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STUDIES ON TREATMENT OF SYNTHETIC RUBBER MANUFACTURING INDUSTRY EFFLUENT BY ISOLATED *PSEUDOMONAS* SPECIES

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ABSTRACT

The study deals with characterisation and treatment of industrial wastewater from a rubber processing industry situated in Chengalpattu, Tamilnadu. For treatment of the wastewater, methods used by the industry were insufficient, so attempts were made to carry out treatment of this effluent by biological means. Characterisation of the effluent was carried out and studied for microbial degradation. Microorganisms were isolated by using selective enrichment technique from petroleum soil. Isolation of microbes was carried from 80% effluent as it gives good results. Two isolates were found having maximum degradation efficiency. Identification of isolates was carried according to Bergey's Manual. The isolates were found *Pseudomonas palleronii* and *Pseudomonas solanacearum.* Identified isolates were studied for their degradation abilities. Optimization of environmental conditions, which affect degradation rate, were carried out and include effect of agitation and aeration, effect of simple nutrients, effect of alkaline pH, effect of mineral salts and use of mixed culture on rate of degradation etc.

INTRODUCTION

Bioremediation is one of the aspects that makes use of microorganisms in waste treatment processes, which have no adverse effects and are ecofriendly. Here attempts are made to isolate microorganisms having ability to degrade particular effluent/wastewater. The cultures are generally isolated from natural environment by using enrichment culture techniques to increase the degradation efficiency.

The rubber industry situated in Chengalpattu is manufacturing synthetic rubber. Raw material used is mainly hydrocarbon-based compounds. In rubber reclaim processing, rubber powder is processed in autoclave. Steam in autoclave is condensed in condenser. The condensed steam is effluent, which requires treatment before disposal. Hydrocarbons are toxic to lower eukaryotes and humans. At low concentration these may cause skin problems. Some hydrocarbons like halogenated and aromatic hydrocarbons are shown to be carcinogenic (Singh 2003) making the treatment necessary. The current chemical treatment procedure employed by the industry was not yielding the desired results, hence they wanted some microbial process capable of treating the waste fully.

Initially, effluent was analysed for its TS, TDS, TSS, TVS, oil and grease, BOD and sulphate content and attempts were made to study microbial degradation. Pure cultures of microbes were isolated, purified and characterized for their degradation capabilities. Optimization of environmental factors influencing degradation was carried out.

MATERIALS AND METHODS

Wastewater sample from rubber industry was collected in plastic cans and stored at room temperature till the analysis was completed. Effluent sample was analysed according to standard methods (APHA 1985).

Enrichment: Attempts were made for isolation of hydrocarbon degrading microorganisms from soil by selective enrichment technique (Atlas 1981). Basal inorganic salt medium (BISM) with increasing concentration of effluent was prepared (Table 1). Enrichment was carried out with 50 mL of BISM + 0.5% effluent, inoculated with 1 g of soil sample. First enrichment flask was incubated at room temperature for 4 days. Gram staining was carried out, and 10 % from that flask was inoculated into further flasks, which had decreasing concentration of BISM and increasing concentration of effluent as 1, 5, 10, 20, 50, 80 and 100 %. Flasks were incubated at room temperature for 4 days. Gram staining showed Gram-negative coccobacilli and Gram-positive cocci.

Table 1: Composition of BISM.

BISM		Modified BISM	
$(NH_4)_2SO_4$	1 g	$(NH_4)_2SO_4$	1 g
K_2HPO_4	1 g	K_2HPO_4	1 g
$MgSO_4.7H_2O$	0.3g	$MgSO_4.7H_2O$	0.3g
$CaCl_2$	0.1g	D.W.	1000mL
$FeSO_4$	0.02 g		
D.W.	1000mL		

BISM was modified for experiment because addition of $FeSO_4$ and $CaCl_2$ to effluent caused precipitation.

Table 2: Characteristics of effluent.

Parameters	Effluent
pH	8.5
Odour	Unpleasant
Colour	Brownish
TS (mg/L)	5700
TDS(mg/L)	4300
TSS(mg/L)	1400
TVS(mg/L)	1700
Oil and grease(mg/L)	7400
BOD(mg/L)	1400
Sulphate(mg/L)	1.809

Isolation: Bacteria were isolated from flask having effluent concentration 80%. Effluent agar was used for isolation. Effluent agar was prepared with 2% agar-agar in 50% of effluent and streaked with loopful of inoculum from pre-enriched 80% of effluent plates, and incubated at room temperature for 3 days. After incubation, 5 different colonies were obtained. These cultures were purified and named as A, D, I, B, N. Efficiency of degradation of every isolate was checked out by inoculating suspension of these 5 isolates separately in 80% effluent (25 mL). After incubation for 4 days, maximum degradation was observed in flasks inoculated with isolates N and D as compared to other isolate (Table 3). So these two isolates were used for identification.

Identification: Pure culture colonies of both the isolates N and D from effluent agar plates were studied for Gram staining. Isolate N was Gram negative, short rods, and isolate D was Gram-negative coccobacilli. Both the isolates were checked for biochemical properties like utilization of glucose, lactose, mannitol, with acid and gas production, gelatin liquefaction, starch hydrolysis, arginine hydrolysis, oxidase activity and catalase activity according to Bergey's Manual of Systematic Bacteriology as per Table 4 (Krieg 1984) Isolates were identified to be of genus *Pseudomonas* and their identification up to species level was carried out according to Bergey's manual. Isolate N was identified to be *Pseudomonas palleronii*, and isolate D as *Pseudomonas solanacearum.*

Evaluation of degradation: Identified isolates were used in degradation of 80% effluent. Evaluation of degradation was carried out by inoculating 80% effluent with 10% of inoculum of each isolates separately in flasks. Flasks were incubated at room temperature for 7 days. After incubation, the

Table 3: Isolation of efficient degrader of effluent depending on degradation rate of each isolates.

Day of incubation	1st	2nd	3rd	4th
Control	+++++++	+++++++	+++++++	+++++++
Isolate A	++++++	+++++	++++	++
Isolate B	++++++	++++++	++++	+++
Isolate D	++++	+++	++	+
Isolate I	++++++	++++	++++	+++
Isolate N	++++	+++	++	+

Note: (+) sign for turbidity of the effluent. Isolates N and D were found to be efficient degraders.

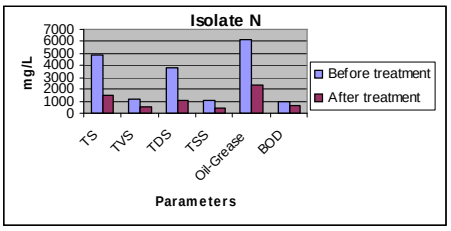

Fig.1: Parameters before and after treatment of the waste with isolate N.

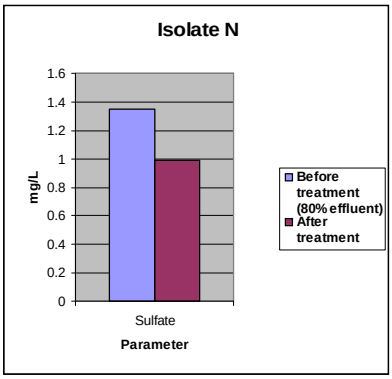

Fig.2: Parameters before and after treatment of the waste with isolate N.

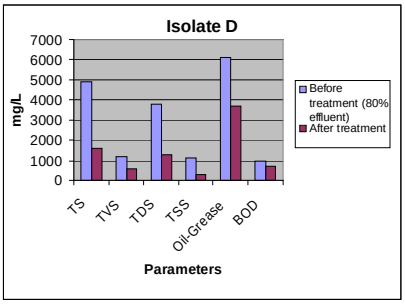

Fig.3: Parameters before and after treatment of the waste with isolate D.

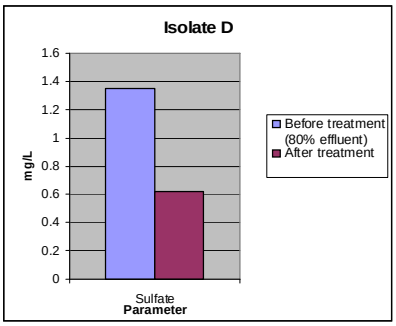

Fig.4: Parameters before and after treatment of the waste with isolate D.

treated effluent was characterized for some important parameters compared with untreated 80% effluent, and % reduction in parameters was calculated (Table 5).

RESULTS AND DISCUSSION

Table 2 shows that rubber industry effluent has high BOD, TS and oil and grease content. The pH of the wastewater was in alkaline range. The alkaline pH is due to amines, trace amount of sulphate diaryldisulfide (DADS) which is generally used for reclaiming synthetic rubber. High TS content shows that this effluent requires treatment.

Optimization of degradation conditions: pH of the effluent was 8.5. Attempts were made to isolate microorganisms in alkaline condition so during enrichment, wastewater was not neutralised. The identified isolates were studied for pH tolerance limit and it was observed that they can tolerate pH up to 10. So neutralisation of effluent was not needed during treatment.

Table 4: Biochemical characteristics of isolates N and D (medium used: effluent agar medium).

Character	Isolate N	Isolate D
Cell size (µm)	2.4	1.6
Motility	motile	motile
Pigment production	-	-
Utilization of glucose	-	-
Utilization of Lactose	-	-
Utilization of Manitol	-	-
Utilization of Xylose	-	-
Indole production	-	-
Gelatin liquefaction	-	-
Starch hydrolysis	-	-
Arginine hydrolysis	-	-
Nitrate reduction	-	-
H_2S production	-	-
Oxidase activity	+	+
Catalase activity	+	+
Lipase activity	+	+

(+) positive, **(-)** negative

To study the effect of other simple organic nutrients, peptone, glucose (1%) and yeast extract (0 .5%) were added and rate of degradation was studied. It was observed that these organic nutrients reduce the rate of degradation.

Table 5: Evaluation of degradation.

Parameters (mg/L)	Before treatment (80% effluent)	After Treatment			
		Isolate N		Isolate D	
		mg/L	%(Reduction)	mg/L	%(Reduction)
TS	4900	1500	69.38	1600	67.34
TVS	1200	500	58.33	600	50
TDS	3800	1100	71.05	1300	65.78
TSS	1100	400	63.63	300	72.72
Oil & Grease	6100	2300	62.29	3700	39.39
BOD	1000	600	40	700	30
Sulphate	1.349	0.987	26	0.616	45.4

However, presence of inorganic nutrients like $(NH_4)_2SO_4$ and H_2PO_4 (1%) in effluent shows increase in degradation rate, i.e., with presence of these minerals salts degradation time was reduced from 4 days to 2 days.

Effect of agitation and aeration: Flasks were inoculated and incubated at room temperature on rotary shaker and other sets of flasks were kept steady. After 3 days, degradation was observed in the flasks kept steady but there was no degradation in flasks kept on rotary shaker.

Effect of mixed culture: 80% effluent (50mL) was taken into 3 flasks. Out of the 3, 2 were inoculated with pure culture of isolates and one with mixed culture of both. After incubation of 3 days, mixed culture flasks did not show degradation as compared to pure culture.

Isolation of plasmid: Ability of isolates to degrade effluent may be due to presence of degradative plasmids. To check the presence of plasmids, agarose gel electrophoresis (Das 2005) was carried out and presence of plasmids in both the isolates was observed. For the experiment ampicillin resistant *E. coli* strain was used as reference.

CONCLUSION

The treatment parameters optimised in these studies were of 7 days as incubation time at room temperature. pH neutralisation of the effluent was not necessary in the treatment procedure. It is observed that without agitation and aeration, it would be possible to degrade the effluent. It was favourable to use pure culture than mixed culture. The volume of inoculum is optimised to 10% v/v. Effluent was diluted to 80% during treatment. Addition of mineral salts like K_2HPO_4 and $(NH_4)_2SO_4$ favours the rate of degradation. However, simple organic nutrients like peptone, glucose (1%) and yeast extract (0.5%) reduce the degradation rate. Treated effluent shows significant reduction in BOD, TS, oil and grease, sulphate and other parameters (Figs. 1, 2, 3 and 4). Microbial treatment with isolates carried out on large scale is favourable, so that after treatment effluent can be safely disposed off into water bodies.

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