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INDUSTRIAL APPROACH TO BIOREMEDIATION: A CRITICAL PERSPECTIVE

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INTRODUCTION

The quality of life on Earth is linked inextricably to the overall quality of the environment. The problems associated with contaminated sites are increasing worldwide, and the estimated number of contaminated sites is significant. It is now widely recognized that contaminated land is a potential threat to human health, and its continual discovery over recent years has led to international efforts to remedy many of these sites, either as a response to the risk of adverse health or environmental effects caused by contamination or to enable the site to be redeveloped for use.

Some technologies have been used for remedy like high-temperature incineration and various types of chemical decomposition (e.g., base-catalyzed dechlorination, UV oxidation). They can be very effective at reducing levels of a range of contaminants, but have several drawbacks, principally their technological complexity, the cost for small-scale application, and the lack of public acceptance, especially for incineration that may increase the exposure to contaminants for both the workers at the site and nearby residents.

Bioremediation is an option that offers the possibility to destroy or render harmless various contaminants using natural biological activity. Bioremediation is the use of living organisms, primarily micro organisms, to degrade the environmental contaminants into less toxic forms. It uses naturally occurring bacteria and fungi or plants to degrade or detoxify substances hazardous to human health and/or the environment. It will not always be suitable, however, as the range of contaminants on which it is effective is limited, the time scales involved are relatively long, and the residual contaminant levels achievable may not always be appropriate. Although the methodologies employed are not technically complex, considerable experience and expertise may be required to design and implement a successful bioremediation program, due to the need to thoroughly assess a site for suitability and to optimize conditions to achieve a satisfactory result.

Bioremediation is defined as the process whereby organic wastes are biologically degraded under controlled conditions to an innocuous state, or to levels below concentration limits established by regulatory authorities. Contaminant compounds are transformed by living organisms through reactions that take place as a part of their metabolic processes. Biodegradation of a compound is often a result of the actions of multiple organisms. For bioremediation to be effective, microorganisms must enzymatically attack the pollutants and convert them to harmless products. Like other technologies, and bioremediation has its limitations. Some contaminants, such as chlorinated organic or high aromatic hydrocarbons, are resistant to microbial attack. They are degraded either slowly or not at all, there are no rules to predict if a contaminant can be degraded. Bioremediation techniques are typically more economical than traditional methods such as incineration, and some pollutants can be treated on site, thus reducing exposure risks for clean-up personnel, or potentially wider exposure as a result of transportation accidents. Since bioremediation is based on natural attenuation the public considers it more acceptable than other technologies.

1)METHODS FOR ANALYSIS AND TREATMENT:

As the project is dealing with treatment of different samples with various types of bacteria, we need to specifically sort out the type of bacteria to be used. This sorting can be done only by knowing the parameters that affect the quality of the samples. Also, knowing these parameters would help us in having a better idea about the quality of water and the amount of reduction needed in the parameters to meet the corresponding limits. Analysis is one better process required to be done before treatment. This can be done under various methods in order to obtain the information regarding the samples and the microorganisms to be used for treatment. This process moves upon different methods that reveal the parameters like pH, Turbidity, Chloride and sulfate salts content, CODs, BODs, suspended and dissolved salts in the sample etc. So following specific methods would reveal the required information correspondingly. The methods used under analysis are as follows:

i) pH Determination:

pH being one of the major factors that affect the quality of the sample and one vital factor that is to be maintained for the growth of micro-organisms has to be accurately analyzed. This makes our decision easy about the type of bacteria to be used. Generally the pH can remain between both acidic and basic ranges. So information from this method gives us the environment present in the sample and hence revealing the type of bacteria to be used that can survive in the sample and treat.

Procedure followed is always started with casual laboratory calibration of pH meter. pH meter is the device that directly displays the pH and temperature of the sample. It makes use of the electrodes present in a small glass container. Calibration is done in the usual way i.e. by using distilled water to calculate the pH which gives out the reference point. The PH displayed on the device is directly used as the pH of the sample. So this can help us in deciding the bacteria i.e. some of them are capable of surviving acidic environment and some the basic ones. So in this way this analysis method helps in determining one vital parameter, pH.

ii) Turbidity:

Turbidity is another factor that decides the quality of the sample. Turbidity is induced by various factors like the salts present in the sample, and other impurities. This gives us the basic information for comparison between different samples as turbidity itself explains the amount of dissolved and suspended salts.

The opalescence in the sample gives us an idea about the content of turbidity. This will be numerically obtained using 'turbid meter'. As this too is a laboratory apparatus, it is needed to be calibrated. This will be done by using distilled water. A small glass container is filled with distilled water and kept into the device which directly gives out the turbidity in numerical under the units of NFU(Nephelo Turbidity Units).The device makes use of the principle of diffraction and transmission of light passed through the glass container. At the time of calibration it's distilled water and in case of turbidity determination it's the sample. So following this procedure by making use of this device would give us the turbidity of the sample, in fact the quality of the sample.

iii) Total Suspended Salts:

As the samples are the ones which are post production wastes, like effluents and sewages, they contain many salts in them in different states i.e. either in dissolved state or in the suspended state. Suspended state is nothing but the particle size of the salts is more than a specified limit in microns. The amount of salts present in such state must be determined, which can be done by following simple method of filtration.

A little amount of sample (around 10 ml) is taken and is filtered using filter paper. After filtration the suspended salts get filtered off and will be collected on the filter paper. As the paper will be weighed before filtration and will be weighed after filtration, the amount of suspended salts present will be obtained, by simple subtraction. Before calculating the weight of filter paper after filtration, it must be dried using an oven. So this gives out the amount of suspended salts in the sample which helps us in determining one useful parameter. This not only determines the quality of the sample but also helps us in deciding about the type of bacteria to be used. TSS is finally calculated by using the formula:

$$\text{TSS} = [(\text{Final wt. of filter paper} - \text{initial wt. of paper}) * 10^6 / \text{volume of sample}] \text{ mg/lit}$$

iv) Total Dissolved Salts:

Similar to total suspended salts content, we have salts in the dissolved state too. Such salts cannot be determined by simple filtration as they are under size to get collected by filtration. But they are of much importance, because they affect the quality of the sample in crucial way, by creating a media with dissolved impurities which in turn affects the environmental conditions for the bacterial growth. Similar procedure as in the case of TSS is followed to determine the dissolved salts content. Moreover many salts get dissolved in the sample as water is UNIVERSAL solvent. Hence it is important to get information regarding TDS.

10 ml of sample is taken into a crucible. The weight of the crucible before introducing the sample must be noted. The crucible filled with certain amount of sample is kept on boiling water pan. The temperature must be around 105 degrees Celsius if kept for 2 hours and around 180 degrees if kept for an hour. The liquid sample gets evaporated in the process leaving the dissolved salts back in the crucible. Simple principle of evaporation is applied here i.e. majorly liquids get evaporated leaving the solid contents back. After keeping it for ample time, the crucible is again weighed after drying it to get rid of the wetness. The amount of suspended salts is then obtained by simple subtraction. TDS is calculated by using the formula:

Total Dissolved Salts content obtained reveals the major amount of complexes and other sort of salts present in the sample.

$$\text{TDS} = [(\text{Final weight of crucible} - \text{Initial weight of crucible}) * 10^6 / \text{volume of sample}] \text{ mg/lit}$$

Chemical Oxygen Demand (COD) :

Chemical Oxygen Demand (COD) is defined as the quantity of a specified oxidant that reacts with a sample under controlled conditions. The quantity of oxidant consumed is expressed in terms of its oxygen equivalence. COD is expressed in mg/L.

COD is often measured as a rapid indicator of organic pollutant in water. It is normally measured in both municipal and industrial wastewater treatment plants and gives an indication of the efficiency of the treatment process. COD is measured on both influent and effluent water. The efficiency of the treatment process is normally expressed as COD Removal, measured as a percentage of the organic matter purified during the cycle.

Both organic and inorganic constituents of the sample are subject to oxidation; however the organic component predominates and is of greater interest. COD is a defined test; digestion time, reagent strength and sample COD concentration all affect the extent of sample oxidation.

COD is often used as a measurement of pollutants in natural and waste waters and to assess the strength of waste such as sewage and industrial effluent waters. COD has further applications in power plant operations, chemical manufacturing, commercial laundries, pulp & paper mills, environmental studies and general education. In potable drinking water plants, COD values should be less than 10 mg/L O₂ at the end of the treatment cycle. This method comprises of 2 steps. First step is to *keeping the sample along with the necessary reagents in COD reactor* and second step involves *the titration of sample with the ferrous ammonium sulfate solution*.

1st step: reagents which will be added to the sample (total of 15 ml made up with water changing with the concentration of sample) are mercuric sulfate, potassium permanganate along with the conc. Sulfuric acid, water and silver sulfate. As the reactions involved in this process are very slow i.e., in order to enhance the above all reactions we need to add them all in one sequence as below.

- i) $HgSO_4$
- ii) $K_2Cr_2O_7$
- iii) H_2SO_4
- iv) Sample
- v) H_2O
- vi) $AgSO_4$

Uses of Reagents:

I) In order **to avoid the interference** the chlorides in the above reaction, **we use mercuric sulfate (half spatula)**. It reacts with the chlorides whichever present in the sample and form the respective chlorides thus by leaving sample undisturbed.

II) **Potassium dichromate(10 ml)** along with conc. Sulfuric acid(25 ml) acts as an oxidant for the sample i.e., they will oxidize the organic matter whatever present in the sample.

III) **Silver sulfate (pinch)**, acts as an agent for precipitating NITRATES!



Figure 1 showing the sample after the addition of all reagents

And then we will keep the entire sample in COD digestion apparatus covered up by condenser the heater, or reactor, is used to obtain fast organic reactions. Specifications for this reactor are written in Standard Method 5520 C. with specific temperature and vessel requirements. Since it is vital that the reaction take place at 150°C ($\pm 2^{\circ}\text{C}$) for 2 hours it is important to ensure accurate pre-heating. The reactor is also equipped with a timer to notify the operator when the reaction is completed.



Figure2 showing sample after taking out from the COD Digestion Apparatus

2nd step: Second step involves the titration of the resultant sample of step 1 with standard ferrous ammonium sulfate solution. Ferric ion is being used as an indicator .titration is stopped when color change takes place from green to brick red. And the sample reading can be noted from the burette.

COD can be calculated by using the following formula

Amount of COD =

$$\text{(Blank reading – sample reading) * N * 1000 * atomic wt of oxygen / vol of sample}$$

Where N is normality of ferrous ammonium sulfate solution and Atomic wt of oxygen is 8.

Blank reading can be obtained when the contents excluding the samples are titrated. By titrating with known concentration of potassium permanganate, normality of ferrous sulfate can be obtained.

v) CHLORIDE TEST :

One of the major types of impurities present in the sample is chloride. Chloride being highly toxic can't be used for general drinking and house hold purposes. So, in order to find out the amount of chlorides in sample, we will be using the chloride test.

Current test comprises of simple titration of sample with the silver nitrate solution. Indicator which will be used here is 4-5 drops of potassium permanganate sol. Titration can be stopped when the color change takes place from yellowish to brick red color.

Principle which is being taken here is the simple formation of chloride salts of silver chloride. Usage of required bacteria is important as some bacteria can enhance the actual chloride content in the sample.

$$\text{Amount of chlorides} = B.R * N * 35.45 * 1000 / \text{vol of sample}$$

Where B.R is burette reading and N is normality of silver nitrate solution.

vi) Sulfate test :

The main types of impurities present in the sample are organic matter, chlorides and sulfate salts. In order to know about content of sulfates present in the sample, we will be carrying out the sulfate test. Samples, if kept untreated for sulfate content will have its huge effects on us. As they are the one main salt in the sample, it needs to be removed. Sulfate test is being carried out in two stages.

In first stage, water bath plays a vital role in the removal of all organic matter in the sample. Sample is taken in a small crucible and the required amount of sulfuric acid depending on the size of the sample. If the sample volume is 10 ml, we will be needing 25 ml of sulfuric acid. Main aim of sulfuric acid is to oxidize all the organic matter and evaporate it.

Then the crucible is kept in water bath at 108 degrees. After keeping it in water bath for about 20 minutes, we need to add the barium chloride powder in order to get the sulphates in precipitate form and then keep it for 2 hours.

The left over sample in crucible is subjected to **second stage** process where vacuum pump is being used. It makes use of vacuum for sucking out all the water present in the sample and we get only sulfate salt on the filter paper.

By calculating the initial and final weights of filter paper, we can calculate the actual sulfate content the sample.

$$\text{Sulfate content} = (\text{final} - \text{initial weight}) * 411.6 * 1000 / \text{vol of sample}$$

The above discussed methods are the ones required to know the quality of the sample i.e. by analysis. So after following the Methods of analysis, we will be with the needed information that helps us to decide the type of bacteria to be used and also we will be with the reference point to which the final results will be compared with. The final result is obtained by different methods like Phyto-remediation, Bioremediation etc. The concerned method of our project being Bioremediation, enough knowledge regarding the method and mechanisms involved was obtained.

Bioremediation is the usage of Microorganisms for treating different parameters of the sample which were briefed in the former context. This is the process in which the sample is inoculated with micro-organisms, preferably bacteria that can act up on various chemicals present in the sample which are making it harmful to the external environment. During our project different samples were inoculated with both aerobic bacteria and anaerobic bacteria. As the bacteria take their time to act up on the sample, the samples were kept for incubation for ample time. Aerobic bacteria act up on the sample relatively fast when compared to anaerobic. The reason behind it is, anaerobic conditions take time to come into action. Aerobic conditions initially prevail in the case of aerobic inoculation though. Hence to get rid of these conditions and to make anaerobic conditions take the part some time has to be used up.

Bioremediation is the industrially followed process which gives out no harm as many chemical methods for treatment does. Using consortium of bacteria i.e. collection of different kinds of bacteria for inoculation would result in variation of results. Many other bacteria present with in the inoculums would affect the final treatment result. Hence it is always preferred to use specific isolated bacteria for the corresponding purpose. Such specificity is only known if pre knowledge and characteristics of various species of bacteria are known. In the other case the treatment process can also be used for finding the characteristics of bacteria by basing the final result of their treatment over the sample.

Generally in laboratory treatment process, 100ml of different samples will be inoculated with 10ml of inoculums that consists of various kinds of bacteria. Similar process is followed for all different samples like Sewage Treatment Process water, Effluent Treatment Process sample, cooling tower water etc. They were inoculated with aerobic bacteria like Mycobacterium, sarcomanus etc. and simultaneously with anaerobic bacteria (10 ml of culture was added to 100 ml sample). Aerobic inoculated samples were kept for incubation on a shaker for around 96hours. In the case of anaerobic bacteria, the inoculated samples were collected in a glass jar an tightly sealed with paraffin before which sodium carbonate was introduced in order to absorb the produced oxygen .Anaerobic bacteria as discussed takes more time than aerobic ones to act. Hence these samples are incubated for 10 days.

After the treatment process, the treated samples are again analyzed following the same methods which were used to analyze the initial samples. All the parameters were analyzed again and compared with that of the initial values. The net changes in all the important parameters like COD, TDS etc. were noted. Later the treated samples were filtered using a filter bed. This filter bed was prepared by making use of three different layers namely fine sand, gravel, charcoal. The COD content was reduced after filtration. This result was obtained after the analysis of final treated and filtered sample.

As the treated ETPs and STPs are needed to be disposed, they need to come down a threshold limit in terms of these parameters. So sampling these ETPs and STPs and treating them for analyzing would help us in finding specific treatment procedures that are effective. When done in large scale

Bioremediation aims at maximum reduction of these factors that prove to be harmful to the external environment. The same process is followed in the large scale though. Bioremediation is one revolutionary process which aimed at treatment of waste in efficient and safe way.

Similar to Bioremediation, another technique which proved to be one better method is also introduced namely BIOREACTOR. This follows the same mechanism as that of bioremediation but got its own advantages. Much detailed explanation about bioreactors will follow.

1) RESULTS AND OBSERVATION

The main aim of performing bio-remediation is to decrease the COD levels in the samples. This can be judged on the basis of values obtained from experimentation before and after the treatment. The initial values must be calculated prior to the treatment, which are used for comparison after the treatment. Not only that, it is necessary to interpret the changes in the values of the parameters with appropriate reasons.

The obtained samples consisted of condensate, processed effluent, STP, etc. Initially various tests were conducted for these samples to obtain the values of pH, turbidity and concentration of chlorides, sulfates, total dissolved solids and total suspended solids. These values are listed in the following table.

Parameters	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
pH	7.52	8.28	9.94	4.81	7.56	8.58
Turbidity (NTU)	4.3	5.4	4.1	4.2	6	33.8
TSS (mg/lit)	20,500	162000	49,800	100000	3,42,000	700
TDS (mg/lit)	2,22,500	98000	4,020	75000	1,86,000	198500

COD	7,050	18816	1,176	4800	1,153.8	561720
Chlorides (mg/lit)	3,424	6248.062	5,498.29	1499.535	4,748.5	11996.28
Sulfates (mg/lit)	8,725.92	2716.5	127.6	2058.6	16,710.96	8643.6

Table 1: Initial values of the parameters of all the samples

These samples were then treated with aerobic and anaerobic consortium separately and maintained at required conditions. After a week, considerable decrease was observed in the color, turbidity and odour of all the samples after this treatment. Parameters of these samples were tested again which are listed below, aerobic and anaerobic separately.

For all the aerobic samples decrease in the cod is observed. The pH change is based on the type of by-products released by the micro-organisms which again depends on the type of organic or chemical matter degraded by them. Accordingly there is a change in the chloride and sulfate concentration because they comprise a large amount of the by-products released.

Parameter	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
pH	9.08	8.3	9.24	8.81	9.27	8.82
Chlorides (mg/lit)	3130	7196.35	4378	1774.44	5430.23	8707.938
Sulfates (mg/lit)	761.46	2247.336	726.06	587.76	3099.35	2219.34
COD	2380	6507.54	553.82	1281.334	1123.2	144150

Table 2: Values of the parameters after aerobic treatment

Similarly, even for the anaerobic samples, the changes in the values of the parameters can be justified as those for the aerobic samples. But a greater decrease in the COD levels for the anaerobic treatment for most of the samples indicates that anaerobic treatment is more effective.

Parameter	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
pH	8.83	8.05	8.71	8.24	8.3	9
Chlorides (mg/lit)	3302	7444.5	4248.68	1649.48	5381	6800.019
Sulfates (mg/lit)	1230	5202.624	1366.512	559.776	3527.41	17435.9
COD	2130	6318.28	482.032	2629.296	1846.15	130696

Table 3: Values of the parameters after anaerobic treatment

After bioremediation, the samples were filtered using a sand filter bed to lessen the color and solid impurities present in them. This process led to the purification of the samples to a much larger extent. After filtration, almost a clear sample was obtained from the sand filter. Again for both the aerobic and anaerobic samples the cod levels were checked.

COD	Aerobic	Anaerobic
Sample 1	1728	1590
Sample 2	4896	5245.3
Sample 3	1324.8	1886.8
Sample 4	1296	1380.78
Sample 5	630.78	1509.43
Sample 6	4838.4	5024.6

Table 4: COD values after filtration throu
AEROBIC SAMPLE BEFORE FILTRATION





AEROBIC SAMPLE AFTER FILTRATION

1) BIOREACTOR: INDUSTRIAL APPROACH TO BIOREMEDIATION

Bioreactor can be defined as any manufactured or engineered device or system that supports a biologically active environment. It can be also seen as a vessel in which a chemical process is carried out which involves organisms or biochemically active substances derived from such organisms. This process can either be aerobic or anaerobic.

TYPES OF BIOREACTOR

- a) Primary level: Air lift Bio reactor
- b) Secondary level: Trickling bed Bio reactor
- c) Moving bed bio film Bio reactor (MBBR)

AIR LIFT BIOREACTOR

Bioreactor in which the reaction medium is kept mixed and gassed by introduction of air or another gas (mixture) at the base of a column-like reactor equipped either with a draught tube or another device.

TRICKLING BED BIOREACTOR

Trickling filter consists of a fixed bed of rocks, sand, coke, gravel, over which sewage or other wastewater flows downward and causes a layer of microbial slime (bio film) to grow, covering the bed of media. Aerobic conditions are maintained by forced air flowing through the bed or natural convection of air if the filter medium is porous. The removal of pollutants from the wastewater stream involves both "*absorption and adsorption of organic compounds by the layer of microbial bio film*".

MOVING BED BIOFILM BIOREACTOR (MBBR)

The moving bed bio film bioreactor works on the "*principle of pressure difference*." The basis of the bioreactor are the bio film carrier elements (Polyethylene/propylene rings) which provide a large protected surface area for the formation of bio film and optimal conditions as well for the bacteria culture to grow and thrive. These polyethylene rings act as a support for the formation of bio film of microbes. Due to pressure difference, rings move (bed moves) and water sample moves as well, which leads to uniform mixing providing more surface area. The cultures added can be aerobic/anaerobic depending upon the action. The bio film takes about a day or two to form and then the action of bacteria takes place on the samples.

CONCLUSION

Getting into the HEALTHY world requires the usage of different Measures that help either in prevention or cure of the factors causing the SICK environment. When concerned about the biological health, it is the case of DRUG USAGE. As usage comes into action only after production, drug production is the emphasized part of the story. This again connects us to the fact that emission of waste is the major part of postproduction and the waste produced in that manner doesn't just remain calm, rather it will prove havoc to the external environment. Hence they need to be treated by efficient processes and so are the methods discussed.

Bioremediation, which is one major process for treatment of industrial effluents, is the method on which our project aimed at. All the analysis procedures discussed in order to make out the parameters that affect the quality of effluent are meant to get a proper way to move with bioremediation. As the detailed explanation of all such methods like TSS, TDS, Turbidity analysis, pH analysis etc. were given, the only point to be made is the methods are sensitive and we end up in great paradox of results. So utmost care must be taken while carrying out the procedure. Let it be the case of apparatus or the analyst. Carrying out the analysis for the samples treated with different types of microorganisms reveals the relatively best inoculums for the required process. Though there are many other chemical and physical treatment procedures, biological treatment methods are always efficient and less harmful.

Main concern in the effluent is always about the COD (Chemical Oxygen Demand) and this is one of the parameter by which the quality of the effluent is decided. As discussed, all the treatment procedures aim at reducing the COD content. Considering the results obtained after the treatment procedures by inoculating aerobic and anaerobic bacteria, the reduction in COD content is drastic in case of aerobically treated sample. This shows that aerobic bacteria can be preferred against anaerobic for the treatment process. ETP (Effluent Treatment Process) sample and STP (Sewage treatment process) samples were the ones which were treated with the inoculums and the results after the treatment, filtration in terms of COD derives that STP samples can easily be treated by using aerobic bacteria. In case of ETP, the concentration levels of the corresponding parameters are high; hence microorganisms couldn't efficiently act up on the sample unlike in the case of STP. In this process of analysis, required knowledge about different aerobic and anaerobic bacteria is obtained. Different environmental conditions for their growth and survival are known too. At the end these procedures proved to be much efficient by using specific type of bacteria for treatment process and led our project of effluent and sewage TREATMENT in better path accounting it under BIOREMEDIATION.

BIOREACTOR as mentioned is one successful implementation of technology in order to treat the given samples i.e. of effluent and sewage. Usage of similar inoculums as in bioremediation and much more complexion in its construction and mechanism made it unique. As the main advantage being the high surface area provision for the treatment by bacteria and ample time for bacteria to act, the technique of Bioreactor is one majorly adaptable process for treatment purpose. The effluents and sewage water, after treatment should get reduced in terms of their parameters to required limit for either using it with in the industry or for disposal into the water bodies. Disposal into water bodies obviously requires less COD levels dissolved salts content when compared with the levels corresponding for re usage in the industry. After all the major concern is to preserve environment and maintain ecological balance.

Finally, it's always the case of Prevention and Preservation which seldom requires cure and keeps us provided with required amenities for better life. Nothing in complex though, it's much simpler when moved on the lines like "PRESERVE THE NATURE".

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GLOSSARY

1. ANALYSIS: it is the process of breaking a complex topic into smaller parts to gain a better understanding of it
2. TREATMENT: Process of removing contaminants from effluent and waste water in order to use it for the domestic purposes.
3. TSS – TOTAL SUSPENDED SALTS present in the sample
4. TDS – TOTAL DISSOLVED SALTS present in the sample
5. COD – CHEMICAL OXYGEN DEMAND is the measure of how much amount of oxidant is reacting with the sample under controlled conditions.
6. BOD – BIOLOGICAL OXYGEN DEMAND
7. BIO REMEDIATION: Use of micro organism metabolism to remove pollutants in the effluent, generally classified as in situ and ex situ.
8. AEROBIC BACTERIA: bacteria which can grow in the presence of air
9. ANAEROBIC BACTERIA: bacteria which can grow in the absence of oxygen