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Research Article

FUNGAL AND ALGAL DIVERSITY IN SUGAR MILL EFFLUENT AND BIOREMEDIATION USING FUNGAL CONSORTIUM

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ABSTRACT

The quality of the air, water, soil, and the entire ecosystem at large has been deteriorated by industrial pollution. One of the most important agro-based industries in India is the sugar mill. The sugar mill is also a great source of contamination too. Biodiversity of fungi and algae in the sugar mill effluent was examined and found fungi such as *Aspergillus flavus, A. fumigatus, A. luchensis, A. niger, A .oryzae, A. terreus, Fusarium oxysporum, Helminthosporium oryzae, Penicillium citrinum* and *Trichoderma* sp and the consortium of the same has the potential to bio remediate the collected sugar mill effluent. Nineteen different algal genera was identified in different purposed sugar mill effluent and external water has served a good nutrient for more number of genera (13 genus) compared with other sources. From Aringar Anna sugar mill, Kurungulam the effluent has been collected and by using the standard methods, the physicochemical properties of the sugar mill effluent was analysed. Bioremediation of the sugar mill effluent is done by immobilizing the fungal consortium as beds in the column. The sugar collected mill effluent was inoculated with immobilized beds with 5% of fungal consortium, and air was passed continuously using an aerator. Under aseptic conditions, the sample was subjected to filtration and the parameters of the physicochemical analysis were estimated after 3 and 6 weeks. A high BOD, COD, TSS, TDS, chloride, sulfate and oil & grease was found in the analysis of the physicochemical properties of the raw sugar mill effluent. After the three weeks of treatment with immobilized fungal consortium, drastic reduction of all the parameters studied were recorded.

Keywords: Sugar mill effluent, Physiochemical parameters, Bioremediation, Fungi, Algae, Biodiversity.

1. INTRODUCTION

Environmental pollution is the unsolved problem in today's modern world. Environmental pollution on account of industrial growth is indispensable. The problem arises with the disposal of industrial water into whether solid, liquid or gaseous is an unsolvable hassle. All these three means of disposing the water has the great potential of polluting the water [1, 2]. Toxic metals are transferred and concentrated in plant tissues from the soil by means of using the effluent from industries and sludge of sewage into the agricultural lands. This has become one of the common practices in India. As a result, plants are getting damaged which directly becomes a hazard for humans and animals. The heavy metals can turn into toxins, above certain concentration and over a narrow range. In Indian economy, the sugar industry plays an important role but it is also responsible for the production of high degree of organic pollution in the aquatic and terrestrial ecosystem with its effluents. The physico-chemical properties of the aquatic bodies which receives the effluent, is altered and thereby it affects the aquatic flora and fauna. The rural and semi urban population uses the stream and river water for agriculture and domestic purposes. When sugar effluent is discharged into the environment, it results in serious health hazard to the population along with fish mortality and damage to the paddy crops as the waste water accumulates into the agricultural land. Sugar mill industry is highly responsible for creating an eminent impact on the rural economy in particular and the economy of country in general. In India, sugar industry rank second among the agro based industries. Sugar industry operates only for 120-200 days in a year and it is seasonal. During the manufacturing of the sugar, a large amount of waste is generated which contains high amounts of suspended solids, organic matters, effluent, sludge, press mud and bagasse [3-6]. Sugar mills play a major role in discharging large amount of effluents thereby polluting the water bodies. Sugar consumption rate is highest in

India, as per the USDA foreign agriculture service statistics. In production of sugar, India has notched up second position following Brazil and India is one among the largest producers of sugar in the world. [7]. In a day, 35,000 kg sugar has been produced by using 110-115m^3 and letting 95-100m^3 of effluent water. There are 453 sugar mills in India and 287.4 million tons of sugar is produced per year. It has been proven that industrial wastes for land application were cost effective. In the present study, the sugar mill effluent is collected and bio remediated by the immobilized fungal algal consortium.

2. MATERIAL AND METHODS

2.1. Sugar mill effluent sample collection

From Aringar Anna sugar mill, Kurungulam, Tamil Nadu the sugar mill effluent was collected. Sterilization was done to the sampling containers before sampling of effluent. As soon as the sample was collected, it was transported to the laboratory for further sampling.

2.2. Isolation and identification of fungi from sugar mill effluent

For the microbial analysis, the industrial effluent was collected in sterile bottles. In the laboratory, by following the procedure described [8] fungal identification is carried out by pour plate method.

2.3. Isolation and Identifications of Alga from sugar mill effluent

To isolate Microalgae from sugar mill effluent, pour plate method in Bristol's medium was adopted. To a sterilized labelled test tube containing 9 parts of distilled water, 1 part of the effluent was added and the serial dilution was carried out in the ratio of 1; 10000 dilution. From each diluted samples, one ml was transferred to a corresponding petri-dishes and the molten sterile Bristol's medium was poured aseptically into the petri-dishes and allowed to solidify in the laminar air flow chamber. The plates were incubated after solidification at room temperature for 2 weeks. Microalgae cultures were pure cultured on the same media after the incubation and based on the standard methods they are identified.

2.4. Analysis of physicochemical characteristics of sugar mill effluent

Different physicochemical parameters like colour, odour, temperature, pH, Turbidity, etc. and physicochemical properties such as total suspended solids (TSS), total dissolved solids (TDS), total hardness, biological oxygen demand (BOD), chemical oxygen demand (COD), chloride, sulphate, and oil & grease were measured from the effluent collected from the sugar mill using standard analytical methods [9].

2.5. Preparation of immobilized fungal consortium

The most effective matrix to enhance the efficiency of sewage treatment was found to be the microbial consortium which is immobilized on the natural coconut coir. According to the procedure of Humera et al. [15], the fungal consortium (*Aspergillus flavus, A. fumigatus, A. luchensis, A. niger, A. oryzae, A. terrreus, Fusarium oxysporum, Helminthosporium oryzae, Penicillium citrinum* and *Trichoderma* sp) was immobilized on natural coconut coir pith. To the flasks containing YEPG medium, the sterile coir piths were added. One piece of each fungal mycelium (1 mm) cultured for 7 days on Saboraud's dextrose broth (Hi media) were added to each culture flask. In a rotary shaker (110 rpm) the samples were incubated for 5 days (26°C).

2.6. Bioremediation of sugar mill effluent using immobilized fungal consortium

The collected sugar mill effluent was inoculated with immobilized fungal mat containing consortium of *Aspergillus flavus, A. fumigatus, A. luchensis, A. niger, A. oryzae, A. terrreus, Fusarium oxysporum, Helminthosporium oryzae, Penicillium citrinum* and *Trichoderma* sp and by using an aerator air was supplied uninterruptedly. Subsequently the samples were filtered after second and third week under aseptic condition and physico-chemical parameters (pH, Temperature, TS, TDS, TSS, BOD, and CODetc.) were estimated.

3. RESULTS

Biodiversity of fungal and algal populations in sugar mill effluents were studied and the fungi identified in the present effluents were *Aspergillus flavus, A. fumigatus, A. luchensis, A. niger, A. oryzae, A. terrreus, Fusarium oxysporum, Helminthosporium oryzae, Penicillium citrinum* and *Trichoderma* sp. There were five genus identified and among the five *Aspergillus* sps were predominant (table 1). As far as algal diversity is concerned, there were 19 different types and *Spirulina platensis* & *Stigonema ocellatum* were detected in all the four types of effluent and hence the sugar mill effluent could serve as a substrate for the production of single cell protein accumulating microalgae (table 2).

The sugar mill wastes contains a high amount of production load particularly, suspended solids, organic matters, press mud and much more 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 such a lot 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. The present study has also evidenced to see the elevated levels of all the 12 parameters studied (table 3) and total dissolved solids and total solids were very high among other parameters studied (table 4,5) and next to that was sulphate concentrations and it was very high in all the four samples analysed (fig. 1).

Table 1: Identification of fungi from various sugar mill effluent 1

S. No	Name of the fungi	Different field soil (CFU/ml)			
		A	B	C	D
1	*Aspergillus flavus*	2	4	3	4
2	*A. fumigatus*	4	2	2	1
3	*A. luchensis*	2	1	3	4
4	*A. niger*	6	5	4	3
5	*Aspergillus oryzae*	5	4	3	-
6	*A. terreus*	6	1	2	3
7	*Fusarium oxysporum*	2	3	2	2
8	*Helminthosporium oryzae*	3	2	3	4
9	*Penicillium citrinum*	4	5	4	3
10	*Trichoderma* sp	7	8	7	3
Total number of species		10	10	10	9
Total number of colonies		41	35	33	27

A -Service water, B- Boiling house water, C- Excess condensate water, D- External water

Table 2: Identification of Algae from various sugar mill effluent

S. No	Name of the microalgae	Different places (CFU/ml)			
		A	B	C	D
1.	*Anabaena azollae*	-	08	-	02
2.	*Chrocococcus limneticus*	07	-	04	03
3.	*Dermocarpa* sp.	05	03	-	-
4.	*Gloeocapsa magma*	-	03	-	-
5.	*Johannesbaptistia*	-	-	07	05
6.	*Gloeothece* sp.	-	06	-	-
7.	*Katagnymene* sp.	09	-	-	09
8.	*Microcoleus* sp.	09	-	10	09
9.	*Myxosarcina* sp.	-	-	05	-
10.	*Nostoc muscorum*	04	02	06	04
11.	*Oscillatoria spongeliae*	07	-	-	07
12.	*Plectonema phormidiuodes*	07	06	-	07
13.	*Pseudanabaena* sp.	13	13	-	13
14.	*Spirulina platensis*	10	10	11	10
15.	*Stigonema ocellatum*	09	14	13	09
16.	*fSymploca* sp.	-	-	07	-
17.	*Synechococcus* sp.	02	-	04	-
18.	*Trichodesmium* sp.	06	-	04	06
19.	*Xenococcus* sp.	07	08	-	07
Total number of colonies		93	73	67	91
Total number of species		12	10	09	13

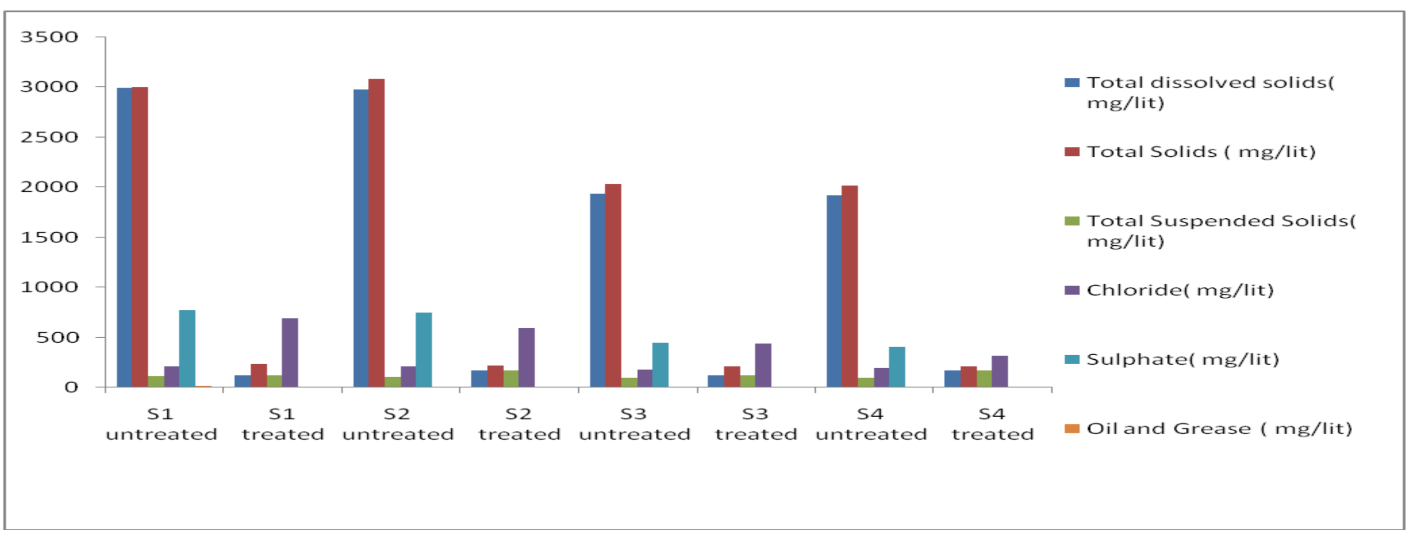
A - Service water, B- Boiling house water, C- Excess condensate water, D- External water

Table 3: Standard by TNPCB (2009) physicochemical properties of sugar mill effluent water

S.NO.	Parameters	Standard by TNPCB (2009)
1.	Colour	Colourless
2.	Temperature °C	40.0
3.	pH	5.5 - 9
4.	Dissolved Oxygen (mg/lit)	0-1.5
5.	Biochemical Oxygen demand (mg/lit)	30
6.	Chemical Oxygen demand (mg/lit)	250
7.	*Total dissolved solids*(mg/lit)	200
8.	Total Solids (mg/lit)	200
9.	Total Suspended Solids(mg/lit)	200
10.	Chloride(mg/lit)	600
11.	Sulphate(mg/lit)	12
12.	Oil and Grease (mg/lit)	-

Table 4: Physiochemical parameters in untreated & treated sugar mill effluent

Parameters	Sample 1		Sample 2		Sample 3		Sample 4	
	untreated	treated	untreated	treated	untreated	treated	untreated	treated
Temperature °C	42	**28**	40	**28**	31	**29**	30	**29**
pH	6.5	**7**	5.7	**7**	5.5	**7**	6.5	**7.4**
Dissolved Oxygen (mg/lit)	129	**1.5**	1.5	**1.3**	252	**1.4**	295	**1.5**
Biochemical Oxygen demand (mg/lit)	97	**27**	92	**30**	85	**29**	80	**28**
Chemical Oxygen demand (mg/lit)	350	**175**	345	**197**	240	**180**	200	**190**
Total dissolved solids (mg/lit)	2990	**120**	2970	**167**	1930	**123**	1920	**167**
Total Solids (mg/lit)	3000	**234**	3075	**218**	2030	**209**	2015	**209**
Total Suspended Solids(mg/lit)	110	**12**	105	**16**	100	**12**	95	**16**
Chloride(mg/lit)	215	**69**	215	**59**	180	**43**	195	**32**
Sulphate(mg/lit)	770	**11**	750	**10**	450	**11**	409	**12**
Oil and Grease (mg/lit)	16	**0**	11	0	10	**0**	9	**0**



A - Service water, B- Boiling house water, C- Excess condensate water, D- External water

Fig. 1: Physiochemical parameters of untreated effluents

The table depicts 19 different algal genera in different purposed sugar mill effluent and external water has served a good nutrient for high number of genera (13 genus) compared with other sources.

Table 5: Statistical analysis

Chemical Parameters	P-value
Dissolved Oxygen (mg/lit)	0.000
Biochemical Oxygen demand (mg/lit)	0.05
Chemical Oxygen demand (mg/lit)	0.041453
Total dissolved solids (mg/lit)	0.002404
Total Solids (mg/lit)	0.004062
Total Suspended Solids (mg/lit)	0.034175
Chloride (mg/lit)	0.013208
Sulphate (mg/lit)	0.004486
Oil and Grease (mg/lit)	0.002555

Hypotheses:

Null Hypothesis: H_0: There is no significant difference in the untreated and treated physio-chemical parameters of paper mill effluent.

Alternate Hypothesis: H_1: There is significant difference in the untreated and treated physio-chemical parameters of paper mill effluent.

Inference:

We reject H_0 (because $p<0.05$) and state that we have significant evidence that there is noteworthy changes in the physiochemical parameters of paper mill effluent after treatments. Moreover the mean values of all physiochemical parameter are less in treated which is in the permissible limits than compared to untreated.

4. DISCUSSION

Sugar industry, the furthermost significant amongst agro centred activities contributing considerably to rural area economy in particular and national economy but the other side of the industrial exploitation leads to generation of greater quantity of waste during the manufacturing of sugar. Sugarcane is the major crop from which sugar can be manufactured in commercial quantity. India is a major sugar producing country [10]. These sugar industries are imparting an important role in the economic growth of the India. Still the waste water released generates a high degree of organic pollution in both water and earthly ecosystems. The physicochemical properties of the receiving water bodies are changed and the aquatic organisms both flora and fauna is affected [4]. Other than the organic pollutants, sugar mill sewage produces insufferable smell and disagreeable color when discharged into the environment without appropriate treatment. Agri-culturalists have been utilizing these sewages for irrigation, found that the development, harvest and soil healthiness were abridged [11-13]. 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 Citrinum*, *Aspergillus flavus, Fusariumm oxysporum, Aspergillus niger* were isolated from sugarcane industrial effluent [14].

In the present study, biodiversity of fungi and algae in sugar mill effluent was studied and compared with previous results. Physicochemical characteristics of the collected sugar mill effluent were analyzed and the results were showed in table 4. The sugar mill effluent was brown in colour and emitted unpleasant smell and is acidic in nature. The maximum temperature of collected sugar mill effluent was 42°C. The maximum amount of TSS and TDS present in collected sugar mill effluent were 110 and 2990 mg/L respectively. It also showed high value of BOD and COD. High amount of chloride (215 mg/L) and sulphate (770 mg/L) and oil & grease was 16mg/l were recorded in the collected sugar mill effluent sample.

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*. [15]. High quality of biological oxidizable organic matter is present in the effluent and hence it shows high BOD value. Oxygen depletion is due to the high content of BOD, whereas due to the insoluble organic and inorganic matter present in the effluent high level of TSS and TDS is present [16]. Due to the presence of high amount of suspended solids, the COD level in the effluent is increased. Several toxic metals such as Copper, Zinc, Lead, Manganese and Iron were in high level in the soil samples collected from the agricultural lands irrigated with effluent.

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 [17]. Similar findings which were recorded in the present study was also reported by Lakshmi and Sundaramoorthy [18]; Rathore *et al*. [19]; Thamizhiniyan *et al*. [20] and Borale and Patil [21]. The variations in physico- chemical properties may be due to the processes involved, raw materials used and chemicals used in the sugar mill. The In the present study, physico-chemical parameters of the immobilized fungal consortium treated effluent was estimated at the third and sixth month and the results were furnished in table 2 and 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*. [22]. 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.

The TSS present in bioremediated sugar mill effluent was 12 mg/L in the third week. The TDS were found to be 120 mg/L, 167mg/L, 123mg/L & 16mg/L in the samples 1 to 4 in the third week. The results of the inorganic contents like chloride & sulphate, revealed reduction after bio remediation of sugar mill effluent. Similar findings were also reported [23, 24, 25]. Thus, it is clear that the sugar mill waste water contains minerals and can be used for agricultural irrigation after giving suitable treatment and proper dilution [26].

In the present study, the physicochemical analysis of sugar mill effluent polluted soil revealed that it contained a high amount of pH, electrical conductivity, nitrogen, phosphorus, potassium, copper, zinc, iron, lead and manganese. At an equivalent time when bacterial isolates were applied as consortia within the sort of immobilized beads, a considerable substantial reduction was observed within the toxicity of metals as observed by Wani *et al*. [27]. The bioremediated soil analysis revealed that it has a reduced amount of pollutants. Further the earlier similar reports by Glick [28]; Prabakar *et al*. [29]; Zhuang *et al*. [30] and Ezhil Bama *et al*. [31] lend support to the present findings.

5. CONCLUSION

This study concluded that physicochemical parameters such as pH, TSS, TDS, BOD, COD, sulfate and hydrocarbons 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 fungal consortium was used for the bioremediation of sugar mill effluent and showed a drastic reduction in the levels of COD, TSS, TDS and other physical properties after 3 weeks of treatment. Application of traditional sugar mill wastewater treatment requires enormous cost and continuous input of chemicals which becomes uneconomical and causes further environmental damage. Bio-treatment offers easy, effective, economical and ecofriendly techniques and utilization of indigenous microbes especially fungi has been found to be very effective. And also the present study evidenced the ability of the sugar mill effluent to support the growth of single cell protein producing microalgae species.

Conflict of interest

The authors declare no conflict of interest.

6. REFERENCES

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