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BIOREMEDIATION OF STEEL INDUSTRIAL EFFLUENTS USING SOIL MICROORGANISMS

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ABSTRACT

Bioremediation is treatment processes that uses naturally occurring microorganisms as well as plants to breakdown, or degrade hazardous substances into less toxic or non toxic substances. Steel Industry is one of nine energy- and waste- intensive industries Major processes in the production of finished steel include coke production, sinter production, iron making, steel making, alloying, casting and shaping, and finishing. During these processes many contaminants like organics, in organics and heavy metals will be released into the effluents that need to be tackled in a safe manner. Bioremediation can be applied on site (*in situ*) or off site (*ex situ*), mediated by mixed microbial consortia and/or pure microbial strains. There are many mechanical and chemical methods for treatment of steel industrial effluents. Also some studies on the bioremediation of industrial effluents are available. The objective of the present study is to conduct preliminary studies using mixed microbial consortia isolated from sewage drain for the bioremediation of steel industrial effluent. The steel industrial effluent collected from a Patancheru Effluent Treatment Ltd., Hyderabad was used in the present study. Physicochemical analysis of the effluent was carried out using standard methods of APHA. Experiments revealed that, upto 95% reduction of BOD and COD was observed by using these mixed microbial consortia. Preliminary isolation studies revealed the presence of four microorganisms namely *Pseudomonas* sps, *Bacillus* sps, *Arthrobacter* sps and *Micrococcus* sps. in the mixed microbial consortia. The study indicates that these microorganisms once characterized can be further used for the bioremediation of industrial effluents.

Keywords: bioremediation, mixed microbial consortia, Patancheru Effluent Treatment Ltd, BOD and COD

INTRODUCTION

Steel is manufactured by the chemical reduction of iron ore, using an integrated steel manufacturing process or a direct reduction process. In the conventional integrated steel manufacturing process, the iron from the blast furnace is converted to steel in a basic oxygen furnace [4]. Major pollutants in wastewaters generated from steel manufacturing include total suspended solids (up to 4,000 mg/l, 1030kg/t), lead (8 mg/l), chromium (5 mg/l), cadmium (0.4 mg/l), zinc (14 mg/l), fluoride (20 mg/l), and oil and grease. The need for treatment of industrial waste-water is required because it causes atmospheric pollution, discoloring and turbidity,

dissolved oxygen depletion, algal and fungal growth, results in sludge deposits etc. The main purpose of treatment processes is to remove the impurities of waste-water and bring the quality of water to the required need standards [6].

The goal in bioremediation is to stimulate microorganisms with nutrients and other chemicals that will enable them to destroy the contaminants [5]. The bioremediation systems in operation today rely on microorganisms native to the contaminated sites, encouraging them to work by supplying them with the optimum levels of nutrients and other chemicals essential for their metabolism. Thus, today's bioremediation systems are limited by the capabilities of the native microbes. However, researchers are currently investigating ways to augment contaminated sites with non-native microbes-including genetically engineered microorganisms-especially suited to degrading the contaminants of concern at particular sites. It is possible that this process, known as bioaugmentation, could expand the range of possibilities for future bioremediation systems.

Regardless of whether the microbes are native or newly introduced to the site, an understanding of how they destroy contaminants is critical to understanding bioremediation. The types of microbial processes that will be employed in the cleanup dictate what nutritional supplements the bioremediation system must supply. Furthermore, the byproducts of microbial processes can provide indicators that the bioremediation is successful. In the present study, bioremediation potential of steel industrial effluent was assessed using local mixed microbial consortia.

MATERIALS AND METHODS:

Collection of the sample:

The sample used in the present bioremediation study is a steel industrial effluent collected from Patancheru Effluent Treatment Limited, Hyderabad.

Physicochemical analysis:

Physicochemical analysis is an important tool in conducting research on the unit process and to find out simple economical and efficient method of treatment. The determination made in physical analysis includes temperature, color, odor and turbidity. Chemical analysis include determination of pH, TDS, SS, DO, COD, BOD, fixed and volatile solids etc. All the chemical analysis of effluent was performed as per standard protocols of APHA [6]. The reagents used for Dissolved oxygen were Manganese sulphate, Alkali Iodide Azide reagent, Conc. H_2SO_4 solution, Starch indicator, Stock Sodium thiosulphate 0.1N, Standard Sodium Thiosulphate 0.025N. The reagents used for Chemical oxygen demand were Sulphuric acid, Standard Potassium dichromate N/4, Ferroin indicator, Standard ferrous Ammonium Sulphate N/10.

COD was calculated as follows:

$$\text{COD mg/lit} = \frac{(A-B) * N * 8000}{\text{Volume of sample in ml}}$$

Where A = ml of Fe $(NH_4)_2(SO_4)_2$ for blank; B = ml of $(NH_4)_2(SO_4)_2$ used for sample and

N = Normality of FAS (Ferrous Ammonium Sulphate).

Chloride analysis was carried out by Argentometric method (APHA). The reagents used for chloride analysis were $K_2Cr_2O_7$ indicator solution, Standard NaCl, 0.014N, Standard $AgNO_3$ titrant, 0.0141N, $Al(OH)_3$ suspension, Phenolphthalein indicator, NaOH-1N, H_2SO_4-1N, H_2O_2-80%. The chloride content was calculated as follows:

$$\text{mg/l } Cl^{-1} = \frac{(A - B) * N * 35450}{\text{Sample in ml}}$$

Where A = Titration for sample in ml; B = Titration for blank in ml & N = Normality of $AgNO_3$

The reagents used for Biochemical oxygen demand are Phosphate Buffer, Magnesium Sulphate, Sodium Sulphate solution 0.025 N, Ferric Chloride, and Calcium Chloride (APHA). The Preparation of Dilution Water and Dilution of Sample for BOD were done as per standard protocols as follows.

Let:

- D_0 = DO in the sample bottle on the 0^{th} day.
- D_1 = DO in the sample bottle on the 5^{th} day.
- C_0 = DO in the blank bottle on the 0^{th} day.
- C_1 = DO in the blank bottle on the 5^{th} day.
- $C_0 - C_1$ = DO depletion in the dilution water alone.
- $D_0 - D_1$ = DO depletion in the sample + Dilution water.
- $(D_0 - D_1) - (C_0 - C_1)$ = DO depletion due to microbes.

BOD was calculated as follows:

$$\text{BOD (mg/l)} = \frac{(D_0 - D_1) - (C_0 - C_1) \text{ mg} * 1000}{\text{Volume of the sample used}}$$

Total Dissolved Solids were calculated as follows:

$$\text{Total dissolved solids (mg/lit)} = \frac{(A - B) * 10^6}{\text{Volume of sample in ml}}$$

Where A = weight of dried residue + dish in gms and B = weight of dish in gms

Total suspended solids were calculated as follows:

$$\text{Total suspended Solids (mg/lit)} = \frac{(A - B) * 10^6}{\text{Volume of sample in ml}}$$

Where: A = weight of filter paper + dried residue in gms; B = weight of filter paper in gms

Isolation of microorganisms:

Soil collected from local sewage drain was used for the isolation of microorganisms. For the initial isolation and cultivation of microorganisms, nutrient agar media was used. One gram of soil was weighed and taken in to a test tube and isolation was done by serial dilution, pour plate and streak plate methods. Pure colonies isolated were characterized using morphological, physiological and biochemical tests [3].

Experimental Design for Degradation studies:

In the present study, BOD and COD parameters were chosen for the treatment as these two parameters are present in very high quantities than standard levels. As the biological treatment studies include the microorganisms use and the retention time, experiment was designed by treating the effluents with the consortium of degradable organisms isolated from the soil samples. Degradation studies were carried out from day one to 10^{th}, 15^{th} and 20^{th} day by estimating the BOD and COD for every 24 hours. All the degradation experiments were conducted by shake flask method. 15 numbers of 500 ml. conical flasks were taken and in each flask 100 ml of steel industrial effluent was added. In each flask, the microbial consortium were added and kept on a shaker. Each day one flask was taken and the treated effluent was used to estimate the BOD and COD values from one to ten days. Studies were also conducted till 15^{th} and 20^{th} day to see if there is any further degradation with longer duration of time. The percent degradation was calculated from the values obtained and the results are tabulated and presented in the results and discussion section.

Biochemical characterization:

Bio-chemical tests are to be performed to characterize microorganisms on in biochemical properties. The biochemical tests performed in the present study were Gram staining, Citrate utilization test, H_2S production, Nitrate reduction test, Indole test, Methyl red test, Voges-proskaeur test, Starch hydrolysis and Gelatin hydrolysis [8].

RESULTS AND DISCUSSION

The present work was carried out to assess the biodegradation potential in terms of percent decrease in highly concentrated BOD and COD values of steel industrial effluent using locally isolated mixed microbial consortia. The influent concentration during the start up was 4620 mg O_2/lit (COD) and 2123 mg O_2/lit (BOD). The initial result of physicochemical analysis of steel industrial effluent is presented in Table – 1. The samples of the influent were collected on the first day of the start up after one hour and after 24 hours. The Chemical Oxygen Demand (COD) and Biochemical Oxygen demand (BOD) of the influent before and after the 24 hrs of the initial start up, was analyzed. Bioremediation of steel industrial effluent by locally isolated mixed microbial consortia was carried out for a period of 20 days. On the first day of analysis, the COD and BOD reduced by about 15.17% and 17.66% respectively (Fig. 1 & 2). The BOD and COD were reduced from 17.66% to 89.63% and 15.17% to 85.77 from 1st day to 10th day respectively. After 10th day, there was nearly 90% and 86% reduction in the BOD and COD values. When 15th and 20th day BOD was measured, it was noticed that the rate of degradation was not significant when compared to the 10th day values suggesting that 10 days retention time may do most of the degradation in the effluent with high strength organic pollution. At the end of 20th day, 92.62% of COD and 95.02% of BOD reduction was observed (Fig. 1 & 2). The detailed results of COD and BOD values are presented in Table – 2. Our results on industrial effluent remediation are in agreement with many similar studies [7,1,9].

Using streak plate and pour plate technique, four bacterial colonies were isolated from the local mixed microbial consortia used in the present study (Fig. 3). They have been identified as *Pseudomonas sps*, *Bacillus sps*, *Arthrobacter sps* and *Micrococcus sps.* by morphological, biochemical & physiological tests presented in Table – 3. Their further identification & characterization is in progress.

Table 1: Physicochemical Analysis of Steel Industry Effluent

S. NO	PHYSICO-CHEMICAL PARAMETERS	VALUES mg/lit
01	pH	8.2
02	Total Dissolved Solids	2730
03	Suspended Solids	262
04	Chlorides as Cl^-	600
05	Sulphates as So_4^-	320
06	COD	4620 mg O_2/lit
07	BOD at 27°C	2123 mg O_2/lit
08	Sulphide as S	0.7
09	Dissolved Phosphate as P	0.9
10	% Sodium	82%
11	Oil and Grease	6

Fig. 1: Graph showing Percent Degradation of COD with time

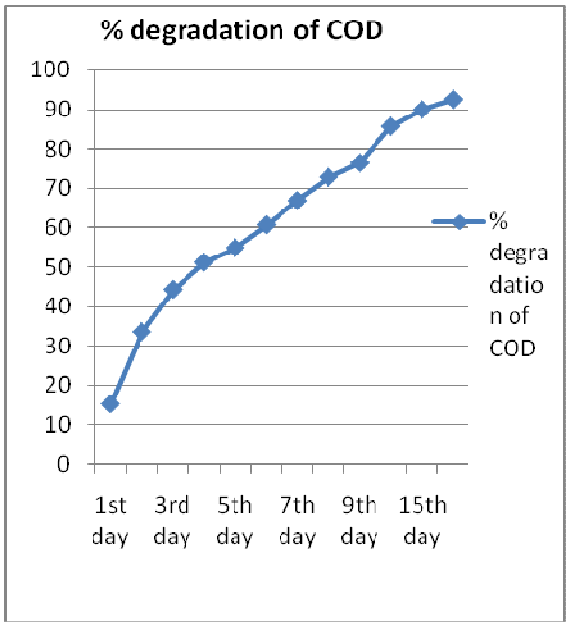

Table 2: Percent Degradation of COD and BOD by mixed consortia of microorganisms

S.NO	NO OF DAYS	% DEGRADATION OF COD	% DEGRADATION OF BOD
01	1st day	15.17	17.66
02	2nd day	33.55	29.15
03	3rd day	44.31	38.71
04	4th day	51.20	49.36
05	5th day	54.91	54.21
06	6th day	60.73	61.84
07	7th day	66.93	66.03
08	8th day	72.84	70.23
09	9th day	76.46	80.31
10	10th day	85.77	89.63
11	15th day	90.05	93.33
12	20th day	92.62	95.02

Fig. 2 Graph showing Percent Degradation of BOD with time

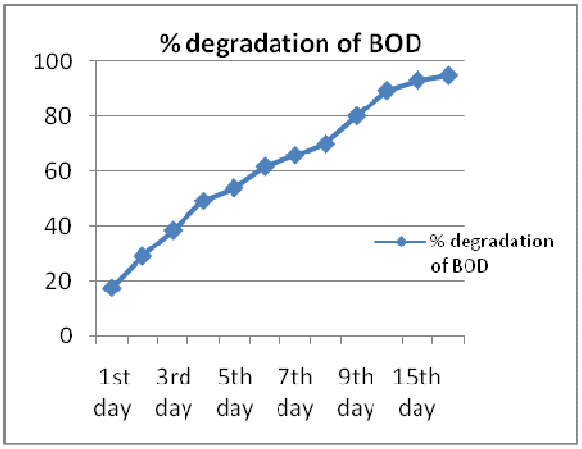

Table 3: Morphological, physiological and biochemical observations of the microbial colonies isolated from the soil

Parameters	A	B	C	D
Size	2	2	2	1
Shape	Circular	Circular	Circular	Circular
Elevation	Flat	Flat	Flat	Flat
Edge	Entire	Entire	Entire	Entire
Color	Orange	Cream	Transparent glossy	Yellow
Surface	Smooth	Smooth	Smooth	Smooth
Gram Reaction	+	-	-	+
Cell Size	2.5	2	2	1
Oxygen Use	Aerobic	Facultative	Facultative	Aerobic
Glucose Use	Yes	Yes	No	No
Endospore(Y/N)	Yes	No		No
Motile(Y/N)	No	Yes		No
Lactose		Positive	Negative	
Glucose		Negative	Negative	
Citrate		Negative	Negative	
H_2S		Negative	Positive	
Indole		Positive	Negative	
Motility		Positive	Negative	
Oxidase			Positive	
Catalase			Positive	
Growth at Temp	33	35	45^0C	40^0 C

A = ***Bacillus*** Sps.
B = ***Escherichia*** Sps.
C = ***Pseudomonas*** Sps.

Fig. 3: Colonies observed by streak plate & pour plate method from mixed consortia
D = *Micrococcus* Sps.

CONCLUSION

Biodegradation of wastewater is reliable and cost-effective method of treatment. The present work encompassed on biological degradation of steel plant effluent using a mixed consortium of soil bacteria. Preliminary physicochemical analysis showed that the effluent contained high levels of BOD and COD than the permissible standard limits which needed to be treated. This

industrial effluent was biodegraded using a mixed consortium of microorganisms for about 20 days at 37^0C by shake flask method. During the first phase i.e., first (1-5 days) of the study, it was brought to around 55%. In the second phase (5-10) days, around 73% of biodegradation was brought up. This reduction of COD and BOD values may be attributed due to the use of mixed consortium rather than a single strain. Comparisons of the results for the first two phases show a high reduction in concentration of BOD and COD ratios. In all up to 95% reduction of BOD and COD was observed by using these naturally occurring consortia of microorganisms. Identification of microorganisms from mixed consortia using biochemical tests revealed the presence of *Bacillus, Pseudomonas, Arthrobacter* and *Micrococcus* species. This preliminary study indicates that these microorganisms can be successfully used in bioremediation process for the removal of industrial effluents. This lab scale study is being extended to the bench top bioreactor so that it can further be scaled up to the industrial level.

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