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"TREATEMENT OF INDUSTRIAL EFFLUENTS IN A BIOREACTOR"

SUBMITTED BY:-

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CERTIFICATE

This is to certify that the project entitled, **"TREATEMENT OF INDUSTRIAL EFFLUENTS IN A BIOREACTOR"** submitted by Sri NAYANA KUMAR BEHERA in partial fulfillments for the requirements for the award of Bachelor of Technology Degree in Chemical Engineering at National Institute of Technology, Rourkela (Deemed University) is an authentic work carried out by him under my supervision and guidance.

To the best of my knowledge, the matter embodied in the project report has not been submitted to any other University / Institute for the award of any Degree or Diploma.

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ABSTRACT

The sources of occurrence of various pollutants from chemical process industries and there harmful effects have been highlighted. Typical composition of wastewater from various sources presented. The methods of treatment of wastewater briefly discussed. Special attention has been paid to the biological treatment mentioning the drawbacks of the traditional methods. The relative advantages of various modern bioreactors working on immobilization technique have been projected. A comparative picture with respect to various modern bioreactors has been presented and the uniqueness of the activated sludge and the fluidized bioreactors in the treatment of wastewater has been emphasized.

Effluent was collected from Rourkela Steel Plant. BOD and COD were then done to measure the oxygen requirement of the effluent. It was then subjected to batch culturing at pH 6.5 to 7.5 and temperature 28 to 30ºC. COD was done on each day of batch culture. The gradual decrease of COD determines the viability of the microorganisms in the batch. After some days of batch culturing plastic beads were inserted so that adsorption over the plastic beads can occur and immobilization can take place. Then SEM was used to know the thickness of the microbes coated over the surface of the beads. Phenol is one of the most common contaminant, the methods of treatment of phenolic wastewater discussed emphasis given on the aerobic biological treatment. Special attention has been paid to the biological treatment. The relative advantages of various modern bioreactors working on immobilization technique have been projected.

Keywords: Biological treatment, BOD, COD, Phenol, Cell Immobilization.

CHAPTER 1

Introduction and Literature Survey

1.1. Fresh Water Crisis

Water is, literally, the source of life on earth. About 70 percent of the earth is water, but only one percent is accessible surface freshwater. The one percent surface fresh water is regularly renewed by rainfall and other means and thus available on a sustainable basis and easily considered accessible for human use. Water is the biggest crisis facing the world today. In India the crisis in terms spread and severity affects one in three people. As per an estimate in 2000, there were 7,800 cubic meters of fresh water available per person annually. It will be 5,100 cubic meters (51,00,000 liters) by 2025. Even this amount is sufficient for human needs, if it were properly distributed. But, equitable distribution is not possible India, which has 16 percent of world's population, 2.45 percent of world's land area and 4 percent of the world's water resources, has already faced with grave drinking water crisis. Water is the single largest problem facing India today. Years of rapid population growth and increasing water consumption for agriculture, industry and municipalities and other areas have strained Indian fresh water resources. In many parts of our country chronic water shortages, loss of arable land, destruction of natural habitats, degradation of environment, and widespread pollution undermine public health and threaten economic and social progress. By 2050 more than 50 percent of population is expected to shift to the cities and the drinking water scarcity will be acute. In the developed world, for example, the United Kingdom must spend close to $60 billion building wastewater treatment plants over the next decade to meet the new European water quality standards. The World Bank has estimated that over the next decade between US $ 600 to 800 billion will be required to meet the total demand for fresh water, including that for sanitation, irrigation and power generation. A water short world is inherently unstable world. Now the world needs another revolution, i.e., a Blue Revolution for conservation and proper maintenance of freshwater.

1.2. Environmental pollution

Environmental pollution is an emerging threat and of great concern in today's context pertaining to its effect on the ecosystem. Water pollution is one of the greatest concerns now a day. In recent years, considerable attention has been paid to industrial wastes discharged to land and surface water. Industrial effluents often contain various toxic metals, harmful dissolved gases, and several organic and inorganic compounds. These may accumulate in soil in excessive quantities in long-term use, ultimately physiologically adverse effects on crop productivity.

The worldwide rise in population and the industrialization during the last few decades have resulted in ecological unbalance and degradation of the natural resources. One of the most essential natural resources, which have been the worst victim of population explosion and growing industrialization, is water. Huge quantity of wastewater generated from human settlement and industrial Sectors accompany the disposal system either as municipal wastewater or industrial wastewater. This wastewater is enriched with varied pollutants and harmful both for human being and the aquatic flora and fauna, finds it way out into the nearly flowing or stationary water bodies and thus makes natural sources of water seriously contaminated.

It has been estimated that over 5 million chemical substances produced by industries have been identified and about 12000 of these are marketed which amount to around half of the total production. Due to discharge of toxic effluents long-term consequence of exposure can cause cancer, delayed nervous damage, malformation in urban children, mutagenic changes, neurological disorders etc. Various acid manufacturing industries discharge acidic effluent, which not only make the land infertile. But make the water of the river acidic also. The high acidity causes stomach diseases and skin ailments in human beings

1.3. Treatment and recycle

Thus it is imperative to purify and recycle wastewater in view of reduced availability and deteriorating water quality. Phenol along with other xenobiotic compounds is one of the most common contaminants present in effluents from chemical process industries. Even at lower

concentration these compounds adversely affect aquatic as well as human life [1-4,8-13]. Also these compounds form complexes with metal ions discharged from other industries, which are carcinogenic in nature. It is water soluble and highly mobile. This imparts medicinal taste and odour even at much lower concentration of 2 μg/l and it is lethal to fish at concentrations of 5-25 mg/l [10]. The maximum permitted concentration level of phenol being 0.5-1 mg/l for industrial wastewater and 1μg/l for drinking water [15,17]. So it is highly essential to save the water resources and aquatic life by removing these compounds from wastewater before disposal.

The main sources of phenolic wastewater are coal chemical plants, oil refineries, petrochemical industries, fibre glass units, explosive manufacture, phenol-based polymerization process, pharmaceuticals, plastic, paints and varnish producing units, textile units making use of organic dyes, anticeptics, antirust products, biocides, photographic chemicals and smelting and related metallurgical operations, etc [2,8-10,17,20].

Table 1: Characteristics of wastewater from process industries

Parameter / Source & amount range, mg/l	From steel industry	From petroleum refinery	From LT coal carbonization	From milk diary plants
PH	8.5-9.5	-	9.0	7.3-9.5
Total solids	175-1300	-	6720	1690-2730
Dissolved solids	125-800	-	5312	920-1660
Suspended solids	50-500	200-400	1408	690-1810
Oils and grease	-	2000-3000	-	290-1390
Chlorides as Cl	-		Nil	104-190
HS and mercaptans	-	10-220	-	-
Nitrogen	800-1400	-	-	62
Sulphates / sulfides	110-220	09	802	Trace
Cyanides	10-50	-	4576	-
Thiocyanates	50-100	-	2840	-
Phenol	500-1000	1500-2000	10240	-
Total alkalinity	-	-	14670	564-610
Phenolphthalein alkalinity	-	-	Nil	152-185
Turbidity	-	-	-	Turbid
BOD	160	100-300	11100ppm	816-3070
COD	790-2450	-	20400ppm	1000-4510

1.4. Hazardous effect of phenol

1.4.1. Phenol :

Fig. 1 structure of phenol

Chemical Formula: C_6H_5OH

Other names: carbolicacid, Benzenol, PhenylicAcid, Hydroxybenzene, Phenic acid.

solubility in water : Phenol has a limited solubility **(**8.3 g/100 ml) at (20 °C) . And is slightly acidic.

Appearance: White Crystalline Solid

It contains a six-membered aromatic ring, bonded directly to a hydroxyl group (-OH).

1.4.2. Uses of Phenol:

- Medical Use: Phenol has antiseptic properties and is used for aseptic (germ-free) techniques in surgery.
- Industrial Use: It is used in the production of drugs i.e(industrial production of aspirin), herbicides, and synthetic resins etc.
- Laboratory Use: Phenol is used along with chloroform (a commonly-used mixture in molecular biology for DNA & RNA purification from proteins).
- Beauty Products: Phenol is also used in the preparation of cosmetics including sunscreens, hair dyes, and skin lightening preparations

1.4.3. Toxicity of Phenol

Acute exposure of phenol causes

- Disorders of central nervous system leading to collapse and coma.
- Hypothermia A reduction in body temperature.

- Acute exposure of phenol can result in myocardial depression.
- Muscle weakness and tremors are also observed.
- Phenol causes a burning effect on skin. Whitening and erosion of the skin may also result due to phenol exposure.
- Exposure to phenol may result in irritation of the eye, corneal whitening and finally blindness.
- Phenol can cause gastrointestinal disturbance, vomiting, weakness, weightlessness and muscle pain.
- It is also suspected that exposure to phenol may cause paralysis, cancer as Phenol and its derivatives are toxic and classified as hazardous materials.

1.4.4. Phenolic Effluent

- Phenol along with other xenobiotic compounds is one of the most common contaminants present in effluents from chemical process industries.
- The main sources of phenolic wastewater are coal chemical plants, oil refineries, petrochemical industries, fiber glass units, explosive manufacture, phenol-based polymerization process, pharmaceuticals, plastic, paints and varnish producing units,
- Textile units making use of organic dyes, antiseptics, antirust products, biocides, photographic chemicals and smelting and related metallurgical operations,
- Various acid manufacturing industries discharge acidic effluent, which not only make the land infertile but make the water of the river also. The high acidity causes stomach diseases and skin ailments in human beings.

1.4.5. Necessity of Phenol degradation

- Phenol is a major human carcinogen and is of considerable health concern, even at low concentration.
- The high toxicity of phenol is a alarming reason itself to purify and recycle phenolic wastewater.
- Many technologies have been investigated for removing and degradation of phenolic compounds in wastewater.

1.4.6. Methods of Phenol Degradation

- Chemical oxidation -Chemical oxidation requires a reactor, which operates at high temperature and high pressure, ultimately huge energy.
- Ion exchange adsorption- In adsorption commonly activated carbon is used which is disposed by incineration. The process of incineration generates many new compounds such as dioxins and furans have very severe consequences on human health.
- Solvent extraction -Solvent extraction there is a danger of contamination of treated water by the solvent. The solvents used for phenol recovery are benzene, isopropyl ethyl and butyl acetate. In addition to the presence of solvent in treated water, the high cost of solvent is another disadvantage.

1.5. Treatment Methods

The conventional methods of treatment of phenolic and nitrate-nitrogen wastewater are largely physical and chemical processes but these processes led to secondary effluent problems due to formation of toxic materials such as cyanates, chlorinated phenols, hydrocarbons, etc. These methods are mainly chlorination, ozonation, solvent extraction, incineration, chemical oxidation, membrane process, coagulation, flocculation, adsorption, ion exchange, reverse osmosis, electrolysis, etc [2,8,9,19].

In solvent extraction there is a danger of contamination of treated water by the solvent. The solvents used for phenol recovery are benzene, isopropyl ethyl and butyl acetate. In addition to the presence of solvent in treated water, the high cost of solvent is another disadvantage. In adsorption commonly activated carbon is used which is disposed by incineration. The process of incineration generates many new compounds such as dioxins and furans have very severe consequences on human health. Chemical oxidation requires a reactor, which operates at high temperature and high pressure, ultimately huge energy [2]. Aerobic and anaerobic biochemical treatment techniques are replacing these methods because of their inherent advantages.

1.5.1. Biodegradation

Biodegradation is a biological treatment method and is attractive due to the potential to almost degrade phenol and overcomes the disadvantages posed by other processes .It produces producing innocuous end products, reduced capital and operating costs, maintain phenol concentrations below the toxic limit.

Advantages:

- It is the most potential method to degrade phenol below the toxic limits
- No harmful byproducts
- Simple to install
- Low capital and operating cost
- Self regulating

Disadvantage:

- Degradation mechanism hardly known
- difficult to control
- slow response time

Features:

- Removes hydrocarbons and BOD/COD in contaminated water through an attached growth biological treatment technology.
- Uses oxygen transfer with a large protected biofilm attachment area to achieve high removal rates.
- Incorporates neutrally-buoyant Media Pac.

Benefits:

- Increases the efficiency of the biological treatment process by increasing the amount of surface area.
- Capable of treating a variety of flow rates and contaminants.
- Minimal maintenance compared to other biological treatment systems.
- The FBBR Media Pac incorporates high surface area and large void spaces that are aggressively sloughed to eliminate biofilm growth and fouling.

The most efficient *Pseudomonas Putida* is capable of using phenol as the sole source of carbon and energy for cell growth and metabolism degrade phenol via metapathway. That is the benzene ring of phenol is dehydroxylated to form catechol derivative and the ring is then opened through meta-oxidation. The final products are molecules that can enter the tricarboxylic acid cycle.

The most common Bio-reactors are (1) Aerated lagoon, (2) Oxidation Ditch, (3) Activated sludge system, (4) Anaerobic digestion system, (5) Oxidation pond, (6) Trickling filters, (7) Rotating discs biological reactors, (8) Basket type bioreactors, (9) Hollow fiber membrane bioreactor, and (10) Fluidized bed bioreactors [1,2,4].

Aerobic processes have several advantages, including a large range of wastewater that can be treated, high degree of BOD removal, acceptability of toxic conditions, simultaneous nitrogen and phosphorous removal, better chlorinated organic contaminants degradation, low solids retention time, and feasible small plants.

1.5.2. Aerobic Degradation

The aerobic biodegradation process is represented by the following equation $C_xH_y + O_2 +$ (microorganisms / nutrient) ----------→ $H_2O + CO_2 +$ biomass. Aerobic treatment of waste is the degradation and purification process in which bacteria that thrive in oxygen-rich environments break down and digest the waste. The mixed aerobic microbial consortium uses the organic carbon present in the effluent as their carbon and energy source. The complex organics finally get converted to microbial biomass (sludge) and carbon dioxide.

1.5.3. Digestion Pathway

During this oxidation process, contaminants and pollutants are broken down into end products such as carbon dioxide, water, nitrates, sulphates and biomass (microorganisms). In the conventional aerobic system, the substrate is used as a source of carbon and energy.

It serves as an electron donor, resulting in bacterial growth. The extent of degradation is correlated with the rate of oxygen consumption in the same substrate. Two enzymes primarily involved in the process are di and mono-oxygenases. The latter enzyme can act both aromatic and aliphatic compounds, while the former can act only on aromatic compounds. Another class of enzymes involved in aerobic condition is peroxidases, which are receiving attention recently for their ability to degrade lignin.

1.5.4. Characteristics of aerobic bioreactors

A large range of waste water can be treated. Purification and resettling required. Can handle low to high CODs. Suitable for both cold and warm effluent. Acceptable to toxic presence of toxic materials to certain extent. Neutralization of alkaline wastewater required. Operated in continuous mode with less stability and control. High oxygen requirement. Degree of BOD removal is also high. Simultaneous nitrogen and phosphorous(nutrients) removal is possible. Posses high degradation rate to Chlorinated organic contaminants. When carrier material is used leads to clogging danger. Volumetric loading rates and solids retention time is low. Maintenance required for aeration systems, sludge treatment. Has odour problems if open systems used. Sludge production is high. Investment cost low to medium. High costs for aeration (power), nutrients, sludge disposal. Small plants are possible.

Aerobic treatment produces greater amount of CO_2 which is let out in the environment to increase the atmospheric green house gas (GHG) content. For aerobic treatment the total, the total output is 2.4 kg CO_2/ kg COD (1.4 kg CO_2/ kg COD due to oxidation of hydrocarbons and rest due to degradation of the pollutants in the wastewater). CIS 1, 2 Dichloroethene (DCE) and vinyl chloride concentrations reduced by an average of 80% in aerobic bioreactor. From the study of the effect of toxic chemicals (inhibitory compounds), namely $CrCl_3$, $FeCl_3$, $NaBO_3$, NaCl, $NaNO_2$, $NaNO_3$, and $CHCl_3$, it is found that the oxygen utilization reduced by the biomass during the metabolism in the aerobic bioreactors. In dye wastewater treatment azo dyes are cleaved to aromatic amines. These amines mineralized by means of aerobic treatment by nonspecific enzymes through hydroxylation and ring opening giving rise to CO_2, H_2O and NH_3 under aerobic conditions. For treatment of tannery water aerobic bioreactors superior in terms of loading and presence of toxic chemicals and sludge produced contaminated only to a small

fraction with chromium. Studies carried out with wastewater from a poultry slaughterhouse showed that COD removal ratio was generally higher in the aerobic bioreactor. Successfully treats the Ploychlorinated Dibenzo Dioxin (PCDD) and Ploychlorinated Dibenzofuran (PCDF). A large number microorganisms that includes Pseudomonas sp., degrade alkanes; mono and poly aromatics, benzene, toluene etc. a part of petroleum hydrocarbon pollution.

The drawbacks are huge amounts of sludge and carbon dioxide production, less stability and control of process, maintenance of aeration and sludge disposal systems, high costs for aeration and sludge disposal, clogging danger when carrer material is used and odour problem in open system.

1.5.5. Advancement of Aerobic Bioreactors in Wastewater Treatment

Over the conventional type free-culture bio-reactors the immobilization cell bioreactors like CSTR, PFR, fluidized bed, air lift type, etc. has the following advantages like continuous reactor operation at any desired liquid throughput without risk of cell washout, protection of cells from toxic substrates, higher growth rate gives high concentration of cells in the reactor, easy cell-treated water separation, enhanced gasliquid mass transfer rate, plug flow operation by maintaining the immobilized cells as a stationary phase [1,2,8-10,14,15,17]. The fluidized bed bioreactors are superior in performance due to immobilization of cells on solid particles reduce the time of treatment, volume of reactor is extremely small, lack of clogging of bio-mass and removal of phenol even at lower concentrations [1,2,4-6,9-19].

1.5.6. Fluidized Bed Bioreactor

Fluidized-bed bioreactors (FBR) have been receiving considerable interest in wastewater treatment. A fluidizedbed bioreactor consists of microorganism coated particles suspended in wastewater which is sufficiently aerated to keep the gas, liquid and the solid particles thoroughly mixed. An FBB, on the other hand, is capable of achieving treatment in low retention time because of the high biomass concentrations that can be achieved .A bioreactor has been successfully applied to an aerobic biological treatment of industrial and domestic wastewaters. Biological fixed films exhibit properties that make them preferable to suspended-cell systems for a wide variety of wastewater treatment applications. These properties include high cell

concentrations, enhanced cell retention due to cell immobilisation and an increased resistance to the detriment effects of toxic shock loadings.

Schematic diagram of Fluidized-bed (1) reservoir; (2) temperature control system; (3) pH control system; (4) liquid rotameter; (5) pump; (6) intermediate reservoir; (7) air distributor; (8) sampling; (9) fluidised section; (10) disengaging section; (11) air rotameter.

Fig .2 Schematic Diagram of Fluidized Bed

1.5.7. Fluidized bed bioreactor for wastewater treatment

This reactor had been successfully applied in the treatment of several kinds of wastewater such as ammonia-nitrogen containing wastewater, photographic processing wastewater, phenolic waste water, coke oven wastewater, and other domestic and industrial wastes. Also used successfully for the reductive biotransformation of mercuric ions to elemental mercury present in the effluents from industrial amalgam process, combustors and power stations [1,2,4-6,9,11-15,18,19].

A fluidized bed bioreactor (FBB) is capable of achieving treatment in low retention time because of the high biomass concentration. FBB offers distinct mechanical advantages, which allow small and high surface area media to be used for biomass growth [1,2,9,13-15]. Fluidization overcomes operating problems such as bed clogging and the high-pressure drop, which would

occur if small and high surface area media were employed in packed-bed operation. Rather than clog with new biomass growth, the fluidized bed simply expands. Thus for a comparable treatment efficiency, the required bioreactor volume is greatly reduced. A further advantage is the possible elimination of the secondary clarifier, although this must be weighed against the medium-biomass separator [13,15].

Conventional FBB are operated in two different ways. In a bioreactor with a heavy (matrix particle density larger than that of liquid) biomass support (e.g. silica sand, coal), fluidization is commonly conducted with an upward co current flow of gas and liquid through a bed of particles. Under fluidization conditions, the bed is fluidized with an upward flow of a liquid counter to the net gravitational force of the particles. Once fluidized, each particle provides a large surface area for biofilm formation and growth. The support media eventually become covered with biofilm and the vast available growth surface afforded by the media results in a biomass concentration approximately an order of magnitude greater than that maintained in a suspended growth system [13,15].

A practical problem, which occurs in the operation of an FBB, is the excessive growth of biomass on support media. This can lead to the channeling of bioparticles in fluidized beds since biomass loading can increase to such extent that the bioparticles began to be carried over from a bioreactor. The problem of over expansion of fluidized bed due to biomass growth has generally been solved by the removal of heavily biomassladen particles from bioreactor, followed by the addition of biomass-free particles. However this solution complicates operation of a bioreactor and introduces the need for additional equipment external to the bioreactor, such as a vibrating screen or an incinerator [13-15].

The degradation of phenolic type liquors, derived from coal processes, in a continuous stirred-tank bioreactor (CSTB), packed-bed bioreactor (PBB) and FBB shown in [15]. The degradation rates of 0.087, 0.053 and 0.012 kg phenol/m^3 were achieved in the FBB, PBB and CSTB respectively.

The nutrients for microbial growth are transported first from bulk phase to the surface of the biofilm, and then transported to the inner regions of the biofilm via diffusion. The limiting mass

transport rate controls the performance of the biofilm reactor [12,15]. From literature it is seen that the external resistance can be neglected in the case of a high fluidization flow rate [12]. In a three-phase fluidized bed bioreactor it is found reaction rate follows first-order kinetics with respect to oxygen and zero-order one with respect to phenol. For chemical and bio-chemical process, where mass transfer is the rate-limiting step, it is important to know the gas hold-up as this is related directly to mass transfer. The gas hold up at high pressures is always larger than that at low pressures, regardless of the liquid velocity and particle size in three-phase fluidization.

1.5.8. Activated sludge

Activated sludge is a process dealing with the treatment of sewage and industrial wastewaters. Atmospheric air or pure oxygen is bubbled through primary treated sewage (or industrial wastewater) combined with organisms to develop a biological floc which reduces the organic content of the sewage. **Activated sludge** is also the name given to the active biological material produced by activated sludge plants and which affects all the purification processes.

Fig. 3 Schematic diagram of an activated sludge process

1.6. Pseudomonas putida

Pseudomonas putida is a gram-negative rod-shaped saprotrophic soil bacterium. It is the first patented organism in the world.

Fig .4 pseudomonas putida

1.7. Immobilization of Microbial Cells

Cells of mixed culture collected from soils containing pollutants or specific culture (pure) isolated from the pollutant containing soil are immobilized in/on solid matrix. The specific cultures such as *Pseudomonas Putida* (NICM, Sp, MTCC, Q5, DSM, KT etc) either psychotropic or mesophilic type, *T cutaneum* R57 used for biodegradation of phenol, Catechol, Azo dyes removal of ionic mercury etc., *Pseudomonas* spp. And Bacillus spp. used for denitrification, green sulfur bacteria for sulfide removal etc. are used for immobilization [2,4-12].

Acclimization of microorganismss is done by increasing the pollutant concentration (say of phenol) gradually during culture preparation. The acclimized culture is used for the immobilization in/on the solid matrix [8].

Immobilization of cells means that the cells have been confined or localized so that it can be reused continuously. These exhibit totally different hydrodynamic characteristics than surrounding environment. Living cells produce enzymes (biological catalysts) to catalyze cellular reactions vital to the organism. The microorganisms are normally immobilized on

natural and synthetic supports [10]. Various types of solid matrices like polyacrylamide gel, Ca alginate, porous glass, plasic beeds, activated carbon, sand, charcoal, diatomaceous earth, cement balls made of coal ash, cellulose, polymeric materials, polymeric ions, chitosan, lignins, chitins, coal, collagens etc. have been used for immobilization of whole cells. In the recent years,
the immobilization of biocatalysts with polyvalent salts of alginic acids has received much attention because of low cost of alginate and the mild conditions of immobilization[8].

Techniques of immobilization are broadly classified into four categories namely covalent bonding, cross-linking (chemical methods), entrapment and adsorption (physical methods). Covalent binding most extensively used technique, where cells or enzymes are covalently linked to the support through the groups in them or through the functional groups in the support material. In the cross-linking technique, the cells are immobilized through chemical cross-linking using homo as well as hetero-bifunctional cross-linking agents. Adsorption is the simplest of all techniques and does not alter the activity of the bound cells. Adsorption involves adhesion or condensation of the cells to the surface of a carrier. The diving force causing immobilization is the combined hydrophobic interactions, hydrogen bonding and salt bridge formation between the adsorbent and cells. Entrapment within gels or fiber is a convenient method for reactions involving low molecular weight substrates and mainly used for immobilization of whole cells. This method is nothing but the polymerization of the unsaturated monomers in the presence of Cells results in the entrapment of the cells within the interstitial spaces of the gel.

CHAPTER 2

EXPERIMENTAL

2.1. Analytical methods for water quality parameters

- BOD-The quantity of oxygen required by the microorganisms for the stabilization of the biological decomposable organic matter. BOD tests measure the molecular oxygen utilized during a specified incubation duration for the biochemical degradation of organic material and the oxygen used to oxidize inorganic material such as ferrous iron and sulfides. The most common BOD test consists of a 5 day period in which a sample is placed in an airtight bottle under controlled conditions temperature (20ºC ± 1ºC), keeping any light from penetrating the sample to prevent photosynthesis. The Dissolved Oxygen (DO) in the sample is measured before and after the 5 day incubation period, and BOD is then calculated as the difference between initial and final DO measurements. BOD can be considered a more "natural" test in determining the oxygen required to oxidize organic matter.

COD

- Chemical oxygen demand test determines the oxygen required for chemical oxydation of organic component as well as the no. of inorganic component with the help of strong oxidant i.e. potassium dichromate.

- Once oxidation is complete, the excess potassium dichromate is titrated with ferrous ammonium sulfate (FAS) until all of the excess oxidizing agent has been reduced to Cr^{3+}. Typically, the oxidation-reduction indicator Ferroin is added during this titration step as well. Once all the excess dichromate has been reduced, the Ferroin indicator changes from blue-green to reddish-brown.

- COD is often preferred for daily analysis since it is inherently more reproducible, accounts for changing conditions and takes a short time to complete.

2.2. Methods to know the phenol concentration in the effluent:

- Spectrophotometric analysis- phenolic materials react with 4- aminoantipyrine in the presence of potassiumferricyanide at a pH of 10 to form a stable antipyrine dye. This can be done with two methods direct photometric method and chloroform extraction method by reading the absorbance at 510nm and 460nm respectively. **Direct photometric** method is used when the phenol conc. in the effluent Is high. **Chloroform extraction** method is used when the phenol conc. in the effluent is low.

2.2.1. SPECTROPHOTOMETRIC METHOD

Scope and application:

1. This method is application to the analysis of drinking, surface and saline water, domestic and industrial waters
2. This method is capable of measuring phenol materials that contains 50ug/l in the aqueous phase using phenol as a standard.
3. This method is not possible to use differentiate between kinds of phenols

Principle:

The distillable phenolic compounds react with 4-aminoantipyrine at pH 10+-0.2 in the presence of potassium ferricyanide to form a colored antipyrine dye . This dye is kept in aqueous solution and the absorbance is measured at 500nm.

Apparatus :

1. Ph meter
2. Spectrophotometer
3. Funnel
4. Filter paper
5. Reparatory funnels,500 or 1,000 ml
6. Membrane filter

Reagent

1. Phosphoric acid solution,1+9: dilute 10ml of 85% H_3PO_4 to 100 ml with distilled water .
2. Copper sulphate solution: dissolve 100g $CUSO_4$ $5H_2O$ in distilled water and dilute to 1 liter.
3. Buffer solution: dissolve 16.9g NH_4CL in 143 ml conc. NH_4OH and dilute to 250 ml with distilled water. Two ml should adjust 100 ml of distilled to PH 10.
4. Aminoantipyrine solution: dissolve 2 g of 4 AAP in distilled water and dilute to 100 ml.
5. Potassium ferricyanide solution: dissolve 8 g of $K_3Fe(CN)_6$ in distilled water and dilute to 100 ml .
6. Stock phenol solution: dissolve 1.0 g phenol in freshly boiled and cooled distilled water and dilute to 100 ml.
7. Working solution A: dilute 10 ml stock phenol solution to 1 liter with distilled water 1 ml =10 ug phenol.
8. Working solution B: dilute 100 ml of working solution A to 1000 ml distilled water 1 ml=1 ug phenol.

2.2.1.1. DIRECT PHOTOMETRIC METHOD

Procedure

1. Using working solution A prepare the following standards in 100 ml volumetric flasks.
2. To 100 ml of distillate or an aliquot to 100 ml or standards, add 2 ml of buffer solution and mix. The pH of the sample and standards should be 10+0.2
3. Add 2.0 ml aminoantipyrine solution and mix.
4. Add 2.0 ml potassium ferricyanide solution and mix.
5. After 15 minutes read absorbance at 510 nm.