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EFFECT OF BACILLUS SUBTILIS AND PSEUDOMONAS PUTIDA ON THE REMOVAL OF POLLUTANTS IN MATCH INDUSTRY WASTEWATER

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ABSTRACT

Due to industrialization and developmental activities nowadays water resources are polluted severely. Among the polluting industries, match industries are playing a major role. Sivakasi is a town situated in Tamil Nadu, India is having a number of match industries. For various processes during the manufacturing of matchsticks harmful chemicals are used. The wastewater generated from the match industries is creating problems due to contamination of groundwater and river water nearby Sivakasi. An attempt was made to treat the wastewater using bacterial species viz., Bacillus subtilis and Pseudomonas putida. Water samples were collected from wells of Sivakasi and analyzed for the parameters viz., BOD, COD, Nitrates, Phosphates, and Sulphates and it was found that all the parameters were exceeding the permissible limit. The water samples were inoculated with a pure culture of Bacillus subtilis and mixed cultures of Bacillus subtilis and Pseudomonas putida at different pH conditions (5.5, 6.5 and 7.5). The results revealed that at pH 7.5 the removal efficiency of the pollutants was higher. Also when the mixed microbial consortium was used the efficiency was more when compared with the inoculation of single bacterial culture. At pH 7.5 with mixed culture the percent removal of various pollutants viz., BOD, COD, Phosphate, Nitrate, and Sulphate was 80, 73, 68, 66 and 62 respectively,

KEYWORDS: *Match Industry Wastewater, Mixed Bacterial Culture, Degradation of Pollutants*

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INTRODUCTION

Almost 70% of water in India has become polluted due to the discharge of domestic sewage and industrial effluents into natural water sources viz., rivers, streams, and lakes. About 90% of the rural population living in India depends on groundwater for domestic use. The improper management of water systems may cause serious problems in availability and quality of water. In India, 75% of illness and 80% of the child mortality is attributed to water pollution.

The town Sivakasi is situated in the Southern portion of Tamil Nadu. It is located at 9.28 North latitude and 77.48 East longitudes. It is located at 100.07m above MSL. The town comprises 6.89km2 in extent and is famous for fireworks, printing press, match works. About 80% of India's fireworks are manufactured here. High explosive products manufactured include trinitrotoluene (M/s Associated Industrial Consultants (India) Private Ltd (1998). Acids viz., sulfuric acid, Nitric acid, oleum required in the manufacturing process are almost always manufactured in-house. Steps in the manufacture involve the use of raw materials viz., toluene, cellulose, Calcium cyanide, picric acid, glycerine nitro benzene

etc.

There are nearly 450 fireworks factories giving direct employment to about 40,000 workers and indirectly about one lakh employment opportunities in various works such as paper tube making, wire cutting, box making, and sale distribution in the countryside. The annual production is about 50,000 tonnes and reached the turnover around Rs, 350 crores according to industrial sources (Thamburaj, 2000). The wastewater from the match industry was found to be containing the pollutants viz., phosphate, sulfate, TOC, potassium etc., (Manonmani et al., 2015). The base contains a smaller amount of phosphorus sesquisulfide. It also contains sulfur, rosin and a small amount of paraffin wax to sustain combustion. A water-soluble dye may be added to give the base a color such as red or blue. It is reported that phosphorus is the most dangerous chemical in match industry effluent (Rajathilagam et al, 2012).

The ground-water in Sivakasi is found to be highly polluted and the people dependent on this water is often affected (Ramu et al. 2009). The groundwater samples of Sivakasi was characterized and it was found that Total hardness, Nitrates, Potassium, Phosphates were exceeding the limit and the concentration of heavy metals viz., Cu, Al and Ba was also high (Sathish Ajay, 2015). If the nutrient-rich water is discharged into the water bodies without proper treatment, it would cause eutrophication ((Heinz et al., 2002) So it is essential to treat the effluent from match industry to reduce the risk. The effluents could be treated by ozonation, chemical coagulation, and adsorption and electrochemical technology. These methods are costly and generating chemical sludge. So it needed to identify a suitable method to degrade the pollutants. The use of bacterial species to degrade the pollutants would be an alternate cost-effective treatment option to solve environmental problems generated by industries. So in the present study, an attempt was made by using bacterial culture to treat the effluent generated by match industry

MATERIALS AND METHODS

The effluent samples were collected from Viswanatham road and Pillayar Koil Street in Sivakasi where the number of match industries is more. The samples were collected between February to March 2017. The samples were collected in sterile containers and transported to the laboratory under refrigerated condition. The samples were preserved below 4^0C in order to prevent microbial degradation.

CHARACTERIZATION OF MATCH INDUSTRY WASTEWATER

The samples were analyzed for various physic-chemical parameters in accordance with the methods prescribed in the Standard Methods of Water and Wastewater Treatment (APHA, 2005).

pH

The pH of the sample was measured using Elico LI 120 equipped with combined glass-calomel electrode. The pH meter was calibrated using buffer solutions.

Sulfates

The sulfate content in the effluent was determined by Turbidimetric method (APHA, 1975). Prior to sulfate determination, suspended solids were removed from the sample by centrifugation. After addition of 0.25ml conditioning reagent (50ml glycerol, 30ml concentrated HCl, 75g NaCl, 100ml ethanol and 300ml deionized water) to 5ml of the sample, an excess amount of finely ground $BaCl_2$ was added and the sample was stirred for 1min on a vortex mixer. The absorbance of the sample was measured at a wavelength of 420nm. The absorbance of the sample was used to

calculate the concentration of sulfate. A calibration curve for the dependency of adsorption on sulfate concentration was obtained using a similar procedure

Nitrates

Nitrate is measured in terms of nitrate concentrations. It was estimated by azo dye method using spectrophotometer at 540nm (Apha, 1998)

Phosphates

The phosphate concentration in the wastewater was determined by Calorimetric Method (APHA, 2012). The absorbance was measured at 540nm.

Chemical Oxygen Demand (COD)

COD of the samples was determined by Open reflux, dichromate titrimetric method as described in standard methods (APHA, 1998).

Bacterial Culture

Pure cultures of bacterial species viz., *Pseudomonas sp.* and *Bacillus sp*. were got from IMTECH, Chandigarh. The bacterial cultures were transferred to Nutrient Broth and incubated for 24hrs at 37^{o}C. Then the cultures were mass multiplied in Nutrient broth. The pH of the medium was maintained at 7.4. After incubation, the cells were harvested by centrifugation in a centrifuge at 10,000rpm for 15 min. The cell biomass was transferred to sterile saline and the cell concentration of each strain was adjusted to an optical density of 0.01(600nm). Every 12 hours OD was determined using the spectrophotometer.

Experimental Setup

The experiment was conducted in a Beaker (Capacity 1000ml, height 158mm and diameter 59mm) by batch mode. Since the Dissolved oxygen level in the wastewater was zero initially, the samples were aerated partially using aquarium pump. After aeration, the pure and mixed cultures of bacteria ($1x10^{9}$cfu/ml) were inoculated into the conical flask and cultured for 72 hours.

Effect of Bacterial Species on Degradation of Pollutants in Wastewater at Different PH

The pH of the wastewater collected from match industry was adjusted to 5.5, 6.5, 7.5 and 8.5 and inoculated with pure and mixed bacterial cultures and kept in incubator shaker. Then samples were taken periodically and determined for the various parameters viz., BOD, COD, phosphate, Nitrate, and sulfates.

RESULTS AND DISCUSSIONS

Characterization of Wastewater Samples

The match industry wastewater collected from two locations of Sivakasi town was characterized and the results are shown in table1

The pH of the wastewater was found to be slightly acidic and it was 5.5. The BOD of match industry wastewater was found to be 288 to 457mg/l (Manonmani et al., 2015). The BOD was 288mg/l and this might be due to the higher solid contents with higher buffering capacity (Chen and Lin, 2000). The concentrations of phosphate, nitrate, and sulfate were

found to be 138, 105 and 610mg/l respectively. The higher concentration of nitrate in the wastewater might be due to the use of nitrogen compounds in the match industry (Manonmani et al, 2015). The wastewater had more phosphate concentration than the permissible limit and it might be due to the use of phosphorus as an important raw material for matchstick manufacturing

Effect of Bacterial Species on Reduction of Pollutants in Wastewater at Different PH

The wastewater was inoculated with pure and mixed cultures of bacterial species and incubated for 72 hours. Then samples were taken after 24hours, 48 hours and 72 hours and analyzed for various parameters. The experiment was conducted at different pH levels and the results are given in tables 2 to 4

At pH 5.5, after 72 hours when the wastewater was inoculated with only *Bacillus subtilis* the reduction of BOD, COD, Phosphate, Nitrate, and Sulphate were found to be 107, 306, 84, 71and 512mg/l respectively. But when mixed cultures of *Bacillus subtilis* and *Pseudomonas putida* were used more reduction was observed.

At pH 6.5 also the reduction was found to be more when mixed cultures were used when compared with the control and single culture. After 72 hours of incubation, the BOD reduction was from 99mg/l to 70mg/l in the case of single culture inoculation and reduction from 77 to 61mg/l when mixed culture was used. For COD reduction also the same trend was observed.

In all the pH conditions maximum reduction of all the pollutants was observed when mixed culture was used when compared with the single culture and control.

The removal efficiency of all the pollutants was calculated and tabulated (Table 5)

At pH 7.5 when mixed culture was used the maximum reduction of BOD, COD, Phosphate, Nitrate, and Sulphate were observed when compared with a single pure culture of *Bacillus subtilis*. For single culture, there was 75% reduction in BOD and for mixed cultures of *Bacillus subtilis* and *Pseudomonas putida* it was 80%. The percent reduction in COD was found to be 53 and 73 for single and mixed culture respectively.

When single culture was used there was 49% reduction in phosphate and for mixed culture, it was 68%. In the case of Nitrate also the same trend was observed. It was 45% and 66% for single and mixed culture, respectively. For single culture, the sulfate reduction was 32% and 62% for mixed culture. Phosphate utilizing bacteria was known to be present in various environments (Illmer and Shimer, 1992). The BOD, COD, Nitrate, Phosphate, and Sulphate in wastewater was removed efficiently when *Bacillus sp*. was used at pH 6.0 (Yokubu etal., 2011). In the present study, the pollutants in the match industry wastewater were removed efficiently at pH7.5 when the bacterial species viz., *Bacillus subtlis* and *Pseudomonas putida* were used as these bacterial species showed better growth at pH 7.0 to 8.0. The same trend was observed when the match industry wastewater was inoculated with mixed bacterial culture (Manonmani et al. 2015)

REFERENCES

1. *APHA 1975. Standard Methods for the Examination of Water and Wastewater, 14th Ed. American Public Health Association, American Water Works Association and Water Environment Federation, Washington, DC, USA.*
2. *APHA 1998. Standard Methods for the Examination of Water and Wastewater, 20th Ed. American Public Health Association, American Water Works Association and Water Environment Federation, Washington, DC, USA.*

3. *APHA 2005. Standard Methods for the Examination of Water and Wastewater, 21th Ed. American Public Health Association, American Water Works Association and Water Environment Federation, Washington, DC, USA.*
4. *Chen, K. C., Lin. Y. H., Chen. W. H and Liu. Y.C.(2002). Degradation of Phenol by PAA– immobilized Candida tropicalis. Enzyme Microbial. Tech. Vol. 31: 490 – 497.*
5. *Henz, M., Aspegren, H, Lacour, J, Nielson, P.H. and Lee. N (2002). Effects of Solids Retention time and Wastewater Characteristics on Biological Phosphorus Removal. Water Sci. and Technol., Vol. 45(6): 137 – 144.*
6. *Illmer and Schimer (1992). Solubilizing Inorganic Phosphates by Microorganisms Isolated from Forest Soils. Soil. Biol.Biochem, Vol. 24: 389 – 395.*
7. *Muralimohan, N., T. Palanisamy, and M. N. Vimaladevi. "Experimental study on removal efficiency of blended coagulants in textile wastewater treatment." International Journal of Research in Engineering & Technology 2.2 (2014): 15-20.*
8. *Manonmani, P, Rama Krishnan. D and Paulpand, P (2015). Biodegradation of Wastewater discharged from dye and match industries in Sivakasi town. Journal of Global Biosciences, Vol.4 (1): 1279 – 1293.*
9. *M/s Associated Industrial Consultants (India) Private Ltd (1998) Report*
10. *Ramu. A, Muthulakshmi. Land Kannan, N.K. (2009). Physico-chemical Characteristics of Groundwater in and around Sivakasi Region. Indian Journal of Environmental protection, Vol.29 (5): 435 – 438.*
11. *Sathish Ajay. P(2015). Study of Fireworks Industry and its Groundwater Contamination in Sivakasi. M.E. Thesis, Anna University, Chennai, Tamil Nadu, India.*
12. *Thamburaj F. 2000. A Brief Report on Sivakasi fireworks Industries Vol. 26(9): 130 – 133.*
13. *Yokubu, S.E, Ajao, A.T, and Adebayo. G.B (2011). Bioremediation of Textile Industrial Effluent Using Mixed Culture. Journal of Microbiology and Biotechnology, Vol. 1(5): 50 – 56.*

APPENDICES

Table 1: Characteristics of Match Industry Wastewater

Sl. No.	Parameters	Values *
1.	pH	5.5
2.	BOD at 27°C(mg/l)	288
3.	COD(mg/l)	525
4.	Phosphate(mg/l)	138
5.	Nitrate(mg/l)	105
6.	Sulphate(mg/l)	610

* Values represent average of 3 determinations

Table 2: Effect of Bacterial Species on the Characteristics of Match Industry Wastewater at pH 5.5

Parameters	Control	*Bacillus Subtilis*			Mixed Culture(*Bacillus Subtilis* and *Pseudomonas Putida*)		
		24h	48h	72h	24h	48h	72h
BOD(mg/l)	260	137	123	107	109	95	79
COD(mg/l)	500	350	333	306	255	236	215
Phosphate(mg/l)	138	99	84	71	76	63	53
Nitrates(mg/l)	105	76	71	66	58	54	51
Sulphate(mg/l)	610	518	512	509	388	381	374

Table 3: Effect of Bacterial Species on the Characteristics of Match Industry Wastewater at pH 6.5

Parameters	Control	*Bacillus Subtilis*			Mixed Culture (*Bacillus Subtilis* and *Pseudomonas Putida*)		
		24h	48h	72h	24h	48h	72h
BOD(mg/l)	260	137	123	107	109	95	79
COD(mg/l)	500	350	333	306	255	236	215
Phosphate(mg/l)	138	99	84	71	76	63	53
Nitrates(mg/l)	105	76	71	66	58	54	51
Sulphate(mg/l)	610	518	512	509	388	381	374

Table 4: Effect of Bacterial Species on the Characteristics of Match Industry Wastewater at pH 7.5

Parameters	Control	*Bacillus Subtilis*			Mixed Culture (*Bacillus Subtilis* and *Pseudomonas Putida*)		
		24h	48h	72h	24h	48h	72h
BOD(mg/l)	260	99	85	70	77	63	61
COD(mg/l)	500	346	315	284	225	204	184
Phosphate(mg/l)	138	73	58	46	52	39	31
Nitrates(mg/l)	105	79	76	71	51	48	43
Sulphate(mg/l)	610	462	458	449	379	373	365

Table 5: Effect of Bacterial Species on the Percent Removal of Pollutants in Match Industry Wastewater at Different pH

Sl. No.	Parameters	Removal Efficiency (%)					
		Bacillus Subtilis			Mixed Culture (*Bacillus Subtilis* and *Pseudomonas Putida*)		
		pH5.5	pH 6.5	pH 7.5	pH5.5	pH 6.5	pH 7.5
1.	BOD at 27°C(mg/l)	50	60	75	63	65	80
2.	COD(mg/l)	33	35	53	53	58	73
3.	Phosphate(mg/l)	30	33	49	50	55	68
4.	Nitrate(mg/l)	33	38	45	49	53	66
5.	Sulphate(mg/l)	21	23	32	42	49	62