ISSN: 0975-766X
CODEN: IJPTFI
*Available Online through* *Research Article*
**www.ijptonline.com**

# BIOREMEDIATION OF SUGAR MILL EFFLUENT BY IMMOBILIZED BACTERIAL CONSORTIUM

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*Received on 08-12-2014* *Accepted on 29-12-2014*

**Abstract**

In the present study, the sugar mill effluent was collected and the physico – chemical properties of the sugar mill effluent was analyzed. The bacterial consortium was immobilized and used for the bioremediation of the collected sugar mill effluent. The collected sugar mill effluent was inoculated containing $10^5$ cfu/ml of bacterial culture, and air was passed continuously using an aerator. After 3 and 6 months, the sample was filtered under aseptic condition and physico-chemical parameters were estimated. The physico- chemical properties showed a drastic reduction in the levels of COD, TSS, TDS, heavy metals and other physical properties after six months of treatment.

**Key words:**

Bioremediation, Bacterial consortium, Heavy metals, Physico-chemical properties, Sugar mill effluent.

## 1. Introduction

The sugar industry is playing an important role in the economic development of the Indian sub continent. The effluents produce a high degree of organic pollution in both aquatic and terrestrial ecosystems. They alter the physico-chemical characteristics of the receiving aquatic bodies and affect aquatic flora and fauna.

Use of industrial effluent and sewage sludge on agricultural land has become a common practice in India as a result of which these toxic metals can be transferred and concentrated into plant tissues from the soil. These metals have damaging effects on plants themselves and may become a health hazard to man and animals.

Bioremediation is a process that uses microorganisms, or their enzymes to return to the natural environment altered by contaminants to its original condition. Heavy metal bioremediation involves removal of heavy metal from waste water and soil through metabolically mediated or physico-chemical pathways. This natural and eco friendly

technology is a low cost technology, aesthetically pleasant, soil organism- friendly, and more importantly, it is able to retain the fertility status of the soil even after the removal of heavy metals.

In the present study, the sugar mill effluent was collected and bioremediated by bacterial consortium (*Bacillus subtilis +Pseudomonas fluorescens).*

## 2. Materials and Methods

### 2.1. Collection of sugar mill effluent sample

Before sampling the effluent, the polythene container was cleaned thoroughly using distilled water. Immediately after the effluent sampling, the effluent sample was taken to the laboratory and stored at room temperature in the laboratory for further analysis using standard methods.

### 2.2. Analysis physico-chemical characteristics of sugar mill effluent

The collected sugar mill effluent was assessed for various physico - chemical parameters like colour, odour, temperature, pH, Electrical conductivity, Turbidity, etc. Physico-chemical properties such as total suspended solids (TSS), total dissolved solids (TDS), total hardness, biological oxygen demand (BOD), chemical oxygen demand (COD), chloride, calcium, magnesium, sulphate, nitrogen, phosphorous, sodium, potassium and heavy metals (Iron, Zinc, Copper, Lead and Manganese) were measured using standard methods

### 2.3. Preparation of immobilized bacterial consortium

The bacterial consortium (*Bacillus subtilis* and *Pseudomonas fluorescens*) was immobilized as beads according to the procedure of Leung *et al*. Two percent sodium alginate solution is prepared in sterile distilled water by heating it to 60°C and mixing it thoroughly on a magnetic stirrer. Later 100ml of the sodium alginate is cooled to room temperature and 10% (10ml culture in 100ml sodium alginate solution) of the cell culture is added, the optimum condition was also studied as described above. The contents were mixed well by vigorous shaking to get a homogenized mixture. In a separate beaker, 100 ml of 0.1M calcium chloride solution was taken. The sodium alginate containing cell culture suspension was extruded drop wise through a syringe and allowed to fall in the beaker containing calcium chloride solution. The beads of sodium alginate gel formed are left in the beaker overnight for hardening. Then beads were washed and stored in distilled water at 28 ± 2°C.

### 2.4. Bioremediation of sugar mill effluent using immobilized bacterial consortium

The collected sugar mill effluent was inoculated with 5% inoculum containing $>10^5$ cfu/ml of bacterial consortium

(*Bacillus subtilis* + *Pseudomonas fluorescens*), and air was passed continuously using an aerator. After 3 and 6 months, the sample was filtered under aseptic condition and physico-chemical parameters (pH, Temperature, TS, TDS, TSS, BOD, COD, Heavy metals, Nitrogen, Phosphate etc) were estimated.

**3. Results and Discussion**

India is a largest sugar producing country. The effluents released produce a high degree of organic pollution in both aquatic and terrestrial ecosystems. Farmers have been using these effluents for irrigation, and found that the growth, yield and soil health were retarded. The life in effluent is highly diverse. Some potential fungal strains such as *Penicillium*, *Alternaria*, *Aspergillus flavus, Fusarium monolifome, Aspergillus niger* were isolated from sugarcane industrial effluent.

The sugar mill wastes contain a high amount of production load particularly, suspended solids, organic matters, pressmud and several air pollutants. Wastewater from sugar mills with its high Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD) and Total Dissolved Solids (TDS) rapidly depletes available oxygen when discharged into water bodies and have adverse impact on aquatic life so much so rendering the receiving water unfit for drinking and domestic purposes, reducing crop yields if used for irrigation, and exacerbating corrosion in water systems and pipe. (Prabahar et al, 2010)

In the present study, physico – chemical characteristics of the collected sugar mill effluent was analyzed and the results were showed in Table - 1. The sugar mill effluent was acidic nature with brown colour and emitted unpleasant smell. The temperature of collected sugar mill effluent was 36°C and the EC was 2.48 $dSm^{-1}$. The TSS and TDS present in collected sugar mill effluent were 379 and 1310 mg/L respectively. It also showed high value of BOD (1069 mg/L) and COD (2260 mg/L). High amount of calcium (293 mg/L), magnesium (299 mg/L), chloride (377 mg/L), sodium (106 mg/L), potassium (125 mg/L), sulphate (430 mg/L), nitrogen (1300 mg/L), phosphorous (6.17 mg/L) were recorded in the collected sugar mill effluent sample.

**Table-1: Physico-chemical properties of collected sugar mill effluent.**

| S.No | Parameters | Values | Standard by TNPCB (2009) |
|---|---|---|---|
| **1.** | Colour | Brown | Colourless |
| **2.** | Odour | Unpleasant | Odourless |
| **3.** | Temperature | 36.0 | 40.0 |

| | | | |
|---|---|---|---|
| **4.** | pH | 4.6 | 5.5-9 |
| **5.** | Electrical conductivity | 2.48 | - |
| **6.** | Total suspended solids(mg/L) | 379 | 200 |
| **7.** | Total dissolved solids(mg/L) | 1310 | 200 |
| **8.** | Total hardness (mg $CaCO_3$/L) | 278 | 250 |
| **9.** | BOD (mg/L) | 1069 | 30 |
| **10.** | COD (mg/L) | 2260 | 250 |
| **11.** | Calcium (mg/L) | 293 | 200 |
| **12.** | Magnesium (mg/L) | 299 | 100 |
| **13.** | Chloride (mg/L) | 377 | 600 |
| **14.** | Sodium (mg/L) | 106 | - |
| **15.** | Potassium (mg/L) | 125 | - |
| **16.** | Sulphate (mg/L) | 430 | 12 |
| **17.** | Nitrogen (mg/L) | 1300 | 600 |
| **18.** | Phosphorous (mg/L) | 6.17 | 10 |
| **19.** | Zinc (mg/L) | 0.89 | 0.01 |

*TNPCB- Tamil Nadu pollution board

Similar findings which were reported by Saranraj and Stella (2012). The variations in physico- chemical properties may be due to the processes involved, raw materials used and chemicals used in the sugar mill. The acidic nature of sugar factory effluent may be due to the release of hydrochloric acid during the process of extraction of sugar from sugar cane as reported by Balagopal *et al*. The presence of high BOD clearly indicated the high quality of biological oxidizable organic matter. The high content of BOD causes oxygen depletion, where as presence of high level of total suspended solids and total dissolved solids might be due to the insoluble organic and inorganic matter present in the effluent (Thamizhiniyan *et al,* 2009). The increased COD levels in the effluent could be due to the presence of high amount of suspended solids.

In addition, the soil samples collected from the agricultural land irrigated with effluents had high level of several toxic metals as Copper, Zinc, Lead, Manganese and Iron. It indicated that the selected effluent used to irrigate agricultural land is not fit for agricultural practices as high metal content may directly or indirectly interfere with the metabolic activities of crop plants by altering the conformation of proteins, for example enzymes, transporters or regulators (Sharma *et al*, 2004).

**Table-2: Physiochemical properties of sugar mill effluent.**

| S. No | Parameters | Values | Standard by TNPCB (2009) | Bioremediated effluent after 3 months |
|---|---|---|---|---|
| 1 | Iron (mg/L) | 17.00 | 1.00 | 8.3 |
| 2 | Copper (mg/L) | 0.436 | 0.01 | 0.95 |
| 3 | Lead (mg/L) | 0.62 | 0.05 | 0.15 |
| 4 | Manganese (mg/L) | 0.075 | 0.01 | 0.01 |

**Table-3: Physiochemical properties of bioremediated sugar mill effluent using immobilized bacterial consortium after 3 months.**

| S.No | Parameters | Bioremediated effluent after 3 months |
|---|---|---|
| **1.** | Colour | Colourless |
| **2.** | Odour | Odourless |
| **3.** | Temperature (°C) | 31 |
| **4.** | pH | 7.0 |
| **5.** | Electrical conductivity (dSm-1) | 2.26 |
| **6.** | TSS (mg/L) | 329 |
| **7.** | TDS (mg/L) | 388 |
| **8.** | BOD (mg/L) | 327 |
| **9.** | COD (mg/L) | 591 |
| **10.** | Oil and grease (mg/L) | 8 |

| | | |
|---|---|---|
| **11.** | Calcium (mg/L) | 271 |
| **12.** | Magnesium (mg/L) | 0.11 |
| **13.** | Chloride (mg/L) | 283 |
| **14.** | Sodium (mg/L) | 65 |
| **15.** | Potassium (mg/L) | 102 |
| **16.** | Sulphate (mg/L) | 95 |
| **17.** | Total Nitrogen (mg/L) | 1021 |
| **18.** | Phosphorous (mg/L) | 6.2 |
| **19.** | Zinc (mg/L) | 0.22 |

-below detection level

In the present study, physico- chemical parameters of the immobilized bacterial consortium (*Bacillus subtilis* + *Pseudomonas fluorescens*) bioremediated effluent was estimated at the third and sixth month and the results were furnished in Table – 2 and Table - 3. After treatment with microbial isolates the effluent turned colourless and odourless, which might be due to the action of microbial isolates. This result was supported by Swamy *et al and* Saranraj *et al*. The pH of the effluent was acidic prior to bio treatment. Later on the pH was changed to neutral due to bioremediation of the effluent.

**Table-4: Physiochemical properties of bioremediated sugar mill effluent using bacterial consortium after 6 months.**

| S. No | Parameters | Bioremediated effluent after 6 months |
|---|---|---|
| 1 | Colour | Colourless |
| 2 | Odour | Odourless |
| 3 | Temperature (°C) | 30 |
| 4 | pH | 7.0 |
| 5 | Electrical conductivity ($dSm^{-1}$) | 2.03 |
| 6 | TSS (mg/L) | 203 |
| 7 | TDS (mg/L) | 176 |

| | | |
|---|---|---|
| 8 | BOD (mg/L) | 93 |
| 9 | COD (mg/L) | 218 |
| 10 | Calcium (mg/L) | 212 |
| 11 | Magnesium (mg/L) | - |
| 12 | Chloride (mg/L) | 278 |
| 13 | Sodium (mg/L) | 56 |
| 14 | Potassium (mg/L) | 94 |
| 15 | Sulphate (mg/L) | 56 |
| 16 | Total Nitrogen (mg/L) | 711 |
| 17 | Phosphorous (mg/L) | 4.4 |
| 18 | Zinc (mg/L) | 0.15 |
| 19 | Iron (mg/L) | 5.5 |
| 20 | Copper (mg/L) | 0.14 |
| 21 | Lead (mg/L) | 0.08 |
| 22 | Manganese (mg/L) | - |

-below detection level

The TSS present in bioremediated sugar mill effluent was 203 mg/L in the sixth month. The TDS were found to be 176 mg/L in the sixth month. BOD with the degree of pollution varied within a span of six months to 93 mg/L and 218 mg/L respectively. Similar findings were also reported by earlier workers. TDS was also found to be high before treatment of sugar mill effluent which may be due to the presence of high salt content and in bioremediated effluent it showed reduction indicating that the microbial isolates have the efficiency to reduce the TDS in the sugar mill effluent. Similar results were observed by Saranraj *et al*.

The results of the inorganic contents like potassium, phosphate, sodium, calcium, sulphate, nitrate and heavy metals (Iron, Zinc, Lead, Manganese and Copper) revealed reduction after bio remediation of sugar mill effluent.

## 4. Conclusion

This study concluded that physico-chemical parameters such as pH, electrical conductivity, TSS, TDS, BOD, COD, chloride, hardness, calcium, magnesium, sulphate and heavy metals were relatively high in the sugar factory effluent and severely affected the environment and water bodies. The immobilized bacterial consortium (*Bacillus subtilis* + *Pseudomonas fluorescens* ) was used for the bioremediation of sugar mill effluent and showed a drastic reduction in the levels of COD, TSS, TDS, heavy metals and other physical properties after six months of treatment. Biotreatment offers easy, effective, and ecofriendly techniques and utilization of bacterial consortium can be applied for correcting the sugar mill effluent treatment.

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