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Cyanide Removal from Steel Plant Wastewater by Strains of *Oscillatoria* sp. and *Chlorella* sp.

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Abstract- Cyanide salts and hydrogen cyanide are used in electroplating, metallurgy, production of organic chemicals, photographic developing, in making plastics, fumigating ships, and some mining processes. Cyanide is a powerful and rapid-acting poison. Since it is safer, effective and highly economical, many scientists made various bioremediational approaches to remove cyanide in past few decades. In present study two tolerant strains of microalgae were isolated and preliminarily used to remove cyanide from industrial wastewater. *Oscillatoria* sp. And unicellular *Chlorella* sp. Were able to remove considerable amount of cyanide and phenol from steel-plant wastewater. For both the strains, pH 7.0 and sucrose as sugar source were most favorable for cyanide removal. Effect of PVF immobilization was also studied.

Keywords: *Oscillatoria; Chlorella;* cyanide; phenol; PVF.

1. INTRODUCTION

Cyanide salts and hydrogen cyanide are used in electroplating, metallurgy, production of organic chemicals, photographic developing, in making plastics, fumigating ships, and some mining processes [1, 2]. Wastes produced by industries processing cyanogenic crops like cassava and bitter almond also contain cyanide compounds originating from decomposition of cyanogenic glucosides [3]. Cyanide is a powerful and rapid-acting poison. Hydrogen cyanide has been used in gas-chamber executions and as a war gas. Exposure to high level of cyanide might harm brain and heart leading to coma and death [1]. Cyanide exerts the toxic effect by inhibiting oxidative enzymes from mediating the process by which oxygen is utilized to complete production of ATP in mitochondria. Cyanide forms a bond with Ferri-cytochrome oxidase and inactivates it. Thus the energy producing step is prevented [4, 5].

Chemical treatment of cyanide by oxidation with Ozone or hydrogen peroxide is highly expensive [5]. There are some drawbacks of such processes. For instance, alkaline chlorination needs careful control of chlorine concentration and may give rise to uncontrolled formation of toxic and biologically persistent organochlorine compounds [2]. The realization of high cost involvement and other problems in cyanide removal or degradation process by chemical or other conventional means has opened the doors leading to investigations in the field of bioremediation. Previously biological methods were considered impractical or impossible because of the general belief that toxic cyanide compounds would inhibit enzymatic activities and thus any organism would be killed. But later on several organisms, which can tolerate or detoxify cyanide, are found [6, 7, 8]. Since application of biological systems is safer, effective and highly economical, many scientists made various approaches to remove cyanide in past few decades [7, 9].

Several attempts were made to study the effect of specific strains of bacteria on cyanide removal from wastewater [3, 4, 10, 11]. Few fungi and algae were also tested for cyanide remediation [12, 13, 14, 15]. In present study two tolerant strains of microalgae were used to remove cyanide from industrial wastewater.

2. MATERIALS AND METHOD

2.1 Wastewater Collection

Wastewater samples were taken from Aeration Tank-I of Bokaro Steel Plant (BSP) and analysed for different physico-chemical and microbial parameters [16]. All the tests were done within 24 hours of collection of wastewater. Temperatures, pH and colour of the samples were recorded at the time of sampling. Cyanide and phenol were also determined by spectrophotometric method according to APHA [16]. Algal scum was scrapped from Aeration Tank-II of BSP and different algal and cyanobacterial strains were isolated by spread plate technique and then identified [17, 18].

2.2 Selection of tolerant strains

The isolated algal and cyanobacterial strains were tested for their independent tolerance of cyanide in Chu-10 and BG-11 media respectively, both in broth and plates. Filter sterilized cyanide at different concentrations were added to medium after autoclaving. Strains were inoculated and observed for growth after 5 days at 28ºC.

2.3 Cyanide removal by algal and cyanobacterial strains in respective media

The selected strains were aseptically inoculated (1 ml. of 5 days' fresh culture grown in respective media) in 100 ml. Chu-10 (algal) and BG-11(cyanobacterial) media spiked with potassium cyanide of 15 mg/l concentrations in 250 ml glass flasks. The experimental sets were kept under incubation for a week at 30ºC and 2000 lux illumination for 16hrs. light and 8 hrs. dark phases along with aeration. Control sets without inoculation were also run. Cyanide removal efficiency of the strains was calculated by subtracting final cyanide concentration in filtrate of inoculated set from that of control set after every 24 hours incubation for a week. Final cyanide estimation was done with filtrate after harvesting cell-masses by centrifugation at 11000 rpm for 15 minutes.

2.4 Effect of pH, added sugars and phenol in media

To study the effects of added sugars, in some sets additional glucose or sucrose (1g/l.) was added. To study the effects of phenol (which could be co-pollutant with cyanide), in some sets additional phenol (50 and 100 mg/l.) was added. For studying pH requirement of the strains pH of the said media was calibrated with buffer solutions prior to inoculation of strains according to Sadasivam and Manickam [19].

2.5 Cyanide and phenol removal potential assessment in wastewater of BSP by biomass

To study the effect of microalgal or cyanobacterial biomass, 500ml. of wastewater (Aeration Tank-I) was inoculated with 8 ml of moderately grown 7 days' biomass of the strains. These biomasses were grown in respective media supplemented with 10mg/l cyanide. Dry weight of the inoculated biomass was approximately 15 mg. Approximately same amount of biomass was dried at 70ºC to estimate the dry weight. To study the effects of added sugars, in some sets additional sucrose (1g/l.) was added.

Immobilization of the strains was done by growing the two strains in respective medium along with approximately 1cm^3 sterile cubic blocks of polyvinyl foam (PVF), under static condition for 10 days. These algal or cyanobacterial biomass blocks (20-22 pieces) were inoculated in 500 ml. wastewater. In both the cases controls (without biomass and with only agar-agar blocks respectively) were also run. Cyanide and phenol removal efficiency of the strains was calculated by subtracting final concentration of cyanide or phenol in filtrate of inoculated set from that of control set. In case of inoculated set, final cyanide estimation was done with filtrate after harvesting cell-masses by centrifugation at 11000 rpm for 15 minutes.

3. RESULTS AND DISCUSSION

Table-1 Physico-chemical and microbiological characteristics of Bokaro Steel Plant wastewater

Characteristics	**Aeration tank-I**
pH	7.8±0.2
Colour	Light brownish
Phenol (mg/l)	91.1±9.3
Cyanide (mg/l)	14.6±4.9
Bacterial Load (cfu/ml)	5.8x10^7
Fungal Load (cfu/ml)	3.5x10^3

Light brown to straw coloured wastewater from Bokaro steel plant (BSP) showed a slightly alkaline to slight acidic pH (Table 1). The pH, Phenol and cyanide contents respectively at Aeration tank-I, BSP were about 7.8, 91.1mg/l, 14.6 mg/l. Phenol content was about 90mg/l. in aeration tank-I. The wastewater of Effluent treatment plant of Bokaro Steel Plant contained very high amount of cyanide and phenol at least at the entry point of aeration tank-I. Out of 3 cyanobacteria of BSP only one and the green alga from BSP were also found to be tolerant to cyanide up to 40 mg/l. Only the green alga and the cyanobacterium which had grown at 40 mg/l. cyanide concentration were taken for further studies.

Oscillatoria sp. ESBSP$_C$-2 and *Chlorella* sp. ESBSP$_A$-5 were taken for these studies. In 15 mg/l cyanide concentration, *Oscillatoria* sp. ESBSP$_C$-2 removed 79.3% cyanide in a week, while the unicellular green alga removed 96.5% (Fig. 1). The rate of cyanide removal or degradation was higher upto third day, thereafter the rate slowed down but maintained overall steady level in both cases. The rate of cyanide removal or degradation was higher up to third day, thereafter the rate slowed down.

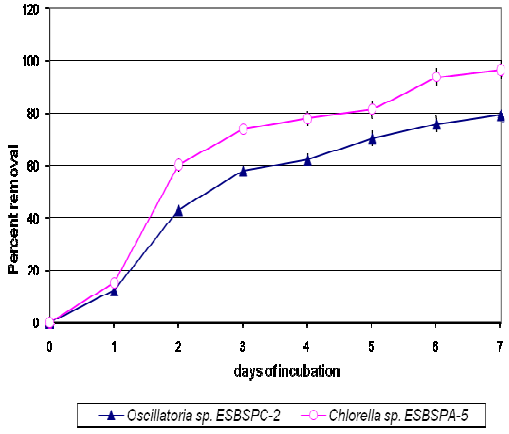

Fig. 1: Cyanide removal efficiencies of *Oscillatoria* sp. ESBSPC-2 and *Chlorella* sp. ESBSPA-5 in respective media with 15 mg/l cyanide in respect with control

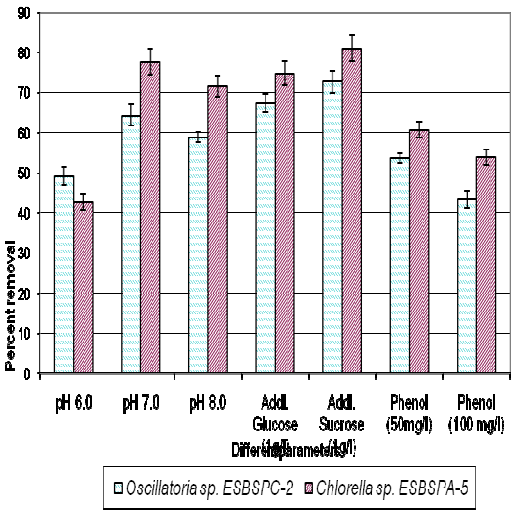

Fig. 2: Effects of different parameters on Cyanide removal efficiencies of *Oscillatoria* sp. and *Chlorella* sp. after 5 days incubation, in respective media with 15 mg/l cyanide in respect with control

For both the strains, pH 7.0 was most favorable for cyanide removal (Fig. 2). *Oscillatoria* sp. $ESBSP_C$-2 removed about 49, 65 and 59 % in 5 days in pH 6.0, 7.0 and 8.0. *Chlorella* sp. $ESBSP_A$-5 removed about 43, 78 and 72 % in 5 days in pH 6.0, 7.0 and 8.0. For both the strains, pH 7.0 was most favorable for cyanide removal. Specially acidic media hampered with cyanide removal as even at pH 6.0 cyanide removal was greatly reduced. In comparison to glucose, sucrose as sugar source was more preferred by the strains. In case of sucrose, removal of about 73 and 81 % was seen respectively in strains *Oscillatoria* sp. $ESBSP_C$-2 and *Chlorella* sp. $ESBSP_A$-5. Presence of phenol reduced the removal percentage in both cases. Supplementary glucose or sucrose enhanced and increased cyanide removal rate, sucrose having better effect. Presence of phenol on the other hand, reduced rate and total percentage of cyanide removal, due to its toxic effect.

By free biomass of *Oscillatoria* sp. $ESBSP_C$-2 cyanide removal was 40.4, 53.3 and 60.2 % day wise for three days incubation without sucrose as additional sugar (Table 2). With sucrose free biomass removed 43.2, 56.7 and 62.4 % cyanide respectively in 1, 2 and 3 days. Phenol removal also gradually increased to 61.7 and 64.8 % respectively in sucrose-free and sucrose-supplemented wastewater. Phenol removal was 36.1, 47.3 and 61.7 % day wise for three days incubation without sucrose as additional sugar. With sucrose, free biomass removed 37.8, 50.8 and 64.8 % cyanide respectively in 1, 2 and 3 days. Immobilized biomass of the said strain showed almost similar rate of cyanide and phenol degradation. By immobilized biomass of *Oscillatoria* sp. $ESBSP_C$-2 cyanide removal was about 43, 52 and 65 % day wise for three days incubation in case of sucrose-free and 45, 56 and 64 % in sucrose-supplemented wastewater respectively. Phenol removal was about 33.6, 54.6, 61.4 %; 41.6, 53 and 59.4 % day-wise in case of sucrose-free and sucrose-supplemented wastewater respectively.

By free biomass of *Chlorella* sp. $ESBSP_A$-5 cyanide removal was 49.8, 59.0 and 62.6 % day wise for three days incubation without sucrose as additional sugar (Table 2). With sucrose free biomass removed 50.9, 61.4 and 65.9 % cyanide respectively in 1, 2 and 3 days. Phenol removal was about 40.7, 54.4, 72.8 %; 41.6, 53.0 and 59.4 % day-wise respectively in sucrose-free and sucrose-supplemented wastewater. Immobilized biomass of the green algal strain showed almost similar rate of phenol degradation but cyanide degradation decreased to some extent. Cyanide removal was 35.3, 45.7 and 53.3 % day wise for three days incubation without sucrose as additional sugar and 38.1, 49.4 and 55.8 % day-wise in sucrose-supplemented wastewater respectively. Phenol removal was about 42.9, 52.3, 70.4 %; 46.6, 56.4 and 73.5 % day-wise respectively in sucrose-free and sucrose-supplemented wastewater. By free biomass of *Oscillatoria* sp. $ESBSP_C$-2, total cyanide removal from BSP wastewater was 60% and phenol removal 62% in three days incubation without sucrose and 62% and 65% respectively with sucrose as additional sugar. For free biomass of *Chlorella* sp. $ESBSP_A$-5 the figures were 62, 66 and 73 and 75% respectively. Immobilization of biomass had increased cyanide removal in case of *Oscillatoria* sp. $ESBSP_C$-2, phenol removal remaining almost the same. But in case of *Chlorella* sp. $ESBSP_A$-5 immobilization of biomass had reduced cyanide removal. Probable explanation might be that in case of *Chlorella* sp., the cells were embedded and trapped within the meshwork of PVF cubes, thus reducing the cyanide removal or degradation rate. While in case of *Oscillatoria* sp. $ESBSP_C$-2, the filaments of the strain were mostly on external sides of the cubes and quite free along with a firm grip to the cubes.

4. CONCLUSION

Cyanide is a potent inhibitor of cellular metabolism and it must be reduced to very low level, generally around 0.1 to 1.0 ppm, before the wastewater can be discharged [3]. Chemical processes used to detoxify cyanide –containing industrial wastewater suffer from many drawbacks. Microbial treatment is an area, which could open a new way to remove cyanide from wastewater. Strains like the above mentioned *Oscillatoria* sp. $ESBSP_C$-2 and *Chlorella sp.* $ESBSP_A$-5 are very promising in this respect. These strains could be used in removing cyanide in immobilized systems from wastewater. Of

course some *in situ* study and up-scaling augmentation are necessary.

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Table-2: Percent removal of cyanide and phenol in respect with control, from BSP wastewater by the biomasses of cyanobacterial strains, both free and PVF-immobilized (with or without additional sucrose).
[Initial Cyanide=16.2 mg/l.; Phenol=81.0 mg/l.]

Strains	Status	Addl. sucrose	Cyanide removal (%)			Phenol removal (%)		
			Incubation time (hours)			Incubation time (hours)		
			24	48	72	24	48	72
Oscillatoria sp. $ESBSP_C$-2	Free	No sucrose	40.37	53.33	60.21	36.10	47.29	61.74
		Sucrose +	43.19	56.67	62.38	37.79	50.73	64.84
	Immobilized	No sucrose	42.99	52.06	64.93	33.61	54.61	61.4
		Sucrose +	45.16	55.71	64.19	41.68	53.02	59.37
Chlorella sp. $ESBSP_A$-5	Free	No sucrose	49.76	59.04	62.60	40.77	54.40	72.87
		Sucrose +	50.93	61.42	65.95	49.35	52.38	70.87
	Immobilized	No sucrose	35.26	45.74	53.28	42.81	52.30	70.42
		Sucrose +	38.16	49.39	55.80	46.62	56.42	73.51