International Journal of Advances in Bio-Informatics and Bio-Technology– IJABB
Volume 1 : Issue 2 *[ISSN 2475-2258]*
Publication Date : 30 September, 2014

Bioaugmentation of Xenobiotic Treatment Activated Sludge during Start-Up and Shock Loadings

Nyuk-Min Chong**;** Jie Liang; Kuei-Yen Lin

***Abstract*—Indigenous microbial biomass, such as the municipal wastewater treatment activated sludge, needs a prolonged acclimation period before the sludge can degrade a xenobiotic organic compound. During acclimation, some of the sludge microorganisms are converted into degraders by acquiring specific genetic coding for metabolism of the xenobiotic organic. At activated sludge system start-up and during xenobiotic shocks, activated sludge microorganisms may not have been acclimated to the newly introduced xenobiotic and thus may fail to treat the influent xenobiotic. The purpose of this study was to investigate the benefits of bioaugmentation to activated sludge during the vulnerable times of xenobiotic treatment start-up and shock-loading. Laboratory activated sludge reactors fed biogenic organics were operated and start-up and shock-loading tests were performed with the steady state activated sludge. 2,4-dichlorophenoxyacetice acid (2,4-D) replaced biogenic influent in the start-up and shock-loading tests, to which 2,4-D influent up-times were continuous and square-wave, respectively. The times required to successfully degraded 2,4-D were compared between a control and a test system, which was augmented with activated sludge previously acclimated to 2,4-D. Test results showed that bioaugmentation was instrumental in shortening start-up and shock recovery times. The start-up times shortened were proportional to the amount of acclimated biomass augmented.**

***Keywords*—Bioaugmentation, activated sludge, xenobiotic, start-up, xenobiotic shock**

I. Introduction

Xenobiotic organic compounds, most of which are man-made through complicated processes into which high amount of energy were input, are stable to chemical and biological decomposition [1]. As a result, most xenobiotic pollutants are persistent, or difficult to treat. Fortunately, majority of xenobiotic organic compounds can become biodegradable after a bacterial population has gained degradation capability through the process of acclimation [2, 3]. After a successful acclimation, some or all microbial cells develop the capability of xenobiotic degradation and the cell previously indigenous to the xenobiotic are converted into degraders [4]. Acclimation of ordinary or indigenous activated sludge thus may be used to obtain the required degraders for the degradation and thus treatment of the target xenobiotic pollutant(s).

When a xenobiotic occurs in the waste-stream of a wastewater treatment plant of any township, the efficiency of an activated sludge treatment plant in treating xenobiotic influent depends on the sludge's capability to degrade the xenobiotic. The capability can be described by the quantity of degrader (capable sludge) present in the sludge [4]. There are instances in which a treatment plant fails its xenobiotic treatment because the plant contains insufficient xenobiotic degraders: 1) During activated sludge start-up for xenobiotic treatment, the biogenic grown (indigenous) activated sludge had not been acclimated; 2) at periodic xenobiotic influent, the existing sludge may be all indigenous, or some microorganisms were previously capable but now have de-acclimated or out-dominated during the time when the xenobiotic was absent from the influent. To improve the performance of an activated sludge plant, bioaugmenting the plant is found effective with microbial biomasses that possess the specialized characteristics, including the capacity to treat xenobiotic pollutants. Study reports on bioaugmentation that showed positive results are found which attest bioaugmentation as a viable technique for the treatment of xenobiotic containing wastewater. Example references that show positive results using bioaugmentation are cited: improvement in hazardous chemical degradation [5], enhancement of target compound removal and more rapid re-acclimation to a toxic compound [6], tolerance against the toxic shock loads [7, 8], and improvement of recovery after toxic exposure [9]. Other bioaugmentation benefits are also reported: counteraction of sludge bulking [10]; adsorption of metal [8], treatment of a pollutant at low concentration [6, 11], rapid start-up and stable performance of municipal wastewater treatment [12], and increased pH tolerance [13].

When the questions on the activated sludge xenobiotic treatment efficiency are addressed, the quantity of degrader grown and maintained in the activated sludge reactor is often the main issue that needs careful examination. Cases or situation in which degraders are lacking or insufficient must be corrected. Among the methods of such correction, external supply of degrader, or bioaugmentation, can be the simplest. Degraders can be converted from ordinary/indigenous activated sludge biomass by acclimation. The quantity of degrader converted may vary with culturing conditions and the nature of their previous encounter with the xenobiotic compound [14, 15]. One of the cultivation methods for degrader is the multiple-time acclimation and degradation of the target xenobiotic. The batch-reactor acclimation method renders a simple and efficient way for degrader cultivation.

The purpose of this study was to examine the usefulness of bioaugmentation to a continuous-flow activated sludge system during its xenobiotic treatment start-up and during xenobiotic shock loading. The augmentation biomass used was a degrader- containing activated sludge that was cultivated from

Nyuk-Min Chong*; Jie Liang; Kuei-Yen Lin
DaYeh University
Taiwan

batch acclimation to the xenobiotic. The benefits of such bioaugmentation were observed for shortening start-up and shock recovery times and the possible increase of degradation rate.

II. Materials and Methods

A. Preparation of Augmentation Biomass

The initial activated sludge seed was obtained from a soil that did not have any record of xenobiotic nor metal (slag) contamination. The mixed culture from soil was grown to a suitable amount on Nutrient Broth (NB Difco 234000) for at lease three (3) subcultures in multiple shake-flasks. The shake-flask biomass was used to seed an indigenous sludge cultivation reactor that was operated in a fed-batch mode. The feed to the fed-batch reactors contained biogenic substrates of sugar (100 mg/l) and peptone (25 mg/l) and minerals: $FeCl_3$, 1.2 mg/l; $CaCl_2$, 12 mg/l; $MgSO_4.7H_2O$, 65 mg/l; NH_4Cl, 125 mg/l; K_2HPO_4, 200.0 mg/l; and KH_2PO_4, 156.6 mg/l.

The xenobiotic compound used as the activated sludge treatment target was 2,4-dichlorphenoxyacetic acid (2,4-D). The biomass used for bioaugmentation was prepared from activated sludge harvested from the fed-batch reactor. The biogenic grown activated sludge was placed in acclimation reaction with 2,4-D. Acclimation was performed in batch type reactors (shake flasks). Culture medium contained mineral nutrients listed above and 2,4-D as the sole carbon and energy source. Acclimation to 2,4-D was intensive with the sludge undergoing acclimation and degradation of 2,4-D for three (3) consecutive times: After a complete degradation, supernatant was discarded and 2,4-D and nutrients were replenished and 2,4-D was allowed to be degraded. The 2,4-D acclimated sludge was designated as A-Sludge.

B. Operations of Experimental Activated Sludge

Experimental activated sludge of the continuous-flow-with-cell-recycle type (CSTR) were operated. The CSTRs were operated with a mean-cell-residence-time (θc) of 10 days and hydraulic residence time (θ) of 8 hours. Ordinarily, the CSTRs were fed an influent (feed media) that contained biogenic substrates and nutrients similar to those listed above for the fed-batch reactor. Influent was introduced to each reactor by an adjustable-speed peristaltic laboratory pump (Cole-Parmer Master-flex) through silicone tubing. Flowrate into a reactor was adjusted to achieve the required θ; θc was achieved by wasting sludge twice a day from the mixed reactor contents; amount of sludge (reactor liquid) wasted each time was $\frac{1}{2}\times\frac{1}{\theta_c}$ of the reactor volume. The reactor liquid volume was refilled to the original level with the clear effluent.

C. Measurements

Method of measurement of 2,4-D was by HPLC. Suspensions with activated sludge solids were filtered (through Millipore Millex GS, pore size 0.22 m). 2,4-D concentrations in the filtrates were measured using HPLC (Agilent 1200 Series). The column used was reverse phase C-18, length 250 mm and ϕ 4.6 mm, with particle size 5µm (Phenomenex Luna 00G-4041-E0). The mobile phase consisted of acetonitrile (CH_3CN) in H_2O (80% v/v), pH adjusted to 4.0 with concentrated H_3PO_3. Flow rate was 1 ml/min. Sample injection volume was 20 µl. Retention time was 3.5-3.8 minutes. 2,4-D was detected by a UV detector at 283 nm.

The concentrations of activated sludge in A-Sludge and in the CSTR measured as suspended solids (SS). Activated sludge samples were filtered through fiber-glass filters (Whatman GF/C), from which SS was determined from the filterable portion after drying (filtered and dried at 103-105°C), according to Standard Methods of SM2540-D [16].

D. Bioaugmentation Schemes

Laboratory activated sludge CSTRs were operated with the influent of biogenic substrates during ordinary time. Steady states were obtained for each CSTR. Start-up tests for xenobiotic treatment were operated with the ordinary CSTR by replacing biogenic influent with 2,4-D, which was input continuously until 2,4-D was considered completely degraded. A shock test was applied to the ordinary CSTR with a single or multiple square-wave input of 2,4-D and a duration of up-time was maintained before the down-time. Bioaugmentation schemes applied to activated sludge system start-ups and shock loadings are listed in Table 1. The performances in 2,4-D treatment of the control and the bioaugmented reactors were compared.

TABLE I. EXPERIMENTAL DESIGN FOR XENOBIOTIC TREATMENT START-UP AND XENOBIOTIC SHOCK LOADING USING BIOAUGMENTATION

	Up-down pattern	*Up-time 2,4-D (mg/l)*	*Bioaugmentation*	*Designation*
Start-up	Continuous up	100	Control (not augmented)	UC
			100 at 0 hr	UB1
			100 at 0 hr, 1, 2, and 3 d	UB1S
			200 at 0 hr	UB2
Shock (square wave)	Single wave: 8 hr up	50	not augmented	SC1
			100 at 0 hr	SB1
	Double wave: 4 hr up-8 hr down-4 hr up		not augmented	SC2
			100 at 0 hr	SB2

a. Augmented with A Sludge to make the concentration (mg/l) in the reactor.

III. Results and Discussion

A. System Start-up

The performance of the activated sludge reactors after xenobiotic 2,4-D became the new influent is shown in Fig. 1a. When 100 mg/l of 2,4-D was continuously influented to the activated sludge reactors, the biogenic cultured (indigenous) activated sludge had not the ability to degrade 2,4-D in the initial period that lasted for several days. For the un-augmented reactors (UC), 2,4-D behaved simply like a non-reactive compound being input into the reactor; 2,4-D concentration in UC reactor increased exponentially and then remained at a constant value of approximately that of the influent. Because activated sludge have the ability to acclimate to 2,4-D after the initial lag time, 2,4-D concentration in the UC effluent was found to decrease to an undetected level after 6 days. This 2,4-D curve shows that, when a xenobiotic is newly input to a continuous-flow activated sludge, the sludge would acclimate to the xenobiotic in a pattern similar to that of a batch-type acclimation.

Fig. 1a also shows that when activated sludge that was previously acclimated to 2,4-D (A-Sludge) was supplemented at start-up, degradation of 2,4-D could occur without an obvious acclimation delay. A-Sludge harbors certain amount of degradative capacity. The performance of A-Sludge indicates that the acclimated sludge was useful in extending its capability and started to degrade the influent 2,4-D at the early stage, while comparatively, the existing indigenous sludge must acclimate to 2,4-D in the reactor. The shortening of start-up time was about 1 day at the augmentation of 100 mg/L of A-Sludge.

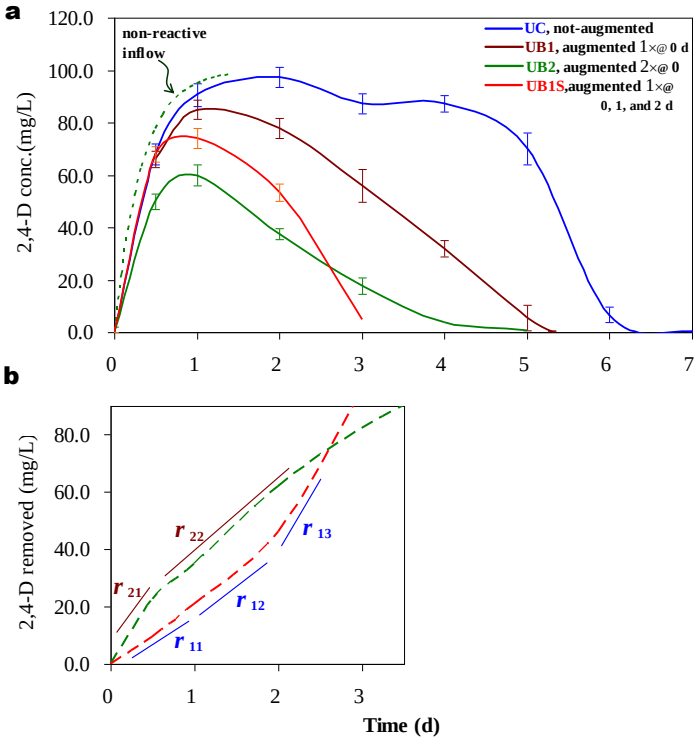

Figure 1. Performance of activated sludge during 2,4-D treatment start-ups, showing the benefits of bioaugmentation. a) 2,4-D concentration in effluent; b) 2,4-D removed (theoretical minus reactor concentrations), indicating rates of 2,4-D removal, by bioaugmentation schemes of UB1S and UB2 (See Table 1 for reactor designations).

When A-Sludge was augmented in higher amount, its degradation performance was shown to have extra benefit. The rates of 2,4-D degradation by the one-time (UB2) and the serial (UB1S) augmentation schemes is shown in Fig. 1b. Bioaugmentation with 2 times (2×) A-Sludge concentration showed an initial degradation rate (r_{21}) of approximately twice that augmented with 1 time (1×) A-Sludge concentration (r_{11}). Furthermore, serial additions of A-Sludge increased degradation rate every time augmentation was made. Degradation rate increased at the 3rd augmentation (r_{13}) to one higher than the one-time 2× augmentation such that the overall start-up time was further shortened. It is logical that degradation rate increased with the amount of degrader present in the reactor. Dynamic model for the calculations of degradation rate of A-Sludge that is mixed with indigenous sludge requires mathematical formulations that involve reactor flow configurations, acclimation process of the indigenous sludge, and most importantly, the quantity of degrader that was initially present in A-Sludge.

The bioaugmentation schemes using acclimated sludge prove a feasible method during a xenobiotic treatment start-up.

B. Shock Loading Recovery

Fig. 2 shows the recoveries from 2,4-D leaks at shock-loadings of 2,4-D. Fig. 2a shows that under a sudden input of 2,4-D that lasted for 8 hr, the un-augmented reactor (SC1) had a 2,4-D curve much in resemblance to that of a non-reactive compound being diluted out from a CSTR. This 2,4-D curve shows that there must be very little, if any, degradation of 2,4-D by the indigenous sludge. By comparison, the augmented reactors (SB1) showed its 2,4-D degradation as early as the 4th hr. The early accumulation of 2,4-D was because rate of input was larger than the degradation rate that could be exerted by the augmented biomass; degradation was prominent after augmentation had gradually counteracted the rate of accumulation of 2,4-D from the influent.

As shown in Fig 2b, recovery from the double-wave shocks were also assisted by the augmentation of acclimated sludge. Degradation was almost completed in 8 hr after 2,4-D influent ceased. Increased degradation rate by A-Sludge in the SB2 reactors resulted in an effluent curve that had a lower 2,4-D concentration compared to the curve of SC1 reactor during all time.

Again, the augmentation schemes using acclimated sludge prove a feasible method for remediation of xenobiotic shocks.

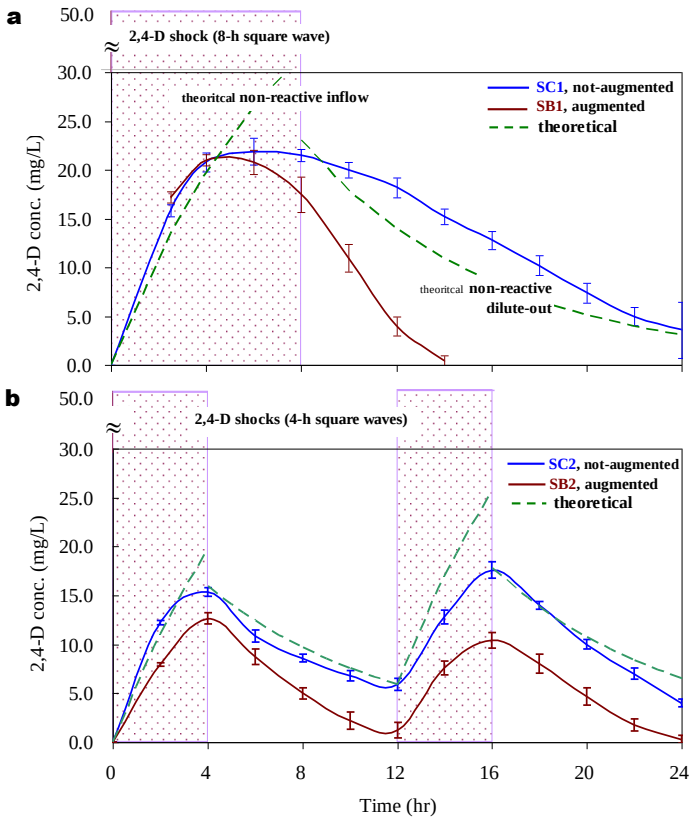

Figure 2. Performance of activated sludge during 2,4-D shock loading and recovery benefits of bioaugmentation. a) single-wave shock, b) double-wave shocks.

IV. Conclusions

Bioaugmentation using activated sludge previously acclimated to the target xenobiotic was affirmed a feasible method to shorten start-up time and shock recovery time. The most doubted difficulty about the usefulness of bioaugmentation is the survival and accustomedness of the added biomass. This skepticism is largely relieved when activated sludge of very similar origin and culture history is used for bioaugmentation, owing to the fact that the added biomass is not totally foreign to that originally exist in the reactors. The batch acclimation method for degrader production is a relatively simple process. The overall benefit of using acclimated sludge for practical bioaugmentation application is promising. A thorough evaluation of the benefits of such bioaugmentation practice can be made weighing the cost of degrader cultivation with the risk of treatment failure penalty.

References

[1] M. Alexander, "Nonbiodegradable and other recalcitrant molecules," Biotechnol. Bioeng., vol 15, pp. 611-620, 1973.

[2] I. Singleton, "Microbial metabolism of xenobiotics: fundamental and applied research," J. Chem. Technol. Biotechnol., vol 59, pp. 9-23, 1994.

[3] G. Buitron, A. Gonzalez, and L. M., Lopez-Marin, "Biodegradation of phenolic compounds by an acclimated activated sludge and isolated bacteria," Water Sci. Technol., vol 37, pp. 371-378, 1998.

[4] N. M. Chong, "Development of a tool for measurement of the degradation capacity of a biomass for a xenobiotic," Enzyme Microb. Technol., vol 37, pp. 467-471, 2005.

[5] R. W. J. Babcock, K. S. Ro, C. C. Hsieh, and M. K. Stenstrom, "Development of an off-line enricher-reactor process for activated sludge degradation of hazardous wastes," Water Environ. Res., vol 64, pp. 782-791, 1992.

[6] S. Y. Leu, R. W. Babcock, C. J. Tzeng, and M. K. Stenstrom, "Modeling the performance of hazardous wastes removal in bioaugmented activated sludge processes," Water Environ. Res., vol 81, pp. 2309-2319, 2009.

[7] A. F. Duque, V. S. Bessa, M. F. Carvalho, and P. M. L. Castro, "Bioaugmentation of a rotating biological contactor for degradation of 2-fluorophenol," Bioresour. Technol., vol 102, pp. 9300-9303, 2011.

[8] S. C. Eleren, and U. Alkan, "Reducing effect of aerobic selector on the toxicity of synthetic organic compounds in activated sludge process," Bioresour. Technol., vol 100, pp. 5714-5720, 2009.

[9] A. E. Schauer-Gimenez, D.H. Zitomer, J. S. Maki, and C. A. Struble, "Bioaugmentation for improved recovery of anaerobic digesters after toxicant exposure," Water Res., vol 44, pp. 3555-3564, 2010.

[10] N. Z. Al-Mutairi, "Aerobic selectors in slaughterhouse activated sludge systems: A preliminary investigation," Bioresour. Technol., vol 100, pp. 50-58, 2009.

[11] K. S. Ro, R. W. Babcock, and M. K. Stenstrom, "Demonstration of bioaugmentation in a fluidized-bed process treating 1-naphthylamine," Water Res., vol 31, pp. 1687-1693, 1997.

[12] J. Guo, J. Wang, D. Cui, L. Wang, F. Ma, C. C. Chang, and J. Yang, "Application of bioaugmentation in the rapid start-up and stable operation of biological processes for municipal wastewater treatment at low temperatures," Bioresour. Technol., vol 101, pp. 6622-6629, 2010.

[13] N.M. Chong, S. L. Pai, and C. H. Chen, "Bioaugmentation of 20a0.n activated sludge receiving pH shock loadings," Bioresour. Technol., vol 59, pp. 235-240, 1997.

[14] N.M. Chong, and Y. S. Chen, "Activated sludge treatment of a xenobiotic with or without a biogenic substrate during start-up and shocks," Bioresour. Technol., vol 98, pp. 3611-3616, 2007.

[15] N.M. Chong, M. Luong, and C.S. Hwu, "Biogenic substrate benefits activated sludge in acclimation to a xenobiotic," Bioresour. Technol., vol 104, pp. 181-186, 2012.

[16] APHA (American Public Health Association), AWWA (American Water Works Association) and WEF (Water Environment Federation), Standard Methods for the Examination of Water and Wastewater, 20th Ed. Washington D.C., U.S.A., 1998.

About Author (s):

Prof. Chong (PhD) has a career in environmental engineering for more than 30 years; his jobs include wastewater treatment plant engineering and research and professorship in universities. Prof. Chong's main area of research is environmental biotechnology - biodegradation of persistent/hazardous organic pollutants. J Liang and KY Lin were Prof. Chong's students, both are now pursuing careers in environmental engineering.