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ISOLATION AND CHARACTERIZATION OF BACTERIA FROM LABORATORY DEVELOPED ACTIVATED SLUDGE USED IN THE TREATMENT OF FOOD INDUSTRY EFFLUENT

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ABSTRACT: Nowadays the developing countries are making their profit with the help of different industries. During production of various valuable products the industries also generate a large amount of waste water which may cause nuisance to the community if it is disposed improperly. Especially, marine food industrial effluent that has high oxygen demand, possibly leads to death of marine population. So by taking this problem into consideration we treated the waste by activated sludge process. The activated sludge process is totally aerobic process. In an activated sludge process aerobic and facultative microorganisms like bacteria, protozoa and rotifers, play a very important role. The isolated bacteria from activated sludge were used to treat waste. Significant reduction in the COD and BOD values were observed when isolates were used in consortium. The initaial COD and BOD values of the waste was 5440mg/lit and 2325mg/lit respectively and after the treatment with consortium for 24 hrs the COD reduced to 430 mg/lit. and BOD to 455mg/lit. So our study will be helpful to minimize the health concerning effects of the wastes generated by industries and also to keep our environment clean and healthy.

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1.INTRODUCTION

Nowadays the developing countries are making their profit with the help of different industries. Different industries such as Tannery, textile, Pharmaceuticals, Chemical industries producing pesticides Paper & pulp industries, Fertilizers, Steel mills, Metal plating industry, Oil refineries, Rubber industries & Food industries. These industries produce their product and sale in the market to get the profit. But during production of various valuable products the industries also generate a large amount of waste water which may cause nuisance to the community when it is disposed improperly. The term Industrial waste is "the liquid waste resulting from manufacturing and industrial process which utilize moderate to large quantities of water."[1,2] The Industries require large amount of water for power generation and cooling purposes and hence the industries tend to establish near water body or bank of river. But due to the rapid industrialization some industries are establishing in rural and agricultural areas, this trend is intensifying the problem of pollution. The seafood industries primarily process saltwater fish (tuna, sardines, pacific whiting, swordfish), mollusks (oysters, clams, scallops), crustaceans (crabs and lobster) and others such as shrimp, octopus etc. are often processed concurrently or seasonally [2]. In most fish processing industries the waste water with substantial amount of soluble, colloidal and particulate forms is produced. Wastewater from seafood processing operations can be very high in dissolved and suspended organic materials. This results in high biological oxygen demand (BOD) and chemical oxygen demand (COD). Fats, oil and grease are also present in high amounts. Often suspended solids and nutrients such as nitrogen and phosphate can be high. Unpleasant odor and high temperature are also issues. Seafood processing wastewater was noted to sometimes contain a high concentration of sodium chloride from boat unloading, processing water and brine solutions [2]. The major types of waste found in seafood processing industrial effluent are blood, offal products, viscera, fins, fish heads, shells, skins, and meat "fines". Organic materials in the wastewater are produced in the majority of these processes. However, most of it originates from the butchering process, which generally produces organic materials such as blood and gut materials. The fats, oil and grease present in the waste may cause harm to the water ecology, the toxic effect of the effluent may also hamper the fertility of the soil. So such a waste must be treated before it is disposed off. There are the following methods to treat the effluent. There are 3 kinds of treatment procedures:-

1. Physical.
2. Chemical.

3. Biological.

Physical treatment: - The physical treatments used to treat sewage same kind of treatment is used for the treatment of Industrial waste [1]. The operations which are used for treatment of waste water in which change is brought about by means of or through the application of physical forces are known as physical unit operations [3].

The unit operations most commonly used in waste water treatment includes

a) Screening.
b) sedimentation
c) flocculation
d) floatation

Chemical treatment:-

The processes which are used for the treatment of waste water with help of chemical reaction are known as chemical unit processes [3].

There are three kinds of processes in chemical treatment

1. chemical precipitation
2. adsorption
3. neutralization

Biological treatment:-

The treatment of industrial waste biologically is most convenient and the cheapest way of treatment and disposal [1]. The main objective of this process is to coagulate and remove the non settleable colloidal solids and to stabilize the organic matter. The major aim of biological process is to reduce the organic content and in many cases the nutrients such as nitrogen and phosphorus [3].

The biological process can satisfactorily employed for waste containing organic other pollutants susceptible to microbiological attack.

The biological treatment is divided in two major parts-

i. Anaerobic Process
ii. Aerobic process
iii. Anaerobic treatment

The biological treatment processes that occur in absence of oxygen and the bacteria those survive only in absence of dissolved oxygen are called as obligate anaerobes.

The various process involved in anaerobic treatment are:-

a) Anaerobic digestion:-

b) Anaerobic contact process

c) Anaerobic filter

d) Anaerobic lagoons

ii)Aerobic treatment:-

Aerobic processes are biological treatment processes that occur in the presence of oxygen. The bacteria those survive only in presence of dissolved are known as obligate aerobes.

There are four methods which are used commonly and those are:-

a) Aerated lagoons:-

b) Trickling filters

c) ACTIVATED SLUDGE PROCESS:-

The main process of our concern was Activated sludge process. The activated sludge process is totally aerobic process. The biological purification of sewage by this process is brought about in presence of sufficient air and bacteria [4]. In the activated sludge process the organisms are mixed thoroughly with organics, so that they can grow and stabilize the organics. The microorganisms grow and clump together (flocculate) to form an active mass of microbial floc called activated sludge. The mixture of activated sludge and waste water in the aeration basin is called as mixed liquor [5]. In an activated sludge process aerobic and facultative microorganisms like bacteria, protozoa and rotifiers etc., play a very important role. For the operation for activated sludge plant it is necessary to keep the primary settling and aeration equipments in efficient condition but also maintain microbial growth and the secondary keeping settling tank in perfect conditions [6].

The four basic functions of activated sludge process are

i. Adsorptive removal of organic pollutants

ii. Flocculation of colloidal and small particulate matter.

iii. Microbial breakdown of complex organic matter.

iv. Settling of activated sludge

i. Adsorptive removal of organic pollutants

The activated sludge plants fall into two broad categories according to the methods or devices used for aeration and biological flocculation.

a) Diffused air units

b) Mechanical aeration units

The main features performed by these units are

 Oxygen transfer to sewage and keep the floc aerobic.

 Circulation of floc through sewage and create contact opportunity between floc and sewage.

It keeps the floc from settling to the tank bottom where it is ineffective and quickly become septic.

ii. Flocculation of colloidal and small particulate matter

A floc means accumulation of dead cells along with living cells. Here the mixture dead and living cells are generated to form the floccules. These floccules help the microorganisms to stick with the sewage particles and carry out their degradation [4].

iii. Microbial breakdown of complex organic matter

The floccules adhered to solid sewage particles contain variety of microorganisms capable of producing various kinds of enzymes, and carry out degradation of complex organic substances such as starch, proteins, lipids and hydrocarbons etc.

iv. Settling of activated sludge

When these floccules settle rapidly then the sludge is said to be active. The flocs are living masses of organisms, food and slime material and are highly active centers of biological life [7]. The sludge which has poor settling characteristics and poor compactability is called as bulked sludge. There are two factors which affects the settling characteristics of activated sludge.

First cause of bulking is growth of filamentous organisms under adverse conditions and second is the bound water in which the bacterial cells composing the floc swell through the addition of water to the extent that their density is reduced.

There are three major factors applicable to the activated sludge process in formulating loading parameters are BOD applied to the waste, quantity of the sewage present in the aeration tank and the period of aeration [8]. The most important characteristic of the process is the activated sludge and measuring sludge activity which can indicate the condition of working plant. Eckenfelder, 1985 & Harbold, 1976 recommended MLSS as an ideal parameter as it is very simple to estimate & fairly accurate. The scientist Eckenfelder has specified following optimum conditions.

The optimum conditions for an activated sludge plant

Sr. No.	Parameter	Optimum condition
1	Waste water retention time	4-8 hrs
2	Depth of tank	12 to 16 feet
3	Biomass (MLSS)	2000 – 3000 mg/lit
4	Dissolved oxygen	4.0

Depending upon hydraulic flow pattern within the continuous reactor the reactors are classified as complete mix type, plug flow type and partially mixed. For conventional activated sludge process the aeration tank should have the following utility (Neeri).

Sr. No.	Parameter	Optimum condition
1	Average MLSS in tank	2000 – 2500 mg/lit
2	Recirculation of sludge	25-50% of average total flow
3	Liquid depth	3.5-4.5 m
4	SVI	<100

Knowledge of SVI is an essential feature of good process control, a well settling sludge may have sludge volume index SVI between 50-100 but an index of 200 is indicative of sludge with poor settling characteristics. Tapered aeration, step aeration, sludge reaeration, flat field process. Krause process, modified aeration and extended aeration are modifications of activated sludge process.A wide variety of microorganisms are found in activated sludge such as bacteria, fungi, algae and protozoa. The most predominant organisms are bacteria. Their diverse biochemical nature makes it possible for them to metabolize most organic matter contained in sewage and industrial waste. There are two major groups of organisms present those which actually utilize the polluting substances present in the waste and those which utilize the byproducts produced by the former ones. The bacteria are primarily non nitrifying, aerobic and sporing, many of which are of Bacillus subtilis group. Nitrifying bacteria are primarily Nitrosomones and Nitrobacter. Most bacteria under proper conditions flocculate. One of the principle causes of zoogleal mass is Zooglea ramigera which has been defined as gram negative non spore forming motile capsulated rod. Other common forms of

bacteria found in the activated sludge include Flavobacterium, Pseudomonas and Filamentous organisms. Fungi are not always present because they generally exist in presence of low oxygen tension, low pH or low nitrogen content. Free swimming protozoa are also present that include Paramoecium, Linnotus and Trichods. A wide variation in microorganism's population between the assimilative and endogenous phases is found in the activated sludge. It is reported that during the assimilative phase 74% of the organisms were of the genera Bacillus. Hence, it was decided to study various types of bacteria present in an activated sludge treating marine food industry waste. Accordingly bacteria were isolated from an activated sludge developed in the laboratory and their various characters were studied. The studies mainly consisted of their morphological cultural biochemical and physiological properties. Their COD and BOD reduction potential was also studied.

2. MATERIALS AND METHODS

3.1 Collection of Sample:

The sample was collected from the marine food industry which is situated in Udyamnagar, Ratnagiri, Maharshatra. The collection of sample of waste water was done in to the pre sterilized plastic bottles [6].

3.2 Development of activated sludge:-

waste water collected from the marine food industry of Ratnagiri region and the waste itself is used as source of organisms. This was taken in a graduated glass cylinder in which liquid diet food and phosphate solution was added and the total volume made up to 200 ml with tap water. Aeration though aerator was done continuously during incubation at room temperature aeration was stopped for a 20 min period every day to allow sludge to settle and the supernatant was discarded it was then restored to the mark with fresh liquid diet, phosphate solution and tap water.Acclimatization of an activated sludge to marine food industry waste was done by addition of waste itself whose proportion in the total was gradually increased daily. The following parameters were studied to maintain progress of development.

1.Mixed liquor suspended solids-

Four milliliters of mixed liquor was withdrawn in to a previously weighed evaporating dish and reweighed after drying at 103 to 105 o C till constant weight attained described in APHA (1980)

2.Sludge volume index(SVI):-

Settling property of an activated sludge is expressed in terms of SVI. To determine this, the volume of sludge settled within 30 min. was determined. The ratio of sludge volume to MLSS of sludge was

expressed as SVI [9]

3.Floc density:-

Floc density was determined by Sedgewick rafter cell as described in Trivedy and Goel1984. Microbial aggregates measuring 30 um or more in any dimensions were consider to be floc.

After maintenance of these parameters the microorganisms were isolated and were characterized by using different biochemical tests.

Potential of whole sludge and each isolate to reduce the COD and BOD:-

Seven flask were taken and in each flask 30 ml sample was added the suspension

of each isolates was adjusted to same optical density and was then introduced in each flsak, same thing was done by using sludge. Then 5 ml of samples were taken out after 6, 12,18 and 24 hrs. the COD and BOD of each sample was determined .

COD (Chemical Oxygen Demand):-

COD was determined by Dichormate reflux method. This was done by oxidizing sample under reflux with potassium dichromate and concentrated sulphuric acid in presence solid mercuric sulphate and silver sulphate. The residual potassium dichromate was titrated against standard ferrous ammonium sulphate with ferrous phenanthorline sulphate as indicator [6]

BOD (Biochemical Oxygen Demand):-

BOD was determined by measuring loss of DO in diluted sample(with aerated distilled water) incubated in a BOD bottle at room temperature for five days. DO was determined by winklers alkali azide iodied method. Sample was collected in special BOD bottles to which prescribed volume of saturated manganous sulphate solution, alkali azide iodied and concentrated sulphuric acid was added. Liberated iodine was titrated against sodium thiosulphate using starch as indicator to directly indicate the dissolve oxygen in mg/lit. [6]

3. RESULTS AND DISCUSSION

4.1 Collection of sample:

The waste sample was collected from the marine food industry which is located in Udyamnagar, Ratnagiri, Maharashtra.

Table 4.1 Details of waste sample collected :

Sr. No.	Type of industry	pH	Temperature
1	Marine food industry, Ratnagiri.	7.2	29^0C

4.2 Development of activated sludge

An activated sludge was developed in the laboratory according to the method as described in Finstein (1972). During this development MLSS, Floc Density, SVI were also checked.

The MLSS after 12 days was found to be 4000 mg/lit.

The floc density and SVI are parameters of activated sludge which affects the settling properties. The sludge volume index after 12 days was found to be 118 and floc density was 4.5 × 105 flocs/lit. Then the sludge was subjected to isolation of organisms from it. From the laboratory developed activated sludge, six bacterial strains were isolated.

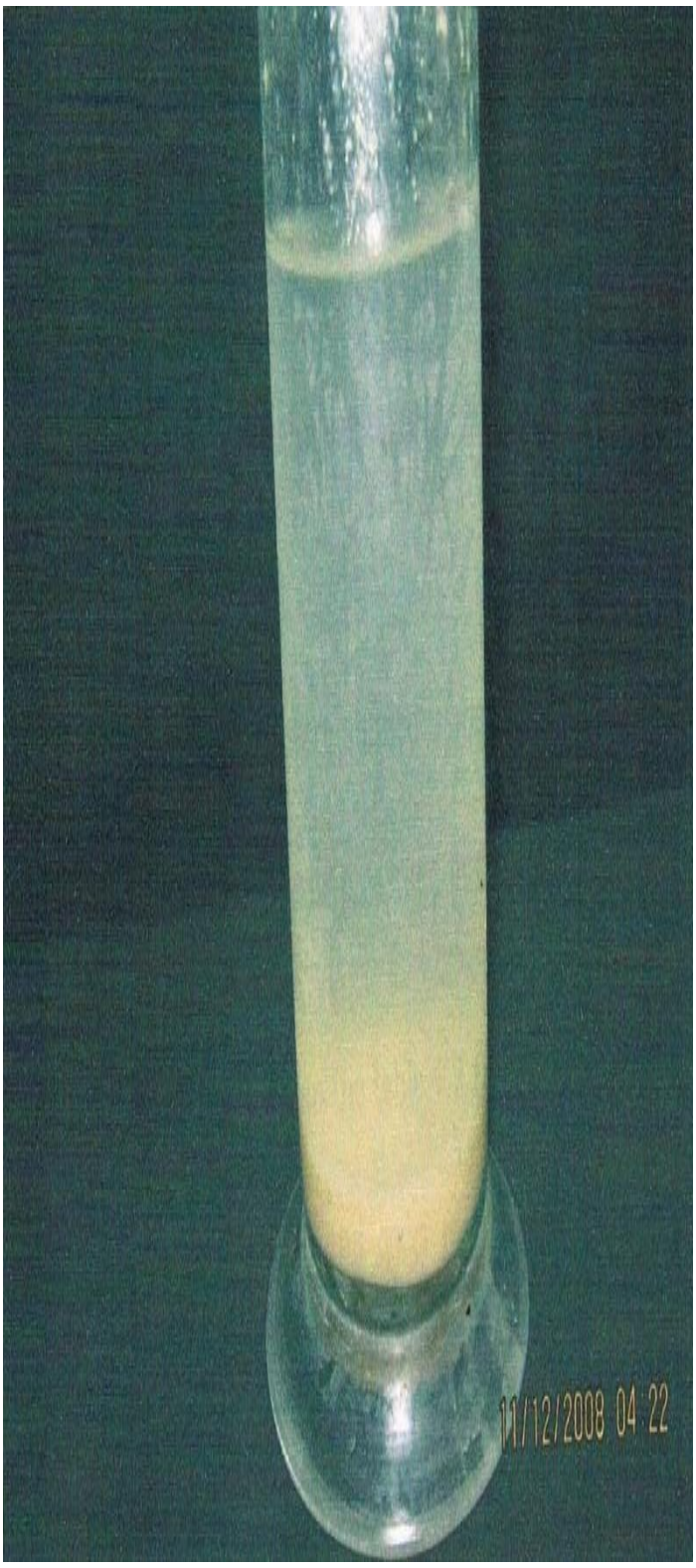

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Table 4.9: Results of COD reduction

Isolate No.	Initial COD mg/lit of effluent	COD after 6 hrs	COD after 12 hrs	COD after18 hrs	COD after 24 hrs
1	5440	3360	2800	2120	1120
2	5440	3640	3280	2280	1360
3	5440	2320	1840	1120	520
4	5440	4840	3960	3040	1640
5	5440	3900	3120	2340	1120
6	5440	3620	2940	1950	950
Combined sludge	5440	2120	1640	950	430

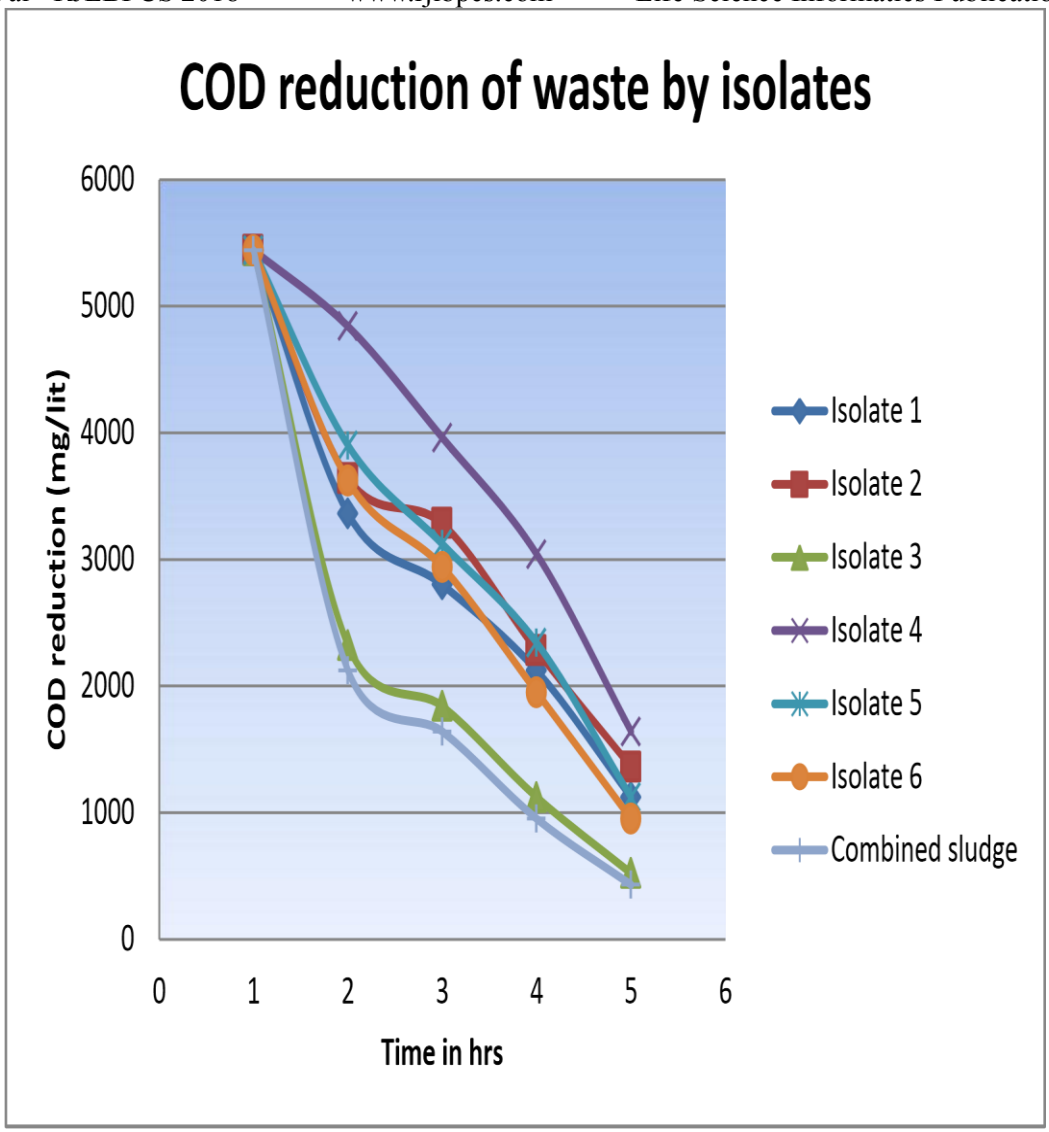

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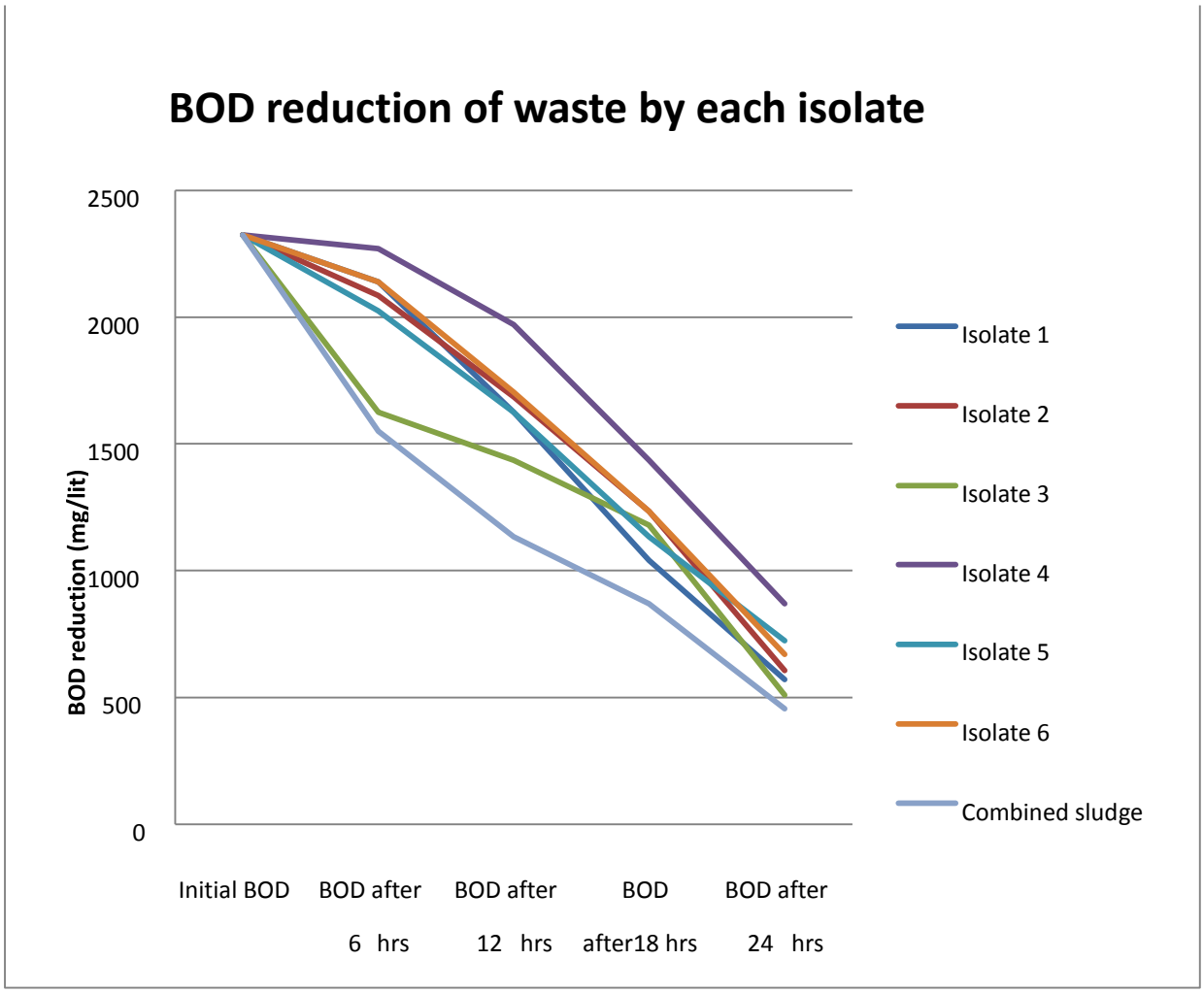
Table NO. –Reduction of BOD

Isolate No.	Initial BOD mg/lit of effluent	BOD after 6 hrs	BOD after 12 hrs	BOD after18 hrs	BOD after 24 hrs
1	2325	2140	1625	1040	570
2	2325	2085	1685	1235	605
3	2325	1625	1435	1180	510
4	2325	2270	1970	1435	870
5	2325	2025	1625	1135	725
6	2325	2140	1705	1235	670
Combined sludge	2325	1550	1135	870	455



4. CONCLUSION

From the laboratory developed activated sludge process six strains were isolated and selected for further studies restricted to characters significant in the activated sludge process. The included their ability to utilize the major components of the waste they would finally act upon glucose, sucrose, maltose, fructose, Arabinose and enzymes like Caseinase, amylase, gelatinase, lipase, urease, catalase, oxidase were studied.In addition their COD reduction and BOD reduction efficiency were also studied.These studies yielded 6 cultures of which 2 isolates can reduce COD and BOD appreciably, however combined sludge was found to be the in all the characters.

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