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EVALUATION OF NOVEL BIO-FRIENDLY TWO-STEP PROCESS IN THE REMOVAL OF HEAVY METALS FROM THE WASTEWATER

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Abstract

Two-step treatment technique was developed for the treatment of water by *Pseudomonas aeroginosa* in a bioreactor in a first phase and then the bacterial treated water was treated with the vetiver grass, cattails and water hyacinth in second phase. Two-step process of bioremediation of 13 days was found to be satisfactory for As, Ba, Cd, Co, Cr, Cu, Hg, Ni, Pb and Zn in compared to the direct treatments with vetiver grass, cattails and water hyacinth in 20 days. As the plants cannot work or tolerate the higher concentrations of heavy metals, so with the first step on an average 52.48% reduction of heavy metals were done within 5 days. It was observed that 100% removal of Pb was found by two-step process of *Pseudomas aeroginosa* with cattails and water hyacinth, respectively in 13 days, while 98.16% removal of Pb was found by direct plant treatment of water hyacinth in 20 days. It was clear that the two-step treatment for vetiver grass, cattails and water hyacinth were found as the most effective treatments.

Introduction

The unsustainable industrialization and the improper disposal of wastewater are the main cause behind the contamination of aquatic environment. The heavy metal, such as Cd, Cu, Cr, Fe and Ni polutan from the industrial wastewater which enter into food chain and ultimately cause the threat for lives (Singare *et al*. 2011). The addition of different chemicals from various industries the characteristics of the wastewater turned into complex in nature (Kumar *et al*. 2008). It is reported that single step phenomenon is time consuming and it is not possible to eradicate the high levels of contaminants as well as it does not give the expected feedback. Biological treatment activities are regarded as the environment friendly methods in wastewater treatment (Lofrano *et al*. 2013). Bacteria could play an effective role in eradicating pollutants specially heavy metals from wastewater (Kumaran *et al*. 2013). Nowadays, plants are being used in wastewater treatment and environmental cleansing as the secondary treatment procedure. In addition, the contaminated soil, sediment and water can be decontaminated by phytoremediation activities (Kokyo *et al*. 2014). Vetiver grass has the large rooting system, rapid growth and has been showed capability in removing contaminants especially heavy metals (Roongtanakiat *et al*. 2007). Water hyacinth (*Eichhornia crassipes)* and Cattails (*Typha latifolia)* are regarded as the best species in industrial wastewater treatments (Sukumaran 2013). The objective of the study is to evaluate the efficiency of the two-step treatment process of removal of heavy metals from the wastewater.

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Materials and Methods

Water samples were collected in plastic container from Gebeng industrial area, Pahang and preserved at low temperature. The selected heavy metals such as As, Ba, Cd, Co, Cr, Cu, Hg, Pb, Ni and Zn were determined by AOAC (2006) method using Inductive Coupled Plasma (ICPMS) spectrometry, Agilent 7500 CX, USA). *Pseudomonas aeroginosa* was isolated and used for the treatment of the water in aerobic bioreactor for 5 days (first phase, Fig. 1). Bacteria were sub-cultured on LB agar medium and preserved at low temperature.

The bioreactor was comprised of one feed tank, one digestion tank and one collection tank. Fifteen liters sample water with 150 ml bacterial culture (3.15×10^6 cells/ml) and 1.5 liters LB broth were added in the bioreactor (Nanda *et al.* 2011). The substrates were stirred continuously by

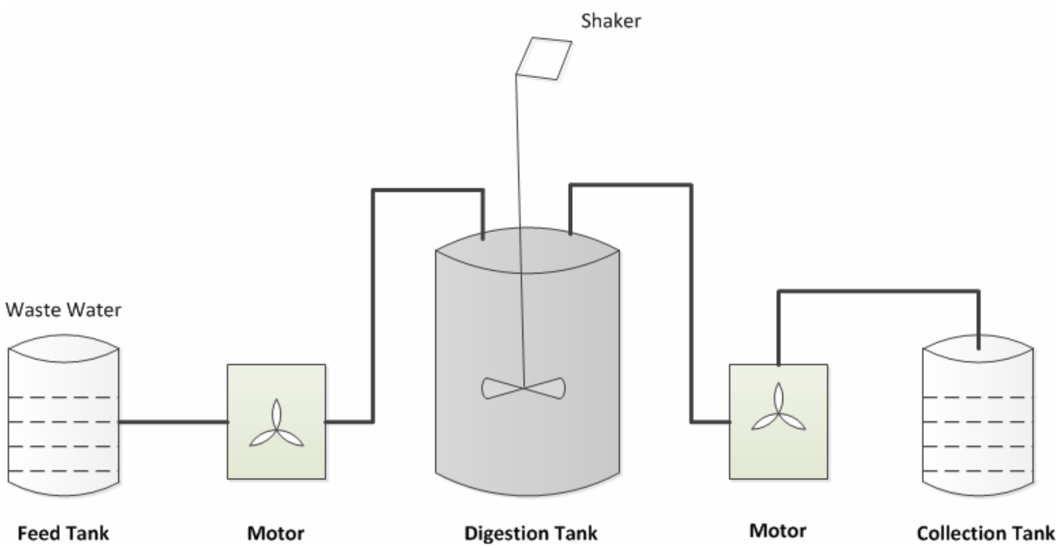

Fig. 1. Design of the aerobic bioreactor plant.

mechanical stirrer at 10 rpm for proper mixing. A control was also made. The treated water was collected by ultra-centrifuge at 30,000 rpm for 20 minutes and heavy metals were measured.

In the experiment sample water was directly treated with vetiver grass (V1, V2, V3 and V4), cattails (C1, C2, C3, and C4) and water hyacinth (WH1, WH2, WH3 and WH4) and conducted for 20 days, while bacterial treated water was also treated with those plants (V1, C1, and WH1) and conducted for 8 days (second phase, Table 1). Initially, all young plants were washed with tap and distilled water and then gently washed with fungicides (Comet M-45, class IV, conc. 2.5 g/l). Finally, all the saplings were kept into distilled water at 14 days for adaptation. Mixed fertilizer (NPK, 21% of each, conc. 1.0 g/l) was added into each experimental pot with seven days interval. All experimental pots were kept under sunlight for 12 hrs/day and the losses of wastewater were filled up with distilled water (Bharti and Banerjee 2012). During the experiment temperature and pH were maintained at 24.56 to 25.61ºC and 7.45 to 7.94, respectively.

Table 1. Treatments of vetiver grass, cattails and water hyacinth.

Treatments	Experimental design
Vetiver grass	V1 = Bacterial treated water (5 days) + fertilizer + vetiver grass (8 days) V2 = 75 % wastewater + fertilizer + vetiver grass (20 days) V3 = 100 % wastewater + fertilizer + vetiver grass (20 days) V4 = 100 % wastewater + vetiver grass (20 days)
Cattails	C1 = Bacterial treated water (5 days) + fertilizer + cattails (8 days) C2 = 75 % wastewater + fertilizer + cattails (20 days) C3 = 100 % wastewater + fertilizer + cattails (20 days) C4 = 100 % wastewater + cattails (20 days)
Water hyacinth	WH1 = Bacterial treated water (5 days)+ fertilizer + water hyacinth (8 days) WH2 = 75% wastewater + fertilizer + water hyacinth (20 days) WH3 = 100 % wastewater + fertilizer + water hyacinth (20 days) WH4 = 100 % wastewater + water hyacinth (20 days)

Removal efficiencies were calculated based on the following formula (1) (Boonsong and Chansiri 2008).

$$\%\ \text{Removal Efficiency} \quad \frac{C_{inp} - C_{tfp}}{C_{inp}} \times 100 \tag{1}$$

where, C_{inp} is initial parameter concentration and C_{tfp} is true final parameter concentration.

Kinetics of heavy metals removal were studied to develop a first order kinetic model and to determine kinetic constant (Pavlostathis *et al.* 1998).

$$\text{Ln}\left(\frac{C_0}{C}\right) = Kt \tag{2}$$

where, K is the constant rate for metal removal, t is time interval. C_0 indicates concentration at initial days and C is concentration at final days.

SPSS version 20.0 was used to do the ANOVA test and the multiple comparisons (removal of metals by different treatments using Tukey (HSD)).

Results and Discussion

Maximum removal (81.74%) of Pb was done by *Pseudomonas aeruginosa*, while minimum (31.02%) was of Ba from the water sample (Table 2). The initial As content of the water was 0.05 mg/l, but after treatment it was changed to 0.02 mg/l and the reduction of As was 54.15%. Nanda *et al*. (2011) exhibited that 34% removal of As and 53% removal of Co by *Pseudomonas* sp.; 44% of Cd and 34% of Cu, respectively removed by S*taphylococcus* sp. and 45% of Hg was removed by *Bacillus* sp. In the study we could reduce 57.06% of Cd, 81.74% of Pb, 53.16% of Ni, 51.72% of Zn, 35.03% of Cu and 54.01% of Co from the water sample, while Kumaran *et al*. (2013) observed 41% of Cd, 87.9% of Pb, 53% of Ni and 49.8% of Zn were removed by *Pseudomonas* sp.

Table 2. Removal of heavy metals from water by *Pseudomonas aeruginosa*.

Metals	Initial (mg/l)	Final (mg/l)	Control (mg/l)	Removal (%)
As	0.05 ± 0.0003	0.02 ± 0.0001	0.05 ± 0.0005	54.15
Ba	0.06 ± 0.0005	0.03 ± 0.0002	0.05 ± 0.0004	31.02
Cd	0.10 ± 0.0006	0.04 ± 0.0002	0.09 ± 0.0008	57.06
Co	0.05 ± 0.0007	0.02 ± 0.0003	0.05 ± 0.0003	54.01
Cr	0.06 ± 0.0006	0.02 ± 0.0002	0.06 ± 0.0005	61.83
Cu	0.09 ± 0.0005	0.05 ± 0.0001	0.08 ± 0.0004	35.03
Hg	0.06 ± 0.0005	0.03 ± 0.0002	0.06 ± 0.0006	45.09
Ni	0.06 ± 0.0006	0.02 ± 0.0001	0.05 ± 0.0003	53.16
Pb	0.07 ± 0.0003	0.01 ± 0.0002	0.07 ± 0.0007	81.74
Zn	0.08 ± 0.0004	0.03 ± 0.0003	0.07 ± 0.0003	51.72

It was observed that *Pseudomonas aeruginosa* could remove at 6.05, 14.19, 21.81, 26.03 and 54.15% of As after 6, 12, 24, 48 and 120 hrs, respectively (Table 3). The same reducing trend was also seen with Ba, Cd, Co, Cr, Cu, Hg, Ni, Pb and Zn after 6, 12, 24, 48 and 120 hrs, respectively (Table 3).

Table 3. Heavy metal removal (%) by *Pseudomonas aeruginosa*.

Parameters	6 hrs	12 hrs	24 hrs	48 hrs	120 hrs
As	6.05	14.19	21.81	26.03	54.15
Ba	4.61	9.71	14.92	19.99	31.02
Cd	7.61	15.09	24.38	32.34	57.06
Co	9.02	13.03	21.66	30.83	54.01
Cr	13.71	25.96	37.20	44.37	61.83
Cu	5.02	11.39	17.63	21.55	35.03
Hg	6.01	12.93	17.52	24.16	45.09
Ni	10.82	17.94	24.71	37.04	53.16
Pb	11.73	20.05	32.18	55.97	81.74
Zn	7.83	13.47	18.39	29.13	51.72

Ajao *et al*. (2011) reported that *Pseudomonas aeruginosa* and *Bacillus subtilis* could remove 20.00, 34.31, 35.32 and 22.95% of Pb, Cu, Zn and Cr, respectively from textile effluent after 5 days and 48.00, 60.77, 85.07 and 50.82% of Pb, Cu, Zn and Cr, respectively after 10 days.

It is important the kinetics of heavy metal removal from wastewater in order to what type of metal removes faster or slower by bacterial treatment. So far, there are few reports on heavy metals removal kinetics. K value of heavy metals was ranked Pb>Cr>Cd>As>Co>Ni>Zn>Hg>Cu>Ba (Fig. 2). Pb was removed faster because the K value was higher than any other metals and followed by Cr, Cd etc., while K value was found lower for Ba, means Ba removed slowly.

The satisfactory R^2 values were found in all metals of the experiments which validate the data obtained in the study (Fig. 2).

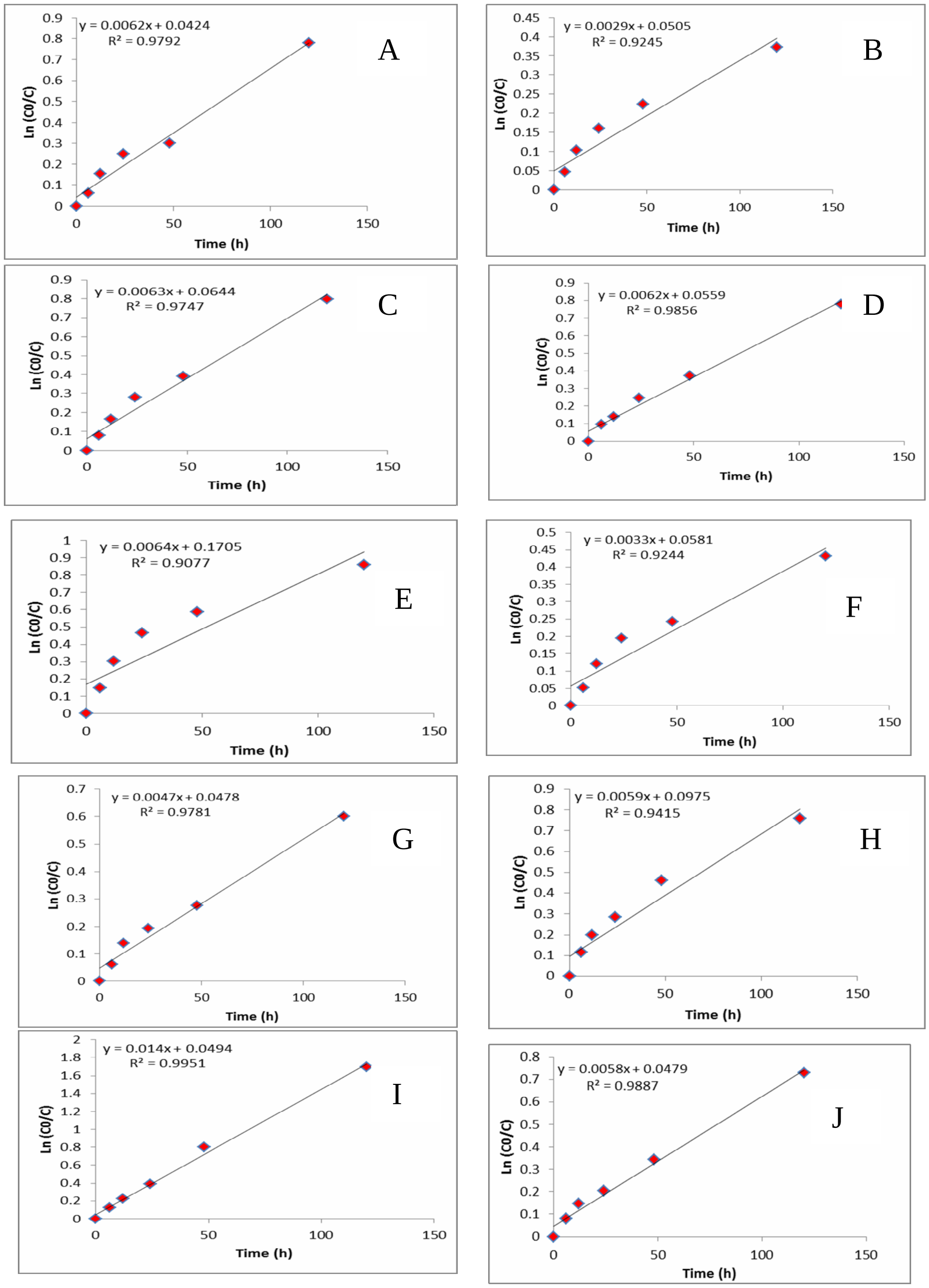

Fig. 2. Kinetics of heavy metals removal by *Pseudomonas aeruginosa*: (A) As, (B) Ba, (C) Cd, (D) Co, (E) Cr, (F) Cu, (G) Hg, (H) Ni, (I) Pb and (J) Zn.

Comparative heavy metal removal efficiency was shown in the Table 4. The removal efficiency of As was found 89.37% in two-step process with V1 after 13 days. On the other hand,

Table 4. Comparative efficiencies (%) between two steps process and the direct treatment.

	Metals	Treatments			
		V1	V2	V3	V4
Vetiver grass	As	89.37	79.58	0.00	0.00
	Ba	80.16	79.06	0.00	0.00
	Cd	95.62	91.32	0.00	0.00
	Co	67.93	63.77	0.00	0.00
	Cr	94.49	91.38	0.00	0.00
	Cu	90.70	89.59	0.00	0.00
	Hg	78.26	61.39	0.00	0.00
	Ni	87.85	84.48	0.00	0.00
	Pb	98.57	75.01	0.00	0.00
	Zn	95.68	92.47	0.00	0.00
		C1	C2	C3	C4
Cattails	As	99.09	80.51	88.80	36.68
	Ba	69.64	60.66	61.64	30.27
	Cd	91.43	70.28	78.13	32.61
	Co	81.92	61.68	72.08	31.54
	Cr	92.67	82.26	90.61	27.59
	Cu	81.69	65.39	77.42	18.83
	Hg	83.17	68.39	73.64	29.88
	Ni	80.45	70.83	76.06	20.36
	Pb	100	70.00	78.25	22.48
	Zn	99.22	96.96	97.81	33.51
		WH1	WH2	WH3	WH4
Water hyacinth	As	90.15	70.61	0.00	0.00
	Ba	65.66	46.87	0.00	0.00
	Cd	100	96.55	0.00	0.00
	Co	77.73	69.91	0.00	0.00
	Cr	90.66	87.18	0.00	0.00
	Cu	82.81	79.22	0.00	0.00
	Hg	93.28	79.30	0.00	0.00
	Ni	76.27	69.93	0.00	0.00
	Pb	100	98.16	0.00	0.00
	Zn	91.72	89.98	0.00	0.00

from the direct treatment with V2 showed 79.58%, while V3 and V4 exhibited no removal due to higher concentrations and toxicity of As that killed the vetiver grass. In the study removal efficiency of As was higher in two step treatment process in comparison with the direct treatment by vetiver grass. The same trend was also observed with Ba, Cd, Co, Cr, Cu, Hg, Ni, Pb and Zn, respectively in two step processes. In case of cattails, the As removal by direct treatment was found 80.51% (C2), 88.80% (C3) and 36.68% (C4) after 20 days, respectively. It was observed that As reduction efficiency was increased with increasing the percentage of wastewater and addition of fertilizer as a source of nutrients and consisted with the Ba, Cd, Co, Cr, Cu, Hg, Ni, Pb and Zn. Kong *et al*. (2003) found that 13 - 58% Hg removal with the pig farm wastewater by vetiver grass. However, combined treatment with *Pseudomonas aeruginosa* and cattails could remove 99.09% (C1) As after 13 days. Yang *et al*. (2006) reported 70.59% Hg reductions by cattails. In experiment with water hyacinth, removal efficiency of As was found 90.15% by two step process and 70.61% after 20 days by direct treatment. So, two step processes combined with *Pseudomonas aeruginosa* and water hyacinth is more effective for the removal of As from the wastewater in comparison with direct treatment with water hyacinth that also followed the removal efficiency with Ba, Cd, Co, Cr, Cu, Hg, Ni, Pb and Zn. However, no removal was observed with WH3 and WH4 due to high concentration and toxicity of As that killed water hyacinth. Sukumaran (2013) reported that cattails can remove As approx. 87.00% after 15 days, while water hyacinth can remove As about 66.08%. Removal of Zn by water hyacinth was reported by Mishra and Trepathi (2009).

Table 5. ANOVA.

		Sum of squares	Degree of freedoms	Mean square	F-test value	Significant (%)
Removal	Between groups	27184.40	7	3883.49	32.78	0.00
	Within groups	8529.60	72	118.47		
	Total	35714.00	79			
Metals	Between groups	0.00	7	0.00	0.00	1.00
	Within groups	660.00	72	9.17		
	Total	660.00	79			

The result exhibited that 98.57, 100 and 100% Pb was removed with V1, C1 and WH1, respectively, while 75.01, 78.25 and 98.16% with V2, C3 and WH2, respectively. Syukor *et al.* (2013) reported similar findings with cattails. Zn removal was observed 95.68, 99.22 and 91.72% with V1, C1 and WH1, while 92.47, 97.81 and 89.98 % with V2, C3 and WH2, respectively.

From the Tables 5 and 6, it is clear that Null hypothesis is rejected here. In case of metal removal, as the p value 0.00 ($p < 0.05$), so there is significance difference between groups and within groups (Table 5). In case of treatments, there are significant differences among treatments. For vetiver grass, V1 is the best treatment because the mean difference between V1 and V2 shows positive value, while the difference between V2 and V1 presents negative value (Table 6). So the treatment of two-step process for vetiver grass (V1) is the effective treatment.

For cattails, C4 is the least treatment, and C1 is the best treatment because the difference between C1 and C4 is high. Moreover, all difference values for C1 (C1 and C2, C1 and C3, and C1 and C4) has been found positive. So, C1 is the best treatment among all cattails treatments. Furthermore, there is significant difference between C1 and C4 because the p value is 0.00 (p <

0.05). For water hyacinth, there is positive difference between WH1 and WH2. Nevertheless, there is a negative difference value between WH2 and WH1. Therefore, WH1 is the best treatment.

Table 6. Multiple comparisons (Removal of metals by different treatments using Tukey HSD).

Treatments	Treatments	Difference	Significant (%)	95% confidence interval	
				Lower bound	Upper bound
V1	V2	7.06	0.83	–8.14	22.25
V2	V1	–7.06	0.83	–22.25	8.14
C1	C2	15.23*	0.05	0.04	30.43
	C3	8.48	0.66	–6.71	23.68
	C4	59.55*	0.00	44.36	74.75
C2	C1	–15.23*	0.05	–30.42	–0.04
	C3	–6.75	0.86	–21.94	8.45
	C4	44.32*	0.00	29.12	59.52
C3	C1	–8.48	0.66	–23.68	6.71
	C2	6.75	0.86	–8.45	21.94
	C4	51.07*	0.00	35.87	66.27
C4	C1	–59.55*	0.00	–74.75	–44.36
	C2	–44.32*	0.00	–59.52	–29.13
	C3	–51.07*	0.00	–66.27	–35.87
WH1	WH2	8.06	0.72	–7.14	23.25
WH2	WH1	–8.06	0.72	–23.25	7.14

Among three plants vetiver grass and water hyacinth were observed less tolerant compared to cattails. It is reported that almost all the plants can be used for secondary treatment. The two-step process was found more effective in removing heavy metals from the industrial wastewater compare to direct treatments with plants.

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