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Study of microflora of sugar industry effluent and their possible role in the degradation of the effluent

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Abstract
Sugar industry is one of the most important agro-based industries and is highly responsible for environmental pollution specially water pollution. In spite of stringent measures, may industries dispose untreated or partially treated effluent in nearby water bodies making water non potable and also affecting flora and fauna of aquatic environment. The available technologies for the treatment of effluent include aerobic, anaerobic and physicochemical methods. Microbial degradation is eco friendly treatment technology.

In the present study effluent samples from three sugar industries located in Aurangabad district were selected. Physicochemical properties were studied. In this study, 22 fungi belonging to 12 genera and 10 bacterial species were isolated The sample having the highest BOD and COD was further selected for microbial treatment using *Aspergillus ustus* -a fungus and *Pseudomonas aeruginosa*- a bacterium under aerobic condition. The degradation of the effluent was monitored in terms of reduction in TDS and COD.

Keywords: sugar industry effluent, BOD, COD, degradation

1. Introduction

Man has made rapid strides in all walks of life with the advances in science. He has utilized science and modern technology for his comfort, pleasure and betterment of living standards. The increasing demand for products coupled with the pressing need to supply them at a faster rate has resulted into increased mass production and industrialization of these products. This has resulted into increasingly larger quantities of inevitable undisposable waster matter which has undeniably caused pollution of atmosphere – land, water and air, by disturbing the equilibrium of natural products, environment and organisms including mankind.

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. Sugarcane is cultivated in over 4 million hectors spread over a wide range of agro-ecological situations both in tropical and subtropical regions[1], (Shahi H.N. 2002) Sugar industry ranks second amongst major agro-based industries in India. Most of the sugar factories are located in Uttar Pradesh, Bihar, Andra Pradesh and Maharashtra. Maharashtra has 102 sugar factories with a production capacity 165.5 tonnes/day [2](Kaul, 52 purkowicz 1998). In India at present there are more than 650 sugar mills producing 15 million tons of sugar and 13 million tons of molasses(Daulata *et al.* 2014). Sugar industry is basically seasonal in nature and operates only for 120 to 200 days in an year. i.e. early November to May or June end. Two states, Maharashtra and Uttar Pradesh alone account for 61% of all India Production[3] (N.Srinivasan 2003, Saranraj and Stella, 2012)

In the present study, effluent samples from three sugar industries located in Aurangabad district, Maharashtra were used. Microflora of the samples was studied and the prominent isolates were used to check the efficiency of degradation.

2. Material and Methods

2.1 Collection of Samples

The water samples were collected directly from the outlet where combined factory effluent is released. Samples were also collected from the sedimentation tanks which provided effluent water for irrigation. The water samples were collected in pre-sterilized glass stopper bottles. The samples were stored by using preservatives i.e. HNO_3 and then kept in refrigerator after being brought to the laboratory as mentioned in APHA 1992.

2.2 Physical examination of the sample

Immediately after collection the sample, pH was determined by using Elico pH meter. TDS of water samples were determined by using probe of 'MAC" water analysis kit. Total solids, TSS were determined by the method of Trivedy and Goel 1986.

2.3 Chemical Examination of the sample

B.O.D.,C.O.D., cadmium, Chromium, Nickel, Chloride, Calcium, Hardness, Magnesium, Zinc, sulphate, Total alkalinity, Oil a0nd grease web` re determined by the method given in "Standard methods for the examination of water and waste water" 18th edition 1992 of "APHA".

2.4. Biological Examination

2.4.1 Isoaltion of bacteria

Bacteria were isolated by using serial dilution technique and using nutrient agar and nutreient agar enriched with waste

water as plating media. The bacteria isolated were identified according Bergey's manual of determinative Bacteriology 8th edition upto genus level.

2.4.2 Isolation of fungi

To study mycoflora of the samples serial dilution method using Potato Dextrose agar,

Czapeks Dox and Sabouraud's agar was used as plating media as given by Ghate 1984.

The fungi isolated by above technique were identified by referring Barnett H.L.,B.B. Hunter 1972. They were identified by classification methods, referring to standard monographs, other relevant literature and taxonomic keys. Observations and camera lucida drawings were done.

2.5 Biodegradation

2.5.1 Bacterial Degradation

Microorganisms play a pivotal role in degrading complex toxic organic compounds and make them available for biological cycle. Organic carbon present in waste water serves as a source of C and energy for microorganisms. Oxygen is the ultimate electron acceptor. Bacteria play a major role in aerobic degradation (Leela lyenger 1998). Chaudhary, Sahoo, Manthan, Rohellain 1998 also recommended *Pseudomonas* for treatment of textile mill effluent. *Pseudomonas* utilizes organic compounds as sole source of nutrients (Murugesh and Sampathkumar 2002) hence it was thought worth to study degradation by using *Pseudomonas*. Extent of degradation was recorded in terms of reduction in TDS after every 48 hrs.

2.5.2 Fugal Degradation

The effluent under study is diluted 1:100 times, sterilized and inoculated with 3 discs of 5mm each of the selected fungal culture were inoculated in the flask. Before and after sterilization TDS is calculated in PPM of the control. For fungus first reading is taken after seven days incubation period and then onwards after every two days.

Degradation of effluent is studied in terms of reduction in TDS by using fungus – *Aspergillus* ustus and a Bacterium – *Pseudomonas* sp.

TDS is measured by probe of "MAC" water analysis kit. Every two day readings were taken. Effluent along with selected organism was incubated upto 30days on a mechanical shaker.

The treated effluent was checked for BOD and COD and % reduction was found out. At the end the waste water was given alum treatment. Such treated effluent was used for phyotoxicity studies.

2.6 Toxicity studies

For confirmation of nontoxic nature of the treated effluent, toxicity assay was carried out using *Sorghum vulgare*. Ten seeds were irrigated by water as a control, the effleunt and the treated effluents, respectively. Three sets of the same experiment were carried out.

3. Results and Discussion

3.1 Collection of Samples

The composite waste water discharged from the various processes was collected as a sample for the study. The samples were collected from from Aurangabad district. The colour of the effluent of was dark brown with a strong smell to milky white with a moderate smell or with a sewage smell. The effluents were either discharged into a nearby water body or used for irrigation. pH of the waste water found to be in the range of 4-6.. Effluent after treatment with oxidation ponds is used for irrigation. Chemicals are added in the tanks where the water is continuously stirred and then passed down to setting tanks from where it is used for irrigation. The untreated water has a dark brown colour and treated water has a light brown colour.

3.2 Physico-chemical Parameter

Effluents were studied for various parameters as shown in Table No. 1

Table 1: Physico –chemical characters of the effluents

Sr. no.	Parameter	Sample 1	Sample 2	Sample 3
1	BOD	55000.0	750.0	340.0
2	COD	94400.0	1888.0	864.0
3	Arsenic	--	--	--
4	Cadmium	---	0.01	---
5	Chromium	0.35	----	-----
6	Nickel	----	----	-----
7	Chloride	6400.0	480.0	510.0
8	Calcium	N.D.	220.0	97.6
9	Hardness	N.D.	760.0	410.0
10	Magnesium	N.D.	327.43	189.42
11	Zinc	11.0	0.330	0.107
12	Sulphate	N.D.	31.4	64.0
13	Total Alkalinity	N.D.	690.0	336.0
14	Oil and Grease	N.D.	5.6	2.4
15	pH	4.76-5.44	5.74	5.90
16	temp°c	27.0	27.1	26.7
17	conductivity	45.6 µmhos	2.5	3.2
18.	ORP	130mv.	-26mv.	0.00
19	TDS	198.3 ppm	197.00ppm	195.00ppm
20	TS	66900 mg/L	1800 mg/l	1900 mg/l

ND- Not Detected

Sugar industry wastewater was characterized by high BOD, COD and TDS (Reddy *et al.* 2014).

3.3 Biological Examination

3.3.1 Isolation of bacteria

Effluent samples inoculated on nutrient agar showed ten different species of Gram positive motile rods belonging to Genus *Bacillus* were isolated. Colonies were opaque, milky white, lemon yellow, dirty white to white. Ten different species of Gram negative motile rod belonging to Genus *Pseudomonas* were isolated. From the work done by various researchers, *Pseudomonas* was selected for further study.

3.3.2 Isolation of fungi

Table 2: Distribution of fungi in the effluent samples

Sr. No.	Name of the fungus	Sample 1	Sample 2	Sample 3
1	*Aspergillus ustus*	+	+	+
2	*Aspergillus clavatus*	+	+	+
3	*Aspergillus fumigatus*	+	+	+

4	*Aspergillus ochraceous*	+	-	-
5	*Aspergillus terreus*	+	-	-
6	*Aspergillus niger*	+	+	+
7	*Aspergillus glaucus*	+	+	+
8	*Alternaria alternata*	+	+	+
9	*Alternaria citri*	+	+	+
10	*Cladosporium oxysporum*	+	+	+
11	*Chloridium*	+	-	-
12	*Curvularia lunata*	+	+	+
13	*Curvularia clavata*	+	-	-
14	*Fusarium moniliforme*	+	+	+
15	*Fusarium oxysporum*	+	+	+
16	*Mucor hiemalis*	+	-	-
17	*Nigrospora*	+	-	-
18	*Penicillium nigricans*	+	+	+
19	*Penicillium implicum*	+	+	+
20	*Phoma*	+	+	+
21	*Rhizopus nigricans*	+	+	+
22	*Trichoderma*	+	-	+

+ Present, _ Absent

Mycoflora of effluent samples showed 60% *Aspergillus ustus*, 51% *Aspergillus niger*, 50 % *Fusarium moniiforme*, 40% *Aspergillus galucus* and 40 % of *Alternaria alternta* species. Amongst the highest occurence of the fungi, *Aspergillus ustus* was selected for further study.

3.4 Biodegradation of the waste

It is observed that nature always tries to develope the organism suited for any changed environment. Bacteria and fungi like any other microorganism adapt to their racial existenc. It was therefore thought worthwhile to survey the microflora with a hope to select the most efficient decomposer. Out of the three effluents the effluent having highest BOD and COD values of that was selected for further study.

Out of the fungi isolated frequently occurring fungi Aspergillus ustus, and a bacterium having a property to degrade wide range of chemical substances, *Pseudomonas* was selected for biodegradation studies. Pseudomonas was reported by various researchers for its good degradation property (S. Murugesh and G. Sampathkumar 2002, R. Gowrishanker, R. Palaniappar and S. Ponpandi 1997.) Fig 1 and 2 showed reduction in TDS by the two selected organisms.

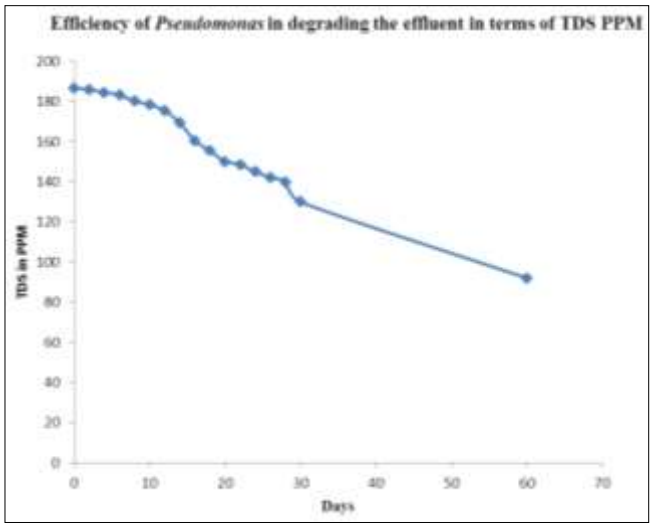

Fig 1. Efficiency of *Pseudomonas* in degrading the effluent in terms of TDS PPM

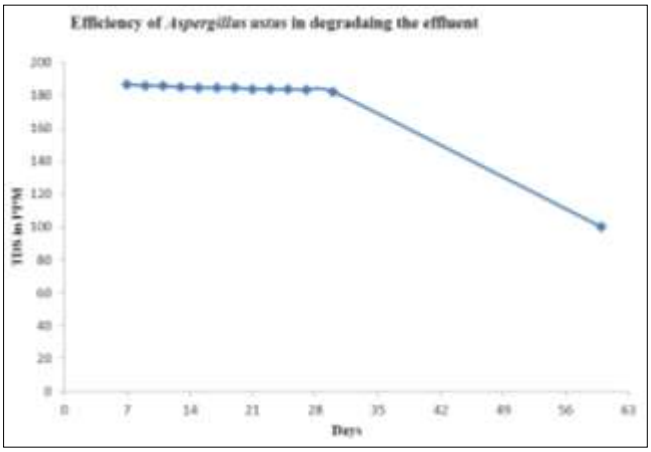

Fig 2: Efficiency of *Aspergillus ustus* in degradaing the effluent

Alongwith reduction in TDS, reduction in BOD and COD was checked. There was a significant reduction of 35% in BOD and 46% in COD. This treated effluent was further treated with a physical treatment by using alum and then used for phyto toxicity studies.

3.5 Toxicity studies

As most of the industries discharge either trated or partially treated effluent into nearby water bodies, it was found essential to check phytotoxicity of the biodegraded effluent in the present study. This treated effluent was tested for phytotoxicity using *Sorghum vulgare* L. Toxicity was tested in terms of root length, shoot length and percent germination. Results of treated effluent as shown in Table 3. From the results it is observed from root and shoot length as well as % germination that highest toxicity of the untreated effluent whereas the treated effluent showed significant increase in root length, shoot length and % germination reflecting non toxic nature.

Table 3: Toxicity assay using *Sorghum vulgare* L.

	Root length	**Shoot length**	**% Germination**
Control	6.64	10.46	100
Untreated effluent	1.5	2.5	5
Treated effluent	6.95	10.51	95

4. Conclusions

- All effluent samples showed very high BOD, COD and chlorides
- All effluent samples were acidic in nature having pH ranging from 4.7 -5.9
- Ten Gram positive motile rods belonging to Genus Bacillus were isolated from the samples as well as Gram negative motile rods of Genus Pseudomonas were isolated.
- Twenty two different fungi belonging to genera *Aspergillus, Alternaria, Cladosporium, Curvularia, Fusarium, Penicillum* etc were isolated from the effluent samples.
- Bacterial and fungal biodegradation of the effluent showed decrease in TDS
- Phytotoxicity studies showed nontoxic nature of the treated wastewater.
- The present studies indicted possibilities of employing bacteria and or fungi under controlled condition to degrade the waste. However, more inputs are needed in order to standardize the method

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