J. Myanmar Acad. Arts Sci. 2019 **Vol. XVII. No.1A**

TREATMENT OF INDUSTRIAL WASTEWATER SAMPLES WITH SEQUENCING BATCH BIOFILM REACTOR

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Abstract

The aim of this research work is to prepare biofilm for the treatment of three industrial wastewater samples from dry cell battery factory, nickel plating factory and leather factory in industrial zones I and II, Mandalay Region, Myanmar by using sequencing batch biofilm reactor. Totally four bacterial strains were isolated from three wastewater samples and identified. Isolated bacterial strains were recultured on sponges that were used as biofilm in this study. Decolourization activity of sequencing batch biofilm reactor was studied and found to be 54.29% (after 72 h treatment), 74.13 % and 53.32 % (after 64 h treatment) for wastewater samples from dry cell battery, nickel plating and leather factories wastewater samples, respectively. The order of removal of metal ions from three industrial wastewater samples by using sequencing batch biofilm reactor was Cr^{3+}> Pb^{2+}> Cd^{2+}. Some physicochemical parameters such as pH, turbidity, total dissolved solids (TDS), dissolved oxygen (DO), chemical oxygen demand (COD) and biochemical oxygen demand (BOD) of industrial wastewater samples before and after treatment with sequencing batch biofilm reactor were also investigated.

Keywords: sequencing batch biofilm reactor, industrial wastewaters, bacterial strains,decolourization activity, heavy metals

Introduction

In the current era of globalization and rapid industrialization, the environmental issues are becoming more and more nuisance for human being. Heavy metals present in wastewater and industrial effluent is major concern of environmental pollution. Removal of heavy metals from the effluent are very important part of the research carried out in environmental field (Dhokpande and Kaware, 2013). The sequencing batch reactor (SBR) can be combined with biofilm growth on the surface of a support material, originating the sequencing batch biofilm reactor (SBBR) (Rajput and Khambete, 2015).

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Sequencing batch biofilm reactor (SBBR) systems, operated in a fill-and-draw mode, have been successful in the treatment of wastewater (Di Iaconi *et al*., 2004; Rodgers *et al*., 2004; Prendergast *et al*., 2005). Biofilm carriers are used for upgrading current wastewater treatment systems. In the SBBRs, the biomass grows as a biofilm on spongy carriers that move freely into the wastewater. In a biofilm process, dissolved organic materials and nutrients are directly absorbed from bulk phase to the biofilm by means of concentration gradient, where dissolved heavy metals are adsorbed onto and into biofilm as a result of interactions between metal ions and the negatively charged microbial surfaces, gradually reducing the aqueous metal concentration (Abu-Bakar *et al*., 2008;Rahman*et al*., 2013).

Microbial biomass can bind heavy metals either actively or passively or by a combination of both processes. Several studies have shown the effects of metal concentration on bacteria resistance to heavy metals and many of these research have proven that high resistance is related to high metal concentration. This means that there is a positive correlation between the metal concentration and bacterial tolerance to heavy metals and it can be effective to have access to a higher efficiency in biological purification of contaminated soils and wastewater samples (Nasrazadani *et al*., 2011).

This study was aimed to remove colour and heavy metals from industrial wastewaters using sequencing batch biofilm reactor.

Materials and Methods

Samples collection

Two selected wastewater samples from dry cell battery factory and nickelplating factory were collected from Industrial zone I in Pyigyitagun Township, Mandalay Region (Figure 1). Another wastewater sample from leather factory was collected from Industrial zone II in Amarapura Township, Mandalay Region (Figure 2). Location sites are shown in Figure 3.

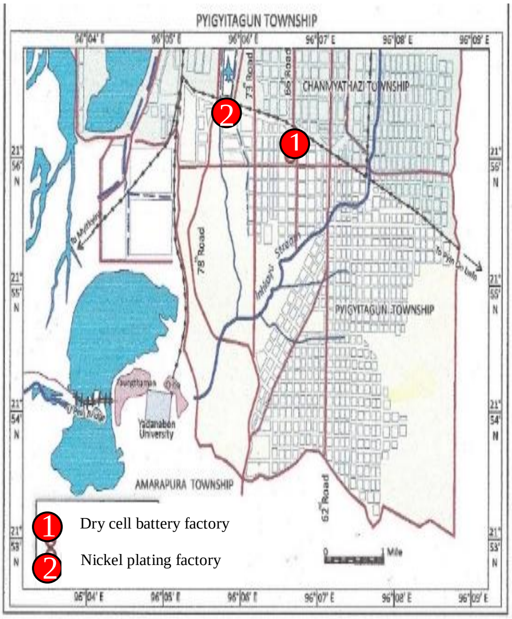


Figure 1: Location map of the study area (1)

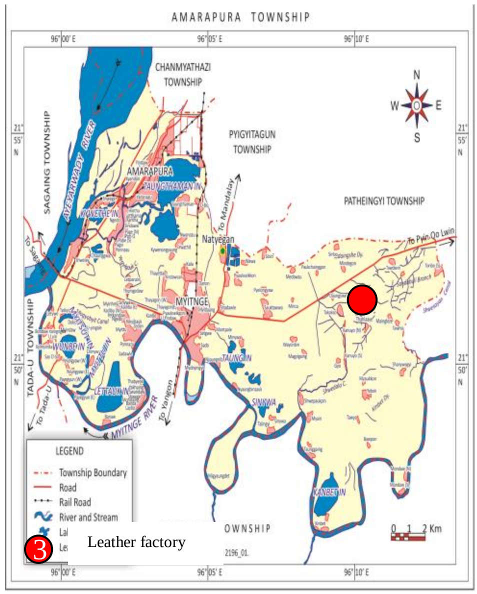


Figure 2: Location map of the study area (2)

(a) (b) (c)

Figure 3: (a) Dry cell battery factory, (b) Nickel plating factory and (c) Leather factory and their drainages

Wastewater Treatment by Using Sequencing Batch Biofilm Reactor

The sequencing batch biofilm reactor technology is a modification of the much popular activated sludge process (ASP) (Dutta and Sarkar, 2015). It was applied the wastewater treatment using with bacterial biofilm. For reducing the colour and toxic metal ions from wastewater effluent, sequencing batch biofilm reactor was used and detected by the activity of indigenous bacterial isolates and thus,is considered to be a suitable system for wastewater treatment in pilot scale. In this study, sequencing batch biofilm reactor was fabricated by steel with the dimension of 33 cm height, 26 cm diameter, total volume capacity of 17.7 L, and working volume of 14 L. The speed of impeller was adjusted at 220 rpm. There are three outlets (1, 2 and 3) in the reactor. Details of the reactor design are described in Table 1 and the reactor and its parts are shown in Figure 4.

Table 1 Reactor Configuration

Specification	Quantity
Diameter of reactor (cm)	26
Height of reactor (cm)	33
Total volume in reactor (L)	17.7
Working volume in reactor (L)	14
Type of support materials used in reactor	polyurethane sponges
Size of polyurethane sponges (cm ×cm × cm)	2.54 ×2.54 × 2.54
Number of polyurethane sponges	250
Total volume of polyurethane sponges (mL)	3769
Impeller speed (rpm)	220
Air flow rate	0.69 dmL/min

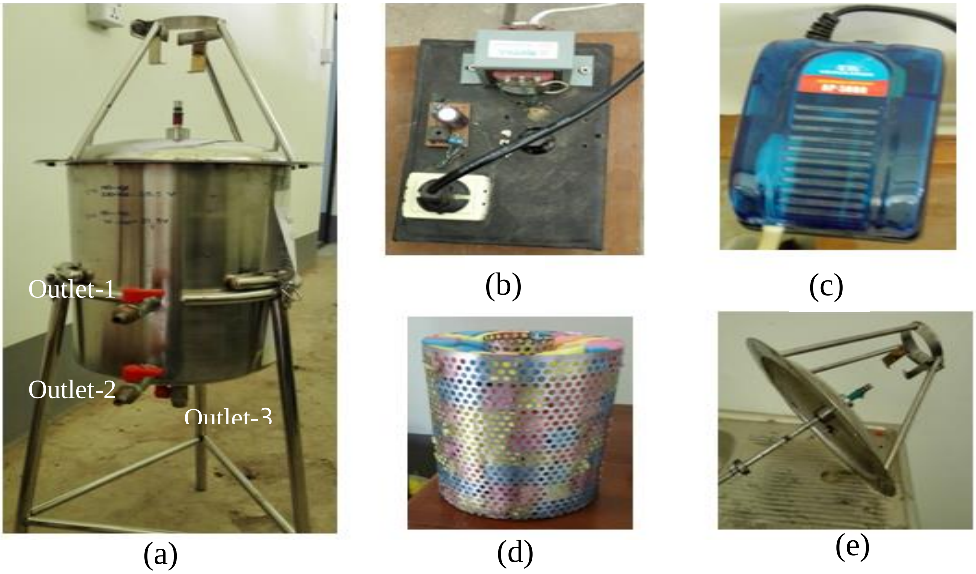


Figure 4: (a) Sequencing batch biofilm reactor and its parts used in this study (b) Impeller speed controller, (c) Air compressor, (d) Solid support container (e) Impeller

Isolation of Bacteria from the Wastewater Samples

To cultivate bacteria from wastewater samples, 1 mL of wastewater sample was directly cultured on nutrient agar in the sterile petri dish. It was incubated overnight at 37ºC. Numerous colonies with different morphologies appeared on the agar plate were obtained. The distinct clear colonies were collected and then, they were separately sub-cultured on nutrient medium to get the pure culture. Then, microscopic morphology of the bacterial isolates were determined using Gram's staining method.

Preparation of Media for Broth Culture

Firstly, 750 mL of distilled water was added in a 800 mL beaker containing 7.5 g of nutrient broth. The beaker was shaken with thermostatic control magnetic stirrer for 10 minto mix the ingredients thoroughly. The same solution was prepared to get 1500 mL nutrient broth. The flasks were plugged with cotton wool, sealed with aluminum foil and then sterilized in an autoclave at 121ºC for 15 min under 15 psi pressure. After cooling the broth solution at room temperature, single colony of selective four bacterial strains were added. Before use, the bacterial strain from the plate was checked by Gram's staining method. Finally, the flasks were incubated at 37 ºC for 48 h. It was used as the mother culture.

Preparation of Stock Solution

$(NH_4)_2SO_4$ (0.75 g), glucose (15 g), yeast (30 g), molasses (750 mL) and distilled water (15 L) were mixed into the pot. Then, the solution was heated for boiling. After cooling the solution at room temperature, it was sterilized by autoclaving at 121ºC for 30 min.

Wastewater Treatment by Sequencing Batch Biofilm Reactor

At first, 250 numbers of polyurethane sponges (size: 2.54 cm × 2.54 cm × 2.54 cm) were filled into the steel solid support container. Then it was sterilized in an autoclave at 121 ºC for 30 min. The mother culture including four bacterial strains was then introduced into the reactor with the ratio of 1:10 culture broth and stock solution. After 3 days incubation for bacteria, the cultured broths in the reactor were drawn out from the sludge disposal. After that, the reactor was filled with the wastewater for the treatment. The treated samples were withdrawn at 4h intervals from the outlet 2. The decolourization activity was measured at 450 nm wavelength by UV spectrophotometer. Moreover, atomic absorption spectrophotometer was used for determination of metal ions (Cd^{2+}, Cr^{3+}, Pb^{2+}) from wastewater samples. The percent decolourization and the removal percentage of metal ions (Cd^{2+}, Cr^{3+}, Pb^{2+}) were computed.

Determination of some Physicochemical Parameters of Three Industrial Wastewater Samples

In this experiment, some physicochemical parameters such as pH, turbidity, total dissolved solids (TDS), dissolved oxygen (DO), chemical oxygen demand (COD) and biological oxygen demand (BOD) of industrial wastewater samples before and after treatment with sequencing batch biofilm reactor were analyzed according to standard analytical procedures (Government of India and Government of the Netherlands, 1999; PASCO,

2010) at Ministry of Agriculture, Livestock and Irrigation Department of Fisheries Aquaculture Division, Freshwater Aquaculture Research Water and Soil Examination Laboratory in Yangon.

Results and Discussion

Isolation and Identification of Bacteria from the Wastewater Sample

Totally four bacterial strains (two bacterial strains from wastewater sample of dry cell battery factory, P_1 and P_2, one bacterial strain from wastewater sample of nickel plating factory, $N_5$and one bacterial strain from wastewater sample of leather factory, L) were isolated. These isolated four bacterial strains were identified by Gram's staining reaction, colonial morphology and microscopic morphology. Colonial morphology and microscopic morphology of isolated strains are shown in Figure 5.

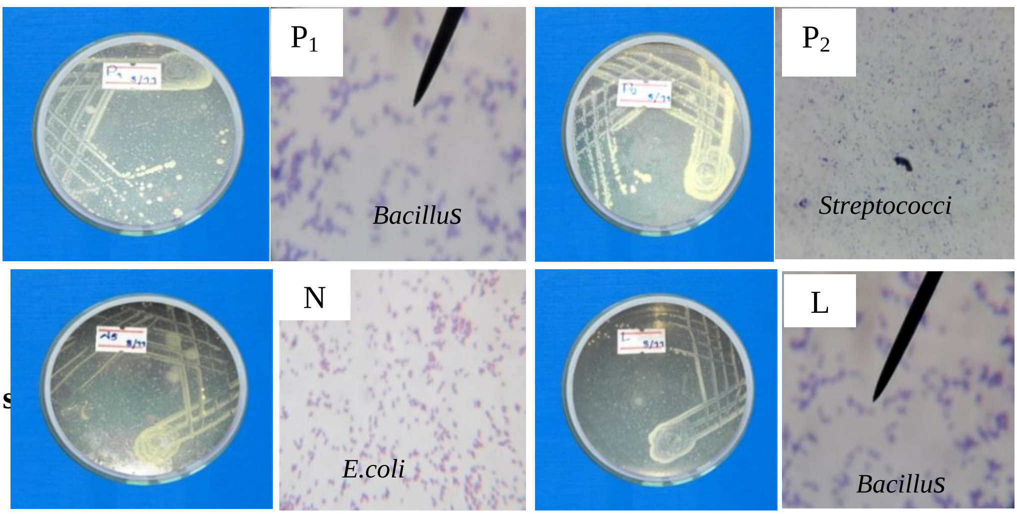


Figure 5: Colonial and microscopic morphology of the bacterial isolate P_1, P_2, $N_5$and L on nutrient media from three industrial wastewater

According to morphology study, two bacterial strains (P_1, P_2) from wastewater sample of dry cell battery factory and one bacterial strain (L) from wastewater sample of leather factory were gram positive (+). Another one bacterial strain (N_5) from wastewater sample of the nickel plating factory was found to be gram negative (-). Among them, one gram positive (+) from wastewater samples of dry cell battery factory (P_1)and leather factory (L) were found to be *Bacillus*. Another gram positive (+) from wastewater sample of the dry cell factory (P_2) was found to be *Streptococci*. One gram negative (-) from wastewater sample of nickel plating factory (N_5) was *E.coli*.

Bacterial Biofilms

The biofilm of each bacterial isolate was prepared for wastewater treatment as shown in Figure 6.

(a) (b) (c)

Figure 6: Process of biofilm (a) Spongy in initial broth culture P_1, P_2, N_5, L (b) Broth culture after 2 days (c) Four biofilms

The sponge and the adsorption of each isolated bacterial strain on sponge under microscopic investigation are shown in Figures 7 and 8 respectively. The adsorption of isolated bacterial strains (P_1, P_2, N_5 and L) on sponge showed different morphologies under microscopic investigation.

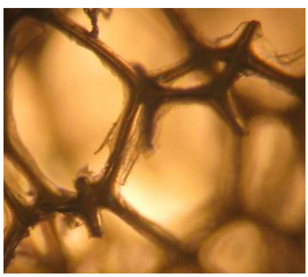
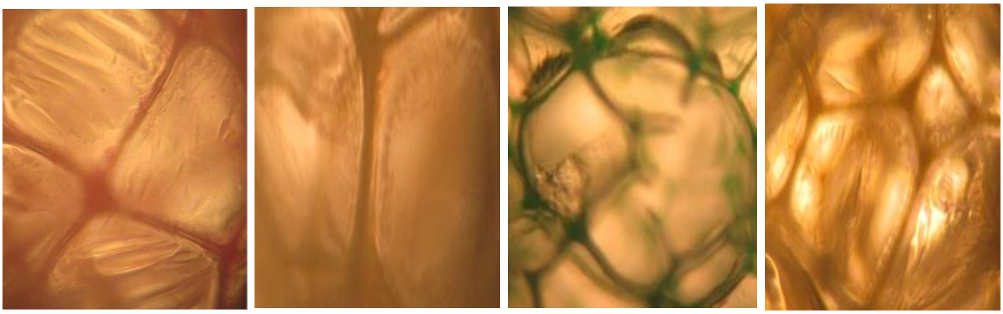


Figure 7: Sponge only under microscopic investigation



(a) (b) (c) (d)

Figure 8: Bacterial adsorption on sponge after 2 days incubation under microscopic investigation (a) *Bacillus* sp. (P_1) (b) *Streptococci* sp. (P_2) *(c) E.coli* (N_5) and (d) *Bacillus* sp. (L)

Wastewater Treatment by Using Sequencing Batch Biofilm Reactor

The decolourization activity and the removal percentage of metal ions after wastewater treatment by using sequencing batch biofilm reactor were investigated in this study. Firstly,the wavelengths of maximum absorption for industrial wastewater samples were determined.As shown in Table 2 and Figure 9, the wavelengths of maximum absorption for industrial wastewater samples from dry cell battery factory, nickel plating factory and leather factory were observed at 450 nm. Thus, the wavelength of 450 nm was chosen for the determination of decolourization activity.

Table 2: Relationship between Absorbance and Wavelength for Three Industrial Wastewater Samples from Factories

Wavelength (nm)	Absorbance of wastewater		
	Dry cell battery factory	Nickel plating factory	Leather factory
400	0.570	0.501	1.23
450	0.665	0.644	1.40
500	0.407	0.388	1.35
550	0.105	0.045	1.27
600	0.365	0.354	1.21
650	0.362	0.354	1.01

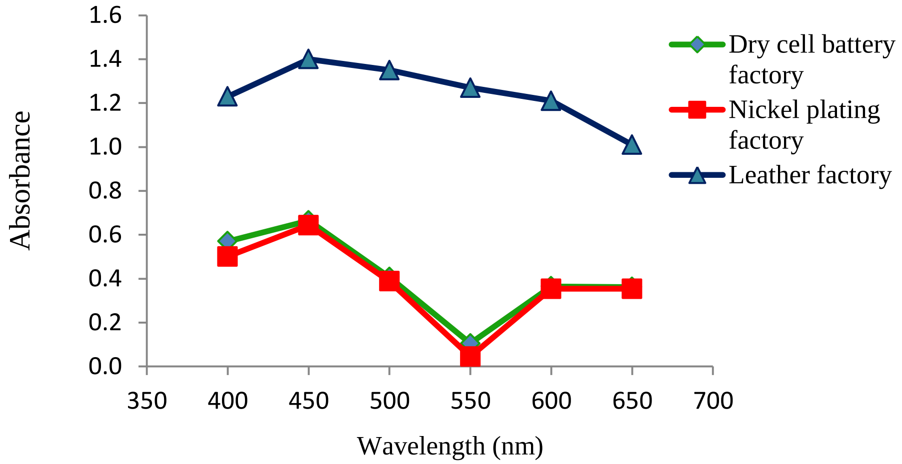


Figure 9: Plot of absorbance as a function of wavelength for three industrial wastewater samples

Decolourization of Wastewater Samples by Using Sequencing Batch Biofilm Reactor

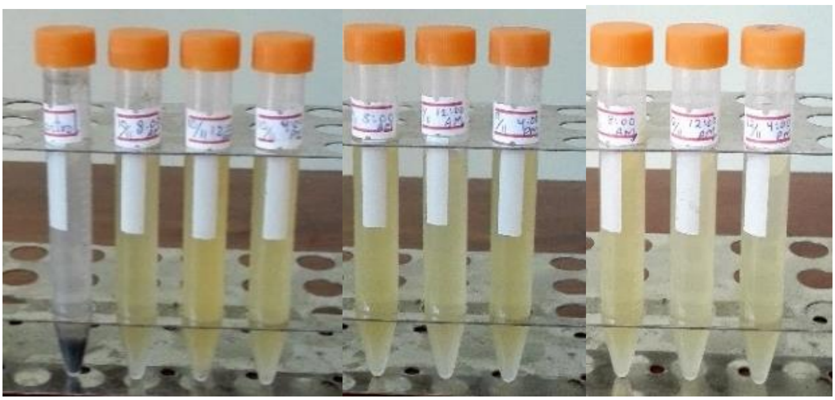
Sequencing batch biofilm reactor was used for the decolourization of the wastewater samples from dry cell battery factory,nickel plating factory and leather factory.

As shown in Table 3 and Figure 10, sequencing batch biofilm reactor could decolourize54.29 % of the wastewater sample of dry cell battery factory after72 h treatment. Moreover, highest decolourization percent was achieved at 74.13% after 64 h treatment for wastewater sample of nickel plating factory as shown in Table 4. The wastewater from leather factory after treatment with sequencing batch biofilm reactor was decolourized53.32 % after 64 h treatment (Table 5).

Table 3: Percent Decolourization of Wastewater Sample of Dry Cell Battery Factory by Using Sequencing Batch Biofilm Reactor

No	Time (h)	Absorbance of treated sample	% Decolourization
1	16	0.408	38.64
2	20	0.208	53.68
3	24	0.424	36.24
4	40	0.314	52.78
5	44	0.458	31.13
6	48	0.350	46.77
7	64	0.107	53.83
8	68	0.320	51.87
9	72	0.104	54.29

Absorbance of control = 0.665



(a) (b) (c) (d) (e) (f) (g) (h) (i) (j)

Figure 10: Decolourization of wastewater sample from dry cell battery factory by using sequencing batch biofilm reactor
(a) Control (b) 16 h (c) 20 h (d) 24 h (e) 40 h (f) 44 h (g) 48 h (h) 64 h (i) 68 h and (j) 72h after treatment

Table 4: Percent Decolourization of Wastewater Sample of Nickel Plating Factory by Using Sequencing Batch Biofilm Reactor

No	Time (h)	Absorbance of treated sample	% Decolourization
1	16	0.191	44.76
2	20	0.142	58.72
3	24	0.122	64.53
4	40	0.096	72.09
5	44	0.095	72.38
6	48	0.094	72.67
7	64	0.089	74.13
8	68	0.093	72.96
9	72	0.191	72.67

Absorbance of control = 0.344

Table 5: Percent Decolourization of Wastewater Sample of Leather Factory by Using Sequencing Batch Biofilm Reactor

No	Time (h)	Absorbance of treated sample	% Decolourization
1	16	1.022	27.00
2	20	0.941	32.79
3	24	0.773	44.78
4	40	0.822	41.29
5	44	0.685	51.07
6	48	0.701	49.93
7	64	0.652	53.32
8	68	0.815	41.78
9	72	0.802	42.71

Absorbance of control = 1.40

Removal of Metal Ions from Wastewater Samples by Using Sequencing Batch Biofilm Reactor

As shown in Table 6 and Figure 11, sequencing batch biofilm reactor could remove metal ions, 21.48 % of Cd^{2+}, 47.72 % of Cr^{3+} and 45.60 % of Pb^{2+} from dry cell battery factory wastewater sample after 72 h treatment. Moreover,sequencing batch biofilm reactor **c**ould remove metal ions,35.76 % of Cd^{2+}, 51.97 % of Cr^{3+} and 42.96 % of Pb^{2+} from nickel plating factory wastewater sample after 72 h treatment (Table 7 and Figure 12). The results from Table 8 and Figure 13 revealed that the bacterial biofilm could reduce 20.33 % of Cd^{2+}, 62.63 % of Cr^{3+} and 40.75 % of $Pb^{2+}$from leather factory wastewater sample after 72 h treatment.

Table 6: Removal Percentage of Cd^{2+}, Cr^{3+} and Pb^{2+} Ions from Wastewater Sample of Dry Cell Battery Factory by Using Sequencing Batch Biofilm Reactor

Metal ions	Untreated (mg/L)	Treated (mg/L)	Removal of metal ions (%)
Cd^{2+}	0.256	0.201	21.48
Cr^{3+}	0.352	0.184	47.72
Pb^{2+}	0.364	0.198	45.60

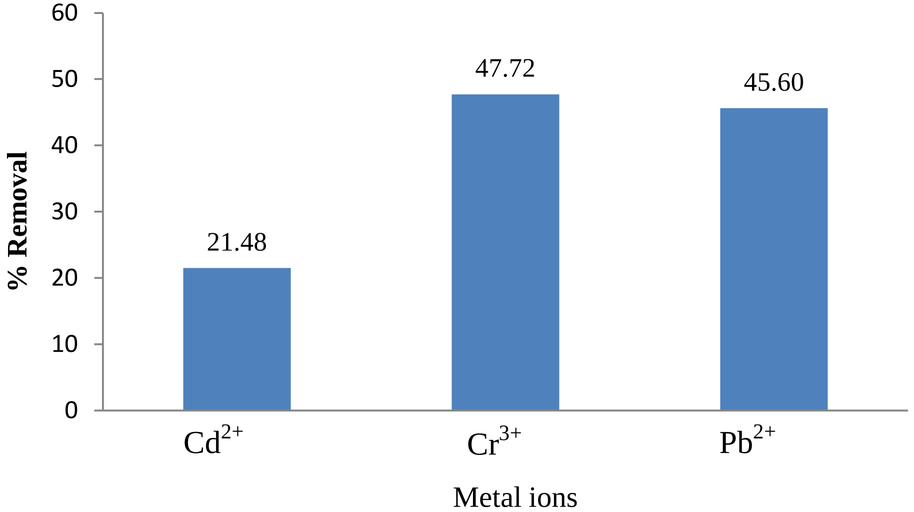


Figure 11: Removal percentage of Cd^{2+}, Cr^{3+} and Pb^{2+}ions from wastewater sample of dry cell battery factory by using sequencing batch biofilm reactor

Table 7: Removal Percentage of Cd^{2+}, Cr^{3+} and Pb^{2+} Ions from Wastewater Sample of Nickel Plating Factory by Using Sequencing Batch Biofilm Reactor

Metal ions	Untreated (mg/L)	Treated (mg/L)	Removal of metal ions (%)
Cd^{2+}	0.316	0.203	35.76
Cr^{3+}	0.356	0.171	51.97
Pb^{2+}	0.284	0.162	42.96

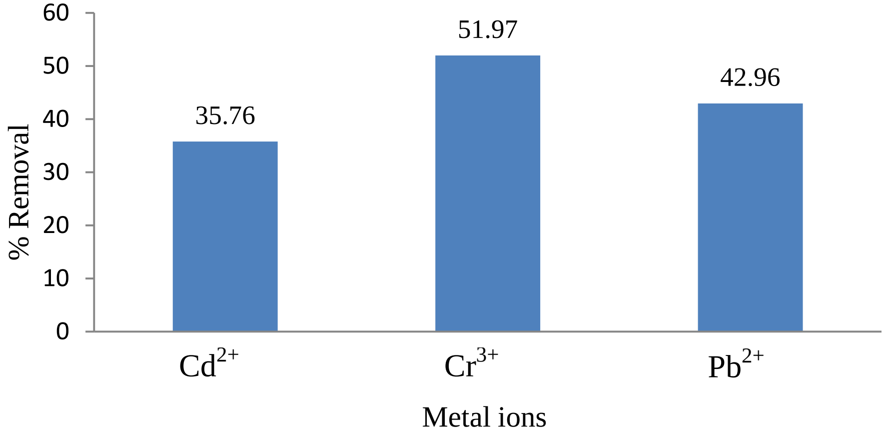


Figure 12: Removal percentage of Cd^{2+}, Cr^{3+} and Pb^{2+} ions from wastewater sample of nickel plating factory by using sequencing batch biofilm reactor

Table 8: Removal Percentage of Cd^{2+}, Cr^{3+} and Pb^{2+} Ions from Wastewater Sample of Leather Factory by Using Sequencing Batch Biofilm Reactor

Metal ions	**Untreated (mg/L)**	**Treated (mg/L)**	**Removal of metal ions (%)**
Cd^{2+}	0.246	0.196	20.33
Cr^{3+}	0.372	0.139	62.63
Pb^{2+}	0.265	0.157	40.75

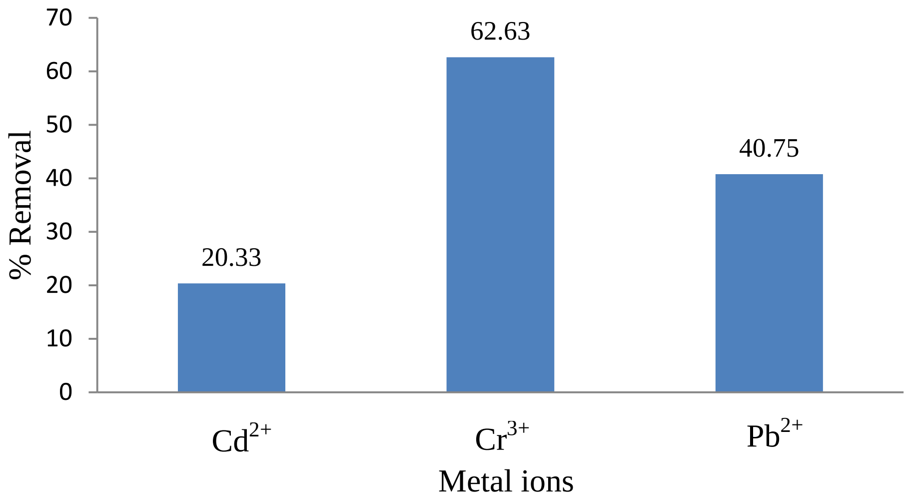


Figure 13: Removal percentage of Cd^{2+}, Cr^{3+} and Pb^{2+}ions from wastewater sample of leather factory by using sequencing batch biofilm reactor

Some Physicochemical Parameters of Three Industrial Wastewater Samples before and after Treatment by Using Sequencing Batch Biofilm Reactor

Some physicochemical parameters such as pH, turbidity, total dissolved solids (TDS), dissolved oxygen (DO), chemical oxygen demand (COD) and biological oxygen demand (BOD) of industrial wastewater samples before and after treatment by using sequencing batch biofilm reactorwere determined and the results are shown in Table 9.

The pH value of untreated wastewater samples from dry cell battery factory, nickel plating factory and leather factory were 8.0, 7.7 and 8.8 respectively. After treatment with sequencing batch biofilm reactor, the pH values of the wastewater samples were reduced to 7.6, 6.9 and 8.5 respectively.

The total dissolved solid (TDS) values of the untreated dry cell battery factory, nickel plating factory and leather factory were found to be 664 mg/L, 828 mg/L and 8588 mg/L respectively, however,after treatment with sequencing batch biofilm reactor TDS values were reduced to 330mg/L, 470 mg/L and 4152 mg/L. About half of the initial TDS values were reduced in wastewater from factories.

The turbidity of untreated wastewater samples from dry cell battery factory, nickel plating factory and leather factory were 101 NTU, 153 NTU and 883 NTU, respectively. After treatment with sequencing batch biofilm reactor the turbidity values of wastewater samples were reduced to greater than half of the initial values (52 NTU, 85 NTU and 313 NTU, respectively) of dry cell battery, nickel plating and leather factories).

Before treatment, the DO values of wastewater samples from dry cell battery factory, nickel plating factory and leather factory were respectively, found to be 1.48 mg/L, 1.47 mg/L and 0.95 mg/L. After treatment with sequencing batch biofilm reactor the DO values of treated wastewater samples from dry cell battery factory, nickel plating factory and leather factory were found to be 3.10mg/L, 2.89 mg/L and 2.20 mg/L, respectively. These DO values of three wastewater samples were found to increase after treatment.

Before treatment, COD values of wastewater samples from dry cell battery factory, nickel plating factory and leather factory were found to be 161 mg/L, 502 mg/L and 3538 mg/L, respectively. After treatment with sequencing batch biofilm reactor COD values of wastewater samples from dry cell battery factory, nickel plating factory and leather factory were found to decrease to 75 mg/L, 215 mg/L and 1230 mg/L, respectively.

In this study, the BOD values of untreated wastewater samples from dry cell battery factory, nickel plating factory and leather factory were 703 mg/L, 340 mg/L and 1875 mg/L, respectively. BOD values of the treated wastewater samples with sequencing batch biofilm reactor were found to reduce to 352 mg/L, 152 mg/L and 785 mg/L, respectively, for dry cell battery, nickel plating and leather factories.

Table 9: Some Physicochemical Parameters of Three Industrial Wastewater Samples before and after Treatment by Using Sequencing Batch Biofilm Reactor

Parameter	Unit	Dry cell battery		Nickel plating factory		Leather factory	
		Untreated	Treated	Untreated	Treated	Untreated	Treated
pH	-	8.0	7.6	7.7	6.9	8.8	8.5
TDS	mg/L	664	330	828	470	8588	4152
Turbidity	NTU	101	52	153	85	883	313
DO	mg/L	1.48	3.10	1.47	2.89	0.95	2.20
COD	mg/L	161	75	502	215	3538	1230
BOD	mg/L	703	352	340	152	1875	785

Conclusion

Four bacterial strains from wastewater samples were isolated and used as biofilm in the reactor in this study. Analysis for decolourization activity by spectrophotometer yielded that sequencing batch biofilm reactor could decolourize 54.29% of wastewater sample of dry cell battery factory after 72 h treatment, 74.13% of wastewater sample of nickel plating factory and 53.32% of wastewater sample of leather factory after 64 h treatment. After 72 h treatment, atomic absorption spectrophotometer analysis showed that sequencing batch biofilm reactor could reduce highest percentages of chromium ion (47.7 % to 62.63 %) followed by lead ion (40.75 % to 45.60 %) and cadminum ion (20.33 % to 35.76 %). According to the experimental data, the sequencing batch biofilm reactor could be applied in the wastewater treatment for removal of Cr^{3+} and Pb^{2+} ions. Moreover, some physicochemical parameters such as pH, turbidity, total dissolved solids (TDS), chemical oxygen demand (COD) and biochemical oxygen demand (BOD) of three industrial wastewater samples were found to decrease after treatment by using sequencing batch biofilm reactor. Thus,sequencing batch biofilm reactor could be effectively applied for the wastewater treatment.

Acknowledgements

The authors would like to thank the Myanmar Academy of Arts and Science for allowing to present this paper and Professor and Head Dr Ye Myint Aung, Department of Chemistry, Pathein University for his kind encouragement.

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