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BIODEGRADATION OF PHENOLS PRESENT IN EFFLUENT WASTEWATER OF STEEL PLANT THROUGH *PSEUDOMONAS PUTIDA*

HARITHA MERUVU[a], B. SRINIVASA RAO[a] and MEENA VANGALAPATI[*]

Center for Biotechnology, Department of Chemical Engineering, College of Engineering, Andhra University, VISAKHAPATNAM – 530 003 (A.P.) INDIA
[a]Environment Management Department, Visakhapatnam Steel Plant, VISAKHAPATNAM – 530 031 (A.P.) INDIA

ABSTRACT

Phenol is a hazardous industrial wastewater pollutant. This project aims at the study of use of *Pseudomonas putida* for the efficient degradation of phenol, which is treated at MBC treatment plant of Steel Plant. Further, the concentration of phenol in a given sample is calculated using Direct Photometric Method following sampling techniques. Another objective is that, the initial concentration of phenol in the effluent before and after treatment using bioremediation techniques chiefly employing *Pseudomonas putida* is also estimated.

Key words: Phenol, *Pseudomonas putida*, Degradation, Visakhaptanam Steel Plant, Mechanical Biological Chemical (MBC) treatment plant.

INTRODUCTION

In service provisions at Visakhapatnam Steel Plant water is chiefly used for watering, chilling, cooling and to equip other ancillaries[1]. Such industrial effluent is rich in chemicals, iron and carbon remains, hence it is treated for reuse or release in adjoining sea-water linked channels. Phenol is usually found in industrial wastewater and is considered a hazardous pollutant[3]. It is an organic hydrocarbon which a benzene ring with one hydroxyl group, commonly present as a contaminant in the effluent released after carbonization of coal. 800 to 900 ppm of phenol is present in the effluent which can be biodegraded by *Pseudomonas putida*. Phenol is considered to be lethal to human beings, it causes severe dermal inflammation and necrosis and has chronic effects on liver, kidney, respiratory,

[*]Author for correspondence; Mo.: 09490187300; E-mail: meena_sekhar09@yahoo.co.in

cardiovascular and central nervous system[4,6]. *Pseudomonas putida* is a gram negative bacterium capable of degrading phenol into catechol and CO_2 phenol is further cleaved into TCA cycle intermediates. Genes coding these enzymes are clustered on the degradative plasmid. This project attempts to assess the biodegradation of phenol by *Pseudomonas putida* .The initial and final concentration of phenol in the effluent are estimated by direct photometry method. The organism is further characterized by various cultural and bio-chemical tests for better understanding of phenol bio-degradation[5].

EXPERIMENTAL

Material and method

Mechanical biological chemical treatment plant (MBC)

The MBC is the basic treatment means available for the treatment of the effluent wastewater generated as a result of various heating and cooling operations pivoted around water being the chief prerequisite. Its various parts, tanks and mode of operation are discussed below. Toxic effluents generated from various sections of steel plant are relieved by MBC through two different lines called W1 and W2. Excess flushing liquor after removal of free ammonia in ammonia column is sent to line W1.W2 is purged water from contaminated water circuit of final gas cooler.MBC is designed to remove tar and oils by mechanical separation methods followed by biological treatment with bacteria in two stages to remove phenol, cyanides and rhodanides. Both W1 and W2 enter the pre-aeration tank, where they get thoroughly mixed with air. On the way to pre-aerator, W1 is cooled in shell and heat exchangers from 100°C-90°C to 50°C-40°C with the help of service water. From the pre-aerator tank mixed effluent is distributed equally to five tar settling tanks where tar and heavy metals get separated due to higher specific gravity[2]. After the tar is removed now the effluent is being mixed with lime solution, before being sent to oil floatater where air is bubbled through the water. Here the oil entrapped in the bubbles is skimmed with oil mechanism forthwith is collected and sent to the “Oil collecting tank by oil discharge channel.This oil-removed effluent is sent to the “equalizing tank” of 1600 Cum capacity. Each equalizing tank is facilitated with P1 (water after 1st stage), P2 (purified water), and lime solution and service water for mixing. Next the water is sent to the first stage aeration tank where mainly phenol degradation occurs by biological oxidation. Overflow from 1st aeration tank is collected in semi purified effluent tank (PI) from where it is pumped to 2nd aeration tank.

In 2nd aeration tank destruction of cyanide and rhodamide occurs by bio-oxidation, furthermore here settling tank, regeneration tank and airlift mechanism is provided to avoid

the sludge loss. Periodically sludge from the bottom of this tank is taken out and sent to sludge drying beds. The bacteria cultivation tank B1 and B2 are provided to store and cultivate both type of bacteria to avoid any unfortunate biological disaster. The effluent of B1 and B2 is checked every day for phenol and cyanide concentration. When the concentration falls below 10 ppm, part of the water from B1 and B2 is filed with fresh effluent and air and phosphorous are supplied from outside for the growth of the bacteria. Continuous air supply must be supplied under any circumstances for the bacteria. Here in the natural bacterial flora of these bacterial tanks and soil cultures, Pseudomonas putida was present and it to be identified precisely by studying the various morphological and biological characteristics and grown in its mixed cultures with predominating concentration by creating favorable conditions for its growth[7].

Determination of phenol concentration-direct photometric method

Procedure

(i) 50 mL of sample is taken in a Kjeldahl's flask and 10 mL of 10% of $CuSO_4$, 10 mL of 1 : 3 H_2SO_4 and few drops of methyl orange are added to it.

(ii) The content in Kjeldahl's flask is steam distilled for nearly one hour to distill out all phenols present. The distillate is condensed and collected in a conical flask.

(iii) The collected distillate is measured and 100 mL is taken in a conical flask and 2.5 mL of 0.5 NH_4OH solutions is added. Immediately pH is adjusted to 7.8-7.9 with phosphate buffer.

(iv) mL of 2% of 4-amino antipyrine solution is added in the conical flask and mixed well. Then 1 mL of 8% $K_3Fe(CN)_6$ solution is added and mixed well. This is kept for minimum 15 minutes.

(v) Spectrophotometer is set to 500 nm. The absorbance of the prepared sample is determined[6].

RESULTS AND DISCUSSION

Mechanical biological chemical treatment plant result

In steel plant effluent initial concentration of phenols is 1093 ppm and after treatment by bioremediation, the phenol content is degraded to 0.56 ppm after 33 days at 37^oC is illustrated in the Table 1.

Table 1: Extent of degradation of phenol versus number of days in mechanical biological chemical treatment plant

Time (days)	Concentration (ppm)
3	1093
6	511.2
9	883.9
12	65.6
15	20.44
18	4.47
21	3.66
24	2.98
27	2.13
30	0.89
33	0.56

Determination of phenol concentration-direct photometric method results

Table 2 gives a list of the various concentrations of phenol solution as studied using photometer.

Table 2: Absorbance levels for various concentrations of phenols

Standard phenol solution (mg) A	Observed absorbency (500 nm) B	Deviation slope
0.1	0.14	-
0.2	0.30	+ 0.02
0.3	0.42	NIL
0.4	0.56	NIL
0.5	0.70	NIL
0.6	0.82	0.02

From this calibration result is calculated as below -

The calibration factor (K) can be calculated as K = A / B, where;

A = mg of phenol sample

B = Observed absorbency

K = 0.1 /0.14 = 0.71

CONCLUSION

The effluent of carbonized coal was estimated for phenol content by "Direct photometric method" before and after bioremediation using *Pseudomonas putida* (obtained from very cheap indigenous mixed bacterial culture), at the Mechanical biological and chemical treatment plant. So, from above results it has been concluded that high content of phenol-1093 ppm is reduced to 0.56 ppm by bio-degradation with *Pseudomonas putida after* 33 days. In the near future it is likely to use mutant forms of *Pseudomonas putida* that could make the process more effective and economical. Even genetically engineered bacteria that are specifically tailor made to suit the needs of a particular industry can be used that could effectively treat the wastewaters specifically saving time and money[9].

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