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# ISOLATION OF BACTERIA FROM IRON AND STEEL INDUSTRY EFFLUENT

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## ABSTRACT

The present study deals with investigate of the essential physico-chemical parameters and isolation, identification of bacteria from the effluent collected from Iron and Steel coolant processing unit in Bharath heavy electrical limited at Trichy. On basis of morphological, biochemical analysis revealed that the isolates were identified as *Pseudomonas sp, Proteus sp, Bacillus sp, Klebsiella sp, Salmonellas sp* present in the coolant effluent. The purpose of this work is to evaluate the effect of several indigenous microorganisms to the effluent in remediating the effluent. Naturally occurring microorganism will be useful to treat the Iron and Steel Industry effluent in Bioremediation treatment processes method.

**KEYWORDS:** Pseudomonas sp, Bacillus sp, Bacteria, effluent.

## INTRODUCTION

The Iron and Steel is one of the most basic materials required for industrialization and plays a vital role in the country's economic growth. From Iron and Steel industry the adequate amount of waste water (effluent) released on water bodies. The main important processing in Iron and Steel industry is cooling machine system – by using water and oil for cooling purpose, to reduce the heat and damage of the machines. The improper disposal of the waste water leads to the environmental pollution, water pollution is one of the greatest concerns now a days. In recent years considerable attention has been paid to industrial wastes discharged to land and surface water. Industrial effluent containing a several organic and inorganic compounds. The coolant unit effluent is normally used in physical treatment it recycled high cost of biomembrane. Bioremediation of effluent treatment will be reducing the

effluent organic and inorganic waste it will be easy and economically reduce the physical treatment cost by reducing the bio membrane frequent usage.[19] Many microbial strains, each capable of degrading a specific compounds, are available commercially for bioremediation. However, indigenous bacteria in the polluted soil and effluent can degrade wide range organic and inorganic pollutants.

In the present study, identifying and isolating bacteria present in the coolants in effluent waste from iron and steel industry, BHEL in Trichy. The microorganism can be reabsorbing the valuable heavy metal in industrial effluent and capable of biodegrading or detoxify the heavy metals usually are already present in contaminated water .[13] Bioremediation technique improve the physical treatment methods and recovery of metals.

## MATERIALS AND METHOD

### Collection of Samples

The effluent samples were collected from four selected coolant processing unit in Iron and Steel industry BHEL at Trichy in Tamil Nadu. They were the following four processing unit Milling (cutting in minute iron chips), Drilling (making hole in iron bars), Cutting (cut the iron bars), Grinding (finishing the iron bars). The effluent samples were collected from these sampling units in sterile plastic bottles was stored transfer aseptically and processed immediately in laboratories as per method.[1] The Samples were used to analysis and microbial study Bergey's (1984) manual method.[9]

### Physiochemical analysis of Iron and Steel Industry coolant effluent samples.

As per APHA (1995) method the effluent collected from milling, Drilling, cutting and grinding units were analyzed for physiochemical parameters.[3] The effluent characterized by various parameters such as Color, Odor, pH, Electrical conductivity, Total suspended solids, Total dissolve solid, chemical oxygen demand (COD), biochemical oxygen demand (BOD), chloride, Sulphate, Fluoride, Iron, Total oil & grease, Chromium.[15],[17] By using AAS, pH meter, UV Spectrometer, Electrical conductivity meter.

### Isolation and identification of Bacteria

Bacterial biodiversity of Iron and Steel Industry coolant effluent samples were studied for six months from Sep (2015) to Feb (2016). Population of bacteria has isolated from the effluent collected from four units by serial dilution technique. Bacteria were identified based on colony characteristics, gram staining method. Morphological studies of the isolates were done

on the basis of colonies, size, color, shape, diameter, elevation and whether opaque, transparent or translucent and by various biochemical studies as given by Bergey's (1984) manual of determinative by bacteriology.[9]

## RESULTS AND DISCUSSION

### Physiochemical analysis of untreated Iron and Steel Industry effluent

The collected coolant effluent samples of Iron & steel Industry was assessed for its physicochemical properties (Table-1.1, 1.2, 1.3, 1.4). Result of analysis of the physiochemical parameters of untreated industrial effluent collected for a period of six months. The result of this study revealed that the color of the untreated industrial were blackish effluent with unpleasant odour.[8],[12] A large number of pollutants can impart color taste and odour to the receiving water bodies. There by making them unaesthetic and unfit or domestic consumption. The pH of the untreated Iron and Steel industry effluent was found to be within the range of 8.2. The untreated iron and steel industry effluent showed higher level of Electrical conductivity value is 5885 above the CPCB permissible limit[5],[6],[7], which could reflect the presence of organic and inorganic substances and salts would have increased the conductivity. The Iron and Steel Industries used water for cooling machine processes that carries away the solid particles and increases the Total suspended solids level in the effluent. The TSS and TDS have a minimum value of 354mg/l (Nov 2015) and3283mg/l (Nov 2015). The presence of TSS and TDS may be due to the insoluble organic and inorganic present in the effluent .[18] The high level of COD was analyzed in grinding unit effluent 3550mg/l. The present investigation revealed high level of COD. This indicates that the untreated effluent is unsuitable for the existence of aquatic organisms. The present study revealed high level of BOD value 1020mg/l. In the untreated effluent due to the presence of considerable amount of organic matter high level of BOD 1662mg/l. The maximum level of chloride and sulphate in the Iron and Steel industry effluent was found to be 890mg/l (Feb 2016) and 680mg/l (Sep2015) respectively. The Iron and Steel industry untreated effluent show the within the range of Iron and Oil and grease value is 54mg/l (Dec 20150) and 16.2mg/l (Sep 2015) respectively. The film of oil that floats over the water body there by distributing processes of photosynthesis in the aquatic plants. oil bioaccmulates in the higher animals and further enters the food chain. The physiochemical properties and heavy metals concentration of the effluent varies depending on the production of sampling station in various industries higher value of chromium 1.87mg/l was found in the untreated effluent of Drilling sampling station.[4][10] The similar value was recorded in various Iron & steel Industry effluent.[10]

**Isolation and identification of Bacterial diversity in Iron and Steel Industry coolant effluent**

Bacterial diversity has not been studied in iron and steel industry effluent. However a few reports are available on the bacterial flora of certain iron and steel industry effluents. The collected effluents were used to study the bacterial biodiversity. The present study 3 bacterial genera isolated from the in Iron and Steel Industry coolant effluent (table-2.2). Less number of bacterial communities was apparently due to the environmental stress caused by the high level of pollutants, which allowed only a restricted number of species that tolerant such conditions.[14]

On basis of morphological, biochemical analysis revealed that the isolates were identified as, *Pseudomonas sp. Proteus sp. Bacillus sp. Klebsilella sp. and Salmonellas sps.*(Table 2&3). Out of these six genera these three species, *Pseudomonas areuginosa, Klebsiella sp, Bacillus sp.* was predominantly present in all the effluent samples collected in study area. Several reports reported that *Pseudomonas areuginosa* also dominant species in heavy metal rich soil as well as in Industrial effluents[11],[16]

It was concluded that bacterial colonies of *Pseudomonas areuginosa* and *Klebsiella sp, Bacillus sp.* can grow successfully in coolant effluent. Several Gram positive bacteria were also known to reduce chromium (III) including several member of the genera *Bacillus sp.* Chromium resistant bacteria capable of reducing chromate have been reported from Chromium polluted environment and they were showing resistance in lower concentration.[2] The selected *Bacillus sp and Pseudomonas sp* will be used further for bioremediation heavy metals and organic pollutants present in the coolant effluent of Iron and Steel Industry coolant effluents.[20]

**Table: 1.1 Physiochemical analysis of Untreated Iron and Steel Industry coolant effluent Sampling Station-Milling**

| **Parameters** | **Sep-2015** | **Oct-2015** | **Nov-2015** | **Dec-2015** | **Jan-2016** | **Feb-2016** |
|---|---|---|---|---|---|---|
| **Colour** | **Black** | **Black** | **Black** | **Black** | **Black** | **Black** |
| **Odour** | **Unpleasant** | **Unpleasant** | **Unpleasant** | **Unpleasant** | **Unpleasant** | **Unpleasant** |
| pH | 8.8 | 8.7 | 8.5 | 8.3 | 8.6 | 8.7 |
| Temperature | 30 | 28 | 27 | 35 | 23 | 26 |
| Electrical conductivity | 5685 | 5347 | 4296 | 4983 | 4752 | 4261 |
| Total Suspended solids | 362 | 817 | 354 | 365 | 1143 | 986 |
| Total Dissolve Solids | 3431 | 4754 | 3283 | 3403 | 5736 | 5112 |
| BOD | 702 | 922 | 960 | 690 | 750 | 870 |

| | | | | | | |
|---|---|---|---|---|---|---|
| COD | 2240 | 2880 | 2920 | 2199 | 2137 | 2447 |
| Chloride | 780 | 685 | 824 | 747 | 690 | 880 |
| Sulphate | 370 | 550 | 410 | 370 | 640 | 440 |
| Fluoride | 1.2 | 1.8 | 2.5 | 1.6 | 1.4 | 1.5 |
| Iron | 3.4 | 4.9 | 4.8 | 5.1 | 4.4 | 3.8 |
| Total oil & grease | 16 | 15.6 | 14.5 | 15.2 | 14.3 | 13.5 |
| Chromium | 1.93 | 2.60 | 2.24 | 1.64 | 1.27 | 1.16 |

**Table: 1.2 Physiochemical analysis of Untreated Iron and Steel Industry coolant effluent Sampling Station-Drilling**

| **Parameters** | **Sep 2015** | **Oct 2015** | **Nov 2015** | **Dec 2015** | **Jan 2016** | **Feb 2016** |
|---|---|---|---|---|---|---|
| **Colour** | **Black** | **Black** | **Black** | **Black** | **Black** | **Black** |
| **Odour** | **Unpleasant** | **Unpleasant** | **Unpleasant** | **unpleasant** | **Unpleasant** | **Unpleasant** |
| pH | 8.5 | 7.8 | 8.6 | 8.5 | 8.7 | 8.5 |
| Temperature | 34 | 27 | 27 | 28 | 27 | 25 |
| Electrical conductivity | 5473 | 4471 | 4542 | 4354 | 4745 | 4285 |
| Total Suspended solids | 751 | 396 | 1122 | 874 | 1357 | 1129 |
| Total Dissolved Solids | 4553 | 3771 | 5384 | 4763 | 5828 | 6253 |
| BOD | 310 | 820 | 924 | 940 | 846 | 970 |
| COD | 756 | 2410 | 2750 | 2850 | 2557 | 2844 |
| Chloride | 810 | 690 | 710 | 642 | 792 | 890 |
| Sulphate | 470 | 360 | 340 | 370 | 570 | 220 |
| Fluoride | 1.6 | 0.8 | 0.6 | 1.5 | 1.8 | 1.4 |
| Iron | 4.2 | 4.5 | 4.4 | 5.3 | 4.6 | 3.2 |
| Total oil & grease | 17.1 | 14.5 | 15.5 | 13.4 | 14.1 | 13.7 |
| Chromium | 1.62 | 1.37 | 1.42 | 1.76 | 1.83 | 1.87 |

**Table: 1.3 Physiochemical analysis of Untreated Iron and Steel Industry coolant effluent Sampling Station-Cutting**

| **Parameters** | **Sep-2015** | **Oct-2015** | **Nov-2015** | **Dec-2015** | **Jan-2016** | **Feb-2016** |
|---|---|---|---|---|---|---|
| **Colour** | **Black** | **Black** | **Black** | **Black** | **Black** | **Black** |
| **Odour** | **Unpleasant** | **Unpleasant** | **Unpleasant** | **Unpleasant** | **Unpleasant** | **Unpleasant** |
| pH | 8.2 | 8.6 | 9.1 | 8.8 | 8.5 | 8.2 |
| Temperature | 34 | 32 | 33 | 27 | 28 | 28 |
| Electrical conductivity | 5420 | 4415 | 4346 | 4557 | 4349 | 4688 |
| Total Suspended solids | 798 | 1023 | 984 | 748 | 992 | 1335 |
| Total Dissolve Solids | 4730 | 5712 | 4987 | 4504 | 5623 | 6117 |
| BOD | 1020 | 930 | 822 | 910 | 893 | 916 |
| COD | 3550 | 2935 | 2615 | 2780 | 2743 | 2915 |
| Chloride | 760 | 810 | 790 | 748 | 852 | 796 |
| Sulphate | 650 | 470 | 420 | 560 | 420 | 340 |
| Fluoride | 0.8 | 0.4 | 1.4 | 1.8 | 1.4 | 1.2 |
| Iron | 3.4 | 5.4 | 4.7 | 4.5 | 5.3 | 3.5 |
| Total oil & grease | 6.2 | 13.5 | 14.6 | 14.3 | 15.3 | 14.6 |
| Chromium | 1.24 | 1.56 | 2.37 | 2.59 | 1.17 | 1.43 |

**Table: 1.4 Physiochemical analysis of Untreated Iron and Steel Industry coolant effluent Sampling Station-Grinding**

| Parameters | Sep-2015 | Oct-2015 | Nov-2015 | Dec-2015 | Jan-2016 | Feb-2016 |
|---|---|---|---|---|---|---|
| **Colour** | **Black** | **Black** | **Black** | **black** | **Black** | **Black** |
| **Odour** | **Unpleasant** | **Unpleasant** | **Unpleasant** | **Unpleasant** | **Unpleasant** | **Unpleasant** |
| pH | 8.5 | 8.2 | 8.7 | 8.4 | 8.3 | 8.6 |
| Temperature | 34 | 36 | 33 | 28 | 28 | 27 |
| Electrical conductivity | 4920 | 4614 | 4745 | 4757 | 4845 | 3488 |
| Total Suspended solids | 985 | 782 | 863 | 961 | 356 | 973 |
| Total Dissolve Solids | 5330 | 4716 | 4884 | 5105 | 4348 | 5216 |
| BOD | 924 | 842 | 810 | 720 | 928 | 785 |
| COD | 2860 | 2617 | 2533 | 2266 | 2825 | 2124 |
| Chloride | 740 | 830 | 860 | 720 | 863 | 870 |
| Sulphate | 680 | 430 | 530 | 580 | 520 | 370 |
| Fluoride | 1.6 | 1.8 | 2.2 | 2.4 | 1.8 | 1.8 |
| Iron | 3.3 | 4.5 | 4.3 | 5.4 | 4.7 | 5.3 |
| Total oil & grease | 15.5 | 12.4 | 12.7 | 12.9 | 13.6 | 13.9 |
| Chromium | 1.24 | 1.56 | 2.37 | 2.59 | 1.17 | 1.43 |

**Table: 2.1 Biochemical characterization of isolated bacterial stains from Coolant effluents**

| Test | Colony-2 | Colony-3 | Colony-4 | Colony-5 | Colony-6 |
|---|---|---|---|---|---|
| **Gram staining** | **Gram negative rods** | **Gram negative rods** | **Gram negative rods** | **Gram positive rods** | **Gram Negative bacilli** |
| Motility | Motile | Non- Motile | Motile | Positive | Motile |
| Catalase | Positive | Positive | Positive | Positive | Positive |
| Oxidase | Negative | Negative | Positive | Negative | Negative |
| Glucose | Positive | Positive | Negative | Positive | Negative |
| Lactose | Positive | Positive | Negative | Negative | Negative |
| Sucrose | Positive | Positive | Negative | Negative | Negative |
| Manitol | Positive | Positive | Positive | Negative | Negative |
| Indole | Positive | Negative | Negative | Negative | Negative |
| Methyl red | Positive | Negative | Negative | Positive | Positive |
| Voges proskauar | Negative | Positive | Negative | Positive | Negative |
| Citrate | Positive | Positive | Positive | Positive | Positive |
| Urease | Negative | Positive | Negative | Negative | Positive |
| TSI | Positive | Negative | Negative | Negative | Positive |
| Strains | *Salmonella Sp* | *Klebsilella sp* | *Pseudomonas sp* | *Bacillus sp* | *Proteus sp* |

**Table-2.2 Number of Isolates From Selected Sampling Units**

| S.no | Effluent sample collection units | Dilution | Number of isolates |
|---|---|---|---|
| 1 | Milling | $10^{-4}$ | *Pseudomonas sp, Salmonella sp,Proteus sp.* |
| 2 | Drilling | $10^{-4}$ | *Pseudomonas sp, Klebsilella sp.* |
| 3 | Cutting | $10^{-4}$ | *Pseudomonas sp. Bacillus sp.* |
| 4 | Grinding | $10^{-4}$ | *Pseudomonas sp, Klebsileella sp, Bacillus sp.* |

## CONCLUSION

In this present study the isolated *Bacillus sp* and *Pseudomonas sp* bacteria were used to assess the bioremediation from Iron and Steel industrial effluent. By using various physic chemical parameters like odour, taste, pH, temperature, BOD,COD, chloride, chromium, fluoride, iron, oil and grease were assessed to show the efficacy the bacterial species and the above results were showed that the *Bacillus sp* and *Pseudomonas sp* are eco- friendly bacteria.

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