

Physicochemical and Microbiological Analysis of Steel Plant Effluents of Visakhapatnam, Andhra Pradesh

K.M. ELIZABETH*, B. GAYATRI, G. TULASI KUMARI and Y. VIMALA
*Department of Microbiology, College of Science
Gandhi Institute of Technology and Management, Visakhapatnam-530 045, India
E-mail: kmelizabeth@yahoo.com*

The physicochemical and microbiological analysis of Visakhapatnam steel plant effluents, both treated and untreated was performed. The physical parameters tested were pH, conductivity, turbidity and temperature; among them, conductivity was beyond the permissible limit before and after the treatment of the effluent. Turbidity was very high in the untreated effluent which was reduced to below the normal limit. The microbiological analysis such as coliform counts and total plate counts had exceeded the normal limit. The total hardness, Ca hardness and Mg hardness, chlorides, total solids, total dissolved solids and ammonia contents were above the permissible limit in both the effluents indicating that the effluents were highly polluted. The bioremediation studies with viable, killed and immobilized non-pathogenic bacteria revealed the fact that there was 76.9%, 97.1% and 95.7% removal of ammonia respectively from treated and untreated effluents. There was 500 mg/L of phenol in untreated effluent which was reduced to < 1 mg/L in treated effluent. Bioremediation could also remove oil and grease to some extent. These results indicate that the treated and untreated effluents of this industry were highly contaminated and bioremediation with non-pathogenic microorganisms was a successful measure of treatment.

Key words: Physicochemical analysis, Steel plant effluent, Bioremediation, Ammonia, Oil and grease.

INTRODUCTION

Visakhapatnam steel plant is located in Gajuwaka, Visakhapatnam, Andhra Pradesh. Every day it discharges tonnes and tonnes of treated effluent into the Bay of Bengal near Appikonda area. Though it is a treated effluent, it still possesses several pollutants beyond the permissible limits. Especially, contaminants like ammonia are around 100 mg/L in treated effluent per day or even more on some other days. But the permissible limit for aquatic life is 2.3 mg/L of ammonia. Earlier we tried to remove ammonia from untreated effluent of this industry with other microorganisms¹. There were several reports on bioremediation of various pollutants²⁻⁵. Therefore as this effluent was very dangerous, it had

drawn our attention to analyze and bioremediate ammonia, phenol, oil and grease from treated and untreated effluents of Visakhapatnam steel plant.

EXPERIMENTAL

Physicochemical analysis of effluent: Both treated and untreated effluents of Visakhapatnam steel plant were collected in sterile containers which were refrigerated immediately after transportation to the Microbiology Laboratory, GITAM, at least until the microbiological, DO and COD tests were completed. All the tests were performed according to the methods of APHA⁶.

Maintenance of bacterial cultures: Four bacterial cultures such as *Bacillus subtilis*, *Staphylococcus* species, *Bacillus cereus* and *Clostridium* species were maintained on nutrient agar slants which were stored at 4°C. Subcultures were made from them to conduct all the experiments.

Estimation of ammonia, phenol and oil and grease: Ammonia and phenol were estimated by Nessler's method and 4-amino antipyrine method⁷ as described earlier¹ by plotting ammonia and phenol standard curves with ammonium chloride and phenol respectively (spectrophotometric method). For estimation of oil and grease petroleum ether method was employed (gravimetric method)⁶.

Bioremediation of ammonia, phenol and oil and grease: This was performed as described earlier¹. Viable bacteria (10^8 /mL), killed bacteria which were dried and powdered (20 mg/mL) and immobilized bacteria in sodium alginate beads (1 g/mL) were incubated with untreated and treated effluents separately for 24 h in a rotary shaker at room temperature. Later the effluent was collected and subjected to these assays. The amount of ammonia, phenol, oil and grease was estimated. Corresponding control effluents without inoculation with bacteria were also incubated simultaneously and the amounts of ammonia, phenol, oil and grease were estimated.

RESULTS AND DISCUSSION

The results are summarized in Tables 1-4. In the present study the pH of the untreated and treated effluents (Table-1) were within the normal limits. The conductivities of untreated and treated effluents were 5.73 and 6.94 milli-mhos respectively suggesting that these increased after treatment and both the values were beyond the permissible limit. The turbidity of untreated effluent (11.8 NTU) was very high which was brought down to 2.9 NTU after treatment. The coliform counts of untreated and treated effluents were 2 and 17 per 100 mL sample respectively indicating that the counts were beyond the lower limit which was increased after treatment. The total plate counts of both the effluents had exceeded the normal limit. The DO content of treated and untreated effluents were greater than 85 and 50 mg/L indicating very high content of dissolved oxygen in both and it was greater in treated effluent indicating the effect of aeration. The COD content of both the effluents was beyond the permissible limit. The total hardness of untreated and treated effluents were 5000 and 8500 mg/L indicating that after treatment the value had increased and both the contents had exceeded the normal limit. Similarly the CaH and MgH of both effluents were beyond the permissible

limit. The chlorides, total solid, total dissolved solids of both effluents and the suspended solids of treated effluent were above the normal limit. The nitrate content was within the limits and the ammonia content was very high in untreated effluent (800 mg/L) and was reduced to 100 mg/L after treatment. The phenol content of untreated effluent was very high, *i.e.*, 500 mg/L, which was reduced to almost 100% after treatment by the industry. These results differ from the results of Nazareth and Mavinkuve⁸, who reported degradation of 19 phenolic compounds by *Fusarium* species. The results of this study differ from our previous results¹ where there was almost 100% removal of ammonia by *Bacillus* species. The oil and grease contents of both the effluents were beyond the permissible limit. The bioremediation of ammonia by viable bacteria (Table-2) showed that *Staphylococcus* species could remove 48 and 62% from untreated and treated effluents respectively.

TABLE-1
PHYSICO-CHEMICAL AND MICROBIOLOGICAL ANALYSIS
OF STEEL PLANT EFFLUENTS, VISAKHAPATNAM

Parameters	Untreated Effluent	Treated Effluent	Permissible Limits
pH	7.01	6.48	6.5-8.5
Conductivity (mMhos)	5.73	6.94	< 2.5
Turbidity (NTU)	11.8	2.9	5.0
Temperature (°C)	29	29	25-27
MPN test (coliforms/100 mL sample)	2	17	0-20
Total plate count (CFU/mL)	2.8×10^{11}	3.51×10^4	< 100
DO (mg/L)	> 50.0	> 85.0	4-8
COD (mg/mL)	1736	1664	100-150
Total hardness (mg/L)	5000	8500	75-150
Ca hardness (mg/L)	3520	6550	75-150
Mg hardness (mg/L)	1480	2050	75-150
Chlorides (mg/L)	3918.7	3381.7	250
Total solids (mg/L)	2600	5700	500-1000
Total dissolved solids (mg/L)	2600	3500	500-1000
Suspended solids (mg/L)	0.0	2200	500-1000
Nitrates (mg/L)	17	16	< 45.0
Ammonia (mg/L)	800	100	50.0
Phenol	500	< 1.0	5.0
Oil and grease	1380	340	50

This bacterium did not remove phenol from untreated effluent which was 500 mg/L. The treated effluent did not contain any phenol. This result indicates that this bacterium had no ability to remove phenol from untreated effluent. Similarly the oil and grease content was very high in untreated and treated effluents (1380 and 340 mg/L) respectively which could not be removed or reduced by it. Highest

percentage removal of ammonia was achieved by viable *B. subtilis*, i.e., 65% in treated effluent. *B. cereus* in its viable form could remove 68.7 and 65% from untreated and treated effluents. Viable *Clostridium* could remove 76.9% of ammonia from treated effluent of steel plant. This bacterium did not remove any phenol from untreated effluent but could remove 35 and 45% of oil and grease from untreated and treated effluents respectively. The biosorption technique (Table-3) showed that there was highest percentage removal of ammonia by *Clostridium* species (97.1%) from treated effluent of steel plant.

TABLE-2
BIOREMEDIATION OF AMMONIA, PHENOL, OIL AND GREASE
BY VIABLE BACTERIA

Organism	No. of cells	Percentage removal of					
		Ammonia		Phenol		Oil and grease	
		UT	T	UT	T	UT	T
<i>Staphylococcus</i> species	10 ⁸	48.0	62.0	0.0	100	0.0	0.0
<i>Bacillus subtilis</i>	10 ⁸	56.0	65.0	0.0	100	0.0	0.0
<i>Bacillus cereus</i>	10 ⁸	68.7	65.0	0.0	100	0.0	0.0
<i>Clostridium</i> species	10 ⁸	67.7	76.9	0.0	100	35	45

Ammonia in UTE = 800 mg/L; in TE = 100 mg/L. Phenol in UTE = 500 mg/L; in TE = 0.0; Oil and grease in UTE = 1380 mg/L; in TE = 340 mg/L. UTE = untreated effluent; TE = treated effluent.

TABLE-3
BIOREMEDIATION OF POLLUTANTS BYBIOSORPTION TECHNIQUE

Organism	Sorbent (mg/mL)	Percentage removal of					
		Ammonia		Phenol		Oil and grease	
		UT	T	UT	T	UT	T
<i>Staphylococcus</i> species	20	83.5	91.2	0.0	100	0.0	0.0
<i>Bacillus subtilis</i>	20	93.4	93.4	0.0	100	0.0	0.0
<i>Bacillus cereus</i>	20	96.0	96.0	0.0	100	0.0	0.0
<i>Clostridium</i> species	20	97.1	97.1	0.0	100	31.0	42.0

Ammonia in UTE = 800 mg/L; in TE = 100 mg/L. Phenol in UTE = 500 mg/L; in TE = 0.0; Oil and grease in UTE = 1380 mg/L; in TE = 340 mg/L. UTE = untreated effluent; TE = treated effluent.

Other bacteria such as *Staphylococcus*, *Bacillus* species and *B. cereus* and *Clostridium* showed 83.5 and 91.2%; 93.4 and 92%; 96.0 and 95.8% and 97.1 and 96% respectively from untreated and treated effluents of Visakhapatnam steel plant. There was 31 and 42% removal of oil and grease by this organism. The ammonia removal was 95.7% by immobilized *Staphylococcus* species from treated effluent (Table-4). Similarly there was highest percentage removal of ammonia, i.e., 82.8% from treated effluent. The immobilized *B. cereus* could remove 87 and 66.2% ammonia by treated and untreated effluent of steel plant.

Clostridium species could remove 57.7 and 92.5% of ammonia by immobilization technique. There was 31 and 42% removal of oil and grease by immobilized *Clostridium* species from untreated and treated effluents respectively indicating that this bacterium had the capacity to remove oil and grease. These results indicate that both treated and untreated effluents of Visakhapatnam steel plant were highly contaminated with chemicals and microorganisms. Bioremediation studies revealed that these organisms can be used to remove ammonia, but not phenol and oil and grease (except *Clostridium* Species).

TABLE-4
BIOREMEDIATION OF POLLUTANTS BY IMMOBILIZATION TECHNIQUE

Organism	Beads (g/mL)	Percentage removal of					
		Ammonia		Phenol		Oil and grease	
		UT	T	UT	T	UT	T
<i>Staphylococcus species</i>	1	58.5	95.7	0.0	100	0.0	0.0
<i>Bacillus subtilis</i>	1	60.7	82.8	0.0	100	0.0	0.0
<i>Bacillus cereus</i>	1	66.2	87.0	0.0	100	0.0	0.0
<i>Clostridium species</i>	1	57.7	92.5	0.0	100	31.0	42.0

Ammonia in UTE = 800 mg/L; in TE = 100 mg/L; Phenol in UTE = 500 mg/L; in TE = 0.0; Oil and grease in UTE = 1380 mg/L; in TE = 340 mg/L. UTE = untreated effluent; TE = treated effluent.

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