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Original Research Article

Rubber processing industry effluent treatment using a bacterial consortium

H.P.Jai Shanker Pillai[1] and K.Girish[2*]

[1]Department of Microbiology, Gulbarga University, Gulbarga - 585 106, Karnataka, India
[2]Postgraduate Department of Microbiology, Maharani's Science College for Women, JLB Road, Mysore – 570 005, Karnataka, India
**Corresponding author*

Keywords

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A B S T R A C T

Wastewater collected from rubber processing industry was characterized for their pollution characteristics. Results of the analysis showed that the total dissolved solids (TDS), total suspended solids (TSS), total solids (TS), ammonia and phosphate were high when compared to effluent discharge standard for industrial wastewater. The biochemical oxygen demand (BOD) and chemical oxygen demand (COD) were 1340 and 2834 mg/l respectively, which showed that the wastewater was high in pollution potentials and need to be treated before discharged to the environment. An investigation of bacteria found in rubber processing wastes was carried out. Four bacterial species were isolated from the wastes and were identified as *Arthrobacter* sp., *Bacillus* sp., *Lactobacillus* sp., and *Pseudomonas* sp. A bacterial consortium was constituted by mixing proportionately these four bacteria and used in effluent aerobic biotreatment. After 15 days of incubation, BOD and COD reduction were 74.03% and 79.92% respectively, which is a significant reduction. Treatment of wastewater also yielded substantial reduction in the TDS (72.63%), TSS (76.1%), ammonia (76.6%) and phosphate (68.75%). From the results of the present study it could be inferred that the constituted consortium could effectively be used for the treatment of effluents from rubber processing industry and this effective effluent treatment method would minimize environmental pollution of rubber industry and make it sustainable and environmental friendly.

Introduction

Natural rubber (NR) processing sector is an industry which produces raw materials used for the manufacture of rubber industrial products (conveyor belts, rubber rollers, etc.), automotive products (fan belts, radiator hoses, etc.), latex products (rubber gloves, toys hygienic products, etc.) and many kinds of adhesives. The major users of natural rubber are tire and footwear industries (Anon, 2007).

However, environmental damages generated from this sector could become big issues. Natural rubber processing sector consumes large volumes of water and energy and uses large amount of chemicals as well as other utilities. It also discharges massive amounts of wastes and effluents (Leong *et al*., 2003). Wastewater is an unavoidable by-product of rubber processing: whatever processing procedures are used for preparing products from latex, there will always be an aqueous liquid as a by-product (Rungruang and Babel, 2008). If the wastewater is put straight into surface waters – wells, streams, lakes or even the sea – without any treatment, it will inevitably pollute that water. The disposal of these effluents into public water bodies can give rise to serious depletion of dissolved oxygen, thus affecting the normal environment supporting the aquatic system (Mohammadi *et al*., 2010). The major chemical component groups of natural rubber waste serum (NRWS) as reported by Lau and Subramaniam (1991) shows that it consists mainly of nitrogenous compounds. In the waste water (effluent) the bulk of the nitrogen component consist mainly of ammoniacal nitrogen as a result of the use of ammonia in the preservation of the latex. The high level of NH_4^+ and other plant nutrients makes it a good medium for algal growth, thus resulting in the eutrophication of water bodies.

The increasing global concern on the environment demands that wastes should be properly managed in order to minimize and possibly eliminate their potential harm to public health and the environment. Biodegradation is the process of utilizing indigenous microorganisms for the degradation of complex organic matter into simpler ones.

Rubber and effluents from rubber processing have been known to support microbial growth (Atagana *et al*. 1999a; Bode *et al*., 2001; Cherian and Jayachandran, 2009). Owing to the need of biological treatment of rubber industry wastes and knowing the fact that various bacteria can grow and degrade the rubber industry wastes, the present study aimed to isolate and characterize indigenous bacteria that can readily degrade the rubber wastes present in the effluents, with a view to developing an effective biological treatment.

Materials and Methods

Sample collection: The wastewater was collected from the discharge unit of the rubber processing sewage system in a can which had been thoroughly washed and rinsed with water. The wastewater sample used for DO (dissolved oxygen) and BOD (biological oxygen demand) determinations were collected directly into dark DO bottles and were added some drops of manganous sulphate solution to fix the dissolved oxygen. Samples were collected by lowering the sterile bottle by means of a string into the tank and covered with the screw cap thereafter. The samples were stored at a temperature of 4^0C until required (usually between 24 and 48 h). The effluents were collected from five different points on the processing - aeration tank, settling tank, backwashed effluent, holding tank and sludge.

Isolation of biodegrading microorganisms: To obtain the culture of bacteria, nutrient agar media was prepared and sterilized in an autoclave. The molten nutrient agar media was poured (approximately 15ml) into each sterilized Petri plates, and allowed to solidify. The effluent was serially diluted and 0.1ml of sample was inoculated on solidified agar media and was spread uniformly with the sterile spreader. These Petri plates were incubated in an incubator for 24 - 48h at 30^oC. The Petri plates were observed after incubation for various bacterial colonies. The colony characteristics such as Gram's reaction, colour, margin, elevation were noted.

Identification of bacteria: The cultures were identified by standard procedures such as morphology, and biochemical characteristics as given by Bergey's Manual of Determinative Bacteriology (Buchanan and Gibbons, 1984). The physiological and biochemical tests were conducted following the methods as described by Cappuccino and Sherman (1999) to identify the bacteria.

Pure culture: To prepare inoculum, the cultures were grown at 30°C on a rotary shaker operating at approximately 120 rpm in the inorganic-salts medium amended with 10% effluent and harvested in early stationary phase by centrifugation at 4°C.

The pellets were washed, the cells were resuspended in buffer, and the entire centrifugation and washing procedure was repeated. The final pellet was resuspended in inorganic salts solution. The bacterial growth was observed.

Acclimatization: Microbial consortium was obtained by mixing proportionately all the four bacterial cultures isolated viz., *Arthrobacter* sp., *Bacillus* sp., *Lactobacillus* sp., and *Pseudomonas* sp.

The consortium was acclimatized by growing it in minimal organic salts medium amended with 10% of rubber processing industry effluent. The minimal medium used in degradation studies contained (mg / ml) KH_2PO_4 – 0.675; Na_2HPO_4 – 5.455; NH_4NO_3 – 0.25; $MgSO_4$ – 0.2; $Ca(NO_3)_2$ – 0.1; and 1 ml mineral solution.

Confirmation of biodegradation: Various parameters such as biological oxygen demand (BOD), chemical oxygen demand (COD), total suspended solids (TSS), total dissolved solids (TDS), ammonia (NH_4^+) and phosphate (PO_4^{3-}) were assayed using standard protocols (APHA, 1995). The effluent was inoculated with 1% inoculum and incubated for 15 days, and estimation was done at the interval of five days.

Results and Discussion

Isolation and Identification of bacteria: Based on the colony characteristics, staining, motility and many biochemical characteristics the bacteria were identified as *Arthrobacter* sp., *Bacillus* sp., *Lactobacillus* sp., and *Pseudomonas* sp. The identification was confirmed through the results of number of biochemical tests as mentioned in table 1.

Biological oxygen Demand (BOD) and Chemical Oxygen Demand (COD): In comparison to the initial levels, both BOD and COD decreased significantly indicating the decrease in the pollutants level. The decrease in the level of chemical oxygen demand indicates the reduction of biologically oxidizable and inert organic materials as a result of the degradation by the bacterial consortium (Table 3). %BOD and %COD reduction were 74.03% and 79.92% respectively.

TDS, TSS, Ammonia and Phosphate: In comparison to the initial levels substantial reduction in the TDS (72.63%), TSS (76.1%), ammonia (76.6%) and phosphate (68.75%) were observed after treatment signifying the degradation of toxic solid components in the effluent by the consortium (Table 3). Reduction in ammonia and phosphate levels indicates that the bacteria and the consortium degrade organic and inorganic constituents. The pH of the effluent changed from acidic to alkaline resulting in reduction of acidity of the effluent.

Rubber processing industries consume large volume of water and chemicals for processing of rubber. A considerable amount of wastewater, effluent is generated, which is very toxic and contain strong colour, a large amount of suspended solids, a highly fluctuating pH, high temperature, COD, BOD etc (Asia and Akporhonor, 2007; Mohammadi *et al*., 2010). Because of these characteristics, treatment of rubber wastewater is an essential requirement before it is being disposed to natural water system (Atagana *et al*., 1999b; Iyagba *et al.,* 2008). Wastes containing high BOD and COD are responsible for a heavy depletion of oxygen levels in the particular sector of the stream or soil (Tariq *et al*., 2006). The BOD and COD concentrations play an important role in the re-use of industrial effluent. It is well known that wastewater from industries contain suspended solids, reduced oxygen, inorganic compounds. In order to minimize the environmental and health hazards, these pollutants need to be brought down to permissible limits for safe disposal of waste water (Manju *et al*., 1998).

The objective of this study was to determine COD and BOD reduction from rubber processing industry effluent by indigenous bacterial treatment. The BOD and COD of the effluents showed that all the effluents had higher BOD and COD values than the permissible limits. Therefore, these effluents needed further elimination of BOD and COD through proper treatment methods before discharge.

Before discharging any industrial effluent the BOD should be removed because it adversely affects the aquatic organisms (Atagana *et al*., 1999b). In the present study a successful reduction of both BOD and COD of effluent from rubber processing industry was observed to a level enough to make the effluent ready to be discharged into the environment, by treating with bacterial consortium. %BOD and %COD reduction were in the range 70-80%, which is a significant reduction. The decrease in BOD and COD is the clear evidence of the better biotreatment efficiency.

Biodegradation is the most environmentally friendly process of utilizing indigenous microorganisms that can be employed for the degradation of complex organic matter into simpler ones. The bacterial consortium employed in this study was isolated from the rubber effluents itself. Rubber and effluents from rubber processing have been known to support microbial growth and has been reported by a number of workers (Atagana *et al*., 1999 a & b; Bode *et al*., 2001; Cherian and Jayachandran, 2009). Bacteria are especially employed for bioremediation as they are generally easier to culture and grow more quickly (Glazer, 1997).

Rubber effluent consists of latex washings and a serum containing proteins, sugars and lipids as well as inorganic and organic salts (Kulkarni and Stantun, 1973; Kulkarni *et al*., 1973). The high level of NH_4^+ and PO_4^{3-} makes it a good medium for algal growth. Failure in reducing these nutrient levels before discharging into the environment lead to accelerated eutrophication of surface waters (Ye *et al*., 1988).

Any treatment system should be able to effectively reduce or eliminate the level of NH_4^+ and PO_4^{3-} compounds in the effluent. In the present study it was observed that the biological treatment of effluents with bacterial consortium reduced the ammonium and phosphorus levels significantly and thus reducing the above mentioned risk. Microbial treatment is also known to reduce the levels of total suspended solids (TSS) and total dissolve solids (TDS) of industrial effluents (Anandapandian *et al*., 2004; Arun *et al*., 2004). Similarly in the present study biological treatment of effluent with bacterial consortium reduced the levels of total suspended solids (TSS) and total dissolve solids (TDS).

Table.1 Characteristics of bacteria used for their identification

Sl. No.	Characteristics	*Arthrobacter* sp.*	*Pseudomonas* sp.	*Bacillus* sp.	*Lactobacillus* sp.
1.	Cell shape	irregular rods with clubbed ends	Rod shaped	Rod shaped	Rod shaped
2.	Gram staining	Gram positive but could be decolorized	Gram negative	Gram positive	Gram positive
3.	Colony on media	Colonies on medium were yellow, round, smooth, convex and 0.5 to 2 mm in diameter	Yellow-greenish colonies, round to slightly irregular, smooth, and 0.5 to 2 mm in diameter	Rough, white, irregular, flat colonies, 2 - 5 mm	Convex, entire, opaque, no pigment, 2 – 5 mm
4.	Motility	+	motile with a polar flagellum	Motile by peritrichous flagella	Non-motile
5.	Spore	--	--	endospore	--
6.	Catalase	+	+	+	--
7.	Oxidase	+	+	+	--
8.	Starch hydrolysis	+	--	+	--
9.	Fermentation	No acid, No gas	No acid, No gas	Acid	Lactose to lactic acid & CO_2
10.	Indole test	--	--	--	--
11.	Methyl red test	--	--	+	+
12.	Voges–Proskauer test	--	--	--	--
13.	Citrate utilization test	--	+	--	--
14.	H_2S production test	--	--	--	--
15.	Urea hydrolysis	--	--	--	--
16.	Nitrate reduction	--	--	+	--
17.	Gelatin hydrolysis	+	+	+	--

***Arthrobacter* sp.: In young cultures, cells were irregular rods with clubbed ends, whereas in older colonies cocci cells could be observed arranged singly, in pairs and in irregular clumps - characteristic of *Arthrobacter* species.

Table.2 Different Microorganisms isolated from different tanks

Organism	A	B	C	D	E
Arthrobacter sp.	+	+	+	+	+
Pseudomonas sp.	+	+	+	+	+
Bacillus sp.	+	+	-	+	-
Lactobacillus sp.	-	-	-	+	+

A = aeration tank; B = settling tank; C = backwashed effluent; D = holding tank; E = sludge.

Table.3 Physico-chemical and organic characteristics of the effluent before and after treatment (after 15 days of Incubation)

Parameters	Consortium (acclimatized)		Percentage reduction
	Before treatment	After treatment	
pH	5.7 ± 0.30	7.5 ± 0.20	
TDS (mg / l)	2240 ± 3.4	613 ± 3.4	72.63%
TSS (mg / l)	3512 ± 4.8	840 ± 2.4	76.10%
TS (mg / l)	5752	1453	74.74%
Ammonia (mg / l)	94 ± 3.0	22 ± 1.5	76.60%
Phosphate (mg / l)	48 ± 2.0	15 ± 1.2	68.75%
BOD (mg / l)	1340 ± 2.0	348 ± 1.3	74.03%
COD (mg / l)	2834 ± 1.9	569 ± 1.8	79.92%

Values are the mean of five replicates ± SE

Traditional disposal method such as ocean dumping is now out of place following numerous incidents of severe negative impacts on the environment after years of disposal. There are physical and chemical methods, which, inspite of costs, do not always ensure that the contaminants are completely removed (Hardman *et al.*, 1993). In recent times there has been a tremendous upsurge in the search for cost-effective and environmently friendly alternatives to traditional methods for dealing with wastes. From the results of the present study it could be inferred that the constituted bacterial consortium could effectively be used for the treatment of effluents from rubber processing industry. However, it is clear that the biodegradative activity of bacteria is a complex one. Further studies are required for understanding the mechanisms of the biodegradation role of these bacteria.

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