Preparation and standardization of biocompost with sugar and distillery industry wastes using selected microbial species

V. Rengaraj¹ and Mazher Sultana*²

¹Research Scholar, Bharathiar University, Coimbatore, Tamilnadu.
²Department of Advanced Zoology & Biotechnology, Presidency College, Chennai, Tamilnadu.

INTRODUCTION

Environmental pollution has been recognized as one of the major problems of the modern world. The increasing demand for water and dwindling supply has made the treatment and reuse of industrial effluents an attractive option. The problem of environmental pollution on account of essential industrial growth is practical terms, the problem of disposal of industrial water, whether solid, liquid or gaseous. All the three types of wastes have potentially of ultimately polluting water [1]. 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 accumulated into plant tissues from soil [5].

Sugar Industry

Sugar industry is one of the most important agro based industries in India and is highly responsible for creating significant impact on rural economy in particular and countries economy in general. Sugar industries rank second among the agro based industries in India. Sugar industry is seasonal in nature and operates only for 120 to 200 days in a year. A significant large amount of waste is generated during the manufacture of sugar and contains a high amount of production load particularly in items of suspended organic matters, effluent, sludge, pressmud and bagasse [6]. Sugar factory effluent produces obnoxious odour and unpleasant color when released into the environment without proper treatment. Farmers

To whom correspondence should be addressed:
V. Rengaraj
E-mail: rengaraj84@gmail.com
have been using these effluents for irrigation, found that the growth, yield and soil health were reduced [7]. The life in effluent is highly diverse and consists of interacting population of microorganisms and effluent fauna and their activities affect physical, chemical and biological characteristics of effluent. Some potential fungal strains such as *Penicillium pinophilum*, *Alternaria gaisen*, *Aspergillus flavus*, *Fusarium monoliforme*, *A.niger* were from sugarcane industrial effluent [8].

**Impact of Sugar Mill Effluent to the Environment**

Sugar industry is the backbone of rural, agricultural and socio-economic development in India. Many industries are directly or indirectly dependent on sugar industry which in turn is responsible for overall development of state. In this context sugarcane production is of vital importance for its products and by-products. Disposal of industrial waste is the major cause of soil pollution. Number of chemical industries, mainly tannery, paper, textiles, atomic and electric power plants discharge pollutants. They contain organic and inorganic as well as non biodegradable material such toxic chemicals affect the soil parameters and there by the soil fertility [9]. Advances in science and technology and the industrial revolution have enabled humans to plot resources. Though industrialization contributes to economical development, most important natural resources like water and soil are commonly polluted with by-products, waste materials and non-utilized parent chemical compounds from the industries which in-turn ultimately affect the agricultural production and food security. All types of revolutions fulfill the needs of growing population on one side and on other side it caused all kinds of pollution which altered the physico-chemical and biological characteristics of the environment [10]. Thus, environmental pollution has become an important global phenomenon, which has demanded attention from all over the countries.

Earlier several microbes were reported to take effective part in bioremediation of industrial wastes. The mechanism by which microorganisms includes biosorption, intracellular accumulation and enzyme-catalyzed transformation [Lloyd, 2002]. On the basis of energetic requirements, biosorption seems to be the most common mechanism. Furthermore, this is the only option where dead cells can be applied as bioremediation agent. Nevertheless, systems with living cells allow more effective bioremediation processes as they can self-replenish and remove metals via different mechanisms (11).

Generally, the effluent is generated from mill house, waste water from boiling house, waste water from boiler blow-down, condenser cooling water and soda and acid wastes [Kolhe, *et al.*, 2000]. Indian sugar mills generate 0.16-0.76 m of waste water for every tonnes of cane crushed by them. The pollution standards stipulate that BOD of waste water should be less than 30 mg/L for disposal into inland surface waters and less than 100 mg/L for disposal on land whereas, the combined sugar mill waste water had a BOD of 1,000 to 1,500 mg/L [12]and[13] reported that the untreated sugar mill effluent is toxic to plants when used for irrigation. The trends in developing countries to use sugar mill effluent as fertilizer has gained much importance as it is considered as a source of organic matter and plant nutrients and serves as good fertilizer. Sugar mill effluent contains considerable amount of potentially harmful substances including soluble salts and heavy metals like Fe, Cu, Zn, Mn, Pb. The long term use of this sugar mill effluent for irrigation which contaminates soil and crops to such an extent that it becomes toxic to plants act and causes deoterioration of soil [14].

The physico-chemical properties such as silt, clay, electrical conductivity, water holding capacity, organic matter and total nitrogen contents, microbial population matter and total nitrogen contents, microbial population samples collected from sugar industry wastes dump than in the non-dump sites [Nagaraju *et al.*, 2009]. Analysis of sugar mill effluents and soil samples had shown high metal content than the permissible limits except Pb. Further, analysis of plant samples have indicated the maximum accumulation of iron (Fe) followed by Mn and Zn in root, shoot, leaves and seeds of wheat and mustard. The physico-chemical analysis showed that the sugar mill effluent was acidic in nature and yellowish in colour. It was rich in total suspended and dissolved solids with large amount of Biological oxygen demand and Chemical oxygen demand. Higher amount of chloride, calcium, magnesium, sodium, potassium and iron were also present in the effluent. These effluents severely affected the plants and soil properties when used for irrigation.

**Sugar press Mud**

Sugar press mud (SPM) is also termed as *press mud cake* or *filter cake*. During the processing of
sugarcane, cane juice contains a large number of impurities which are in the form of precipitates and these impurities are separated using filtration process. Both types of filtration processes i.e. batch type filter presses or rotary vacuum filtration process or produces cake. SPM produced during extraction of sugar as an impurity has multiplex uses like as a fertilizer, animal feed and industrial use as a building lime after calcinations process. Composition of SPM varies with different industries by the following factors:
(a) Classification methods
(b) Variety of cane
(c) Locality
(d) Mill efficiency
(e) Soil type
(f) Nutrients available

This SPM is produced at a rate of 7-9% of total weight of sugar cane in Carbonation industries and 3-5 % in sulfitation industries. Silicon, Iron, Manganese, Calcium, MgO & P2O5 is also detected in some appreciable amount in SPM. Compost fertilizer is always evaluated by the percentage of organic matters in the final product. Organic matter contains all types of fiber, wax, crude proteins sugar, and all other carbon containing components available in the final product. These sugar industries are playing an important role in the economic development of the India but the effluents released produce a high degree of organic pollution in both aquatic and terrestrial ecosystems. They alter the Physicochemical characteristics of the receiving aquatic bodies and affect aquatic flora and fauna. Bioremediation, the use of microorganisms to degrade environmental contaminants, is among these new technologies. Bioremediation has numerous applications, including clean-up of ground water, soils, lagoons, sludge and process-waste streams. Out of several methods that are used in the treatment of industrial effluents, biotreatment offers a cheaper and environmentally friendlier alternative for biological degradation of industrial effluents. Microbial treated industrial effluents contains an array of plant nutrients, trace elements, rich in silica which is essential for siliciculous plants like rice and sugarcane, for non lodging and also for improving resistance to pests and diseases. Industrial wastes for land application have been proven to be a cost effective [15]. The beneficial use of treated effluents for agriculture is the major reuse application worldwide. Untreated effluent used for irrigation is highly toxic to the plant, fish and other aquatic life. The need for economical, effective and safe methods for disposal of pollutant in effluent has resulted in the search for unconventional materials that may be helpful in reducing the pollutant in the effluent. Reuse of treated effluent that is normally discharged to the environment from sugar mill waste water treatment plants is receiving an increasing attention as a reliable water resource [16-19].

OBJECTIVES
Main aim of this work was to prepare and standardize the biocompost using the selected microbial species with the following objectives.
• To analyze the physical and chemical properties of the pressmud.
• To analyse the physical and chemical properties of the spent wash.
• To optimize the biocompost preparation with sugar and distillery industry wastes using selected microbial species.
• To analyze the efficacy of selected microbial species in the biocompost preparations by Insilico method.

MATERIALS AND METHODS
For the present study the pressmud and distillery industry wastes were collected at M/S Rajshree Sugars and Chemicals Limited, Varadaraj Nagar, Theni district, Tamilnadu, India, used sterile polythene container and brought to the laboratory to analyse the physical and chemical properties by following the standard method of APHA (2005). The biocompost was prepared from the pressmud and spent wash by mixed in specific ratio with and without of selected microbial species and it was characterized by following the standard method. Further the efficacy of selected microbial species in the Biocompost preparations was studied by Insilico method and used the following bioinformatics Tools and Databases are used:
RESULTS
Table 1. Characteristics and % of nutrients present in of press mud

<table>
<thead>
<tr>
<th>S.No</th>
<th>Parameter</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Organic carbon</td>
<td>34.5-42%</td>
</tr>
<tr>
<td>2.</td>
<td>Nitrogen</td>
<td>0.47-1.05%</td>
</tr>
<tr>
<td>3.</td>
<td>Phosphorus</td>
<td>2.31-3.01%</td>
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<td>4.</td>
<td>Calcium</td>
<td>0.83-1.98%</td>
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<td>5.</td>
<td>Potassium</td>
<td>0.48-0.84%</td>
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<td>6.</td>
<td>Magnesium</td>
<td>0.05-0.25%</td>
</tr>
<tr>
<td>7.</td>
<td>Sulphur</td>
<td>0.22-0.31%</td>
</tr>
<tr>
<td>8.</td>
<td>Copper</td>
<td>35-200 mg/kg</td>
</tr>
<tr>
<td>9.</td>
<td>Zinc</td>
<td>47-215 mg/kg</td>
</tr>
<tr>
<td>10.</td>
<td>Manganese</td>
<td>163-625 mg/kg</td>
</tr>
<tr>
<td>11.</td>
<td>Iron</td>
<td>250-9500 mg/kg</td>
</tr>
<tr>
<td>12.</td>
<td>pH</td>
<td>6.5-7.3</td>
</tr>
</tbody>
</table>

Biological Parameter
Table 2. Changes in microbial communities due to application of spent wash

| Windrows | Days | 30 | | 60 | | 90 | |
|-----------|------|----|----|----|----|----|
|           | Bacteria (10^5) | Fungi (10^3) | Actinomyctes (10^4) | Bacteria (10^5) | Fungi (10^3) | Actinomyctes (10^4) | Bacteria (10^5) | Fungi (10^3) | Actinomyctes (10^4) |
| W1        | 15.6 | 10.7 | 11.7 | 18.7 | 13.5 | 11.9 | 15.1 | 10.1 | 12.6 |
| W2        | 15.8 | 10.8 | 11.9 | 18.9 | 13.6 | 12.1 | 14.8 | 9.9  | 12.7 |
| W3        | 15.8 | 11.1 | 12.1 | 19.2 | 14.2 | 12.3 | 14.4 | 9.8  | 12.9 |
| W4        | 16.3 | 11.4 | 12.4 | 19.7 | 14.7 | 12.4 | 14.3 | 9.8  | 13.3 |
| W5        | 16.5 | 11.5 | 12.7 | 20.1 | 15.0 | 12.6 | 13.9 | 9.6  | 13.5 |
| W6        | 16.7 | 11.6 | 12.7 | 20.6 | 15.4 | 12.9 | 13.7 | 9.5  | 13.5 |
| W7        | 16.2 | 11.3 | 12.2 | 19.9 | 14.7 | 12.2 | 13.3 | 9.3  | 13.8 |
| W8        | 15.1 | 11.0 | 11.9 | 18.2 | 13.4 | 11.8 | 13.1 | 9.0  | 13.9 |
| W9        | 14.5 | 10.1 | 11.4 | 17.4 | 13.1 | 11.1 | 12.5 | 8.4  | 14.6 |

Values are in the CFU/g of the sample.

Fig-1a: The physical and chemical properties of the compost (pH and EC) after spent wash application on 90th day of composting from windrows (1-9).
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**Fig 1.** 3 Dimensional structure of *Bacillus circulans*

**Fig 2.** 3 Dimensional structure of *Pseudomonas aeruginosa*
DISCUSSION

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 sugar industry effluent which is untreated highly toxic to plants and it is not permissible for irrigation.

The bacterial consortium (Bacillus circulans and Pseudomonas aeruginosa) 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 treatment. Application of traditional sugar mill wastewater treatment requires enormous cost and continuous input of chemicals which becomes uneconomical and causes further environmental damage. Biotreatment offers easy, effective, and
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economical and ecofriendly techniques and utilization of immobilized bacterial consortium can be applied for fine tuning of sugar mill effluent treatment. The treated effluents of industry are not highly polluted and they satisfy the ISI standard values and therefore can be used for irrigation purpose. The detailed study pertaining to the above aspect will be discussed in the light of existing literature. From the results obtained from this study, we identified the conserved regions and also found out the intra molecular details like the amino acids properties of the biodegradable microbe’s sequence. These orthologs genes share sequence homology. The protein structure prediction results show the secondary structure view and the structural properties in various colors. This result explains the molecular secondary structural information of Bacillus circulans and Pseudomonas aeruginosa of the selected proteins. This would be useful in the structure based drug designing and Microbial informatics studies in future. All the above results were discussed.

CONCLUSION
In the present investigation used the sugar and distillery industry wastes to prepare the biocompost with the selected microbial species and found the physical and chemical properties of biocompost. Based on the above study these wastes may be considered as cheapest sources for plant growth. The Insilico study indicates that the Metabolic pathway information of Bacillus circulans and Pseudomonas aeruginosa which was identified from gene/protein sequences analysis have a potential significance in the Biodegradation of sugar and Distillery industry wastes for Biocompost preparation.

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