemical reactions to bring about a chemical change in the properties of the contaminants of interest. The chemical properties are altered under the action of wide variety of microorganisms to cause the decomposition of the specific compounds, within the bulk waste stream. Often the decomposition of the organic compound is not complete and low molecular weight compounds such as aldehydes, ketones, and organic acids are formed. However, these compounds are usually of low toxicity to microbial or aquatic life and are further biodegraded easily under proper conditions. This process may be achieved aerobically or an aerobically in a number of ways. The most widely used aerobic processes are trickling filters, rotating biological contactors (RBC), activated sludge process and their modified versions. The anaerobic process is used both in the treatment of specific wastewater and in sludge conditioning(Belhateche, 1995).

Rubber Industry

Hevea brasiliensis, is the most important commercial source of natural rubber, which is also found in the latex of over 895 species of plants belonging to 311 genera of 79 families. Economic analysis show that during the last 30 years the demand for rubber in various countries is proportional to the Gross National Product. Nearly 65% of all rubber is consumed for the automobile tyres and tubes in developed countries. In

addition to tyres, a modern automobile has more than 300 components made out of rubber and many of these are processed from natural rubber. Use of natural rubber in hoses, footwear, battery boxes, foam mattresses, balloons, toys etc are also well known. Further natural rubber finds extensive use in soil stabilization in vibration, absorption and in road making. Rubber statistics, (Rubber Board. Government of India, Kottayam) presented in table 1.1 and 1.2, clearly indicate the growing importance of rubber in the commercial sector in recent years.

Processing of Natural Rubber Latex

Natural rubber latex is a milky white dispersion of rubber in water, which is harvested by the process of tapping. The latex that flows out from the rubber trees, on tapping, is channelled into a container attached to them. Coconut shells and polythene cups are popularly used as containers. Latex collected in these cups is transfered to clean buckets, two to three hours after tapping. The different kinds of latex harvested from rubber plantations are highly susceptible to bacterial action due to contamination on keeping. Therefore, latex is processed for safe storage and marketing.

Marketable Forms of Natural Rubber

The latex from rubber plantations is processed and marketed as:

1. Processed latex and latex concentrates.

2 Ribbed sheet rubber.

3. Crepe rubber.

4. Technically specified block rubber.

Processing into Preserved Latex or Latex Concentrates

Latex is a white or slightly yellowish opaque liquid with a specific gravity varying between 0.974 and 0.986. It is a weak lyophilic colloidal system of spherical or pear shaped rubber globules suspended in an aqueous serum. The rubber globule is surrounded by a protective layer of proteins and phospholipids, which imparts the prophyllic colloidal nature to latex, and the stability of the latex is attributed to the negative charge present on the protective layer. It also contains a variety of non rubber constituents, both organic and inorganic, in addition to rubber. Although the proportion of these constituents may vary with clones, nutrition, climate etc, in general, the composition of latex is as follows (Rubber and its cultivation, 1992)

Rubber	30 - 40%
Protein	2 - 2.5%
Sugar	1 - 1.5%
Water	55 - 60%

Fresh latex, as it comes out from the tree, is slightly alkaline or neutral. It turns acidic rapidly, due to the bacterial action The formation of organic acids neutralizes the negative charge on rubber particles and the latex gradually gets coagulated on storage. Therefore, fresh latex cannot be kept for long time without precoagulation. Ammonia, sodium sulphite and formalin are used as anticoagulants for providing shelf life.

Preservation

The collected latex is maintained free from contamination by the addition of preservatives. Although the most widely used preservative is 0.7% ammonia, a variety of other substances like sodium pentachlorophenate, EDTA, boric acid etc can also be used along with low level of ammonia (0.2%), for effective latex preservation. Field latex preserved with a suitable preservative is termed as preserved field latex.

Latex Concentrates

Preserved latex concentrate, an important raw material, has wide application. Three major methods are commercially practised for processing latex into preserved latex concentrates .

Latex Concentration by Creaming

The processing of latex into creamed concentrates involves the mixing of a creaming agent such as ammonium alginate or tamarind seed powder, with properly

preserved field latex, and allowing the latex to separate into two layers, an upper layer of concentrated latex and lower level of serum containing very little rubber.

Processing of Latex into Ribbed Sheet Rubbers

Latex is coagulated in suitable containers into thin slabs of coagulum and sheeted through a set of smooth rollers followed by a grooved set and dried to obtain ribbed sheet rubbers.

The two methods mentioned above are slow and the most efficient method is centrifugation. Presently over 90 percent of the concentrated latex is produced by centrifugation.

Latex Concentration by Centrifugation

The processing of latex into latex concentrates by centrifugation Fig.1.1 involves the separation of preserved field latex into two fractions, one containing concentrated latex of 50-60% dry rubber and the other containing 5-10% dry rubber, leaving behind the liquid (Rubber and its cultivation, 1992).

Skim Latex

The important factors governing the composition of skim latex produced are the type of centrifuge, DRC of field latex and efficiency of operation. Normally the rubber

content of the skim latex varies between 2.5 and 10 percent. Skim latex contains about two- thirds of the total serum of the latex The average size of its rubber particles is smaller than that of field latex since the larger particles separate more readily into the concentrate fraction. In addition to the water soluble substances in serum, latex contains proteins which are mainly distributed as adsorbed film on the surface of the rubber particles. As these particles are small, protein content per unit weight of rubber is more in the case of skim latex. This not only renders coagulation more difficult, but also has a marked effect on the properties of rubber. Skim rubber is usually recovered by the addition of sulphuric acid. The coagulum obtained is processed into creepe or sheet by conventional means, taking special care to give thorough mixing. Skim rubber is a low grade rubber and not used in the manufacture of products which require good service properties (Radhakrishana Pillai, 1980).

Pollution due to Natural Rubber Latex Centrifugation Effluent

During the processing of natural rubber an enormous quantity of water is used, which gets loaded with traces of rubber and substances like carbohydrates, proteins and lipids. The effluent has high Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) (Table 1.3). While the various microorganisms including harmful ones, proliferate using these substances and causes high BOD, the presence of organic matter leads to high COD, and both together cause objectionable odour. Considerable reduction in BOD and COD is essential to avoid pollution.

The quality and quantity of rubber factory effluent depend on the type of rubber processed and the size and organisation of the factory. On an average, 20 litres of effluent is generated for every kilogram of processed rubber. Most of the treatment plants employed by the rubber industries do not meet the discharge requirements (Sarkar and Gadgil, 1996). The aerobic nature of the bacterial growth in the effluent often result in the depletion of the dissolved oxygen level of natural water courses, which consequently result in anaerobiosis, and death of aerobic system and the destruction of the water course in terms of social and amenity value.

Several methods such as treatment of latex centrifugation effluent in aerobic pond coupled with stabilization pond, treatment with *Chlorella vulgaris*, seeding the effluent with *Chlorella vulgaris* and maintaining the tank aerated in light, and treatment with a two stage anaerobic system are reported (Jayachandran *et al*, 1994 a).

In a preliminary study, conducted earlier, it was observed that the rubber latex centrifugation effluent can be treated with immobilized *Acinetobacter sp* in a packed bed reactor under continuous process. During the first cycle of operation the percentage of reduction in Chemical Oxygen Demand (COD) was 44%. However, on repeated cycles, the efficiency decreased. This decrease in the efficiency could be due to the high amount of suspended solids which might have clogged the pores of the immobilized beads on subsequent passage of the effluent. Hence, an initial coagulation was carried out with a view to reduce the high amount of suspended solids.

Rotating biological contactor (RBC) is a fixed film bioreactor in which a microbial film is allowed to develop upon the circular disc surface mounted on the rotating shaft. The microbial film formed on the surface of the disc is aerated during the exposed phase of the cycle and nutrients are absorbed during the submerged phase.

The activated sludge process and its modified version have become the most widely utilized biological process for waste water treatment. The activated sludge process is a suspended growth reactor that operates in aerobic metabolism mode. The process involves mixing up of the waste stream with preformed biomass in an aeration tank. The biological reactor must be combined with a sedimentation process to remove the suspended biomass, frequently called as mixed liquour suspended solids. A portion of the biomass is recycled to control the amount of biomass in the bioreacator. The term "activated" is to imply that the microorganisms are conditioned and ready for substrate sorption and degradation.

Coagulation of skim latex is the final stage in the latex concentration which produces the latex centrifugation effluent. The presently used method of coagulation is chemical coagulation with con H_2SO_4. The acid content along with the protein present, reduces the quality of the skim rubber. Hence, an attempt was made to develop a suitable bioprocess for the coagulation of skim latex with a view not only towards improvement of rubber quality but also to reduce environmental pollution.

1.2 SCOPE OF THE PRESENT STUDY

Many of the existing methods for the treatment of rubber latex centrifugation effluent are not only unsatisfactory in their efficiency to effect near perfect treatment in bringing down the COD to optimum level, but also time consuming and need a large landspace. As the rate of effluent generation is extremely high (20 litres for kilogram of rubber) there is a need for development of efficient system,capable of rapid reduction of COD and BOD.

Though the organic load of the rubber effluent is very high, it does not contain much processed chemicals and therefore it can be considered as a 'biological effluent'. Further, the ratio of the Chemical Oxygen Demand to Biological Oxygen Demand (COD/BOD) of this effluent remain almost as a constant value. According to Montgomery (1967), estimation of BOD is not ideally suited for studies on process design, treatability, control of treatment plants, setting standards for treated effluents and assessing the effect of polluting discharges on the oxygen resources of receiving waters. Hence in the present study COD was measured to determine the impact of treatment system on the effluent.

In the present study, attempts were made to evaluate the efficiencies of certain methods such as packed bed reactor using immobilized microbial cells, rotating biological contactor (RBC) and activated sludge process, for rapid and efficient treatment of natural rubber latex centrifugation effluent. In addition, studies were also carried out to

develop a suitable bioprocess for the coagulation of skim latex, as an alternative to the presently used acid coagulation process towards reducing the pollution load, besides recovering quality rubber.

Accordingly, the specific objectives of the present study included the following:

(i) To improve the treatment of rubber latex centrifugation effluent using immobilized *Acinetobacter* sp. isolated from rubber effluent, in packed bed reactor, under continuous process.

(ii) To design a rotating biological contactor [RBC] for large scale treatment of the effluent.

(iii) To evaluate an activated sludge system for the treatment of rubber latex centrifugation effluent.

(iv) To develop a suitable bioprocess for the coagulation of skim latex towards reduction of pollutants in the effluent.

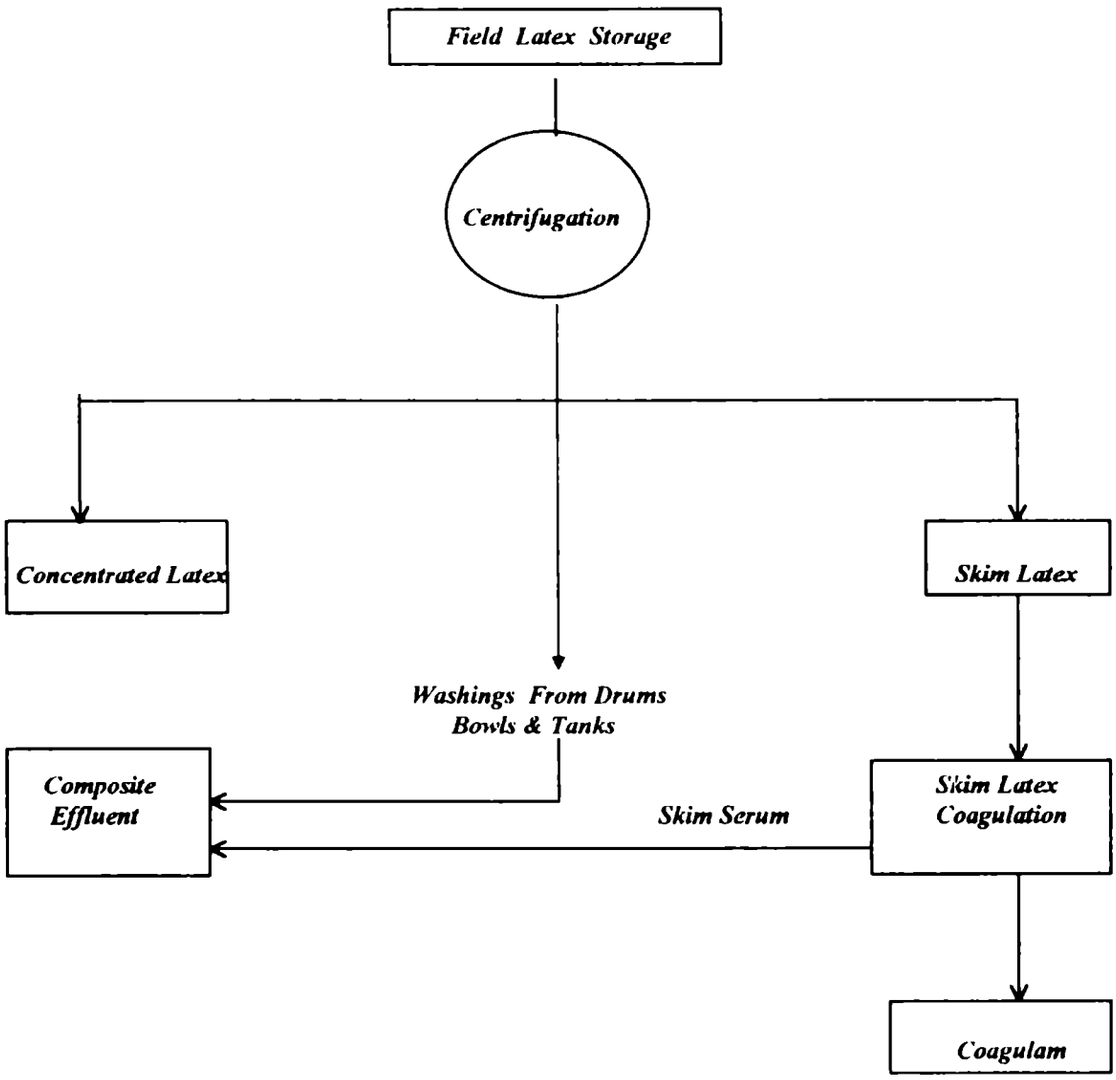

Fig. 1.1 **Schematic representation of processing of natural rubber latex.**

Table 1.1 Natural rubber production in major rubber producing countries.

Countries	Cultivation Area (Hectares) x 10^5	Annual production (Tonnes) x 10^5	Productivity (Kg/Hectare)
India	5.16	4.64	1265
Malaysia	18.37	11.01	1220
Thailand	19.39	17.21	1080
Srilanka	1.62	1.04	862
Indonesia	32.4	13.61	746
China	6.03	3.35	700

Source - Rubber Board, Govt. of India, Kottayam, 1994.

Table 1. 2 Production of natural rubber in India

States	Cultivation area (Hectares)	Annual production (Tonnes)
Kerala	443300	442830
Tripura	19252	2966
Tamil Nadu	17430	15065
Karnataka	14955	9700
Assam	10122	315
Meghalaya	4550	167
Nagaland	1450	11
Manipur	1253	96
Missoram	979	57
Andaman and Nikobar Islands	960	462
Goa	936	120
Orissa	219	--
Maharashtra	91	11
Arunachal Pradesh	75	15
West Bengal	27	--
Madhya Pradesh	8	--

Source - Rubber Board, Government of India, Kottayam, 1994.

Table 1.3 Physico-chemical and biological characteristics of rubber latex centrifugation effluent

Characteristics	Value*
pH	2.5 - 4.5
Temperature	30°C
Chemical oxygen demand (COD)	28 g/l
Biological Oxygen Demand (BOD)	6 g/l
Suspended solids	70 g/l
Total dissolved solids	20 g/l
Total nitrogen	1.8 g/l
Ammoniacal nitrogen	0.7 g/l
Protein	4 mg/ml
Sugar	0.4 mg/ml
Heterotrophic bacterial population	1200 CFU/ml

* Mean values of 50 analyses.

CHAPTER - 2

REVIEW OF LITERATURE

CHAPTER - 2

REVIEW OF LITERATURE

Biotechnological processes rely on microbial communities to transform and remediate undesirable industrial wastes. (Schneegurt and Kulpa, 1998). They are nonpolluting, economical and offer possibility of *in situ* treatment (Kenneth *et al* ,1994). A number of biological treatment methods such as activated sludge system (Borja *et al*, 1994; Ratsake *et al*, 1996) trickling filters (Eighmy *et al*, 1992) rotating biological cantactor (Tyagi *et al*, 1993) stabilization ponds (Moeller and Calkins, 1980), hollow fiber reactor (Liese *et al*, 1990), sequential batch reactor (Cohen *et al*,1997), fluidised bed technology (Johannes and Werner, 1997) and upflow anaerobic sludge blanket reactor (Christian *et al*, 1997) were reported to be efficient in the treatment of many industrial wastewaters.

2.1 NATURAL RUBBER LATEX

Hevea latex within the tree is sterile. However, after tapping, as the latex flows along the tapping cut and spout into the cup, it is contaminated with microorganisms which undergo a rapid growth not only due to the tropical conditions and short generation time (Taysum, 1959) but also due to the wide variety of non rubber constituents such as several aminoacids (Ng Tet Sooi, 1960). To keep the latex fluid sterile, it is a common practice to add with preservatives (Cook, 1960). Hevea latex is a

complex cytoplasmic system consisting of a dispersion of rubber particles in water phase. Its biochemical complexity is indicated by the presence of about 4% non-rubber, comprising mainly of protein, aminoacids, lipids, quebrachitol, carbohydrates and a variety of inorganic ions (Archer *et al*,1963). The contaminants multiply rapidly under tropical conditions at the expense of the non-rubber constituents producing acids (John 1966 a, b). Latex from small holdings is less stable primarily due to a higher level of non-rubber compounds contributed by intensive tapping (Bealing, 1968). The preservatives added to the field latex protect latex from contamination by virtue of their role as enzyme inhibitors and bactericidal properties (Krishnaswamy, 1969). Improvement in the control of fatty acid formation in field latex was achieved by combining ammoniation with an appropriate storage temperature (John, 1972). Better control over microbial proliferation was effected by the addition of a secondary preservative, hydrazine hydrate, to ammonia (John *et al*,1976).

A practical method of treating the latex effluent, before discharge, had been attempted by the Rubber Research Institute of Malaysia for a long time. Several methods of treatment were carried out in laboratory scale (Molesworth, 1958). A biological treatment, trickling filtration, was selected for the pilot plant operation (Molesworth,1961). Overall efficiency and the design status of trickling filter in the treatment of skim serum was also studied (Gale,1961). Holding the effluent in anaerobic pond coupled with stabilization pond (Muthurajah *et al*, 1973) for a period of 15 days for anaerobic digestion and 9 days of stay in stabilization tank was reported as an efficient method of treatment. Subjecting the column effluent to aeration and daylight along

with seeding of inoculum containing *chlorella* sp. as dominant species, for 5 days brought about sufficient purification of the effluent, such that it was dischargeable into waterways without much harm (Kulkarni *et al*, 1973 a, b). A detailed account of the microbiological status of the effluent in various steps in the stabilization pond process and possibilities of further improvement in the treatment of block rubber effluent (John *et al*,1974) and extensive analysis and pre-treatment steps for anaerobic/aerobic ponding system were reported (Ponniah *et al*, 1975). Lab studies revealed that for anaerobic/aerobic treatment of acidic latex concentrate effluent, a retention period of 30 days in anerobic and a minimum of 5 days in aerobic system was necessary (Ponniah *et al*, 1975).

Though anaerobic/ aerobic ponding systems seem to be the most economical biological process, there are certain drawbacks. For instance, the anaerobic/aerobic ponding system requires large land space and the treatment requires more time. This form of treatment may be impractical for factories situated in urban areas.

Indian reports on latex centrifugation effluent treatment is limited to investigations carried out at Rubber Research Institute of India , Kottayam and Regional Research Laboratory, Thiruvananthapuram.

Seeding the effluent with *Chlorella vulgaris* and maintaining the tanks aerated in light were effective in promoting rich algal growth and reduction in pollution load (Jacob Mathew *et al*,1988). Further effluent from sheet rubber factory was reported to be

utilized for Single Cell Protein (SCP) production and irrigation (Jacob Mathew *et al*, 1988).

Attempts to develop and anaerobic two stage treatment system for rubber waste effluent showed a COD removal efficiency of 60% after 10 days of hydraulic retention period (Ashok Pandey *et al*, 1988). Comparative studies on the controlled and uncontrolled start-up of the anaerobic treatment of natural rubber effluent indicated that a pH controlled start-up was promising than an uncontrolled one. However, the methane content of the gas was very low (30-40%) and the average retention time was 30 days (Ashok Pandey *et al*, 1990).

2.2 IMMOBILIZED SYSTEMS IN WASTE WATER MANAGEMENT

Infact, the 'trickling filter' and 'activated sludge' process of wastewater treatment employ microorganisms immobilized by natural means. Microbial cells naturally adhere to colonize on any solid surface immersed in an aquatic environment, forming a biofilm (Characklis *et al*,1983). Immobilized cells have a major role to play in the removal of organic pollutants from industrial effluent or from partially treated sewage effluent. Immobilization of microbial cells and their use in wastewater treatment has been extensively reviewed (Cheetham and Bucke, 1984). The scientific and technical aspects of using immobilized microbial cells for environmental application were also investigated (Cassidy *et al*,1996). The formation of immobilized biomass offer many advantages for the proper operation of wastewater systems. By immobilizing the biomass, the liquid

retention time can be uncoupled from the biomass retention time which enables higher rate conversion of organic and inorganic compounds in relatively small bioreactor. In addition, biomass is easily separated from the purified wastewater by settling (Alfons and Stephanic, 1997).

Many organic and inorganic materials have been used as support materials for immobilizing microorganisms. Both alginate and carrageenan were suggested to be better polymers for immobilizing microbial cells compared to toxic polyacrylamide (Cheetham and Bucke, 1984). Algae immobilized on the surface of chitosan flakes were shown to remove nitrogen and phosphorous from industrial effluents (Chevalier and de la Noue, 1985).

Tampion and Tampion (1987) stated that almost any type of suppport materials could be emperically tried ranging from natural crushed rocks, porous solid wastes, ceramics and other heat treated inorganics to natural organic materials and synthetic plastics in almost every conceivable physical shape and in the whole spectrum of reactor designs. They further concluded that, for wastewater treatement, it is much more important to get the reactor design and flow characteristics right with attention to the gross physical features of the support material.

Cells immobilized in polyvinyl alcohol has also been considered for enhancing nitrification in waste treatment (Myoga *et al*, 1991). The effect of immobilizing materials on the activity of nitrfifying bacteria and removal of ammoniacal nitrogen from

wastewater by immobilized nitrifying bacteria were investigated using six urethane prepolymers (Sumino *et al*, 1992). The efficiency of soil microalgae, *Chlorella vulgaris* and *Scenedesmus bijugalus* entraped in calcium alginate beads for the removal of ammoniacal nitrogen and orthophosphate phosphorous has been reported (Megharaj *et al*, 1992). The denitrifier *Pseudomonas stutzeri* entraped in chitosan beads was incubated under denitrifying conditions in a column receiving a contineous supply of full growth medium (Nussinovitch, 1996).

Immobilized microbial systems were also proved to be efficient in the degradation and removal of many pollutants. Immobilized bacteria capable of degrading phenolic compounds has been reported (Bettmann and Rehm, 1985). Although lower rates of phenol degradation were achieved by an immobilized consortium of methanogens, they were able to tolerate higher phenol concentrations (Dwyer *et al*, 1986). Multistep reactions were carried out with immobilized microorganisms for the removal of phenolic compounds from wastewater (Bisping and Rehm, 1988). Immobilized bacteria were also capable of degrading p-nitrophenol in an aqueous waste stream. (Heitkamp *et al*, 1990).

The efficiency of the immobilized cells in decontaminating water or wastewater containing xenobiotics compounds has been reported (Crawford and O' Reilly, 1989). Immobilized *Phanerochete chrysosporium* was used for bleaching of pulp and paper mill effluent (Singh and Marwaha, 1992). The effect of the presence of supplementary glucose or acetate on the growth and pyridine degrading activity of freely suspended and calcium alginate immobilized *Pimelobacter* sp. was investigated (Rhee *et al*,1996).

An organophosphate degrading soil isolates of *Pseudomonas* sp immobilized in alginate beads has been reported (Ramanathan and Lalitha Kumari, 1996).

Fluidised bed bioreactors, with microorganisms immobilized on various support materials, have been widely used for biological wastewater treatment (Livingston, 1991). Batch biological treatment of synthetic wastewater in a fluidized bed contaning wire mesh sponge particles was reported (Kargi and Eljisleyen, 1995).

Immobilized activated sludge has been tried for treating many industrial effluents. An activated sludge process using immobilized microorganism could reduce the treatment time of wastewater and the amount of excess sludge (Sumino *et al*, 1985; 1986). Activated sludge has been reported to be immobilized with acrylamide (Sumino *et al*, 1987), polyvinyl alcohol (Hashimoto and Furukawa, 1987) polyelectrolyte complexes (Kokufuta *et al*, 1987) and calcium alginate (Sofer *et al*, 1990). It was also coagulated to obtain primary particles to get protection from the toxic effects of immobilizing agent and was immobilized in acrylamide (Sumino *et al*, 1991). Nitrifying bacteria, obtained by enrichment culture of activated sludge was immobilized in porous pellets of urethane gel for the removal of ammoniacal nitrogen from wastewater (Sumino *et al*,1992).

2.3 CHEMICAL COAGULATION

Chemical coagulation is the process of adding chemicals to destabilize particles in the colloidal dispersion, while flocculation is their subsequent enmeshment, by gentle and

prolonged mixing to form discrete visible suspended solids that settle under gravity alone and are easily filterable. The process of initial chemical coagulation has been successfully applied in treating fishery wastewater (Nishide, 1977). Inorganic coagulants were also successfully used in the treatment of seafood processing wastewater (Johnson and Gallager, 1984). Since a large amount of organic matter and other contaminats in wastewater are associated with particles which may have a negative effect on biodegradation process, it is reasonable to start the wastewater treatment process with a good particle separation method (Odegard, 1988). Use of different inorganic coagulants in the treatment of effluent from leather processing industries has resulted in the significant reduction of suspended solids and BOD (Basu and Chakraborthy, 1990). The chemical coagulation was tried in the treatment of rubber latex cetrifugation effluent, where various coagulatnts like alum, aluminium sulphate, lime, ferrous sulphate, ferric chloride and aluminium chloride were observed to be effective in the reduction of COD and suspended solids and consequently contributed to reduced environmental pollution (Madhu *et al*, 1991). Requirements of pretreatment with coagulants for leather tanning industrial wastewater has been emphasized (Tunay *et al*, 1994) The application and scope of physical treatment like coagulation in the industrial wastewater treatment has also been reviewed (Pols and Harmsen, 1994). An emperical relation between the reduction in suspended solids and COD as a result of coagulation, has been reported (Sahoo *et al*, 1997). Coagulation studies on wastewater from vanaspathi manufacturing plant was also reported (Srivastava *et al*, 1997).

2.4 ROTATING BIOLOGICAL CONTACTOR

A rotating biological contactor is an example of a fixed film bioreactor. Although this process was conceived in Germany at the beginning of this century and introduced in the United States in the 1920's, it was marketed only in the 1960's (Huang and Bates, 1980).

The advantages offered by RBC include short residence time, low operation and maintenance costs, and production of readily dewatered sludge that settles rapidly (Weng and Molof, 1974). Biofilm development on a surface is the net result of several physical, chemical and microbiological processes (Characklis, 1981). Biofilm developement on RBC comprises a complex and diverse microbial community made of eubacteria, filamentous bacteria, protozoa, metazoa etc (Hitdlebaugh and Miller, 1981). The floral succession on the RBC surfaces was similar to that observed in activated sludge (Kinner and Curds, 1989).

Zahid and Ganczarczyk (1994) reviewed the structure of RBC biofilm in detail. A laboratory- scale model of a rotating biological contactor was used to investigate the variations in the structural features of the biofilm formed in four consecutive compartments of the modal during both the early and late stages of the 'biofilm' development Microtome sectioning of biofilm showed that in the early stages of growth, the biofilm was predominantly occupied by nonfilamentous bacteria and some protozoa. However, the biofilm in later stages were mostly filamentous and biofilm

porosity decreased with depth in the biofilm, and from one compartment to another. The structure of biofilm involved in wastewater treatment systems are complex than that was previously thought and required the application of modern biological techniques to get a better understanding of the biofilm (Alfons and Stephanic, 1997).

The attachment properties of nitrifying and heterotrophic biofilm developed in laboratory scale rotating biological contactors were studied by measuring the development of biofilm thickness, biofilm density, activity and detachment caused by shear stress (Oga *et al*, 1991). The physical and attachment properties of the RBC biofilm, as well as unit performance, is adversely influenced by the presence of organic particles in the feed. It is believed that the entrapment of the particulate organics by the biofilm matrix causes a subsequent development of oxygen depleted zones and structural flaws within the biofilm (Zahid and Ganczarczyk, 1996). There are several similarities and dissimilarities between microbial films and microbial flocs, the two forms of microbial aggregation. For both the forms of aggregation, availability of metabolic substrates is the major factor for dispersion and for development of external surface. However, microbial films are generated in a more compact way and the presence of solid surface carriers substantially increases the possibility of packing more microorganisms into a unit volume of biological reactors (Ganczarczyk, 1996).

Secondary treatment achieved with RBC on an industrial scale was evaluated in detail (Bogert, 1982). The application of full scale anaerobic/aerobic rotating biological contactors for treating brewary wastewater was investigated (Ware and Pescod, 1989).

RBC was found to be capable of removing pathogens (Sagy and Kott, 1990). Biodegradation of a petroleum refinery wastewater and kinetic parameters were studied in RBC Polyurethane foam (PUF) as a porous biomass support medium was attached on each side of RBC discs. The removal efficiency of total chemical oxygen demand and oil and grease were above 90% and 85% respectively. The results obtained with the RBC-PUF system in general were better than that with a conventional RBC (Tyagi *et al*, 1993). The application of membrane bioreactors for the treatment of municipal and industrial wastewater was also investigated (Brindle and Stephenson, 1996). A modified RBC with four stages was tried for the treatment of synthetic tapioca wastewaters. The discs were modified by attaching porous nechlon sheets to enhance biofilm area (Radwan and Ramanujam, 1996).

2.5. ACTIVATED SLUDGE SYSTEM

The first report on 'Activated Sludge System' was made in 1914 (Arden and Lockett, 1914). Microscopic examination supports the idea that flocs are composed of aggregates of living organisams (William *et al*, 1970) together with other meterials, both organic and inorganic (Forster, 1971). A single floc may contain millions of bacteria and is thus very large compared to an individual bacterium. Protozons are also associated with the flocs (Hughes *et al*, 1972) and have a marked effect on the stability. (Curds and Cuckburn , 1970). Ciliated protozoa in sewage treatment plants produce clear effluent of good quality because of their ability to feed on bacteria and suspended particles, and to induce flocculation (Wheale and Williamson, 1980). Crazing by protozoa induced several

bacterial responses. Selection pressure by competition and predation seemed to explain extinction of specific bacteria (Mallory *et al*, 1983: Goldstein *et al*, 1985). Positive effects of protozoa on carbon mineralization by bacteria were also considered specifically in the context of wastewater treatment (Ratsake *et al*, 1996).

The qualitative and quantitative analysis of the bacterial flora involved in the activated sludge flocs were also carried out. It was observed that as the oxygen level in the flocs is diffusion limited, the number of active aerobic bacteria decreases and the floc size increases (Hanel, 1988). The total aerobic bacterial counts in standared activated sludge are in the order of 10^8 CFU/mg of sludge. The majority of the bacteria isolated fom activated sludge were identified as *Comamonas, Pseudomonas* species (Hiraishi *et al*, 1989).

Madoni (1993) suggested the use of numerically based method called the Sludge Biotic Index (SBI) to define the biological quantity of activated sludge on an objective basis. A model was proposed which correlated sludge retention time and microfauna populations based on data collected form three activated sludge facilities over one year (Salvado, 1994). Most Probable Number (MPN) method was also used to quantify denitrifying and volatile fatty acid (VFA) utilizing bacteria from a biological nutrient removal plant (Kavanaugh and Randwall, 1994).

A micromanipulation technique was introduced for assisting the isolation and identification of filamentous bacteria from activated sludge samples (Hornshy and Horan,

1994) A method to distinguish between viable biomass, dead organic and inorganic meterials in the activated sludges was suggested based on the quantitative determination of DNA. (Liebeskind and Dohmann, 1994)

Activated sludge system could be specifically used for the removal of nitrogen, phosphorous and also for the treatment of many industrial effuents. A prototype aerated anoxic biological nitrification-denitrification process consisting of nitrate recycle to aerated selector zones and anoxic zones that contained a dense array of fine bubble diffusers was suggested (Albertson and Stensel, 1994). Removal of chlorinated phenolic from bleached kralt mill wastewaters was assessed using parallel laboratory scale activated sludge (Hall and Randle, 1994). Blow heat condensates generated from air pollution control equipment were found to have adverse effect at an onsite activated sludge treatment plant through disruption of the mixed liquor settleability (Barton and Drake, 1994). The treatment of the photoprocessing wastewaters were addressed in many papers (Pavlostathis and Jungee, 1994, Pavlostasthis and Morrison, 1994). Protecting the biological treatment systems at a petrochemical complex was accomplished by regulation of hydraulic and pollutant loads, through the use of a multiple stage treatment process (Rebdun and Galil,1994). Treatment of a high strength phenolic wastewater was attributted by a combined wet air oxidation/activated sludge system (Lin and Chuang, 1994). Kinetics of black olive wastewater tratment by activated sludge (Borja *et al*, 1994), the degradation of 2-4, dichloro phenoxyacetic acid and nitrogen conversion (Xin-hui Xing *et al*, 1995) and tretment of fishery wastewater with activated sludge has been reported (Gonazalez, 1996).

Activated sludge system and its application such as Pasveer Ditch system, Pure Oxygen Activated system, Deep Shaft system, Tower Biology System, Bioloke- B system and TSU system are well documented along with the evaluation of 'fill and draw' acclimatization (Vries *et al*, 1990). Operation of full strength activated sludge plants were reviewed with regard to theoretical consideration (Tench, 1994). Dyanamic modelling of the activated sludge was also attempted (Andrews,1994; Argaman, 1995). Also, the practice of manipulating activated sludge reaction environment to obtain maximum nitrogen removal has been optimized using cyclic activated sludge technology (Goronszy *et al*, 1997). A detailed study was conducted in two phases to monitor the effect of variation in hydraulic retention time and C/N ratio of the rheological characteristics of activated sludge (Sudhir Kumar and Gupta, 1998).

2.6 BIOLOGICAL COAGULATION OF SKIM LATEX

Reports on biocoagulation of skim latex are rather scanty. Skim latex is obtained along with the concentrated rubber latex (Radhakrishna Pillai, 1980). In acid coagulation, the acid content along with the coagulated rubber reduces its quality and shows some tendency to scroch (Naunton,1961). Further, acid coagulation leads to the generation of highly acidic latex centrifugation effluent (Jayachandran *et al*, 1994 a,b). Earlier, biological coagulation of Hevea latex was tried with the addition of waste carbohydrates which enhanced the natural coagulation of latex by indigenous microbial population and also led to the production of malodorous rubber (John, 1966). Incorporation of anionic surfactant reduced coagulation time considerably (John and Pillai, 1971).

However, these reports did not consider biocoagulation in respect of controlling pollution in the effluents, which is, infact, a major concern of waste disposal and environmental management. Further, no reports are available on the use of whole cells of microorganisms for biocoagulation purpose.

2.7. IMPORTANCE OF *ACINETOBACTER* SP

The genus *Acinetobacter* as originally proposed by Brisou and Prevot (1954) comprises a heterogeneous collection of bacteria which differ phenotypically and genotypically. The availability of a strain of *Acinetobacter* competent for transformation has made it possible to demonstrate the genetic relatedness of a large variety of gram negative, oxidase negative bacteria (Elliot, 1972). Genetics and physiology of *Acinetobacter* sp was also reviewed (Elliot, 1978). The protential of the *Acinetobacter* sp for polyhydroxybutyrate production (Rees *et al*, 1993) and also for phosphate removal (Duncan *et al*, 1988) were reported. Characterisation of *Acinetobacter* type strains and isolates obtained from wastewater treatment plant was attempted by PCR - Fingerprinting technique (Wiedmann *et al*, 1994). The growth and viability determination of the immobilized *Acinetobacter* cells (Muyima and Cloete, 1995) and metabolism of the *Acinetobacter* cells responsible for enhanced biological phosphorous removal from wastewater(Van *et al*, 1997) has been reported. Members of the genus *Acinetobacter* are a subject of intense research, because these ubiquitous organisms are gaining increasing importance in medicine, biotechnology and environment (Wiedmann *et al*, 1994)

CHAPTER - 3

TREATMENT OF THE RUBBER LATEX CENTRIFUGATION EFFLUENT USING IMMOBILIZED *ACINETOBACTER* SP. BTJR-10 IN PACKED BED REACTOR

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3.1 INTRODUCTION

Immobilized cells have long been an integral part of wastewater treatment plants and processes developed for treating effluents containing organic pollutants. Both conventional methods of sewage treatment, trickling filter and activated sludge system involve the use of immobilized microbial cells, as biocatalysts, for the removal of carbonaceous and nitrogenous wastes. In trickling filters the cells are immobilzed in their own polymer matrix on to surface, usually of stone or manmade plastic, in the form of 'biofilm. In an activated sludge system, cells bind to each other in a polymer matrix through physical force interaction, to form 'flocs'. The end result, in both cases, is that cells are retained *in situ* at high cell densities so that even at high flow rates an effective treatment of sewage is made possible.

Immobilization of microbial cells represents the transfer of cells from a free state to a state in which they are confined and localized in a certain defined region of space with the retention of catalytic activity. So that the cells can be used repeatedly and continuously (Klein and Wagner, 1983). Use of immobilized cells has many advantages

over the use of freely suspended cells. Important among these are the capability of reusing immobilized cells and the ease with which the cells can be seperated from the reaction mixture, thus preventing contamination of the product stream. Other advantages include the ability to disperse the cells evenly by immobilization so as to minimize diffusional restriction on the rates of reaction, and the ease with which immobilized cells can be used to exploit the kinetic features of continuously stirred and packed bed types of reactors. Minimization of product inhibition is a feature of the packed bed reactor and it allows fully continuous process thereby avoiding the changing condition associated with batch operation or with the use of free cells. These advantages also usually result in an increase in the volumetric activity of the system.

Of the several methods of immobilization of cells available, the most popular approach is to entrap the cells in polymeric materials, such as alginate, carrageenan or polyacrylamide. Owing to the toxicity and detrimental effect of polyacrylamide on cell viability, natural algal polysaccharides such as alginate and carrageenan have been the polymers of choice for microbial cell immobilization (Cheetham and Bucke, 1984).

Entrapment with calcium alginate is one of the simplest method of immobilizing cells and it is widely employed in laboratory and pilot scale studies including the construction of reactors for waste degradation. Although it is not a perfect immobilization method, when compared with other methods, yet it appears to be one of the best (Cheetham and Bucke 1984).

Packed bed reactor is characterised by small size and high productivity even in the presence of product inhibition, low void volume, and ease of automation. However, it do suffer from a number of disadvantages. Lack of easy access can make catalyst replacement awkward and environmental control difficult. Fabrication and commissioning cost are high although running cost may be low. Particulate, colloidal, or high viscosity substrate streams tend to block packed bed rector, and, in addition, unless the catalyst is incompressible, channelling or blocking of flow through the catalytic bed may occur. Any mixing is dependant upon the flowrate and thus such reactors are susceptible to diffusional limitation as a result of poor mixing. Increasing flow rate to overcome this problem will reduce productivity and conversion, unless either the reactor length is increased or some recycling of the stream is introduced.

Large amount of the organic matter and other contaminants in waste water are associated with particles which have a negative effect on biodegradation. Hence, it seems reasonable to begin with particle separation process (Odegard, 1988).

Sedimentation, of course, is the most commonly used sewage separation unit process, both for pre-treatment and in the later stages of the process train. Sedimentation is however only effective for the removal of particles larger than about 50 μm on a practical basis. A major part of the particles, larger than 1μm may however be separated by sedimentation subsequent to coagulation.

In coagulation operation, a chemical substance is added to an organic colloidal suspension to cause its destabilisation by the reduction of forces that keep them apart. It involves the reduction of surface charges responsible for particle repulsion, which causes agglomeration. Particles of larger size are then settled and clarified effluent is obtained. Several substances may be used as coagulants such as aluminium sulphate ferric chloride or ferric sulphate as well as organic coagulants (Nishide 1977; Johnson and Gallager, 1984; Ziminska, 1985). Fish scales are reported to be used effectively as an organic wastewater coagulant. (Hood and Zall, 1980).

The coagulation process often referred to as 'chemical treatment' involves coagulation of particles, precipitation of soluble substances like phosphates and a flocculation step during which the coagulated particles are aggregated as flocs and later the flocs are removed by sedimentation process.

It was observed earlier that the rubber latex centrifugation effluent could be treated in a packed bed reactor containing immobilized *Acinetobacter* sp. BTJR-10 under continuous process (Jayachandran *et al*, 1994 a). During the operation the percentage of reduction in COD was 44%. Whereas the efficiency decreased on repeated cycles probably due to the high amount of suspended solids in the effluent which might have clogged the pores of the immobilized beads on subsequent passage of the effluent. Hence, an initial coagulation was carried out with a view to reduce the high amount of suspended solids and improve the efficiency of treatment by immobilized viable cells (Jayachandran and Chandrasekaran, 1997)

3.2 MATERIALS AND METHODS

3.2.1 *RUBBER LATEX CENTRIFUGATION EFFLUENT*

3.2.1.1 *Source*

The raw effluent was collected from a local latex centrifugation plant where, the average rate of generation of the effluent was 6,000 l/day.

3.2.1.2. *Collection and Transportation of the Sample*

The samples were collected from the settling tank, in the early hours of the day, when the wastewater from the skim latex coagulation unit was pumped into it. Samples for microbiological analysis, and for the physico-chemical studies and other experiments were collected respectively in sterile containers and 10 litre carboys, and were transported to the laboratory immediately. The microbiological analysis was done within 3 hours of collection. The samples were maintained at 4°C for minimizing the change in physico-chemical properties, until used.

3.2.1.3 *Characteristics of the Wastewater*

3.2.1.3.1 Colour and Odour

Colour and odour of the effluent samples were assessed by sensory evaluation, immediately after collection, in the field itself.

3.2.1.3.2 *Temperature*

Temperature of the samples were recorded immediately, after the collection , using a sensitive (1/10) thermometer (0-110°C)

3.2.1.3.3. *Total Solids (TS)*

Total solids (both dissolved and suspended solids) present in the effluent were determined based on the methods suggested by APHA (1989).

3.2.1.3.4 *Total Suspended Solids (TSS)*

1. A known volume of sample (100ml) was filtered through a tared gooch crucible ignited to constant weight (W_1) and the crucible was dried at 130°C for one hour.

2. Later, the crucible with the contents was cooled in a dessicator and the weight of the crucible was recorded (W_2).

3. Suspended solids were calculated using the formula

$$\text{Total suspended solids (mg/l)} = \frac{W_2 - W_1 \times 10^6}{\text{Volume of sample}}$$

3.2.1.3.5. Total Dissolved Solids (TDS)

1 A known volume of filtrate (100ml) obtained from the above experiment was taken in a tared dish ignited to constant weight (W_1).

2 The dish with the contents was dried at 130°C for 1h, cooled in a dessicator and weighed (W_2).

3. Dissolved solids were calculated using the formula.

$$\text{Total dissolved solids (mg/l)} = \frac{(W_2-W_1) \times 10^6}{\text{Volume of sample}}$$

3.2.1.3.6. pH

Measurement of pH was carried out using a pH meter (Systronics digital pH meter).

3.2.1.3.7. Dissolved Oxygen (DO)

Dissolved oxygen was determined by the azide modification of Winkler's method (APHA 1989).

1. As soon as the water samples were collected in the BOD bottles, 2ml each of manganese sulphate and alkaline azide solution were added, closed without any air bubble and mixed thoroughly.

2. To the bottle containing well settled precipitate, 2ml of con H_2SO_4 was added, and mixed gently so that the precipitate was dissolved completely.

3 100ml of the solution was then titrated against standard sodium thiosulphate solution using starch as indicator.

4 The endpoint was the disappearance of blue colour.

5 The DO was calculated using the following formula.

$$\text{DO in mg/l} = \frac{K \times \text{Vol. of } Na_2SO_3 \times \text{N. of } Na_2SO_3 \times 1000 \times 8}{\text{Volume of sample}}$$

$$K = \frac{\text{Volume of the bottle}}{\text{Volume of bottle - Volume of reagent added}}$$

3.2.1.3.8. Biochemical Oxygen Demand

Biochemical oxygen demand was estimated according to APHA (1989).

1. The collected samples were diluted before incubation to bring the oxygen demand and supply into an appropriate balance. One litre of distilled water was mixed with nutrients: 1ml each of buffer, calcium chloride, magnesium sulphate and ferric chloride.

2. Samples were neutralized to pH 6.5-7.5 with 1 M H_2SO_4 or1 M NaOH.

3. The DO of the sample was determined initially and after 5 days of incubation in a BOD incubator at 20°C.

4. A blank was also carried out simultaneously.

5. The BOD_5 was then calculated by the following formula.

$$BOD_5 \text{ at } 20^{\circ}C \text{ in mg/l} = (D_0-D_5) - (C_0-C_5) \times \text{Dilution factor}$$

$$\text{Dilution factor} = \frac{1000}{\text{Vol.of sample}}$$

where D_0 - DO content of the sample on the 1st day

D_5 - DO content of the sample on the 5th day

C_0 - DO content of the blank on the 1st day

C_5 - DO content of the blank on the 5th day

3.2.1.3.9. Chemical Oxygen Demand (COD)

Chemical oxygen demand (COD) was determined following APHA (1989)

1 10 ml of the sample was diluted to 500ml using distilled water.

2. 50 ml of the diluted sample was taken in a round bottamed flask for COD determination.

3. 1g $HgSO_4$ was added to the above sample to overcome the difficulties caused by chloride ions.

4. 5ml of con H_2SO_4 was added to dissolve the $HgSO_4$.

5. 1 g of $HgSO_4$ was then added to the above mixture as a catalyst.

6. To the above solution 25ml of 0.25N, Pottassium dichromate was added.

7. The R.B. flask was attached to the condenser and the water was allowed to flow.

8. 70ml of con H_2SO_4 was added through the open end of the condenser and swirling was continued while the acid was being added.

9. The contents in the flask were refluxed for 2h, cooled, washed out into a 500 ml and was suitably diluted and made upto 140ml.

10. 3-4 drops of ferroin indicator was added and the contents were titrated against ferrous ammonium sulphate (0.25N).

11 The endpoint of the titration was the first sharp colour change from blue-green to reddish brown.

12. A blank was also run simultaneously in the same manner using distilled water .

13. The COD was then calculated using the formula

$$\text{COD mg/l} = \frac{(A-B) \times N \text{ of } Fe(NH_4)_2SO_4 \times 8 \times 1000}{\text{Volume of sample}}$$

where A - Volume of $Fe(NH_4)_2 SO_4$ consumed for blank (ml)

B - Volume of $Fe(NH_4)_2SO_4$ consumed for sample (ml)

3.2.1.3.10 *Total Nitrogen*

Total nitrogen was estimated by adding together the organic nitrogen and ammoniacal nitrogen contents, which were determined as mentioned under sections 3.2.1.3.11 and 3.2.1.3.12 respectively and expressed as mg/l.

3.2.1.3.11. Organic Nitrogen

Organic nitrogen was determined according to APHA (1989) employing the Kjeldahl method .

1 100ml of the sutably diluted sample was taken in a 300 ml Kjeldahl flask and added with 4ml of Conc. H_2SO_4, 10 drops of 10% $CuSO_4$ solution (0.3ml) 10g of solid K_2SO_4 and 1ml of 10% NaCl solution. Then the flask was heated to avoid loss through fuming.

2 When water was boiled off, the sample become dark due to the decomposition of organic matter by Conc. H_2SO_4. As the digestion proceeded the colour of the sample turned green. The heating process was continued further for 30 minutes.

3. The flask was cooled and the volume was made upto 100ml

4. 20 ml of the digested mixture was taken in a distillation flask and 10ml of 10N NaOH was added.

5. 5ml of boric acid mixed indicator was added to the steamed out end of the distillation flask. (4% solution of boric acid was prepared by dissolving 4g of boric acid in 100ml warm distilled water. An alcoholic solution of bromocresol green (0.5%) and methylred was mixed in 2:1 ratio. 5ml of mixed indicator was then added to 100 ml of boric acid). When the colour turned blue, the pH was adjusted with 0.0IN HCl until the colour changed to faint pink. The distillate was collected through the outlet upto a total volume of 25ml in the container.

6 When the solution turned blue, due to dissolution of ammonia, it was titrated with 0.01N HCl.

7 At the endpoint, when the colour turned to brown, the volume of HCl was noted. The same procedure was followed for distilled water which was used as a blank.

The calculation was done as mentioned below

$$\text{Kjeldhal - N mg/l} = \frac{(a-b) \times \text{N.of HCL} \times 1000 \times 14 \times d}{\text{ml of sample distilled}}$$

Where a - ml of HCl used with sample

b - ml of HCl used with distilled water

d - is dilution factor

3.2.1.3.12 *Ammoniacal Nitrogen*

Ammoniacal nitrogen in the sample was estimated following the method of APHA (1989).

1. 100ml of the suitably diluted sample was taken in the distillation flask and 1ml of borax buffer solution was added.

2. Steam was generated in the boiling flask

3. 10ml of boric acid mixed indicator solution was taken in 100 ml flask and was placed below the condenser.

4. Ammonia was distilled off and was absorbed in the boric acid mixed indicator solution. The distillation was continued until nearly 40 ml of distillate was collected. The solution then turned into blue colour due to absorption of NH_3.

5. The distillate was cooled and titrated against 0.0IN HCl. At the end point, the colour changed to faint pink. The volume of HCl used was noted.

Calculation was done as follows.

$$\text{Ammoniacal nitrogen mg/l} = \frac{(a-b) \times \text{N. of HCl} \times 1000 \times 14}{\text{ml of sample}}$$

a - ml of HCl used with sample

b - ml of HCl used with blank

3.2.1.3.13. Protein and Total Sugar Estimation

Protein was estimated employing Lowrys method (1951) and total sugar was estimated by phenol sulphuric acid method (Dubois et al, 1956).

3.2.1.3.14. Total Heterotrophic Bacterial Population (THB)

Total heterotrophic bacterial population present in the freshly collected effluent samples was enumerated as colony forming unit (CFU) employing the standard pourplate technique.

3.2.2. MICROORGANISM

Acinetobacter sp. BTJR -10 isolated from the latex centrifugation effluent and available in the culture collection of the Department of Biotechnology, CUSAT, Cochin-22 was used in the present study. The strain was maintained on Nutrient Agar. (High Media) (Jayachandran *et al*, 1994 a, b).

3.2.3 *PREPARATION OF IMMOBILIZED VIABLE CELLS (IVC)*

Bacterial cells were immobilized by gel entrapment method using sodium alginate (Mohan dass 1992; Jayachandran *et al*, 1994 a) as detailed below:

1. 100 ml of sodium alginate solution was prepared by slow addition of the 4 g of dry powder to the distilled water, while being stirred. The stirring was continued for a further period of one hour and warmed at 60°C to ensure camplete dissolution of the powder. The solution was then left to stand for about an hour to allow the air bubbles to escape.

2. Under sterile conditions, 20ml of the prepared cell slurry (1.0 OD)was mixed with 40 ml of sodium alginate solution gently at 1:2 ratio.

3. This sodium alginate cell slurry mixture was then extruded dropwise, through a 10 ml syringe from a height of about 10cm into an excess 0.2 M $CaCl_2$ solution, taken in a large beaker.

4. Beads (4mm diameter) formed of calcium alginate entrapped with cells were allowed to remain for hardening of beads in the $CaCl_2$ solution for 1 hour.

5 After washing with physiological saline three times, the beads were used for further studies.

3.2.4 *ACTIVATION OF IMMOBILIZED VIABLE CELLS*

The immobilized viable (IVC) beads were activated for achieving maximal activity using the latex effluent (Mohandass, 1992). Prepared immobilized cell beads were taken in large 500ml beaker and immersed with latex effluent for varying time intervals. Optimal activation time that promoted maximal activity, by immobilized cells was determined in terms of percentage reduction of COD which was estimated as mentioned under section 3.2.1.3.9.

3.2.5 *RETENTION TIME*

Optimum retention time required for maximal activity was estimated by incubating the immobilized cells in beads with latex effluent for varying periods and tested their

activity in terms of percentage reduction of COD, estimated as mentioned under section 3.2.1.3.9.

3.2.6 *TREATMENT OF EFFLUENT USING IMMOBILISED ACINETOBACTER SP. UNDER CONTINUOUS PROCESS IN A PACKED BED REACTOR*

Immobilized cells in calcium alginate beads were packed to a height of 150 mm in a glass column (Dia, 41mm) with glass wool at the bottom and stabilized with latex effluent. The air bubbles formed during packing were eliminated by gently tapping the glass column and the void volume in the packed bed reactor was determined. After activating the beads for optimum time (optimised as mentioned under sectoion 3.2.4) with latex effluent, the immobilized cells in the packed bed reactor were exposed to latex effluent at the rate of '7ml/h' using a peristaltic pump (Miclins, India). Effluent samples were analysed for residual COD at regular intervals as detailed under section 3.2.1.3.9 The results are expressed in terms of percentage reduction in COD (Jayachandran *et al*, 1994 a).

3.2.7. *CHEMICAL COAGULATION OF THE EFFLUENT*

The effluent was subjected to chemical coagulation using aluminium sulphate, alum and ferric sulphate at different dosages (2-6g/l) and at pH 3 (pH of the effluent) and at pH.7 (Vermani and Narula, 1989). The efficiency of the coagulation was expressed in terms of percentage reduction in COD (Madhu *et al*,1991).

3. 3 RESULTS

Fresh latex centrifugation effluent had high level of COD, BOD, Suspended solids, Total dissolved solids and a pH in the range 2.5-4.5 (Table 1.3) indicating a heavy organic load. *Acinetobacter* sp. isolated from the latex effluent was observed to be efficient in treating the same (Jayachandran *et al*, 1994 a). When the effluent was subjected to continuous treatment by immobilized *Acinetobacter* sp. in a packed bed reactor (characteristics given in Table 3.2) the percentage reduction in COD was 44%. Inspite of recycling of the effluent in the packed bed reactor, there was only a marginal enhancement of (49%) COD reduction (Table 3.3).

Hence, the effluent was subjected to an initial coagulation using different coagulants viz aluminium sulphate, alum and ferric sulphate at different dosages of (2-6 g/l) and at pH 3 and pH 7.

Coagulation with ferric sulphate and alum showed maximum efficiency at a concentration of 5g/l compared to aluminium sulphate which showed maximum efficiency at a concentration of 4g/l (Fig. 3.1). After optimizing the dosage of the coagulants, the coagulation was repeated at two different selected pH of '3' and '7'. At pH 3 (pH of the effluent) coagulation with alum showed a better COD reduction of 15% compared to 1% and 7% achieved, respectively, with aluminium sulphate and ferric sulphate. At pH 7, coagulation with ferric sulphate yielded an enhanced percentage of reduction (58%) compared with aluminium sulphate (36%) and alum (37%) (Table3.1).

When the effluent was treated with immobilized *Acinetobacter* sp. BTJR -10, in a packed bed reactor under continuous process, after an initial coagulation with alum at pH 3, an average of 50% COD reduction was achieved. Whereas, treatment after initial coagulation with ferric sulphate at 5 g/l and at pH 7 yielded a COD reduction of 77%. Hence ferric sulphate was selected as the suitable coagulant at a dosage of 5 g/l and at pH 7. A comparison of the average percentage reduction obtained during the different treatment processes is shown in Table 3.3.

3.4. DISCUSSION

The latex centrifugation effluent ihas a heavy organic load and high amount of suspended solids. It was observed that *Acinetobacter* sp. isolated from the latex effluent, can be used for the rapid treatment of the same (Jayachandran *et al* 1994 a). When the effluent was subjected to continuous treatment in the packed bed reactor containing calcium alginate immobilized *Acinetobacter* sp. 44% COD reduction was achieved in 6hr. Whereas further recycling of the effluent gave only 49% reduction in COD. This may be due to the clogging of the pores of immobilized beads which might have caused diffusional limitation. The high amount of suspended solids present in the effluent testify this fact. Infact an initial coagulation is an essential step in the efficient treatment of the effluents having high amount of suspended solids (Odegaard, 1988). Hence chemical coagulation was tried with three coagulants viz ferric sulphate, alum, aluminium sulphate.

During coagulation, the coagulant species are readily absorbed on to the surface of the suspended solids. This decreases the electrical charges of the suspended solids resulting in the destabilization of the particles followed by agglomeration. Mixing or turbulence promotes collision and results in enhanced coagulation. The visible result of coagulation is the formation of a deposit in the form of porous gelation flakes that settle at the bottom of the vessel.

A certain minimum dose of the coagulant must be added for effective coagulation. Hence, different dosages of 2 - 6 g/l were tried for all the selected coagulants. While the optimum dosage of aluminium sulphate was 4 g/l, it was 5 g/l for both ferric sulphate and alum. Usually additional dosage is required for increased turbidity, but the relation is not linear. While a smaller dose is required for very high turbidities with variable particle size, very low turbidities are difficult to remove. In addition the process is influenced by a number of interrelated factors such as pH. colour, mineral content and composition, temperature, duration and degree of agitation and the nature of coagulant used. Therefore, the optimum dose and condition can not be predicted on the basis of results of physical and chemical analysis of water and must be determined experimentally (Vermani and Narula, 1989).

'pH' is a critical factor which effects the coagulation process. Coagulation of the effluent was carried out at pH 3 and also at pH 7. pH 3 was that of the effluent and coagulation was tried at this pH to know whether the pH neutralisation step can be

avoided or not. However, the percentage of COD reduction obtained at pH 7 was relatively higher.

When the initially coagulated effluent was subjected to continuous treatment process. by *Acinetobacter* sp. BTJR - 10, under the same conditions of the previous study (Jayachandran *et al*, 1994a) the efficiency of the 'COD' removal from the effluent increased to 77% for coagulation with ferric sulphate at a dosage of 5 g/l and at pH 7. The coagulation process removed the suspended solids and hence, could have attributed to limited clogging of pores. Consequently this might have resulted in the enhancement of the reduction in COD.

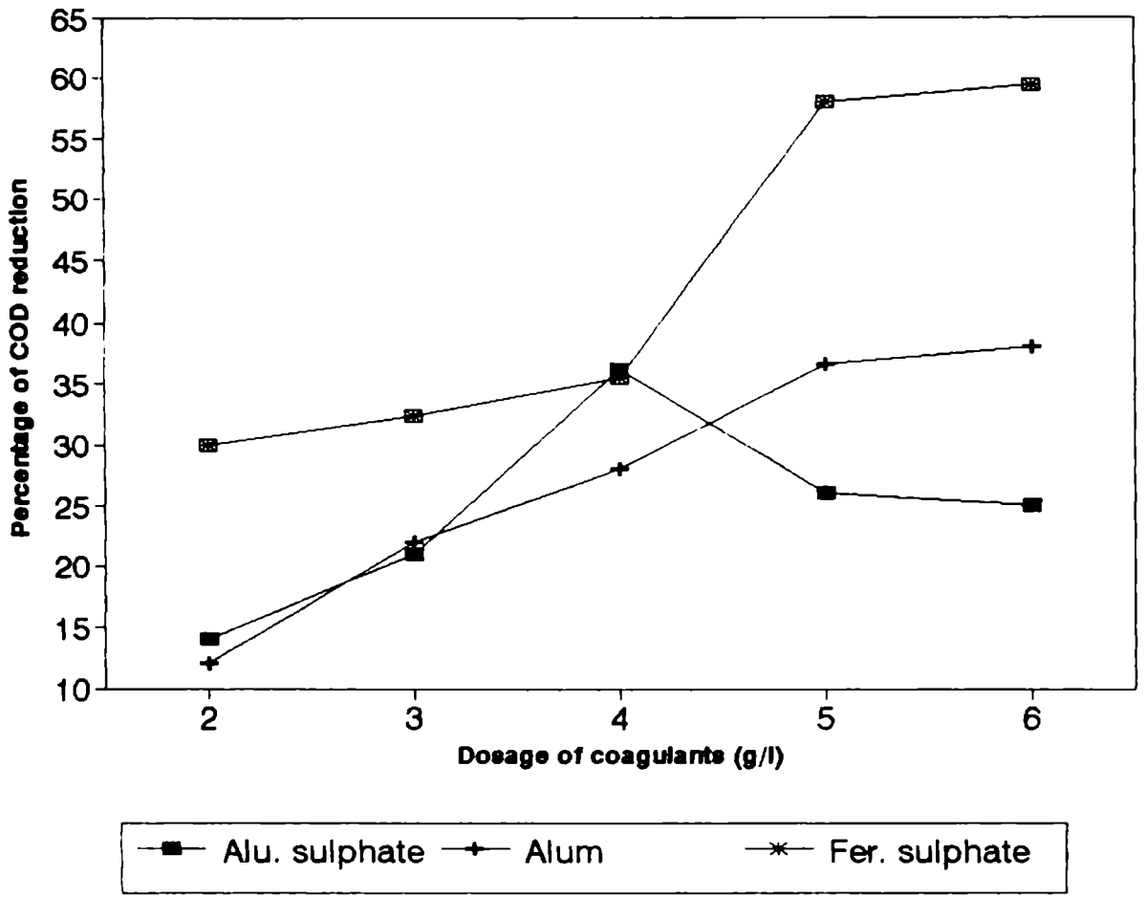


Fig. 3.1 Effect of different chemical coagulants on the reduction of chemical oxygen demand (COD) of the rubber latex centrifugation effluent.

Table 3.1 Percentage reduction in chemical oxygen demand (COD) of the effluent on chemical coagulation at different pH

Coagulants	Dosage used g/l	% reduction in COD	
		*pH 3	pH 7
Aluminium sulphate	4	1	36
Alum	5	15	37
Ferric sulphate	5	7	58

* pH of the raw effluent.

Table 3. 2 Characteristics of the packed bed reactor maintained during the continuous treatment of the effluent.

Diameter of the column	4.14 cm
Bed height	15 .00cm
Total volume	201. 8 cm^3
Void volume	50 .00cm^3
Size of the bead	4.00 mm
Number of beads (approximate)	5000

Table 3. 3 Effect of initial coagulation on the % reduction of chemical oxygen demand (COD) during the continuous treatment of the effluent using *Acinetobacter* sp. in a packed bed reactor.

Process adopted	*% reduction of COD obtained
Continuous treatment without initial coagulation	44
Continuous treatment with recycling and without coagulation	49
**Continuous treatment with an initial coagulation at pH - 3 using alum	50
***Continuous treatment with an initial coagulation at pH - 7 using ferric sulphate	77

* Mean of triplicate.

** At pH 3 alum was found to be better coagulant.

*** At pH 7 ferric sulphate was found to be better coagulant.

CHAPTER - 4

TREATMENT OF THE RUBBER LATEX CENTRIFUGATION EFFLUENT IN ROTATING BIOLOGICAL CONTACTOR (RBC)

CHAPTER-4

TREATMENT OF RUBBER LATEX CENTRIFUGATION EFFLUENT IN ROTATING BIOLOGICAL CONTACTOR (RBC)

4.1 INTRODUCTION

The Rotating Biological Contactor (RBC) consists of closely placed circular discs that are partially submerged in the wastewater. Discs are mounted on a shaft usually running horizontal with the tank's flow direction. These discs, usually made up of an inert material provide support media for biomass. Discs rotate slowly, alternatively contacting biomass with waste water and then with gas phase. The rotation also creates shearing forces for removing excess solids from the discs. The sloughed solids are maintained in suspension, so they can be carried from the unit to a clarifier. RBC units can be compartmentalized with baffles which can be fixed at the bottom of the vessel. RBC can provide high treatment efficiencies with lower energy input than suspended biomass system, such as activated sludge system. Since aeration does not occur by air bubbling, foaming and floating, sludge problems are essentially eliminated. (Cookson, 1994).

The main advantages offered by RBC are short residence times, low operation and maintenance costs and production of a readily dewatered sludge that settles rapidly(Weng and Molof,1974).

When the RBC- discs are rotated at a low RPM there is an initial adsorption of microorganism to the discs surface to form (1-4) mm thick biofilm that is responsible for the organic removal. Biofilm which develops an RBC comprises a complex and diverse microbial community made of eubacteria, filamentous bacteria, protozoa and metazoa. Commonly observed filamentous organisms include *Sphaerotilus*, *Beggiatoa*, *Nocardia*, and filamentous algae such as *Oscillatoria* (Pretorius, 1971; Torpey *et al*, 1971; Hitdlebaugh and Miller,1982). Biofilm examination by transmission electron microscopy showed that *Sphaerotilus* contained many poly-B-hydroxybutyrate inclusions and storage of excess carbon by the bacteria. These inclusions accounted for 11% to 20% of the dryweight of thin bacteria (Rouf and Stokes, 1962). Scanning electron microscopy showed that the RBC biofilm was composed of two layers; an outer whitish layer containing *Beggiatoa* filaments and an inner black layer containing *Desulfovibrio*, a sulfate reducing bacteria (Alleman *et al*, 1982).

The microbial succession on RBC surface is similar to that observed in activated sludge (Kinner and Curds, 1989). Bacterial colonization is followed by protozoan flagellates and small amoeba, free swimming bacteriavorous ciliates, nematodes, stalked ciliates and rotifers. After reaching a certain thickness the biofilm sloughs off and the sloughed material ultimately reaches the final clarifier.

The rotating biological contactor is essentially a plug flow reactor without recycling. The substrate removal is effected due to microbial metabolism, both in film phase and . mixed liquid phase. However, the mixed liquid suspended solid concentration

in the liquid phase of the reactor is always very low, and the hydraulic retention time is very short. Interest in the application of rotating biological contactors (RBCs) for the aerobic biological treatment of wastewater has been growing markedly since its first application. The principal reasons are the operational simplicity, robustness and low energy consumption of the system. However with the increasing demand for a pollutant-free environment; optimization of the RBC performance has become an important goal. In the present study, a laboratory scale rotating biological contactor was designed and treatment of the effluent is tried with a view to achieve effective, economic and large scale treatment of the effluent.

4.2 MATERIALS AND METHODS

4.2.1 *ROTATING BIOLOGICAL CONTACTOR (RBC)*

Rotating biological contactor was designed such that it can be rotated at low RPM (1-10) through the effluent, allowing only 40% of the discs surfaces to be under submerged condition.

Stainless Steel (S.S) rod of 8mm dia was used as the shaft. The bushes which are to be placed in between the discs were made up of medium steel (M.S) rod of 20mm dia and 1cm length. The internal dia of the bushes was selected so as to fit for 8mm dia shaft. The bushes were tightened to the shaft by means of two brass screws and to the corresponding discs by a lateral screw.

Circular discs were made up of perspex glass of 3mm thickness and 25cm dia. The discs were rubbed uniformly with emery paper of standard size: Emery paper '80', '50' and '36' were used to achieve minimum, medium and maximum roughness. The discs were arranged on the shaft with bush of 1cm in between them.

The half cylindrical vessel was also made up of perspex glass of 2mm thickness. Perspex glass of less thickness was selected as it was more easy to bend the sheet, under heat treatement. After bending the sheet to the cylindrical shape, stripes of 6mm thickness perspex glass were closely pasted in a lengthwise manner along the bottom of the vessel in order to increase the strength. The vessel was of 30cm in length and 26cm in width and was divided into four compartments of equal size by means of baffles of 3mm thickness and 8cm height.

The shaft with the closely placed discs was fitted to the vessel in such a way that there were five discs per compartment (Plate 1). The height of the shaft was 13cm above the bottom of the vessel. The distance between the discs, between the disc and the sides of the vessel and between disc and baffles were all set at 1cm

A drainage tap with regulatory valve was fitted to the bottom of the vessel. The inlet and outlet were positioned in such a way to get a 40% contact between the disc and effluent. The exact position corresponding to 40% contact was calculated as follows;

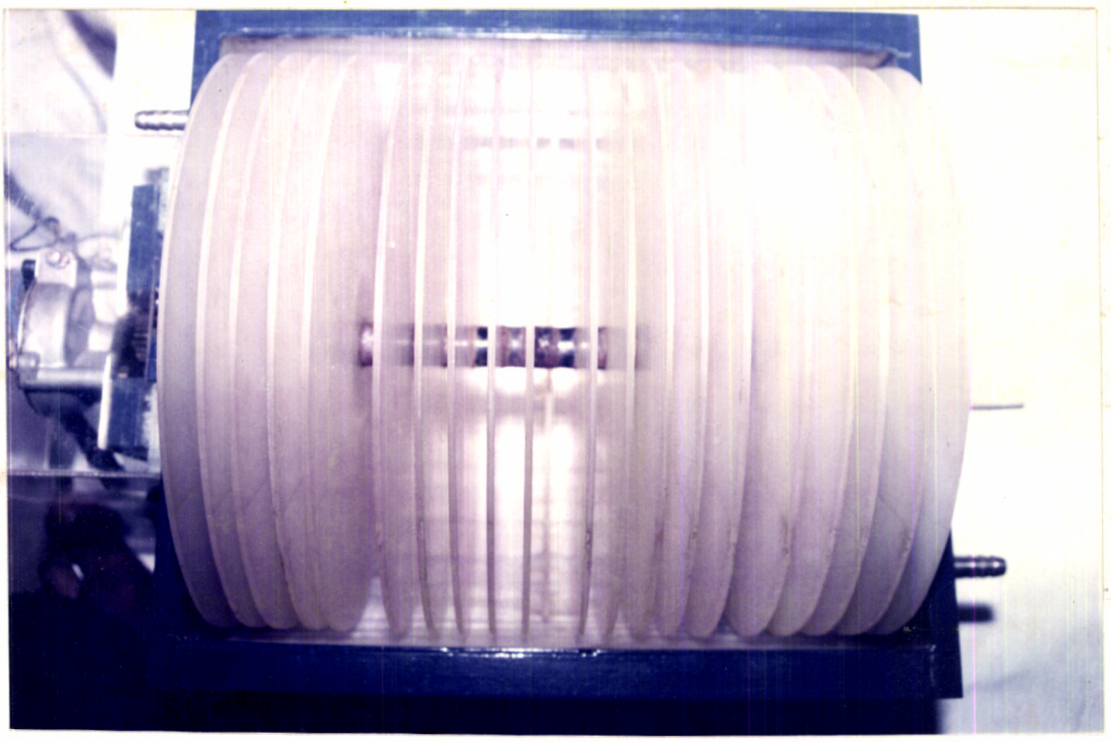
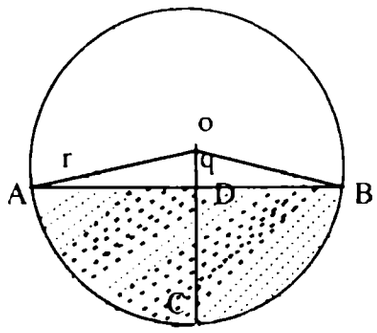


Plate 1 Photograph of the Rotating Biological Contactor (RBC) showing the shaft with closely placed discs



r = 25 / 2 = 12.5cm

Area of the circle = πr^2

Area of the shaded portion = 0. $4\pi r^2$

ie, ADBC

Let it be 40% of the disc

Area of the ΔOAB = ½ AB x OD

= AD x OD

= r Sinθ /2 rCosθ /2 = r^2 Sinθ /2 Cosθ /2

= r^2 / 2 Sinθ

'θ' angle in radius

Sinθ /2 = AD/r AD = r Sinθ /2

Similarly OD = ? $\quad \text{Cos}\theta/2 = OD/r$

$\quad OD = r\,\text{Cos}\theta/2$

Area of the sector OACB = $\pi r^2\theta / 2\pi$

$= \theta r^2 / 2$

Area of OABC = Area of OAB + Area of ABC

$= r^2 / 2\,\text{Sin}\theta + 0.4\pi r^2$

ie, $\theta r^2 / 2 = 0.4\pi r^2 + r^2 / 2\,\text{Sin}\theta$

$\theta = 0.8\pi + \text{Sin}\theta$

By integration θ = 161.85° (2.8248 radius)

$OD = r\,\text{Cos}\theta/2 = 25/2 \times \text{Cos}161.85/2$

$= 1.9716\text{cm}$

Hence the exact 40% is 1.9716 cm below the position of the shaft.

The shaft with the discs was fitted to the trough through bearings with a 'pushfit arrangement' on both the ends. One end of the shaft was connected to a motor (Maruthi Wiper motor) with a reducing gear so as to get the RPM in the range 1-10 (Plate 2).

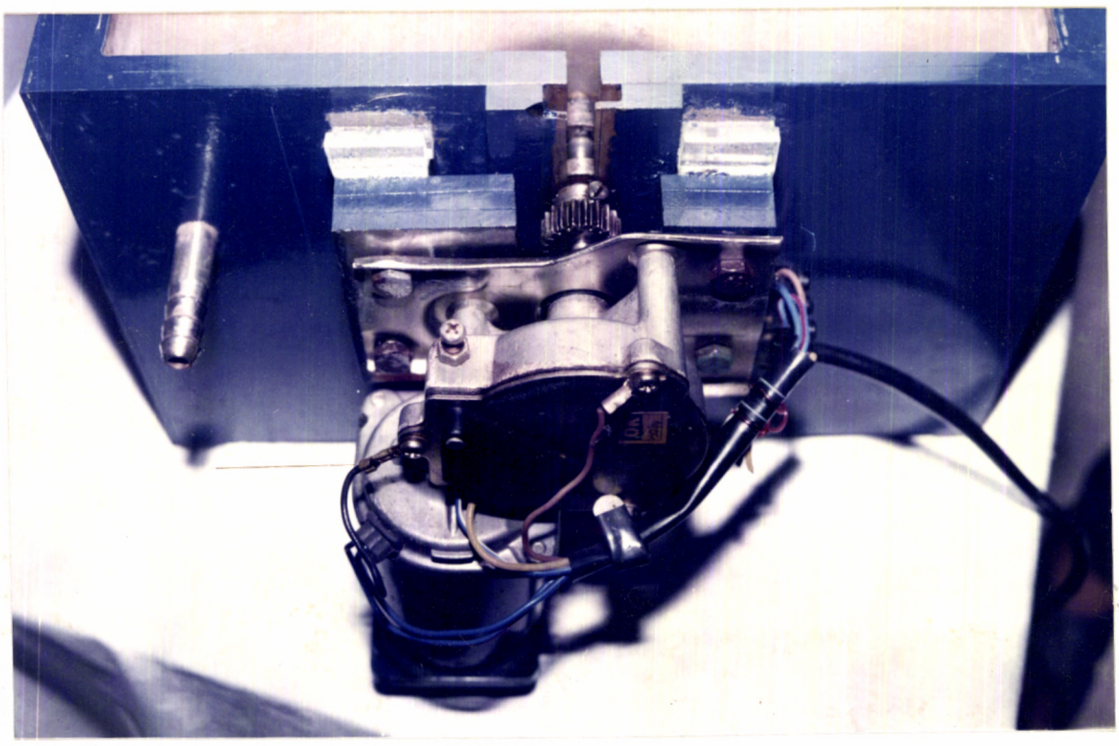


Plate 2 Photograph of the Rotating Biological Contactor (RBC) showing the motor, gear system and push-fit arrangement of the shaft.

The pushfit arrangement and the connection to the motor were fabricated in such a way that the shaft with the discs can be very easily lifted up from the vessel at any time. The entire motor, gear and other electrical connections were enclosed using perspex glass cover of 2mm thickness. This was to protect the motor and other electrical connections from any spillage of the effluent during the operation of the reactor. The cover was placed in suitably made sockets, for convenient removal of them, whenever it was necessary.

Electronic control unit was also fabricated with provisions for regulating RPM, on/off switch, forward/backward rotation selector, and low speed/high speed selector. The entire assembly of the rotating biological contactor along with the control unit is shown in the plate 3.

A small circular disc of 5cm dia was fitted to the RBC disc, towards the edge through brass screws, for easy biomass estimation (attached disc). The ratio of the available area of one disc surface to the area of the attached disc was also noted. This fabricated RBC was pretreated with dilute acetone solution and was repeatedly (5 times) washed with distilled water.

4.2.2 *PRE-RUN OF THE REACTOR*

RBC was set into operation in aqueous medium at different RPMs in the range 1-10 and at different time intervals and the change in the dissolved oxygen of aqueous

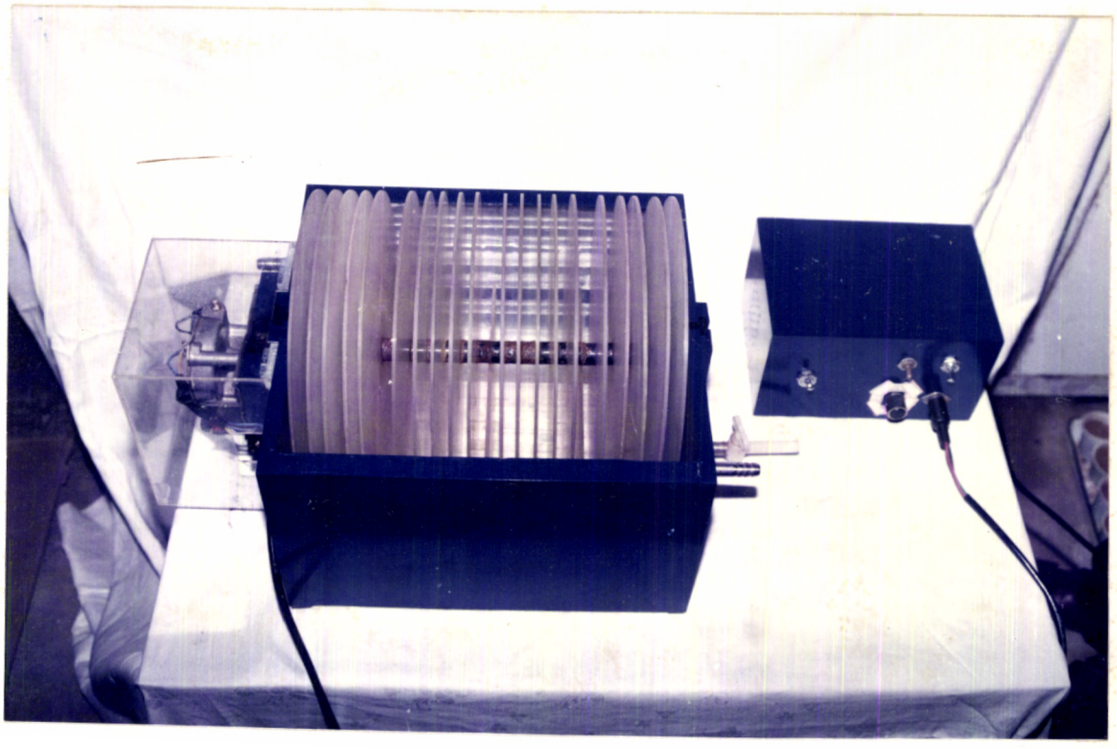


Plate 3. Photograph showing the assembly of Rotating Biological Contactor (RBC) along with the control unit.

solution was noted continuously. Later, RBC was set into operation in uninoculated rubber latex centrifugation effluent, at different RPMs in the range 1-10, and the percentage reduction in COD was recorded as mentioned under section 3.2.1.3.9.

4.2.3. *INOCULUM*

4.2.3.1. Mixed Culture Seed inoculum

'Mixed culture seed inoculum' was prepared from the same rubber latex centrifugation effluent, after subjecting it to vigorous aeration for 30 days with the supply of synthetic waste containing glucose and other growth nutrients. Later, the effluent was subjected to filtration (Whatman filter paper. 42) and the filtrate was used as the ' mixed culture seed inoculum' for treatment of the effluent with RBC.

4.2.3.2. Acinetobacter sp. BTJR - 10

Source of the organism is as explained under section 3.2.2

4.2.4. *BIOMASS ESTIMATION*

The reactor was operated in effluent with mixed culture seed inoculum, overnight, at selected RPM of '2' and the biomass on the disc was estimated. Different methods, such as 'scrapping', 'cotton swabbing', and 'attached disc' (Zahid and

Ganczarczyk, 1993) were employed to collect the biomass from RBC discs. Biomass developed on the RBC discs was estimated in terms of dry cell weight. In scrapping and cotton swabbing method, biomass from a uniform circular area of 5cm diameter (6 samples from 6 different locations) was either scrapped out or subjected to cotton swabbing, dipped into sterile physiological saline and washed with the same. The cell suspension was filtered through a preweighed millipore filter (2mm), dried to constant weight and weighed. In the attached disc method, after overnight incubation the attached discs were removed and the biomass was scrapped as detailed above, followed by washing with sterile saline and estimation in terms of dry cell weight.

The above methods were compared and the one which was more convenient, accurate and gave more consistant result was selected as the most suitable method.

4.2.5 *OPTIMIZATION OF THE CONDITIONS FOR MAXIMUM BIOMASS FORMATION ON THE RBC DISC*

The factors affecting the formation of biomass on the RBC discs such as pH, rpm, incubation period and also surface nature of the disc were studied by operating the RBC in effluent with 'mixed culture seed inoculum'.

Optimal pH for maximal biomass adhesion on the RBC disc was determined by operating the reactor at various pH of the effluent (pH 3, 7, 10, and 12) with mixed

culture seed inoculum. The rpm was maintained at '2' and the operation was continued for 48h.

Effect of rpm on biomass formation was also studied by operating the reactor with mixed culture seed inoculum, at various rpm such as 1,2,3, and 4, at optimized pH of 7 for 48h.

Effect of incubation period on the biomass formation was studied by operating the reactor with mixed culture seed inoculum, at optimised pH of 7 and also at 2 rpm for 24 h, 48h, 72h and 96h.

Nature of the disc surface is also an important factor which effects the biomass adhesion on the disc. The reactor operation was carried out with mixed culture seed inoculum at minimum roughness (MiR), medium roughness (MeR) and at maximum roughness, (MaR) at the optimized conditions of pH, RPM and incubation period (Plate 4). In all the cases the biomass was estimated using attached disc method as mentioned under the section 4. 2. 4 .

4.2.6. *EFFLUENT TREATMENT IN RBC*

Treatment of the rubber latex centrifugation effluent (pH was brought to 7 before treatment) was done with Rotating Biological Contactor using mixed culture seed inoculum and also with *Acinetobacter* sp. BTJR - 10 separately at all the three roughness

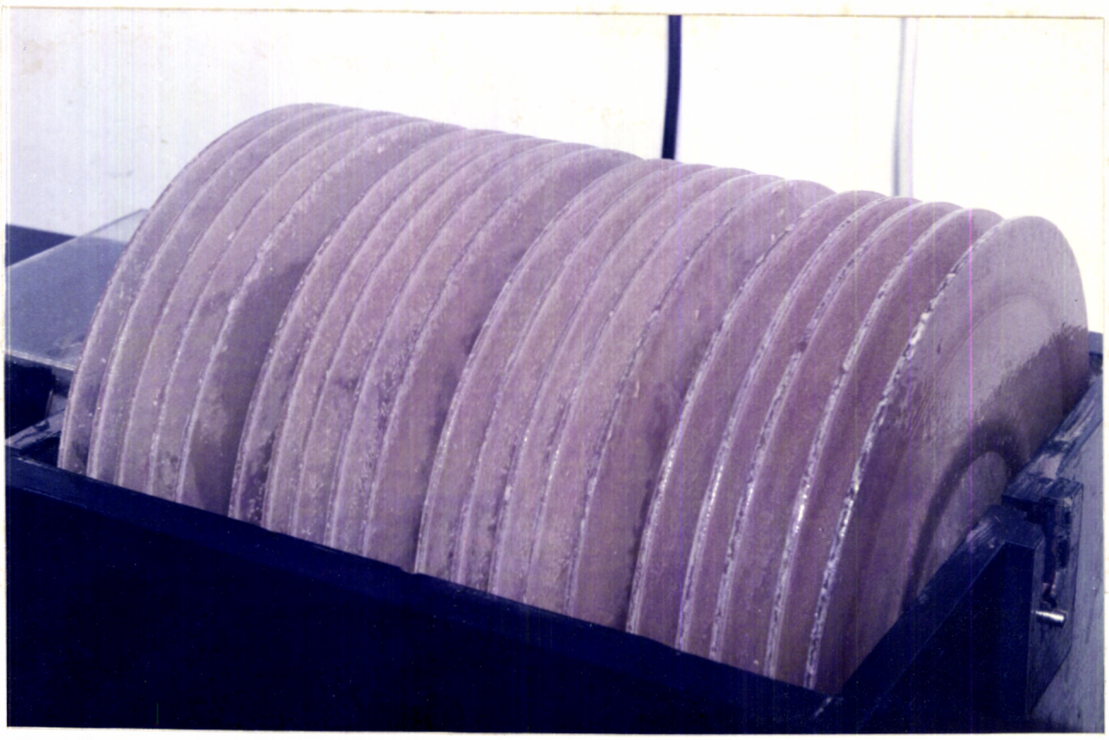


Plate 4. Photograph of Rotating Biological Contactor (RBC) showing the biofilm on the disc after attaining steady state with mixed culture seed inoculum at maximum roughness.

of the discs (MiR, MeR, MaR). The RBC was filled with the effluent, inoculated and was operated at optimum conditions of pH 7, Rpm 2, and incubation period 48h, for maximal biofilm formation. Later, steady state situation was achieved after operating the reactor continuously for further 10 days. The concentration of organic carbon was maintained in terms of COD, at an average organic loading rate of 15g COD/m^2/day corresponding to a hydraulic loading rate of 0.5m^3/m^2/day. Reduction in COD was monitored everyday till steady state was reached.

After attaining the steady state, the fresh effluent was passed into the reactor at different flow rates of 5ml/min, 10ml/min and 15ml/min and the percentage reduction in COD of the effluent after the corresponding retention time of 16.3h, 8.3h and 5.5h was calculated. The same experiment was repeated at all the three MiR, MeR and MaR roughness of the disc separately with mixed culture seed inoculum and *Acinetobacter* sp (Plate 5).

4.3 RESULTS

RBC designed in this study was of 5 l capacity and consisted of four compartments of equal size divided by bottom placed baffles of 8cm height. The shaft with rotating discs were connected to the reactor in such a way that there were '5' discs per compartment at 1cm apart. Thus, totally there were 20 discs and the distance between the discs and baffles, and also between the discs and the sides of the reactor were set at 1cm distance. Only 40% of the rotating disc was in contact with the effluent

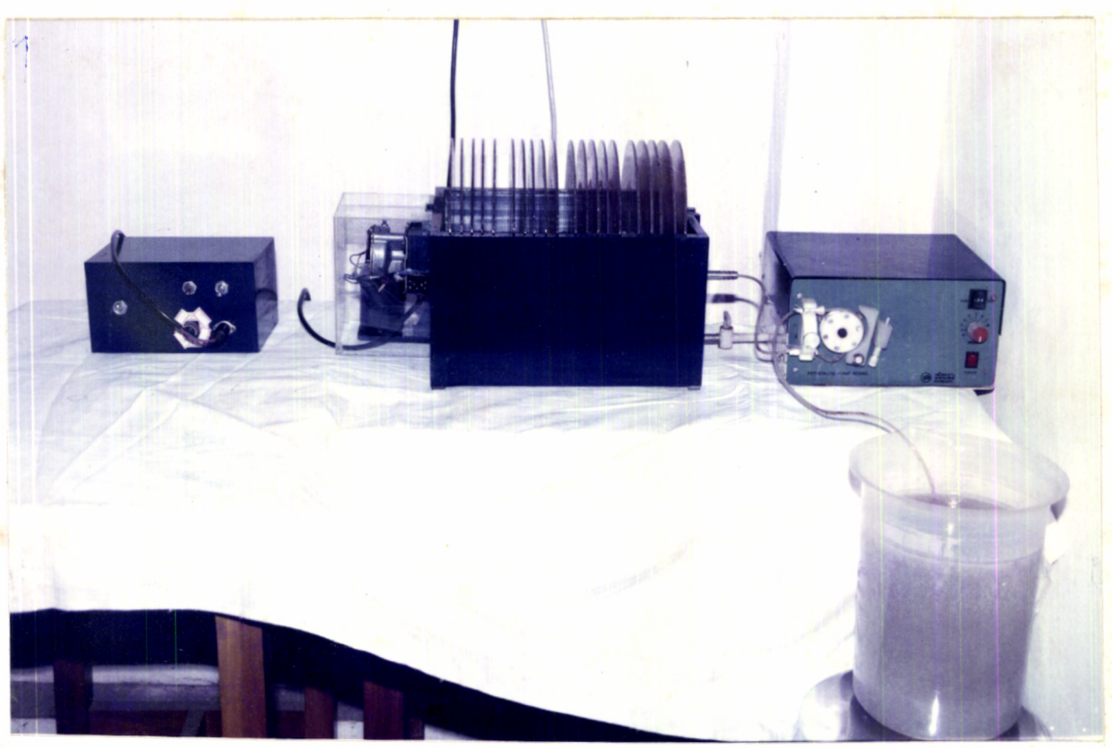


Plate 5 Photograph of Rotating Biological Contactor (RBC) showing continuous operation with rubber latex centrifugation effluent

at a time and hence the total effective area per one disc side surface was 478.43cm^2 giving a total effective surface area of 1.9m^2. One end of the shaft was connected to the motor which was able to supply RPM in the range 1-10.

When the RBC was set into operation with aqueous solution at different RPM maximum increase from to 6-8 mg/l in the dissolved oxygen was observed within 2h of operation at all the rpm (1-10) tried. When the RBC was put into operation with unseeded effluent, at different rpm in the range (1-10) for 2 hours; a maximum of 6.5% reduction in COD was obtained at 2rpm and thereafter, there was no significant increase in the reduction of COD along with increase in rpm. (Fig. 4.1)

Out of the different methods tried for collection of microbial biomass cotton swabbing yeilded biomass in the range of 4.1µg - 4.9µg/ml while for scrapping method, cell densities for the 6 trials were in the range of 3.6µg - 4.1µg/ml. Whereas biomass estimation using 'attached disc' showed comparatively higher concentration of biomass and was in the range of 42 - 44µg/ml. (Fig. 4.2).

Biomass formation on the RBC disc at pH 3 was comparatively less than that at pH 7 Increasing the pH to alkaline conditions (pH 10 or pH 12) did not cause much change in the biomass adhered on to the disc (Fig. 4.3).

In evaluating the effect of rpm on biomass formation on the RBC discs it was observed that a maximum biomass on the disc was at 1rpm and further increase in the rpm reduced the cell density on the RBC discs (Fig. 4.4)

From the data presented in Fig 4.5 it is inferred that maximum biomass could be adsorbed on to discs after 48h and further incubation did not enhance biomass concentration. (Fig. 4.5).

The roughness of the disc surface also affected biomass formation on the disc. The biomass adhesion on the RBC discs increased along with increase in the roughness from minimum to maximum (Fig. 4.6).

After attaining suitable biofilm formation, the reactor was put into operation for achieving steady state conditions. In all the cases a steady state condition was achieved after 8 days. At minimum roughness, when *Acinetobacter* sp. BTJR -10 was used as inoculum, the maximum reduction in COD obtained after steady state was 35% compared to 39% obtained with 'mixed culture seed inoculum'(Fig. 4.7).

Whereas at medium roughness the maximum reduction in COD obtained after steady state was 43% with *Acinetobacter* sp. as inoculum, compared to 47% COD reduction with mixed culture seed inoculum (Fig.4.8)

On attaining the steady state, at maximum roughness, COD reduction obtained was 48% and 52%, respectively with *Acinetobacter* sp. and with mixed culture seed inoculum (Fig.4.9).

After attaining steady state the effluent was passed into the reactor at different flow rates (5ml/min, 10 ml/min and 15ml/min) which subsequently effected retention times of 16.3h, 8.3h and 5.5h and hydraulic loading rates of $3.7m^3/m^2/day$, $7.5m^3/m^2/day$, and 11.3 $m^3/m^2/day$ (Table 4.1) at all the roughness, MiR, MeR and MaR separately with *Acinetobacter* sp. and mixed culture seed inoculum. The percent COD reduction obtained in all the cases were calculated.

Results presented in the Fig. 4.10 indicated that at the minimum roughness, as the flow rates increased from 5 to 15 ml/min, the percent reduction in COD decreased from 34-30, when *Acinetobacter* sp. was used as inoculum, and from 37 to 30 for the mixed culture seed inoculum (Fig. 4.10).

At medium roughness of the discs, as the flow rate increased from 5 to 15 ml/min, the percentage of COD reduction decreased from 41 to 37 for *Acinetobacter* sp. and from 46 to 40 for mixed culture seed inoculum (Fig. 4.11).

Similarly for maximum roughness, along with the increase in the flow rate from 5 to 15 ml/m, the percentage of COD reduction decreased from 46-40 for *Acinetobacter* sp. and from 47 to 33 for mixed culture seed inoculum. (Fig. 4.12)

In all the cases, mixed culture seed inoculum showed better reduction in COD than *Acinetobacter* sp. Moreover, as the roughness of the disc surface is increased, the efficiency of the reactor also increased due to increased cell adhession and hence high biomass activity. Where as, when the flow rate was increased from 5 to 15 ml/min, the percentage of COD reduction decreased due to decrease in the retention time,along with increase in the organic loading rate.

4.4 DISCUSSION

The rotating biological contactor is one of the recent method of biological treatment of wastes. The microbial film grows and covers the entire available surface of the discs. The film adsorbs the organic pollutants during submerged period of rotation cycle and a major amount of oxygen transfer occurs, when the biofilm is exposed to the atmosphere during the other half of the rotating cycle. However, the substrate utilization within the microbial film is a continuous process. The process of 'sloughing' also continues, thus maintaing a constant thickness of the microbial film on the discs. Therefore a RBC is a continuous flow aerated fixed film reactor. While the discs offer necessary surface area for biological growth, the aeration is provided by its rotation. The performance variables in the RBC are the biofilm density, rpm of the discs, submergence, hydraulic loading rate etc.(Oga *et al*,1991). Increased rate of revolution of the disc is believed to contribute more oxygen supply to the system. However the effect of 'rpm' is also found to be negligible under the normal range of operating conditions (Rao, 1987).

Hence in the operating conditions of 1-10 rpm there was not much increase in either the dissolved oxygen content of aqueous solution or in the percentage reduction of COD of the effluent during the pre-runs.

'Attached disc method' was observed to be convenient and more accurate for biomass estimation. The values obtained for biomass estimation in '6' trials were more consistant and were in a higher range. While optimizing the condition for ideal biofilm formation, it was observed that the biomass formation on the discs was very less at pH 3 compared to that at pH 7, whereas the alkaline pH did not affect the biomass formation on the disc. Although the biomass on the disc surface was more at 1 rpm, 2 rpm was optimal for obtaining maximum percentage of COD reduction during the trial runs. Hence 2 rpm was selected for further studies. While optimizing the incubation period, it was observed that the maximum formation of biomass on the disc starts from 48h incubation period.

Studies on the treatment of the effluent with RBC at various roughness of the disc, 'MiR', 'MeR' and 'MaR' suggested that the roughness of the disc surface influenced biomass formation which increased along with the increase in the roughness of the disc from minimum to maximum.

Similarly when the roughness of the disc surface was increased, the percentage of the COD reduction of the effluent also increased in the case of both mixed culture seed inoculum and *Acinetobacter* sp. showing a better activity of the biomass at increased

roughness. The efficiency of the reactor in terms of the COD removal increased along with the increase in the disc surface roughness indicating that a suitable modification of the disc surface such as Aero surf (Sack *et al*, 1986) and polyurethane attachment (Tyagi *et al*, 1994) could result in an enhanced removal of organic content from the effluent.

Infact the usual operation of a rotating biological contactor is with a mixed culture seed inoculum collected usually from the aeration tank of an activated sludge system. This inoculum will be inclusive of all types micro organism including protozoa, and metazoa. Whereas, when a single organism is used as an inoculum, the diversity of the biomass formed on the disc is reduced and hence the efficiency is also reduced. At all the three roughness of the disc, the mixed culture seed inoculum was efficient than the single organism. However the difference was only marginal.

Maximum treatment effliciency obtained with RBC was only 52% indicating partial treatment of the effluent. This might be due to the high organic loading rate used in the operation of the reactor (Stanbury *et al*, 1995). Further the studies conducted at various hydraulic loading rates showed the impact of organic loading rate on the 'COD removal efficiency of the reactor. Higher loading rate could attribute to the biofilim of increased thickness on the RBC disc which might have inhibited effective diffusion to (Zhang and Bishop, 1994) Because of the high organic content of the effluent even at low hydraulic loading rate, the organic loading rate is very high. But at lower organic loading rates it may take a longer period to get a steady state and hence also for the

treatment. One alternative is there to dilute the effluent, but then the reactor is to be operated for a longer time and hence not economical. As the rubber latex centrifugation effluent has high organic load, even the partial treatment itself is significant. Suitable modification of the reactor and use of improved and better inoculum may contribute to further increase in the efficiency of the reactor.

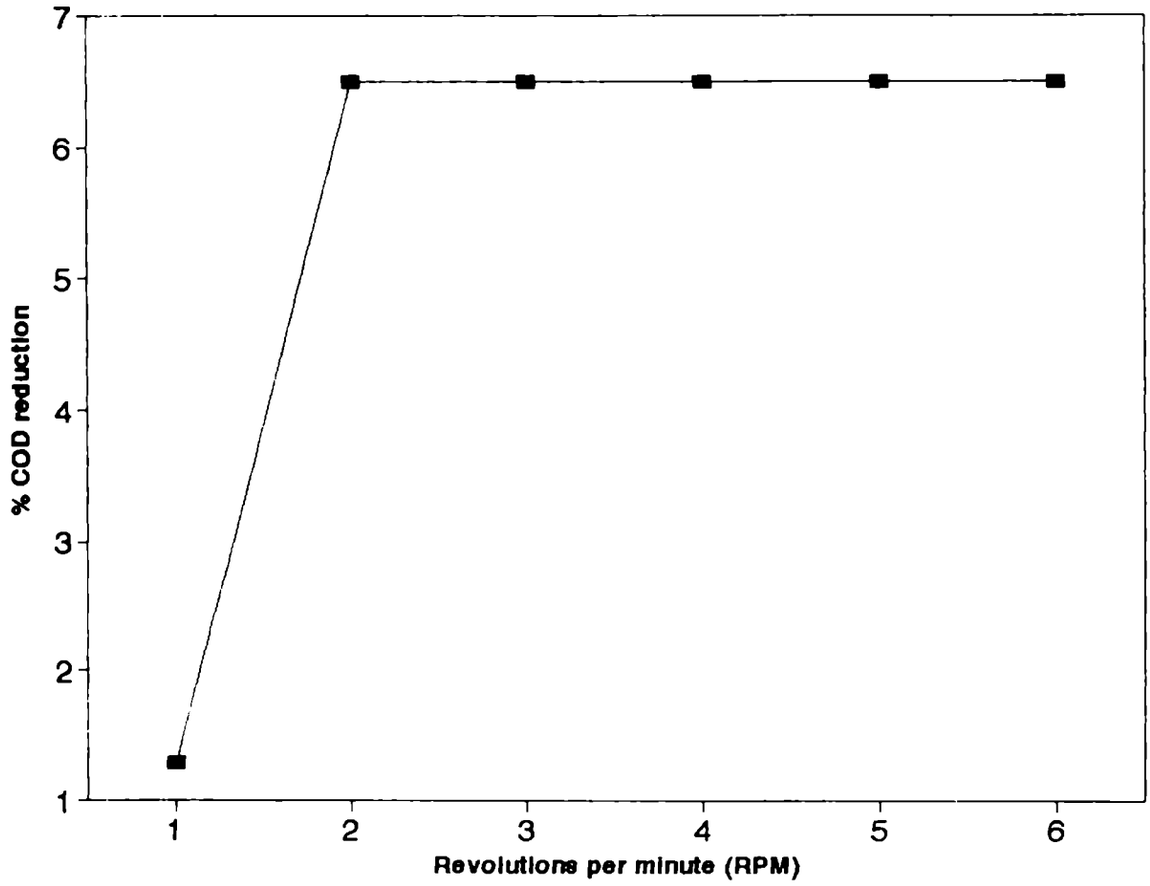


Fig. 4.1 Effect of revolutions per minute (RPM) on the chemical oxygen demand (COD) of the unseeded effluent with rotating biological contactor

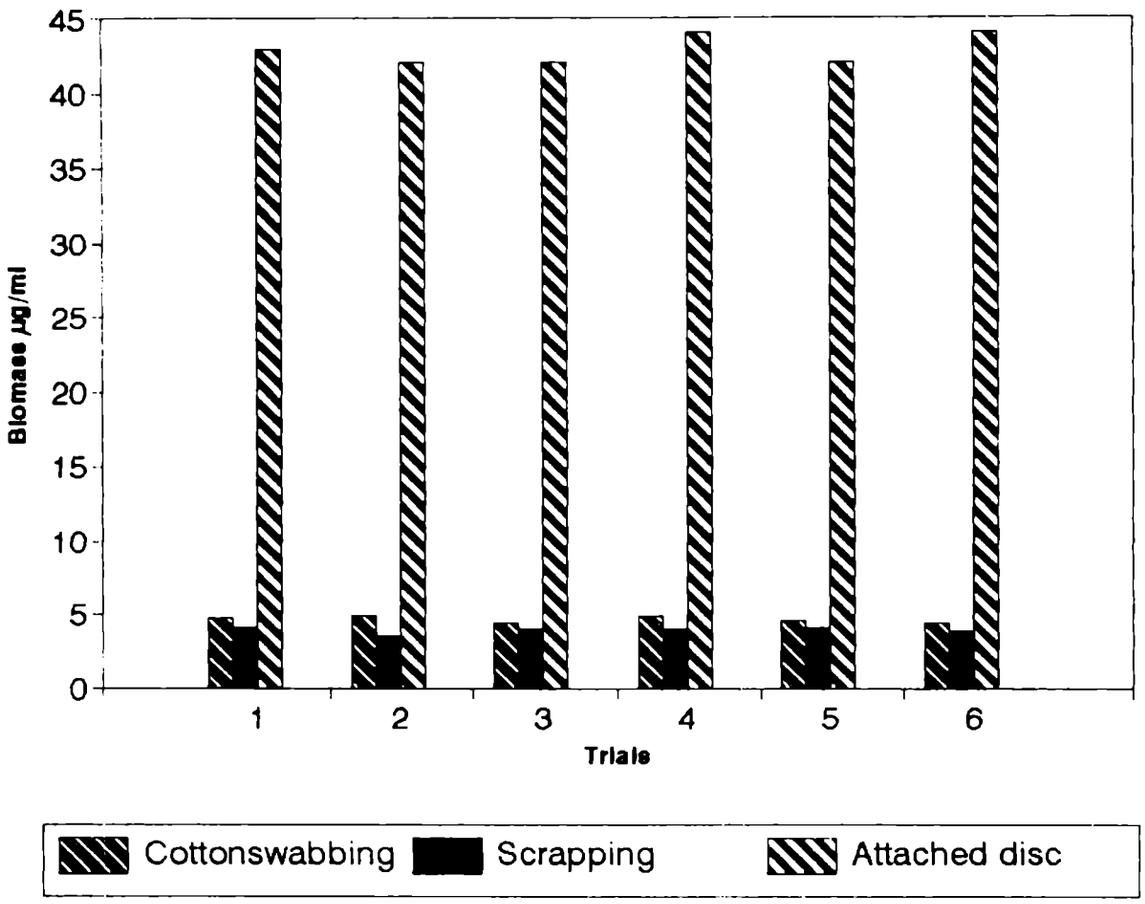


Fig. 4.2 Comparison of the different methods tried for the biomass collection from rotating biological contactor disc during the treatment with rubber latex centrifugation effluent

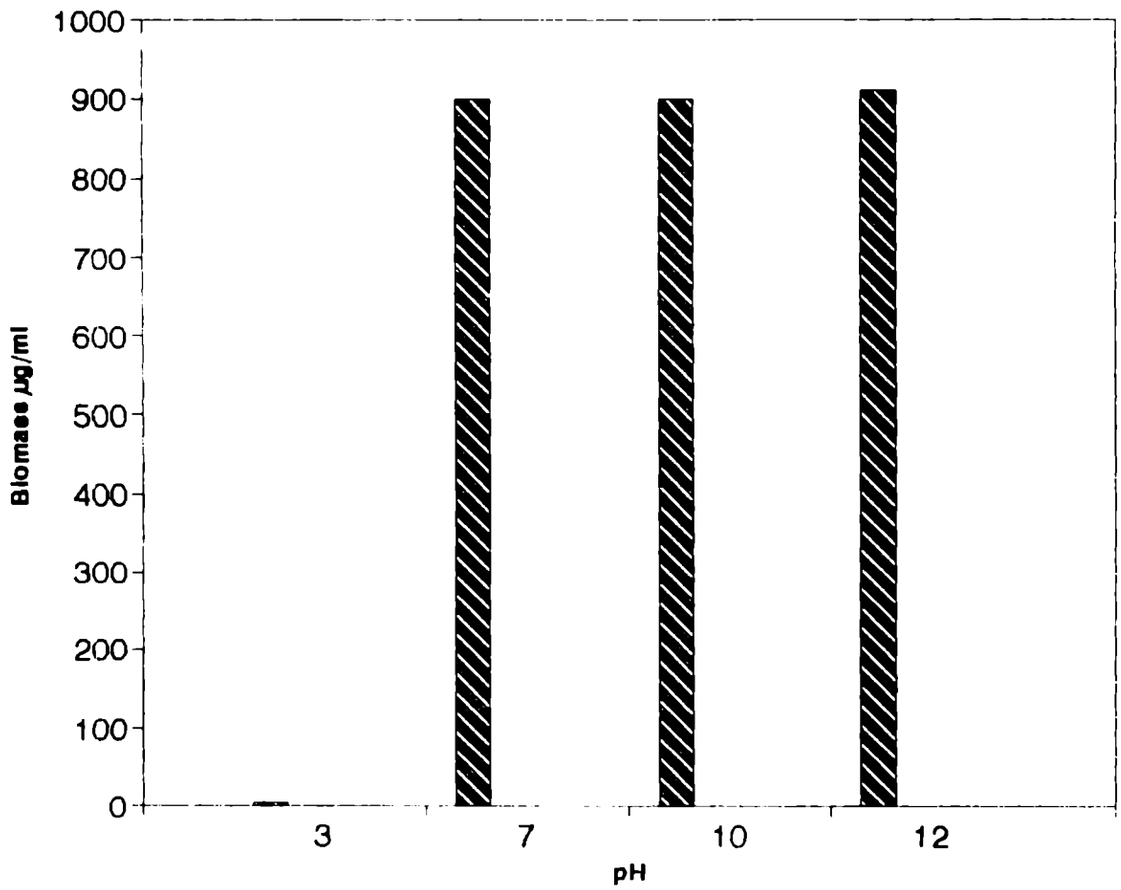


Fig. 4.3 Effect of pH on biomass formation on the rotating biological contactor disc during the treatment with rubber latex centrifugation effluent

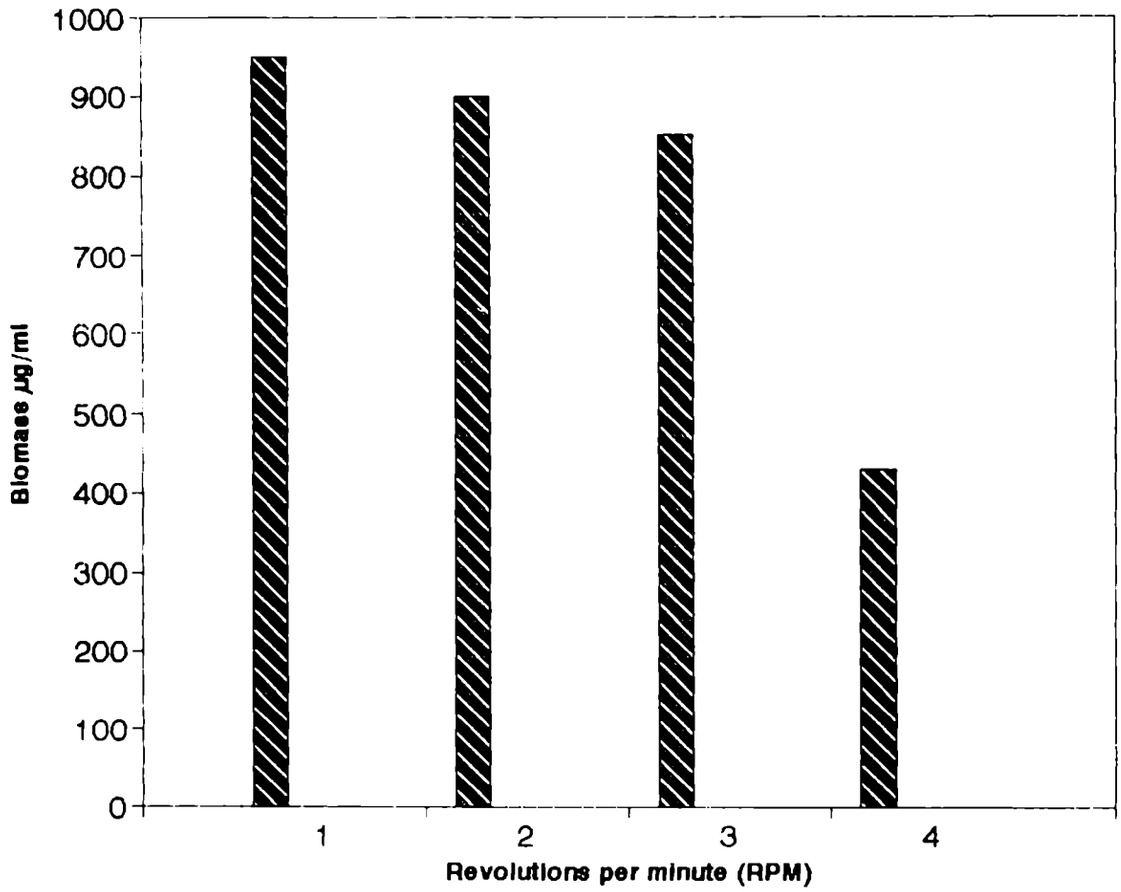


Fig. 4.4 Effect of revolutions per minute of the rotating biological contactor disc on the biomass formation during the treatment with rubber latex centrifugation effluent

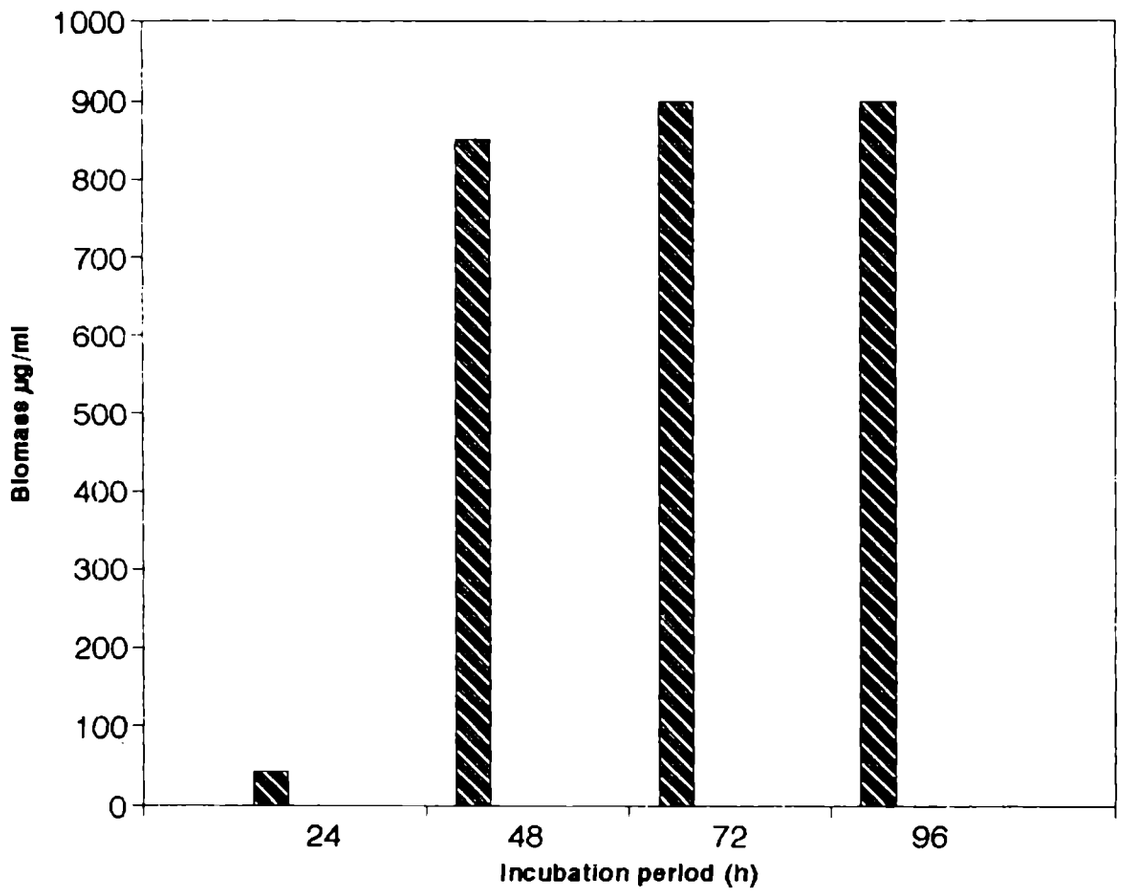
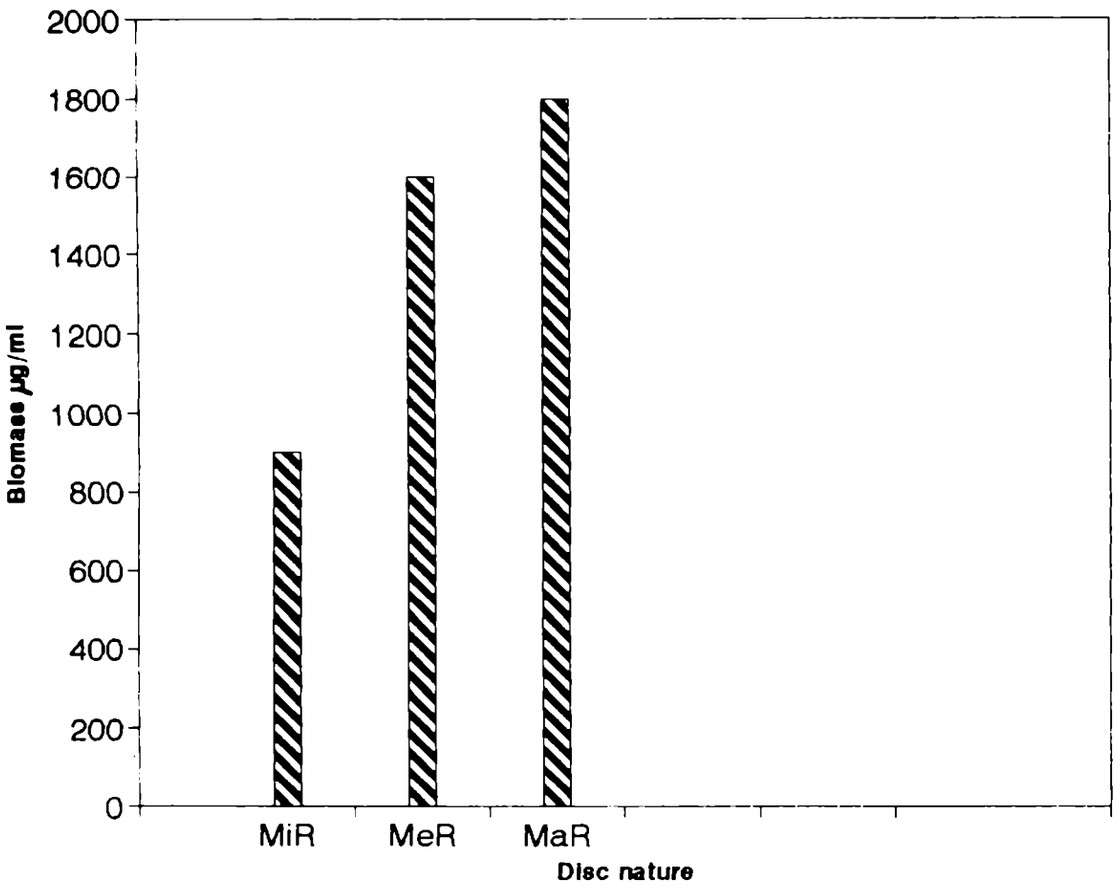


Fig. 4.5 Effect of incubation period on the biomass formation on the rotating biological contactor disc during the treatment with rubber latex centrifugation effluent



MiR-Minimum roughness; MeR- Medium roughness; MaR-Maximum roughness

Fig.4.6 Effect of nature of rotating biological contactor disc on the biomass formation during the treatment with rubber latex centrifugation effluent

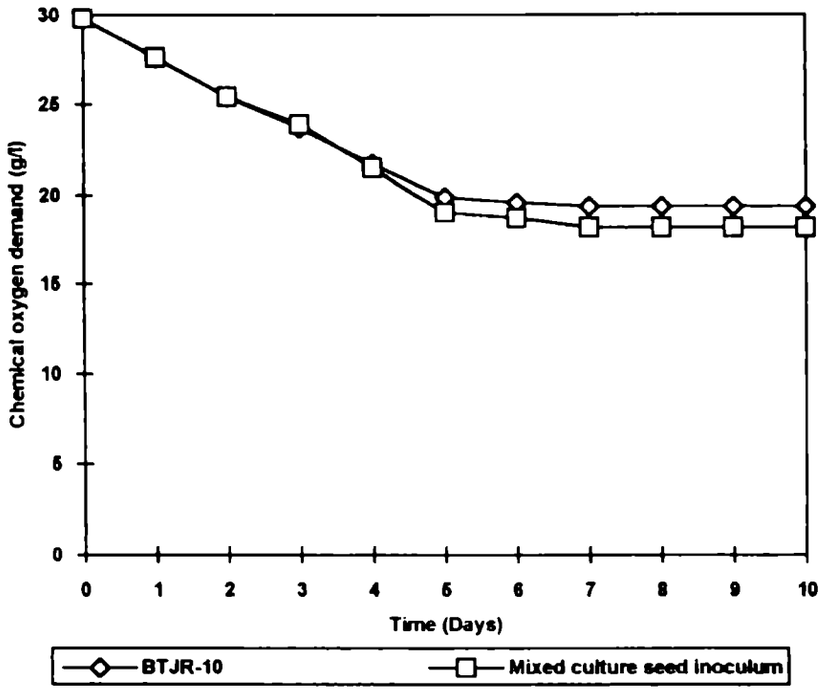


Fig 4.7 Steady state operation at minimum roughness of the rotating biological contactor discs using *Acinetobacter* sp. BTJR-10 and mixed culture seed inoculum for the treatment of rubber latex centrifugation effluent

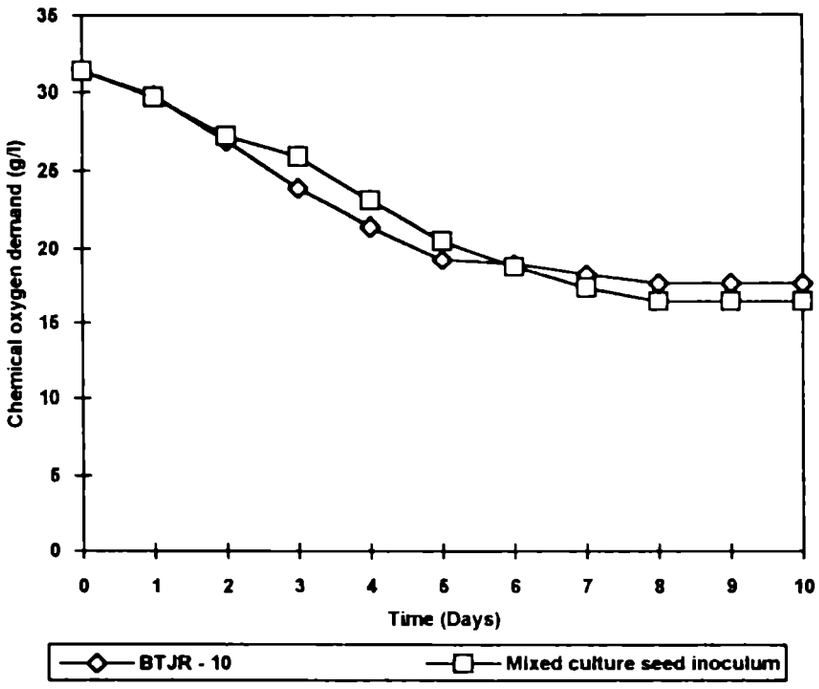


Fig 4.8 Steady state operation of at medium roughness of the rotating biological contactor discs using *Acinetobacter* sp. BTJR-10 and mixed culture seed inoculum for the treatement of rubber latex centrifugation effluent

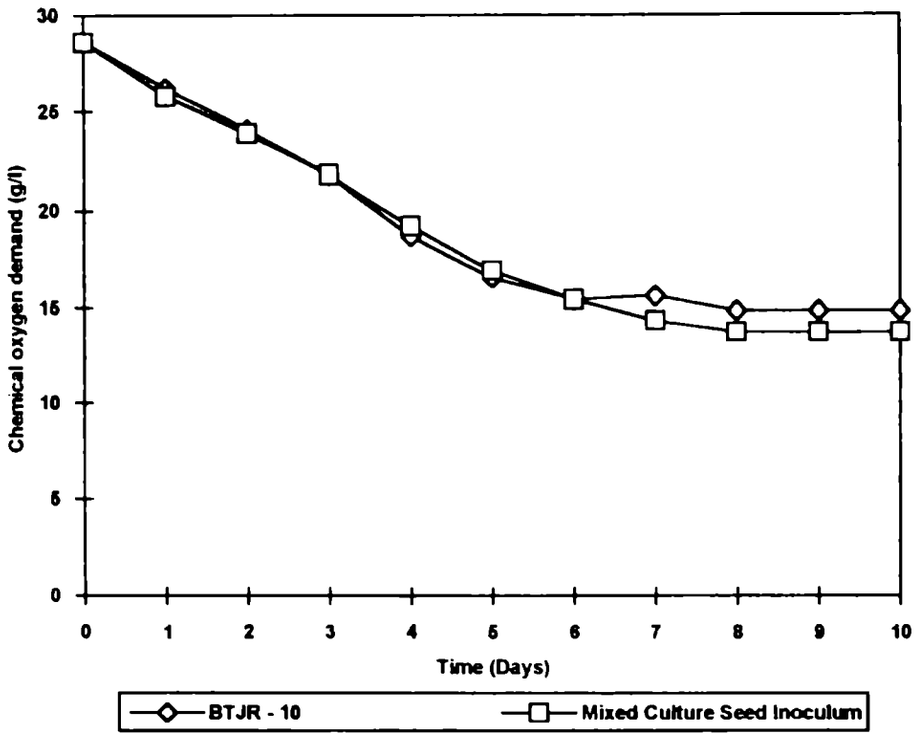


Fig 4.9 Steady state operation at maximum roughness of the rotating biological contactor discs using *Acinetobacter* sp. BTJR -10 and mixed culture seed inoculum for the treatment of rubber latex centrifugation effluent

Table 4.1 Characteristics of the rotating biological contactor performance at different flow rates of the rubber latex centrifugation effluent

Flow rate (ml/min)	Retention time (hour)	Hydraulic loading rate ($m^3/day/m^2$)	Organic loading rate COD ($g/day/m^2$)
5	16.3	3.7	111
10	8.3	7.5	221
15	5.5	11.3	330

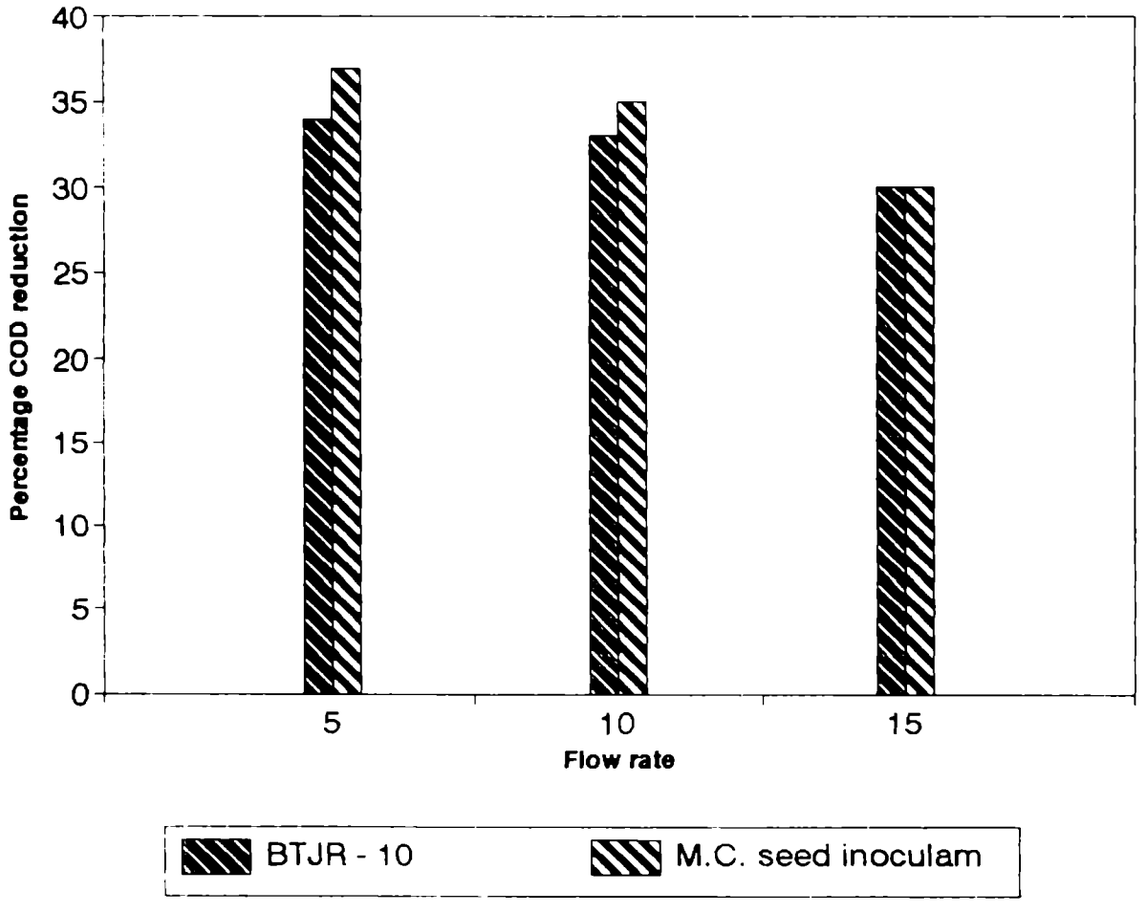


Fig.4.10 Treatment of the rubber latex centrifugation effluent at different flow rates with rotating biological contactor (RBC) using *Acinetobacter* sp. BTJR-10 and also with mixed culture seed inoculum at minimum roughness of the disc

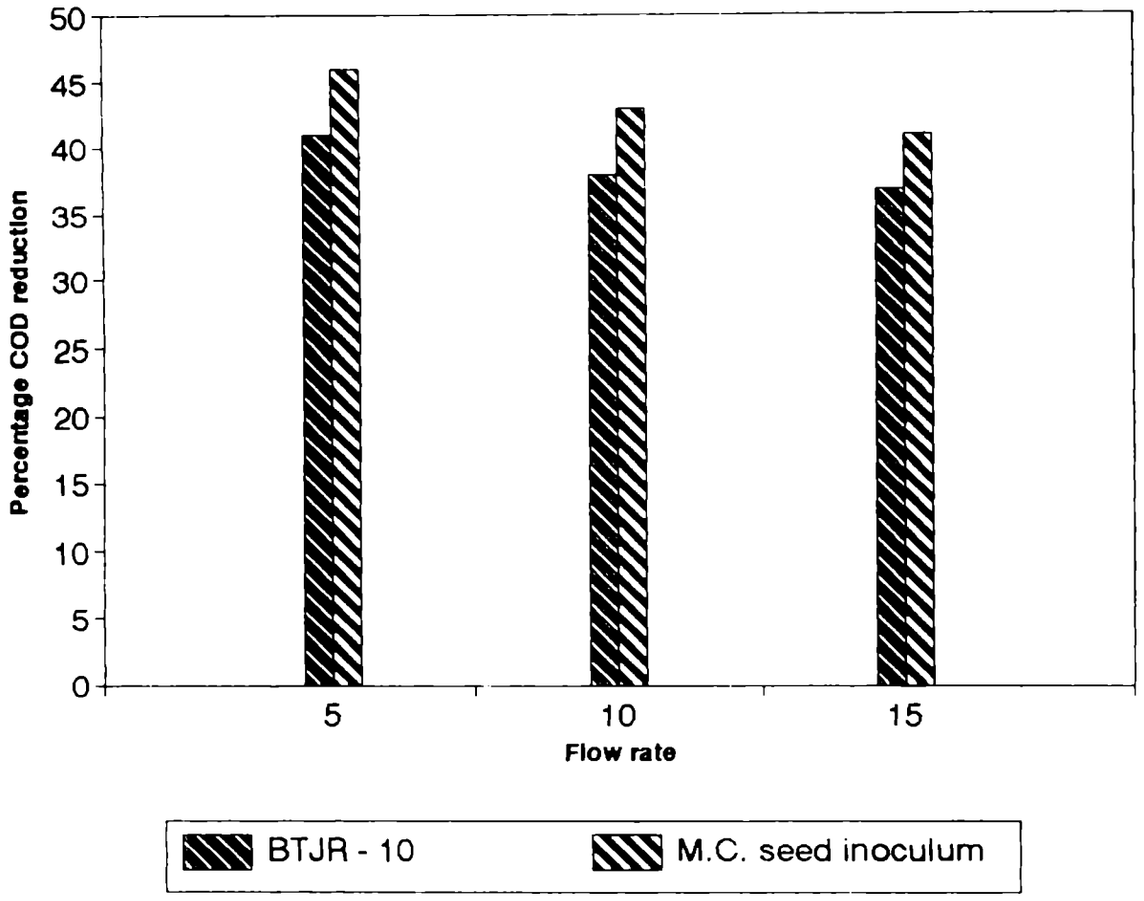


Fig.4.11 Treatment of the rubber latex centrifugation effluent at different flow rates with rotating biological contactor (RBC) using *Acinetobacter* sp. BTJR-10 and also with mixed culture seed inoculum at medium roughness of the disc

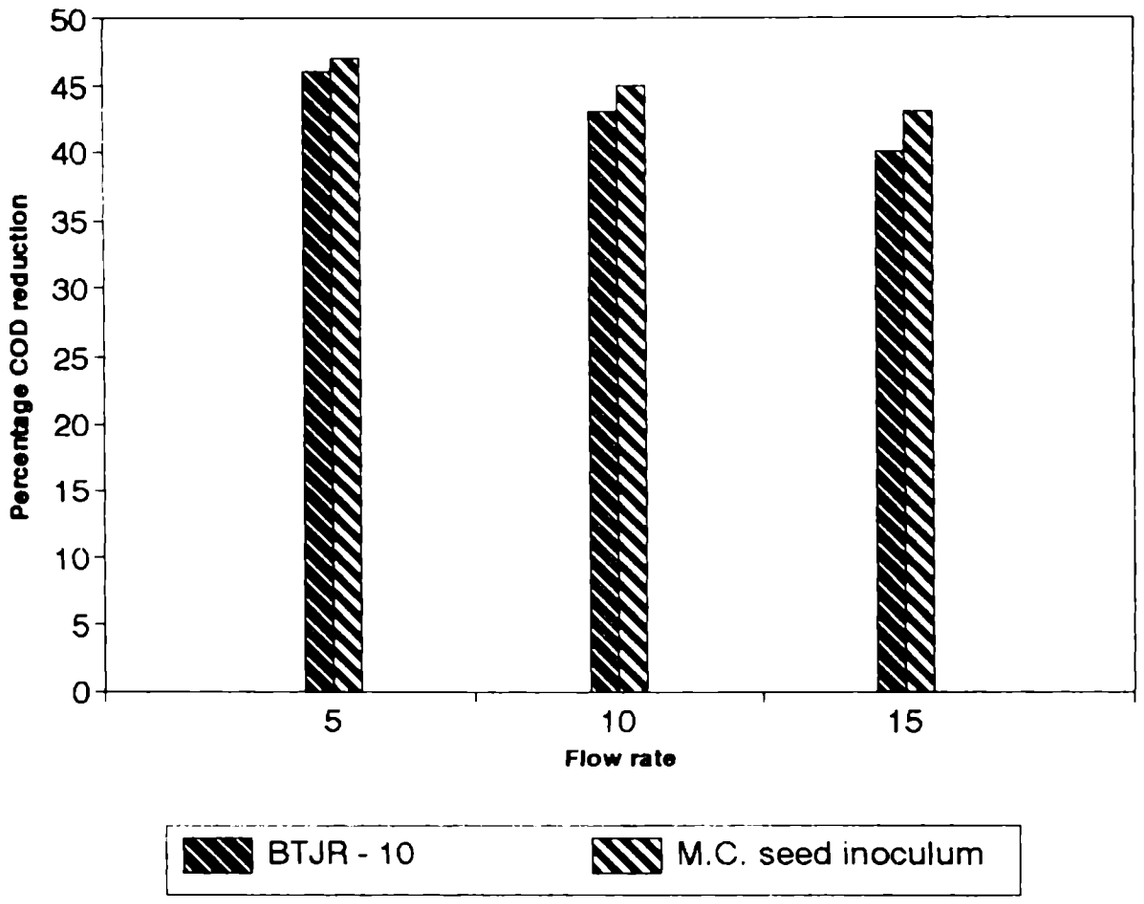


Fig.4.12 Treatment of the rubber latex centrifugation effluent at different flow rates with rotating biological contactor (RBC) using *Acinetobacter* sp. BTJR-10 and also with mixed culture seed inoculum at maximum roughness of the disc

CHAPTER - 5

TREATMENT OF THE RUBBER LATEX CENTRIFUGATION EFFLUENT WITH ACTIVATED SLUDGE SYSTEM

CHAPTER 5

TREATMENT OF RUBBER LATEX CENTRIFUGATION EFFLUENT WITH ACTIVATED SLUDGE SYSTEM

5.1. INTRODUCTION

Activated sludge process, a suspended growth process, that was started in England at the turn of the century and since then the process has been adopted worldwide as a secondary biological treatment for both domestic and industrial wastewaters. This process consists, essentially, of an aerobic treatment that oxidize organic matter to CO_2, H_2O, NH_4 and new cell biomass.

The essential features of the process are: an aeration stage, sedimentation stage and sludge recycle system. Wastewater, after primary treatment, enters the aeration tank where the organic matter is brought into intimate contact with the sludge from the secondary clarifier. This sludge is heavily loaded with microorganism which are in active state of growth. Air is introduced in the tank either in the form of bubbles through diffusers or by surface aerators. The microorganism, utilize the oxygen in the air and convert the organic matter into stabilized low energy compounds and synthesize new bacterial cells. The effluent from the aeration tank containing the flocculant microbial mass, known as the sludge, is separated in a settling tank, sometime called as a secondary settler or a clarifier. In the settling tank, the separated sludge exists without contact with

the organic matter and becomes activated. A portion of the activated sludge is recycled to the aeration tank as the seed and the rest is wasted. If all the activated sludge is recycled then the bacterial mass would keep on increasing to a stage where the system gets clogged with the solids. It is, therefore, necessary to 'waste' some of the microorganisms, and this wasted sludge is later processed and disposed.

Some operational parameters commonly used in activated sludge system include Mixed Liquor Suspended Solids (MLSS), Mixed Liquor Volatile Suspended Solids, (MLVSS), Food to Micro-organism (F/M) ratio, Hydraulic Retention Time (HRT), and Sludge Volume Index (SVI).

MLSS is the total amount of organic and mineral suspended solids including microorganisms in the mixed liquor. The organic portion of the MLSS is represented by MLVSS which comprises non microbial organic matter as well as dead and live micro-organisms and cellular debris. F/M ratio indicates the organic load into the activated sludge system and is expressed in Kilogram BOD/ Kilogram MLSS per day. The SVI represents settleability of the biomass and the HRT is the average time spent by the influent liquid in the aeration tank of the activated sludge process.

There are many modifications to the activated sludge process such as extended aeration, high rate aeration, contact aeration and others. Except for various flow regimes the major differences in these process modifications are the F/M ratio, retentiontime and changes in aeration pattern.

The activated sludge flocs contain microbial cells as well as inorganic particles. The various micro-organisms observed in the activated sludge flocs include bacteria fungi, protozoa, rotifers etc.

Bacteria constitute the major component of the activated sludge flocs. More than 300 strains of bacteria were found in activated sludge. They are responsible for the oxidation of organic matter and for nutrient transformation. They also produce ploysaccaharides and other polymeric materials, that aid in floccutation of microbial biomass. The major genera found in the flocs are species of Zoogloea, *Pseudomonas*, *Flavobacterium*, *Alcaligenes* *Bacillus*, *Achromobacter*, *Corynebacterium*, *Brevibacterium* and *Acinetobacter*, as well as filamentous microorganisms (Hiraishi *et al*, 1989).

Activated sludge does not usually favour growth of fungi, although some fungal filaments are occasionally observed in activated sludge flocs. Fungi may grow abundantly under specific condition of low pH, toxicity and nitrogen deficient wastes. The predominant genera found in activated sludge are *Geotrichum* *Penicillium*. *Cephalosporium*, *Cladosporium* and *Alternaria* (Pipes and Cooke. 1969; Tomlinson and Williams, 1975)

Protozoa are significant predators of bacteria in activated sludge as well as in natural aquatic environments. The protozoans often found in activated sludge generally include ciliates, flagellates and rhizopoda.

Rotifers are also found in activated sludge flocs The main role of rotifers in activated sludge system is that they help in the removal of freely suspended bacteria and also they contribute to floc formation by producing faecal pellets sorrounded by mucus.

Because of the multiplicity of species in the mixed biocenosis of the activated sludge, the system can adopt well to seasonal changes in temperature, the influence of toxic substances, pH values deviating from neutral salt concentration, anaerobic phases and changes in the composition of the wastewater.

Activated sludge process and it's modifications offer very effective methods for treating even high strength wastewater such as effluent from food processing industries (Gostick *et al*, 1990). The activated sludge system was effecient in treating the effluents such as brewery wastewater (Vriens *et al*, 1990), black olive wastewater (Borja *et al*, 1994) and also 2-4 dichloro phenoxy acetic acid containing wastewater (Xin - hui Xing *et al*,1995) Hence in the present study the efficiency of the activated sludge system was evaluated towards effective and large scale treatment of the rubber latex centrifugation effluent

5.2 MATERIALS AND METHODS

5.2.1 *ACTIVATED SLUDGE INOCULUM*

Activated sludge seed used in the present study was collected from the aeration tank of a nearby rubber latex centrifugation effluent plant.

5.2.2 *TREATMENT WITH ACTVATED SLUDGE INOCULUM*

The initially activated sludge was acclimatized in a reactor of 5 l capacity at 30°C by 'fill and draw' operation for almost 30 days. The duration of one acclimatization run was the time interval for maximal COD reduction (Borja *et al*, 1994). MLSS, SVI and COD of the activated sludge system at the start of the acclimatization process was noted In each cycle, half of the supernatant, settled for 30 minutes in the reactor, was drawn before fresh effluent of the same volume was added. (Xin - hui Xing *et al*, 1995).

pH of the effluent was adjusted to 7, once a day, using 1 N NaOH. Air supply was maintained with two air spargers. During the acclimatization process the changes in the COD and MLSS were monitored and the process was continued upto 30 days. SVI was also routinely determined and was kept constant. After attaining the steady state the activated sludge system was put into operation for treatment of the latex centrifugation effluent and the reduction in COD was monitored as mentioned under section 3.2.1.3.9.

5.2.3 *TREATMENT OF THE EFFLUENT WITH IMMOBILISED CELLS*

5.2.3.1 Immobilization of Activated Sludge

Activated sludge (explained in section 5.2.1), for immobilization, was maintained for 30 days with a supply of synthetic wastewater and later immobilized by tube

polymerization method using agar, and by entrapment method with calcium alginate (Sumino *et al*, 1991).

5.2.3.1.1 Immobilization of activated sludge in agar by tube polymerisation method

Immobilization of activated sludge in agar by tube polymerisation method was carried out as detailed below (Matsunaga *et al*, 1980; Sumino *et al*, 1991).

1 100mg agar was dissolved in 4.5ml of 0.9 NaCl (w/v) by heating to 100°C and then cooled to 50°C.

2. 0.5 ml of the activated sludge slurry (10mg dry weight/100ml) in 0.9 NaCl (w/v) was added to the above solution and mixed well.

3. The mixture was immediately passed through a polyvinyl chloride tube with inner diameter of 3mm and left for above 10minutes at 5°C in order to get an elastic gel containing activated sludge.

4. The gel was extruded from the poly vinyl chloride tube and cut at a length equal to its diameter, thus yielding pellets of uniform size and was stored in 0.1M sodium phosphate buffer of pH 7 until required.

5.2.3.1.2 Immobilization of activated sludge in calcium alginate by entrapment method

Activated sludge was immobilized in calcium alginate as described under section 3.2.3.

5.2.3.2 Co-*immobilization of activated sludge and Acinetobacter sp. BTJR -10*

The activated sludge prepared under section 5.2.3.1 and the *Acinetobacter* sp. BTJR-10 mentioned under section 3.2.2 were mixed and immobilized by entrapment technique using calcium alginate as detailed under section 3.2.3.

5.2.3.3 Preparation of Immobilized Viable Cells (IVC)

Immobilized viable cells were prepared as mentioned under the section 3.2.3.

5.2.3.4 Activation of Immobilized Viable Cells

The immobilized viable cell beads containing either activated sludge *Acinetobacter* sp. or both were activated for achieving maximal activity using the latex effluent (Mohandass, 1992). Prepared immobilized cell beads were taken in large 500ml beaker and immersed with latex effluent for varying time intervals. Optimum activation time that promoted maximal activity was determined by estimating the percentage reduction of COD, as described under section 3.2.4.

5.2.3.5 Retention Time

Optimum retention time required for maximal activity by IVC was estimated, after incubation with latex effluent for varying periods, in terms of percentage reduction of COD, as mentioned under section 3.2.5.

5.2.3.6 Batch process treatment of the effluent

Batch process treatment of the latex effluent by immobilized viable cells was carried out in 500ml conical flask using 200 beads of immobilized cells. After activation (optimum activation time), the immobilized cells were exposed to latex effluent for varying retention periods. At the end of the retention period the effluent was decanted out and the residual COD was estimated as mentioned under section 3.2.1.3.9. The entire study was conducted at room temperature (28±2°C).

5.2.3.7 Continuous treatment of rubber latex centrifugation effluent by immobilized cells

Continuous treatment of latex effluent was conducted in a packed bed reactor using immobilized activated sludge as mentioned under section 5.2.3.1.2 and with co-immobilized activated sludge and *Acinetobacter* sp. BTJR-10 as mentioned under section 5.2.3.2. Immobilized cells entraped in calcium alginate beads were packed in a glass column (4.14cm) upto a height of 15cm. Effluent was passed from the bottom of the column upwards using a peristaltic pump (Miclins) at different flow rates of lml/min,

2ml/min, 4ml/min and 6ml/min. Samples were collected from the top after treatment and analysed for residual COD. Control experiments were also run using beads without immobilized cells Half life of the packed bed reactor was calculated for both and compared.

5.2.4. *ANALYTICAL METHODS*

COD, BOD, MLSS, MLVSS, SVI etc were estimated following the standard procedures (APHA, 1989)

5.2.4.1 Estimation of COD

COD was estimated as detailed under section 3.2.1.3.9

5.2.4.2 Estimation of BOD

BOD was determined as per the procedure mentioned under the section 3.2.1.3.8.

5.2.4.3 Estimation of Mixed Liquor Suspended Solids (MLSS)

MLSS represents the total content of the aeration tank. It was determined by filtering an aliquot (100ml) of mixed liquor, drying the filter at 105°C and determining the weight of solids in the sample.

5.2.4.4. Estimation of Mixed Liquor Volatile Suspended Solids (MLVSS)

MLVSS which represents the organic portion of the MLSS was determined by heating the dried filtered samples at 600-650°C.

5.2.4.5. Food to Microorganisms Ratio (F/M)

F/M ratio indicates the organic load into the activated sludge system and was determined as kilogram BOD per kilogram of MLSS per day.

5.2.4.6. Sludge Volume Index (SVI)

Mixed liquor drawn from the aeration tank was introduced into a 1 l graduated cylinder and allowed to settle for 30 minutes and the settled volume (SV) was noted.

$$\text{SVI (ml/g)} = \frac{\text{SV x 1000}}{\text{MLSS}}$$

5.3 RESULTS

Activated sludge system developed in the present study had, 9430mg/l MLSS 32,000 mg/l COD, 7430 mg/l MLVSS and 21ml/g SVI at the start of acclimatization (Table5.1). 48h was considered as the period for one cycle of operation. The

acclimatization was performed through fill and draw mechanism and the F/M ratio maintained was '0.173'. SVI was also maintained constant throughout the operation. Acclimatization process was continued upto 30 days and the changes in the MLSS and COD were monitored (Fig 5.1 and 5.2). From the Figure it was inferred that a constant percentage of COD reduction was achieved after 18 days of operation which continued upto 30 days, maximum of 69% COD reduction was attained at steady state. Whereas, when the system was put into operation after steady state the percentage of COD reduction was to 67.5%, 69.4% and 67.5% respectively for 1st, 2nd and 3rd cycles.

Under batch process treatment of the latex effluent with agar immobilized activated sludge, (optimum activation time - 4h, Fig. 5.3) there was 38.7% COD reduction in 2h. Further increase in the retention time upto 24h did not produce any enhancement in the COD removal (Table 5.2). Similarly on bacth treatment of the effluent with alginate immobilized activated sludge (optimum activation time - 6h, Fig. 5.4) a COD reduction of 39% was achieved in 2h and further increase in the retention time upto 24h promoted a marginal rise to 41% reduction in COD (Table 5.3). The parameters selected for the continuous treatment of the effluent with packed bed reactor using alginate immobilized activated sludge is given in the Table 3.3. The percentage of reduction in COD for the flow rates tried were 1ml/min, 2ml/min, 4ml/min and 6ml/min were 56, 54, 52 and 38 respectively (Table 5.4). Half life of the packed bed reactor was determined by monitoring the performance of the packed bed reactor over a period of 10 days. Half life of the reactor was 5 days (Fig. 5.5).

During batch process treatment of the effluent, using co-immobilized activated sludge and *Acinetobacter* sp. (optimum activation time - 6h, Fig. 5.6) a COD reduction of 50% was achieved in 2h. Further increase in the retention time, to 24h, resulted only a marginal increase (52%) in the % reduction of COD (Table 5.5).

Whereas, on continuous treatment of the effluent, in a packed bed reactor, containing co-immoblized activated sludge and *Acinetobacter* sp. BTJR-10, 60%, 59%, 56% and 44% reduction in COD, respectively, for the flow rates 1ml/min, 2ml/min, 4ml/min and 6ml/min were observed (Table 5.6). The half life of the packed bed reactor, monitored over a 10 days period was 3 days (Fig. 5.7).

5.4 DISCUSSION

The activated sludge system developed in this study was almost a conventional system. The acclimatization was carried out for 30 days through a 'fill and draw' mechanism. Typically this operation consisted of filling a tank with wastewater, aerating the contents of the tank and then allowing quiescent settlement. Finally a clear liquid was drawn from the upper part of the tank leaving activated sludge at the bottom of the tank. After the excess sludge was removed, the system was ready for the next batch wise cycle.

During the acclimatization process the sludge developed was subjected to intimate contact with the fresh effluent during each cycle such that the sludge gets acclimatized to the nature of the effluent. This process was continued until a steady state

was achieved, where the activated sludge showed a steady nature in reducing the organic load of the effluent. The system was then put into operation for treating the effluent.

The activated sludge system developed in the present study required 30 days for attaining a steady state and the maximum percentage of COD reduction obtained was 69%. As the effluent has heavy organic load, there is a chance for the maximum production of excess sludge. Due to the high biomass synthesis the system upsets easily. Hence, the treatment efficiency is low and the nutrient requirement are at their highest (Vries *et al*, 1990).

MLSS of the system at the beginning of acclimatization was also very high. This may be due to the high amount of suspended solids in the effluent. However, after acclimatization, when the steady state was reached, MLSS got stabilized to a value of 3000 mg/l.

During the batch treatment of the effluent using immobilized activated sludge there was 39% COD reduction at 2h treatment. Further increase in the contact time to 24h did not enhance COD reduction. This could be attributed to the high amount of suspended solids in the effluent which could have clogged the pores of the beads and inhibited microbial activity. The same trend was observed with batch treatment of the effluent using co-immobilized activated sludge and *Acinetobacter* sp. where the percentage of COD reduction obtained after 2h of treatment was higher (50%). Possibly

this may be due to the cumulative effect of activated sludge and *Acinetobacter* sp. However the stability of the co-immobilized beads were considerably reduced.

In continuous treatment with packed bed reactor using activated sludge the maximum percentage of COD reduction attained was 56%, at residence time of 50 min, and at 1ml/min flow rate. When the flow rate was increased, the percentage of COD reduction decreased, since there was less contact time at higher flow rates. However, a flow rate of 4ml/min effected 52% reduction COD at contact time of 12.5 min. Similarly, when the continuous treatment was done with co-immobilized activated sludge and *Acinetobacter* sp. in the packed bed reactor, the maximum percentage of COD reduction obtained was 60% in 50 minutes of retention time at 1ml/min flow rate. Although the percentage of COD reduction at higher flow rates was less, yet at 6ml/min flow rate, there was a considerable reduction (44%) in COD. But the half life of the packed bed reactor with co-immobilized activated sludge and *Acinetobacter* sp. was considerably less (3 days) compared to that of the packed bed reactor with immobilized activated sludge alone(5 days).

The results obtained in the present study clearly suggest that packed bed reactor, with immobilized activated sludge and co-immobilized cells of activated sludge and *Acinetobacter* sp. BTJR-10, is a good method for the rapid treatment of rubber latex centrifugation effluent. Infact, the efficiency observed with both the cases is better than the previous report with packed bed reactor containing immobilized *Acinetobacter* sp. where a COD reduction of 44% was observed in 6h hydraulic retention time at 7ml/h

flow rate (Jayachandran and Chandrasekaran,1994 a). The most significant observation made in the present study using activated sludge is that the packed bed with identical process conditions requires a less hydraulic retention time at a higher flow rate to effect a better reduction in the organic load of the effluent. A comparison of the performance of three Packed Bed Reactors (PBR) ie PBR with *Acinetobacter* sp. PBR with immobilized Activated sludge and also with co-immobilized activated sludge and *Acinetobacter* sp. is given along with that of RBC and Activated sludge in the Table 5.7. Though the Packed Bed Reactor offered better method of treating the effluent in terms of % COD reduction obtained, the reactor is very much succepetible to diffusional limitations due to high amount of suspended solids in the effluent. Hence, continuous treatment using Packed Bed Reactor cannot be suggested as a stable system for the single step purification of the effluent. However this system could be fruitful one, when it is preceeded by chemical coagulation. (Jayachandran and Chandrasekaran, 1997).

Table 5.1 General characteristics of the activated sludge system used for the treatment of rubber latex centrifugation effluent.

* Total volume	1 L
Settled volume(SV)	200ml
Mixed Liquor Suspended Solids(MLSS)	9.4g/l
* Sludge Volume Index (SVI)	21ml/g
Mixed Liquor Volatile Suspended Solids(MLVSS)	7.4g/l
* Food/Microorganism ratio(F/M)	0.17g BOD/g MLSS/day

* Kept constant during the process of acclimatization

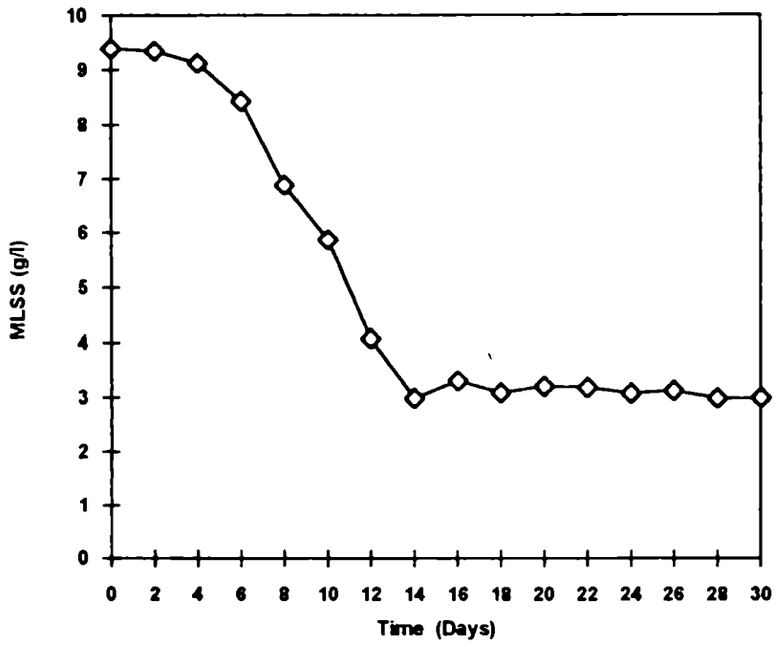


Fig 5.1 Variation in Mixed Liquor Suspended Solids (MLSS) during the acclimatization of the activated sludge system in the treatment of rubber latex centrifugation effluent

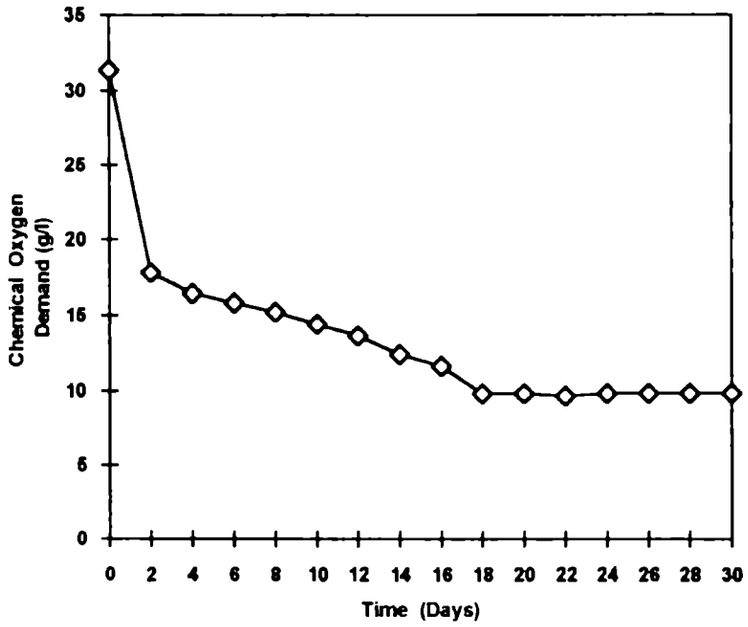


Fig 5.2 Variation in Chemical Oxygen Demand (COD) during the acclimatization of the activated sludge system

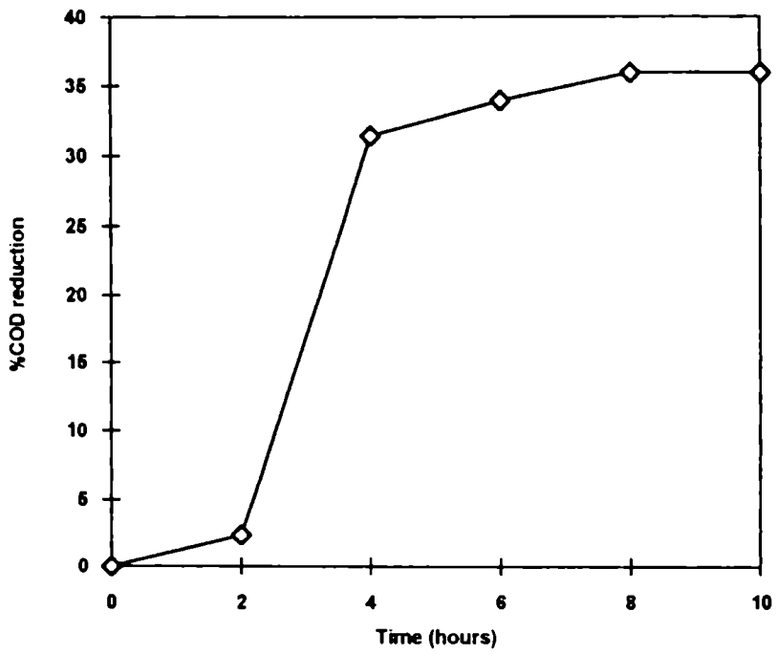


Fig 5.3 Optimization of the activation time for the activated sludge immobilized in agar

Table 5.2 Treatment of the effluent with activated sludge immobilized in agar under batch process

Retention time (hours)	Percentage of COD reduction of the effluent
2	38.7
4	39.3
6	39.3
8	38
10	38
24	38

*** Optimum activation time - 4h**

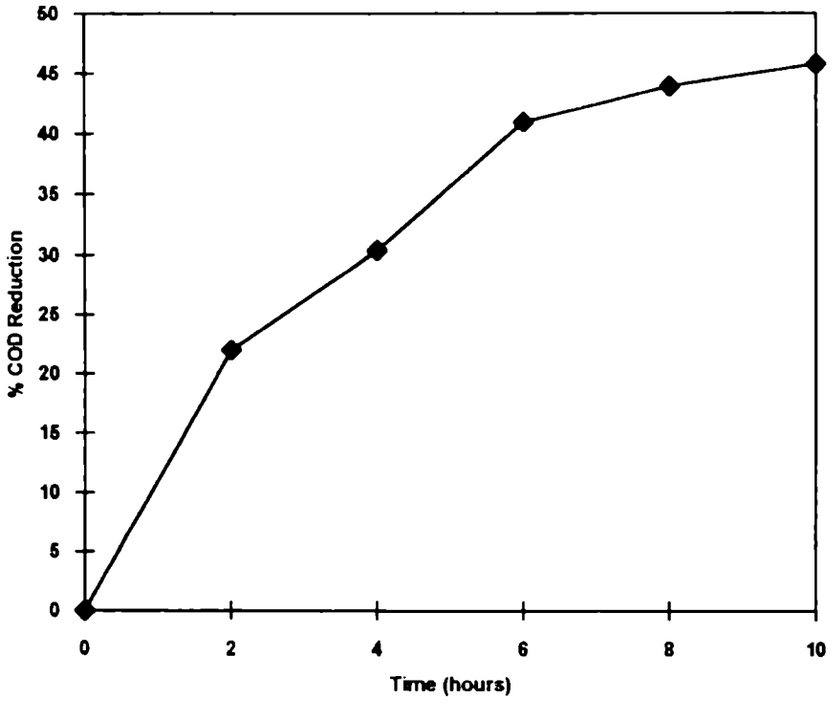


Fig 5.4 Optimization of the activation time for the activated sludge immobilized in calcium alginate

Table 5.3 Treatment of the Effluent with calcium alginate immobilized activated sludge under batch process

Retention time (hours)	Percentage of COD Reduction in the effluent
2	39
4	39
6	39
8	38
10	38
24	41

*** Optimum activation time - 6h**

Table 5.4 Treatment of the effluent with calcium alginate immobilized activated sludge in a packed bed reactor (PBR) under continuous process

Flow rate (ml/min)	% COD reduction	Retention time (minutes)
1	56	50
2	54	25
4	52	12.5
6	38	8.3

Optimum activation time - 6h

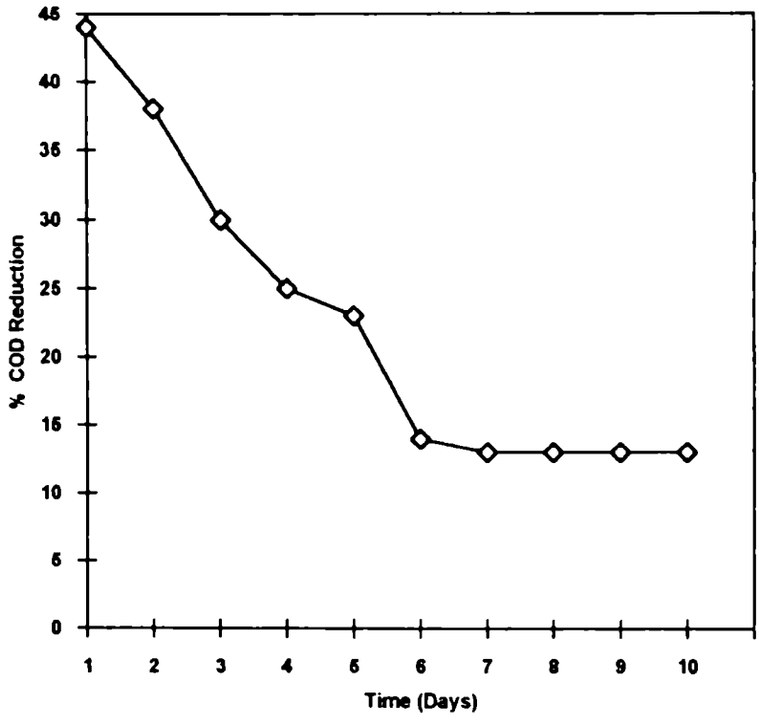


Fig 5.5 Performance of the packed bed reactor (PBR) with calcium alginate immobilized activated sludge in treating the rubber latex centrifugation effluent under continuous process

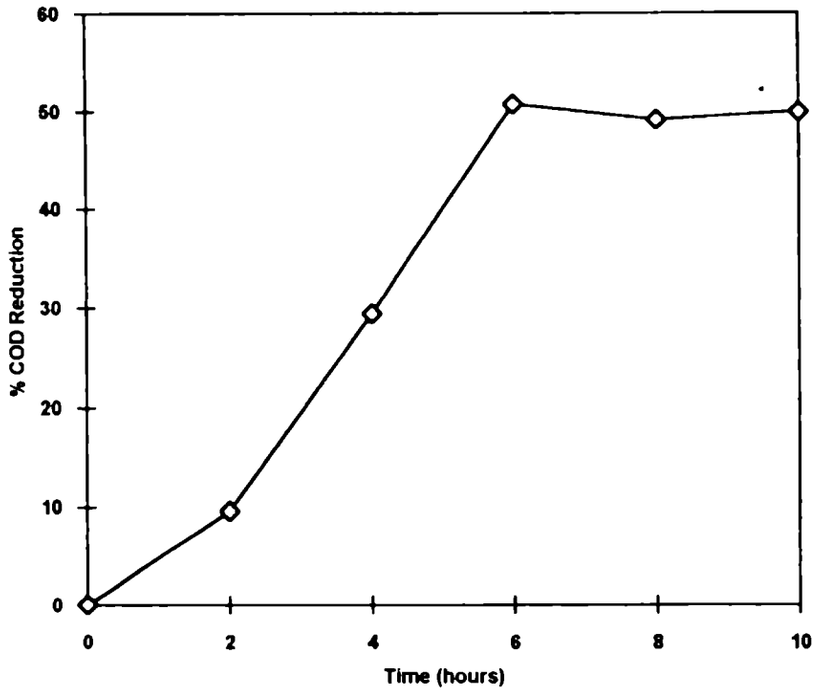


Fig 5.6 Optimization of the activation time for the calcium alginate co-immobilized activated sludge and *Acinetobacter* sp. BTJR-10

Table 5.5 Treatment of the effluent with calcium alginate co-immobilized activated sludge + *Acinetobacter* sp. under batch process

Retention time (hours)	Percentage of COD reduction of the effluent
2	50
4	50
6	52
8	51
10	51
24	52

Optimum activation time - 6h

Table 5.6 Treatment of the effluent with calcium alginate co- immobilized activated sludge + *Acinetobacter* sp. using packed bed reactor (PBR) under continuous process.

Flow rate (ml/min)	% COD reduction	Retention time (minutes)
1	60	50
2	59	25
4	56	12.5
6	44	8.3

Optimum activation time - 6h

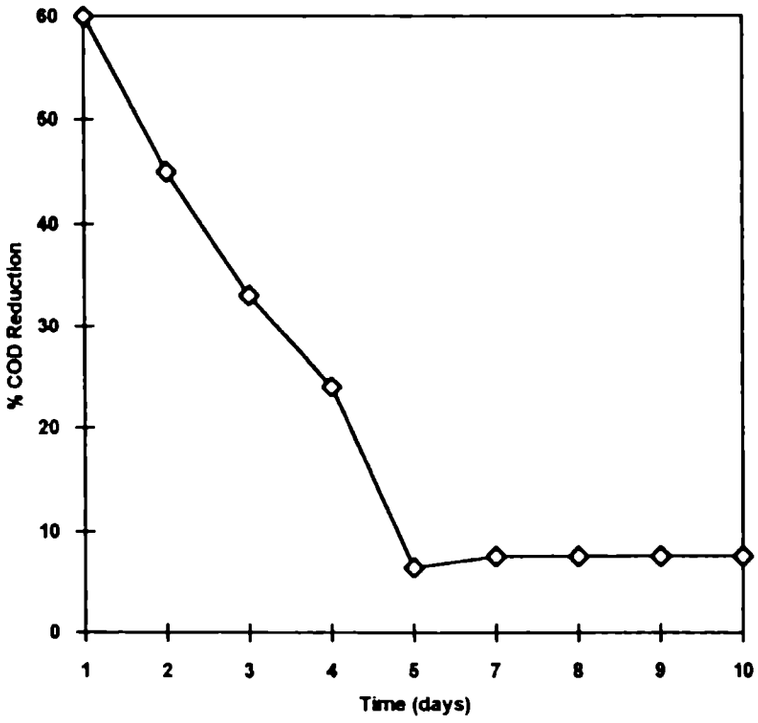


Fig 5.7 Performance of the packed bed reactor (PBR) with calcium alginate co-immobilized activated sludge and *Acinetobacter* sp. BTJR-10 in treating the rubber latex centrifugation effluent under continuous process.

Table 5.7 Comparison of the different processes tried as a single step method for the treatment of Rubber Latex Centrifugation effluent.

Methods tried	Average retention time	Percentage of COD reduction in the effluent
Packed Bed Reactor with *Acinetobacter* sp. BTJR-10	6 h	44
Chemical coagulation (using ferric sulphate)	2 h	58
Rotating Biological Contactor (At max. roughness and 5ml/min rate)	16 h	47
Activated Sludge System	48 h	69
Packed Bed Reactor with immobilized activated sludge	50 min	56
Packed Bed Reactor with co-immobilized activated sludge and *Acinetobacter* sp. BTJR-10	50min	60

CHAPTER - 6

BIOLOGICAL COAGULATION OF SKIM LATEX

CHAPTER - 6

BIOLOGICAL COAGULATION OF SKIM LATEX

6.1 INTRODUCTION

Skim latex, containing 2.5-10% of rubber, is obtained along with the concentrated rubber latex as an equal fraction in volume during centrifugation of the field latex. Protein and other non-rubber constituents, which have specific gravities higher than that of rubber, also migrate into the skim fraction during centrifugation and not only reduce the quality of the rubber but also affect the coagulation process. (Radhakrishna Pillai, 1980)

The important factors governing the composition of skim latex produced are the type of centrifuge, dry rubber content of field latex and efficiency of operation. Normally skim latex contains about two-thirds of the total serum from the field latex. Since the larger particles sperate more readily into concentrated fraction, the average size of the rubber particles in skim latex is smaller than that of field latex. In addition to the water soluble substances in serum, latex contains proteins which are mainly distributed as adsorbed film on the surface of the rubber particles and as the particles are smaller the protein content per unit weight of the rubber is more in the case of skim latex. This not only renders coagulation more difficult but also has a marked effect on the properties of the rubber. Skim latex also contains ammonia which adds to the cost and difficulty of

acid coagulation. With increasing efficiency in concentration the DRC of the skim fraction decreases and the difficulty and cost of the recovery process increase.

The usual method of recovery of skim rubber is by coagulation with sulphuric acid. In acid coagulation, the acid content of the coagulated rubber reduces its quality and shows some tendency to scorch (Naunton, 1961). Further, acid coagulation leads to generation of highly acidic latex centrifugation effluent (Jayachandran *et al*, 1994) which necessitates additional treatment before discharge to the environment inorder to avoid pollution. Coagulation of the skim latex is also performed using enzymes such as trypsin where enzymatic deprotenization of the skim latex can also be achieved. However, this process is not economical as it needs large quantity of enzyme and necessiates further steps such as deammoniation and addition of sodium thiosulphate and formic acid. Information on the coagulation of skim latex using microorganism is not available in the literature and hence the efficiency of whole cells of bacteria for the biocoagulation of skim latex was undertaken with a view, not only towards improvement of rubber quality but also, to reduce environmental pollution due to highly acidic rubber latex centrifugation effluent. To the best of our knowledge this study is the first report of this kind where whole cells of bacteria were applied for the coagulation of rubber (Jayachandran and Chandrsekaran, 1998).

6.2 MATERIALS AND METHODS

6.2.1 *SAMPLE*

From a local centrifugation plant, skim latex was collected in clean containers from the skim latex collection tank, after it was pumped from the latex centrifugation unit. Samples were used fresh immediately after collection.

6.2.2 *MICROORGANISM*

Acinetobacter sp BTJR-10, isolated from highly acidic rubber latex centrifugation effluent (section 3.2.2), was grown in 300ml of nutrient broth (HI) which contained g/l: peptone, 5; NaCl, 5; beef extract, 1.5 and yeast extract, 1.5; pH 7. 4 at 28°C and 150 rpm on a rotary shaker. After 18h, cells were harvested, at 4°C and at 10000g under sterile conditions. Cells were suspended in physiological saline, after washing with the same, and used for inoculation purposes.

6.2.3 *COAGULATION OF SKIM LATEX*

Coagulation studies were carried out in 500ml conical flasks at various dilutions of skim latex. Dilution of the skim latex was made with distilled water. Inoculation of the skim latex (200ml in each flask) was done at 1% (v/v) level using cell suspension prepared at various cell concentrations. After inoculation, the flasks were incubated at

28°C for 48h till a maximum quantity of the coagulated skim rubber was formed over the liquid surface. The experiment was simultaneously carried out using sulphuric acid (chemical coagulation) at the selected dilution rates. Samples were withdrawn at intervals, and analysed for dry rubber content (DRC), nitrogen content and chemical oxygen demand (COD).

6.2.4 *ESTIMATION OF DRY RUBBER CONTENT (DRC)*

Dry rubber content (DRC) of the skim rubber was estimated as follows (Indian Standard specification for natural rubber latex, 1985).

Required quantity of the well mixed latex sample was accurately weighed by difference from a weighing bottle. The sample was diluted to the necessary concentration with water and coagulated with a suitable coagulating agent. When the serum was clear small particles of coagulum were collected by rubbing with main bulk. The coagulum was washed with running water, and pressed to expell water to obtain a uniform sheet not exceeding 2mm in thickness either by hand roller or mechanical rollers. The coagulum was dried at a temperature of approximately 70 ± 2°C until there was no white patches. The coagulum was dried in a dessicator and weighed. Drying operation was repeated until the loss in mass is less than 1mg. The dry rubber content of the sample was calculated using the following equation

$$\text{DRC of the sample} = \frac{M_1 \times 100}{M_2}$$

M_1 - Mass in gram of dry coagulum

M_2 - Mass in gram of the sample taken for the test.

6.2.5 *ESTIMATION OF TOTAL NITROGEN CONTENT*

Total nitrogen content of the skim rubber was estimated as follows (Indian Standards, Specification for natural rubber latex, 1968).

Skim rubber, 0.1g was accurately weighed and added to a micro-Kjeldahl's flask. To this 0.65g of the catalyst mixture, containing of 30 parts of potassium sulphate, 4 parts of $CuSO_4.5H_2O$, and 1 part selenium, was added along with 3ml of Conc. H_2SO_4. The mixture was boiled gently for about half an hour. When the digest became clear green in colour with no yellow tint, 10ml of boric acid was added to the steamed out receiver of the distillation apparatus, followed by two drops of indicator. The receiver was placed under the condenser so that the end of the condenser was dipped below the surface of the boric acid solution. Approximately 10ml of concentrated sodium hydroxide solution was added to the distillation flask and washed with 5ml of water. Steam was passed from generating flask for four minutes and then the receiver was lowered until the tip of the condenser was well above the level of acid. Distillation was continued for one minute and then washed the end with water into the distillate. The final volume of the solution in the receiver was between 30 and 35 ml. Immediately the

contents of the receiver was titrated with standard sulphuric acid, till the colour of the indicator just turned to violet from green.

A blank determination using the same quantities of reagents and condition of test was performed and corrected accordingly.

The nitrogen content of the rubber sample was calculated as given below

$$\text{Nitrogen content percent by weight} = \frac{0.014 \times N(V_2 - V_1) \times 100}{W}$$

N - Normality of the titrant

$V_2 - V_1$ Volume in ml for the test portion minus volume in ml for the blank portion

W - Weight in gram of test portion.

6.2.6 *CHEMICAL OXYGEN DEMAND*

Chemical oxygen demand of the residual samples was estimated as detailed under the section 3.2.1.3.9

... medium concentrations compared to dilutions of 1:2 (v/v) (5.6% and 5.4% w/v, respectively, for 6.4 and 8.0 mg drycell/ml) and 1:100(v/v), (5.0% and 5.6% w/v, respectively, for 6.4 and 8.0 mg drycell/ml). Relatively, the nitrogen content of the skim

6.3 RESULTS

Fresh skim latex, used in the present study, had 9-10% (w/v) of dry rubber content, 9-11% (w/v) protein and a slightly alkaline pH (9.1) due to dissolved ammonia. Initially the optimal dilution of skim latex and the inoculation concentration which could support maximal coagulation of dry rubber was determined by carrying out coagulation at various dilutions of skim latex and cell concentrations. Results presented in Table 6.1 indicated that biocoagulation a dilution of 1:2, 1:10 and 1:20 (v/v) and with cell suspension containing 6.4 mg and 8.0 mg dry cell/ml as inoculum, at 1% (v/v) level effected coagulation yielding a dry rubber content of 5 - 8.3% after incubation for 48 h. The concentration of the skim latex in the sample inhibited bacterial activity at lower dilutions and hence the yield of rubber was less at dilution 1:1 and 1:2 (v/v) compared to 1:10 (v/v) dilution.

The experiment was repeated with inoculum concentrations of *6.4* and *8.0* mg drycell /ml at 1% (v/v), for selecting the ideal dilution of skim latex and the optimum inoculum concentrates, required for enhanced rubber yield. Three dilution viz 1:2, 1:10 and 1:100 (v/v) were tried. Yield of rubber, nitrogen content of the skim rubber and COD of the residual effluent were analysed after 48h. Maximal yield of rubber in terms of dry rubber content (8.3% w/v) was obtained at 1:10 (v/v) dilution rate irrespective of the inoculum concentrations compared to dilutions of 1:2 (v/v) (5.6% and 5.4% w/v, respectively, for 6.4 and 8.0 mg drycell/ml) and 1:100(v/v), (5.0% and 5.6% w/v, respectively, for 6.4 and 8.0 mg drycell/ml). Relatively, the nitrogen content of the skim

rubber was less 1.5% (w/v) at 1:10(v/v) dilution for the inoculum with 6.4 mg dry cell/ml compared to the inoculum with 8.0 mg drycell/ml (1.6%w/v). At 1:2 (v/v) dilution irrespective of the concentration of the inoculum tried the nitrogen content was same (1.7% w/v). Whereas at 1:100 (v/v) dilution the nitrogen content was 1.6 and 1.8% (w/v), respectively, for the inoculum with 6.4 and 8.0mg drycell/ml. COD of the residual effluent was 0.4 g/l for the dilution 1:10(v/v), irrespective of the concentration of the inoculum. While the dilution 1:2 (v/v) resulted in relatively a high level of COD in the effluent (10.0 and 9.5 g/l, respectively, for 6.4 and 8.0 mg drycell/ml), the 1:100 (v/v) dilution gave COD values of 2.7 and 2.0g/l, respectively for 6.4 and 8.0 mg dry cell/ml.

Chemical coagulation with sulphuric acid, with the same dilution and incubation periods supported only 6.8% (w/v) yield of rubber and the nitrogen content was comparatively higher (1.7% w/v). Moreover, the residual effluent recorded 2.2g COD/l (Table 6.2). The low nitrogen content of the biologically coagulated skim rubber, compared to that of chemically coagulated skim rubber, indicated less protein content and hence better quality. Further the pH of the residual effluent was in the range of 6.5-6.6 after biological coagulation compared to that with chemical coagulation (highly acid pH 2.8).

A comparative time course study on the effect of biological and acid coagulation was also carried out and the results are presented in Table 6.3. From the results it was inferred that maximal coagulation of rubber in terms of dry rubber content (8.3% w/v) was effected through biocoagulation after 48h compared to chemical coagulation. With

chemical coagulation a maximum dry rubber content of 6.7% w/v was recorded after 6h of coagulation and further incubation did not enhance the dry rubber content. Similarly, the COD levels in the residual effluent of biocoagulation process showed rapid decline with increase in incubation time and reduction in COD up to 0.4g/l was observed after 42h, unlike with chemical coagulation (2.2g/l).

6.4. DISCUSSION

From the present study, it is evident that coagulation of skim latex can be effectively done with whole cells of bacteria and *Acinetobacter* sp. BTJR-10 has the potential for probable application in the rubber manufacturing industry, for recovering maximal rubber from skim latex with improved quality by virtue of reduced protein content in the rubber compared to that obtained with acid coagulation. Moreover the biocoagulation process assures economic wastewater management in terms of contributing to a low level of COD in the effluent. In fact, chemical coagulation results in a highly acidic effluent which warrants additional neutralisation of pH before the effluent is discharged to the environment, whereas, biocoagulation which resulted in very mild acidic addition (pH 6.5) in the effluent, hardly requires intense treatment before its discharge. It is to be noted that the same strain was reported earlier as a potential strain for the treatment of latex centrifugation effluent (Jayachandran *et al*, 1994) and is now being observed to be efficient for coagulation of skim latex the process which generates this effluent.

Considering the above facts it is evident that for skim latex, biocoagulation offers a better alternative for chemical coagulation in termsof the COD of the residual effluent and also in terms of the quality of the skim rubber obtained, despite the fact that it takes longer time. However, further studies on the role of active principles involved in the biocoagulation process, identification of the genes and overexpression of the same could definitely lead to an economic bioprocess.

Table 6.1 Estimation of rubber yield (in terms of dry rubber content) at various dilutions of skim latex and at different inoculum concentrations.

Rubber yield expressed as % w/v

Dilution factor of the skim latex (v/v)	Inoculum concentration (mgdc/ml)				
	1.6	3.2	4.8	6.4	8.0
1.1	2.7	2.9	3.2	4.9	3.4
1.2	3.7	4.0	4.2	5.6	5.4
1.10	4.2	4.7	5.0	8.3	8.3
1.20	1.8	2.0	3.5	5.0	5.5
1.100	0.7	0.7	0.8	0.8	1.4

Inoculation at 1% (v/v);

48 h incubation;

mgdc/ml is mg dry cell/ml.

Table 6.2 Analysis of the chemical coagulation of the skim latex.

Dilution factor of the skim latex (v/v)	**COD of the residual effluent (g/l)**	**Nitrogen content of the skim rubber (%w/v)**	**Dry rubber content of the skim rubber (% w/v)**
1:2	46.7	1.8	1.6
1:10	2.2	1.7	6.8
1:00	1.3	1.7	6.6

Incubation for 48h.

Table 6. 3 Comparison of the yield of rubber and COD of the residual effluent after biological coagulation with *Acinetobacter* sp. and chemical coagulation with sulphuric acid

Period of coagulation (h)	Dry rubber content of the skim rubber (% w/v)		COD of the residual effluent (g/l)	
	Biological * coagulation	Chemical coagulation	Biological coagulation	Chemical coagulation
6	0.0	6.7	21.0	2.2
12	0.0	6.7	19.9	2.2
18	0.0	6.7	18.9	2.2
24	1.2	6.7	15.4	2.2
30	3.6	6.7	10.4	2.2
36	6.5	6.7	3.0	2.2
42	8.1	6.7	1.0	2.2
48	8.2	6.7	0.4	2.2
54	8.3	6.7	0.4	2.2

Dilution of the skim latex used, 1:10 (v/v)

* For biological coagulation cell suspension with 6.4 mg dry cell/ml was used as inoculum, at 1%(v/v).

CHAPTER - 7

SUMMARY AND CONCLUSIONS

CHAPTER 7

SUMMARY AND CONCLUSIONS

7.1 SUMMARY

7.1.1 The rubber latex centrifugation effluent generated during the different unit operations of latex centrifugation is highly acidic (pH 2.5 - 4.5) has high COD (28,000 mg/l) BOD (6000 mg/l) and sustain some microbial growth.

7.1.2 *Acinetobacter* sp. BTJR -10. isolated from the rubber latex centrifugation effluent is capable of treating the same. Under immobilized state this strain could bring about 44% reduction in COD during continuous treatment of the effluent in a Packed Bed Reactor. Recycling of the effluent in the Packed Bed Reactor led to enhancement in the percentage of COD reduction upto 49%.

7.1.3. The effluent was subjected to an initial coagulation using ferric sulphate, alum and aluminium sulphate as coagulants at pH 3 and pH 7. Initial coagulation of the effluent at pH 7 using ferric sulphate resulted in enhanced percentage of reduction in COD (77%) on continuous treatment with immobilized *Acinetobacter* sp BTJR-10 in Packed Bed Reactor whereas in the case of alum, maximal percentage reduction of COD was 50% at pH 3.

7.1.4. A laboratory scale Rotating Biological Contactor (RBC) of 5l capacity was designed, and treatment studies were conducted The optimum RPM of the RBC for better reduction of the organic load of the effluent was selected as 2 RPM. Among the different methods tried for biomass estimation on RBC disc, attached disc method was efficient and hence selected for routine analysis. The optimum conditions for the formation of biomass on disc were pH 7, RPM 2, incubation - period 48h. Efficiency of RBC for COD reduction in effluent was evaluated at three specific roughness of the RBC disc viz Minimum, Medium and Maximum, using *Acinetobacter* sp. and mixed culture seed inoculum. A steady state condition in RBC was achieved at all roughness, seperately for both 'mixed culture seed inoculum' and *Acinetobacter* sp. within 10 days of continuous operation. At minimum roughness of the RBC discs the maximum percentage of COD reduction, for mixed culture seed inoculum was 37% and for *Acinetobacter* sp. it was 35%. At medium roughness of the RBC discs, the maximum percentage of COD reduction obtained was 47% for mixed culture seed inoculum and 43% for *Acinetobacter* sp. Whereas, at maximum roughness of the RBC discs the percentage of COD reduction obtained at steady state for mixed culture seed inoculum was 52% and for *Acinetobacter* sp. it was 48%.

7.1.5. After attaining steady state the RBC was operated at different flow rates of the effluent (5ml/min, 10ml/min, and 15ml/min) at each roughness of the discs using mixed culture seed inoculum and *Acinetobacter* sp. separately. At all roughness of the disc, a maximum percentage of COD reduction was obtained at 5ml/min

flow rate and also the mixed culture seed inoculum gave a better enhancement in COD reduction.

7.1.6 An activated sludge system was developed for the treatment of rubber latex centrifugation effluent. Steady state operation of the activated sludge system was attained after a 'fill and draw' operation for 25-30 days. A maximum of 69% COD reduction was obtained at the steady state of activated sludge system with the optimum hydraulic retention time of 48h.

7.1.7 Activated sludge, under immobilized condition in both tube polymerised agar and alginate effected 39% reduction in COD in 2h retetion time during batch treatment. Whereas under continuous treatment there was enhanced reduction of COD (56%) in 50 minutes of retention time.

7.1.8. Co-immobilized activated sludge and *Acinetobacter* sp. BTJR - 10 recorded a maximum COD reduction of 60% in 50 minutes of residence time on continuous treatment in a Packed Bed Reactor.

7.1.9 Biocoagulation of skim latex was done with *Acinetobacter* sp. BTJR - 10. Under selected conditions of 48h incubation, 1:10 dilution rate for skim latex and at a cell concentration of 6.4 mg dry cell/ml, biocoagulation was effective such that the rubber yield was 8.342% and the COD of the residual solution was only 406 4mg/l.

7.1.10. Whereas the chemical coagulation of the skim latex at the same dilution resulted in only 6.75% rubber yield and the COD of the left out effluent was 2235.2 mg/l. However, the time required for chemical coagulation was only 6h

7.2. CONCLUSIONS

As the rubber latex centrifugation effluent is having high amount of suspended solids, clogging of the pores of the immobilized beads and hence diffusional limitation is a major drawback. Thus Packed Bed Reactor with immobilized cells although gave promising results for the rapid treatment of the effluent, cannot be used as a single step method for the latex effluent treatment. However, an initial coagulation prior to treatment with Packed Bed Reactor may solve the limitations of this method in industrial scale application

A major outcome of the present study is the recognition of Rotating Biological Contactor (RBC) for the treatment of the rubber latex centrifugation effluent. Using RBC, partial treatment of the effluent can be achieved, comparatively on a large scale and also at higher organic loading rate. However, the efficiency was found to the significantly affected by the surface nature of the RBC discs. Modification of the reactor or an improved inoculum may facilitate safe disposal of latex effluent without the need for larger land space and longer treatment period.

Activated sludge system, showed tremendous potential for application in the treatment of the rubber latex centrifugation effluent, towards healthier environment once scaling up studies are conducted. The process is not only economic, but also holds great promise for the large scale treatment of the effluent as a conventional system.

In general it was observed that Packed Bed Reactor with immobilized bacterial cells, Rotating Biological Contactor and Activated Sludge System hold ample scope for the safe disposal of the latex effluent.

Almost no information is available in the use of bacterial cells for the coagulation of skim latex. The dual role of the *Acinetobacter* sp. BTJR -10, not only in reducing the organic load of the effluent but also yielding rubber of good quality through bioprocess is very much encouraging and promising. Considering the fact that there exists a need for improvement in the quality of rubber, and safe and economic waste water management in the rubber production industries, biocoagulation process could become an effective alternative or additional process for attaining maximum yield of rubber. Of course, there is ample scope for the improvement of the process through strain improvement programme. It is concluded that quality rubber could be obtained from skim latex along with generation of the effluent with minimum COD load towards economic management of rubber latex centrifugation effluent using whole cells of bacteria and suitable bioprocess.

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