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A Comparative Analysis of Coir and Sugar Mill Effluents Treated with Endophytic Organisms Isolated from *Oryza sativa*

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Abstract: The objective of the present study was to investigate the biodegradation capacity of endophytic organisms isolated from *Oryza sativa* shoots on industrial effluent. A comparative analysis was done on coir and sugar mill effluent. Physico-chemical parameters and microbial screening were conducted. The endophytic organisms were isolated from shoots in flowering stages of *Oryza sativa*—both bacteria and fungi—which were co-cultured for a period of 10 days. After the biofilm formation they were introduced into the coir and sugar mill effluents and the physico-chemical parameters were analysed. The results from this study indicate that there was reduction in BOD, COD, chloride, sulphide, calcium, magnesium, sodium and potassium in coir effluent and pronounced decrease in sugar mill effluent which was found to be proved statistically significant. The endophytic organisms were identified by morphological and biochemical parameters and further confirmed with RAPD analysis.

Key words: Endophytic organisms, biofilm, effluent, RAPD.

Introduction

Environmental problems are one of the most important concerns of society especially the wastes produced by industries. The industries chosen for the present study were coir effluent and sugar mill effluent. Enormous volumes of effluent are generated at different stages from these industries, thus manufacturing copious amounts of chemicals and waste.

Indian coir industry is an important cottage industry contributing significantly to the economy of the country. India accounts for more than two-thirds of the world production of coir and coir products. The immature husks of coconut are suspended in a river or water-filled pit for up to 10 months. During this time micro-organisms break down the plant tissues surrounding the fibres to loosen them, a process known as retting. The coconut husk retting effluent is discharged from the retting ground once in two months at the rate of 1000 m^3 with high salinity and BOD (Biology Oxygen Demand) (Ravindranath and Sarma, 1995).

Sugar industry is considered to be one of the best organized industrial sectors in the country, and it is also among the leading economic enterprises. India is one of the largest producers of sugar in the world. Sugar mills consume around 1500-2000 litres of water and generates about 1000 litres of wastewater per day. The sugar mill effluent has a BOD of 1000-1500 mg/litre. The pollution standards stipulate that BOD of effluent should be less than 30 mg/litre for disposal into inland surface water and less than 100 mg/litre for disposal on land (Ramachandran, 1996).

As these industries find use in various ways it is essential to improve the production at the same time

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keeping in mind to control the environmental pollution caused by this industry. The increase in pollution problem can be solved by most recent technology biofilm engineering process. This is the most recent technique in reducing pollution (Premkumar and Daniel Jai Pandiyan, 2007).

Materials and Methods

Collection of Samples

The wastewater sample was collected in Sakthi coir fibre production unit, Rajakamangalam, Kanyakumari district. The wastewater sample was collected once a week from Arignar Anna sugar mill, Kurungulam, Thanjavur.

Microbial Analysis

Isolation and characterization of bacteria: The bacteria from the coir and sugar mill effluent were isolated by standard techniques. The pure culture thus obtained was used for identification by morphological and biochemical analysis and results were compared with Bergey's Manual of Systematic Bacteriology.

Water Analysis

Physico-chemical parameters were conducted for both the effluents before and after treatment with biofilm. Parameters like pH, electrical conductivity, bicarbonate, sulphide, potassium, sodium, residual sodium carbonate and sodium adsorption ratio, chloride (APHA, 1980), calcium and magnesium (Heron and Mackereth, 1955) were measured.

Biodegradation by Biofilm

Collection of Plant Material

Leaves from plants of *Oryza sativa* of healthy flowering stages were collected from paddy research centre, Tamil Nadu Rice Research Institute, Thanjavur district, Tamil Nadu. The collected leaves were placed in autoclaved polypropylene bags with ice and transported to the laboratory in sterile conditions. Rice varieties chosen were: IR Middle stage, AD-06244 Middle stage, Chinnaponnie Mature stage, Chinnaponnie Middle stage, IR-66 Initial stage and ADT-47 Final stage.

Isolation of Endophytic Microbes

The leaves were surface sterilized with 70% ethanol for five minutes and then treated with 0.2% mercuric chloride for 30 seconds. The efficacy of sterilization was checked by rolling the leaves on 0.1% Trypic Soy Agar (TSA) plates. The leaves were homogenized with a sterilized mortar and pestle in phosphate buffered saline. Different dilutions were placed on plates containing semisolid N-free medium consisting of malic acid (5 g), in 0.2N KOH, 1.64% Fe-EDTA solution (4 ml), Agar (2 g) and dis. water one litre.

To isolate pure cultures, bacterial isolates were sub-cultured on TSA plates and incubated at 34°C. Morphological and biochemical analyses and results were compared with Bergey's Manual of Systematic Bacteriology. Fungal isolates were sub-cultured on Sabouraud Dextrose Agar (SDA) plates and microscopic identification was done.

Co-culturing of Fungi and Bacteria

Isolated bacteria and fungi were maintained in Yeast Mannitol Broth (YMB). Cultures were incubated on a rotary shaker at 28°C. After 10 days, each culture was transferred to 50 ml centrifuge tubes and centrifuged at 4025xg for 20 min. Bacterial-fungal co-cultures were then prepared. From 6-day old bacterial cultures, 2 ml was inoculated into 50 ml of concentrated YMB. They were inoculated with 50 ml of spore suspensions of fungal cultures to form the co-cultures (Jayasinghearachchi and Seneviratne, 2004). The cultures were incubated at 28°C on a rotary shaker. At day 7 a loop of the broth culture was removed from each flask using sterilized inoculating needle. Gram staining and lactophenol cotton blue staining was performed. At day 10 the pH of the co-cultures was measured by using the pH meter and the co-cultured organisms were used for treatment of coir industry and sugar mill industrial effluents.

Treatment of Effluents with Endophytic Organism to Form Biofilm

From the co-cultured sample 10 ml of low acidic pH supernatant was poured into the 100 ml of sterilized industrial effluent. Then, the sample was placed for three days. After treatment of organism 1st and 3rd day the physico-chemical parameters were checked before and after introduction of the endophytic organisms.

RAPD Analysis

Isolation of DNA was done by the standard method of Sambrook et al., 1989.

Statistical Analysis

Students 't' test was used to compare the two samples (Zar, 1974).

Discussion

The sugar mill effluent was found to have higher organic load with black colour and foul odour when compared to coir effluent which was brownish to black with an unpleasant odour. The physico-chemical parameters of the sugar mill effluent are found higher than that of coir effluent, which might be due to the difference in the raw material and the different stages in processing. The effluent strategy is presented in Tables 1 and 2.

Morphological and biochemical tests (Bandra et al., 2006) were conducted which were compared with Bergy's manual. The identified organisms from coir effluent were *Serratia* sp, *Proteus* sp, *Pseudomonas* sp, *Escherichia* sp, *Bacillus* sp, *Micrococcus* and *Staphylococcus* sp. The organisms isolated from sugar mill effluent were *Pseudomonas* sp, *Klebsiella* sp, *Proteus* sp, *Erwinia* sp, *Yersinia* sp, *Shigella* sp, *Enterobacter* sp, *Escherichia* sp and *Streptococcus* sp.

The endophytic bacteria isolated from *Oryza sativa* are *Pseudomonas* sp, *Klebsiella* sp, *Serratia* sp, Rhizobium fungi such as *Aspergillus niger*, *Aspergillus flavus* and *Cladosporium* which were co-cultured to form biofilm and used to treat both coir effluent and sugar mill effluent.

After introduction of biofilm the results of coir effluent showed decreased level in all the physico-chemical parameters at a duration of both 24 hrs and 72 hrs (Refer Tables 1 and 2). In the sugar industry effluent analysis a similar trend of reduction in the level of all the parameters was recorded with a more pronounced decrease in 72 hrs of treatment. This decrease in all the parameters can be attributed to the fact the co-culturing of bacteria and fungi contribute to the bio-degrading capacity as found in other similar studies.

Table 1: Physico-chemical parameters of coir effluent before and after introduction of endophytic organisms

S. No.	*Physico-chemical parameters*	*Raw effluent*	*Time duration*	
			24 hours	*72 hours*
1.	pH	6.9	4.20	5.14
2.	EC dsm^{-1}	9.1	1.9	1.8
3.	BOD mg/L	34.4	20.32	16.4
4.	COD mg/L	92.1	87.5	73.4
5.	Bicarbonate mg/L	2.4	5.8	3.7
6.	Chloride mg/L	38.0	13.5	13.1
7.	Sulphide mg/L	99.1	0.68	0.10
8.	Calcium mg/L	4.7	1.7	1.0
9.	Magnesium mg/L	30.1	2.8	2.4
10.	Sodium mg/L	17.2	11.13	10.27
11.	Potassium mg/L	75.6	3.51	3.33
12.	RSC mg/L	-32.4	-1.3	Nil
13.	SAR mg/L	32.0	6.9	5.8

The calculated value of $|t|=1.01<t_{0.01}=1.81$, so the null hypothesis is accepted.

Table 2: Physico-chemical parameters of coir effluent before and after introduction of endophytic organisms

S. No.	*Physico-chemical parameters*	*Raw effluent*	*Time duration*	
			24 hours	*72 hours*
1.	pH	8.5	4.48	3.75
2.	EC (dsm^{-1})	4.0	0.73	0.63
3.	BOD mg/L	141	129.3	98.3
4.	COD mg/L	248.6	140.2	112.1
5.	Bicarbonate (mg/L)	175.21	6.7	3.4
6.	Chloride (mg/L)	160.35	9.9	4.8
7.	Sulphide (mg/L)	155.27	3.33	2.80
8.	Calcium (mg/L)	169.78	11.5	10.7
9.	Magnesium (mg/L)	140.67	2.3	11.8
10.	Sodium (mg/L)	135.08	4.9	3.01
11.	Potassium (mg/L)	95.71	1.59	1.69
12.	RSC (mg/L)	40.00	3.56	2.20
13.	SAR mg/L	50.00	8.7	6.3

The calculated value of $|t| = 0.33 < t_{0.05} = 2.26$, Null hypothesis – accepted.

Seneviratne et al. (2006) suggested that co-culturing of bacteria and fungi enhanced bio-degradation be compared to their monoculture. In the present study there is a decrease in the pollution load which was found to be more pronounced in the sugar mill effluent at 72 hrs after the introduction of endophytic organisms which was proved statistically (Refer Tables 1 and 2).

RAPD analyses were carried out with purified genomic DNA of *Pseudomonas, Klebsiella, Serratia* and *Rhizobium*. The optimal conditions were selected for obtaining accurate amplified band profiles with Rhizobium primers. Results revealed that the DNA band pattern of the *Rhizobium* is similar with the leader Sequence (Ref Figure 1).

RAPD-PCR analysis is used for the identification of endophytic bacterial strains. Zembrzuska (1995) reported that *Pseudomonas* sp isolated from *Oryza sativa* showed different band patterns when they were analyzed using RAPD. Similar results were observed in this present study. By comparing with the earlier reports the present study biofilm shows better degradation of coir effluent and sugar mill effluent.

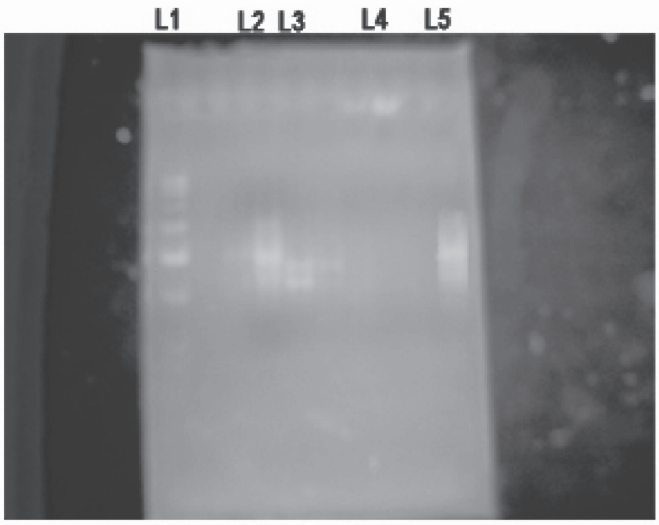

Figure 1: Genomic DNA isolation of endophytic bacteria isolates.
Lane 1: *Pseudomonas* sps; Lane 2: Primer; Lane 3: *Klebsiella* sps; Lane 4: *Serratia* sps; Lane 5: *Rhizobium* sps.

Conclusion

It is crucial to develop an eco-friendly low cost process for decolourization of coloured effluent as an eco-friendly alternative. It can be concluded that endophytic organisms can be employed in the biodegradation of effluents effectively.

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