Simultaneous Removal of Nitrogen, Carbon and Phosphorous from Wastewater Using a Simple Bioreactor

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Abstract: *The removals of carbon (C), nitrogen (N) and phosphorous (P) from wastewater in multi stage reactors have been widely practiced worldwide. However, simultaneous removal of C and nutrients has proven to be difficult. A laboratory scale bioreactor was operated to treat synthetic wastewater. At mean temperature of 28° and HRT of 8 h, an average COD removal efficiency and BOD removal efficiencies were 65.0 ± 26.0 % and 87.8 ± 6.0%, respectively. The total N removal efficiency and P removal efficiency were 86.8 ± 13.9 % and 89.7 ± 4.6%, respectively. The sudden increase in substrate loading rate to the reactor cause drastically decreases in reactor performances. The results of this study revealed that the bioreactor was feasible to use to treat C, N, and P simultaneously at low substrate loading rates.*

Keywords: *Carbon, nitrogen, phosphorus, simultaneous removal, simple bioreactor*

1. INTRODUCTION

Although nitrogen (N), phosphorous (P), and organic carbon (C) are required compounds in both living beings and plants, presence of these compound in excessive amount in the environments cause severe issues on human and other organisms and on ecosystem. Wastewater from various sources is one of the main sources of C, N, and P which released into natural environment. Presence of biodegradable C in the natural water system causes limitation of oxygen in water bodies and degradation of natural aquatic environment. The excess release of nutrients such as N and P into natural water bodies cause in eutrophication and bad water quality, making the tread on the health health and living environment (Schindler, 2006; Howarth & Marino, 2006). For example, excess N and P input leads to eutrophication, in which dissolved oxygen is depleted, septic conditions are caused and odor problem arises in water bodies. Moreover, ammonia is toxic to aquatic life and nitrate cause several health effects, such as methemoglobinemia and vitamin A shortage.

Therefore, stringent water quality standards for C, N and P are established to human health and aquatic environment. Biological approaches have been widely used in wastewater treatment plants for removal of N and P (Ahmed et al., 2008; Hano et al., 1992; Martin et al., 2006). N is mainly removed from wastewaters by biological processes. The biological N removal technology is consisted of two main processes namely, nitrification and denitrification processes (Wiesmann, 2004). In the nitrification process the ammonium (NH_4^+) is converted first into nitrite (NO_2^-) and then into nitrate (NO_3^-) by ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB), respectively. Both of above steps were taken place in aerobic conditions. During the denitrification process produced NO_3^- is reduced to nitrogen gas (N_2) by heterotrophic denitrifiers with the presence of organic carbon as energy source under anoxic condition (Zhao et al., 2013). Biological P removal process requires basically two operational steps namely anaerobic and aerobic. The most responsible group microorganism for biological P removal is Phosphorus Accumulating Organisms (PAO). PAOs release P into the mixed liquor in the anaerobic stage and they uptake P in the aerobic stage. The amount of P uptake in the aerobic phase is higher than the P release in the anaerobic stage resulting P accumulation in PAOs.

However, such conventional biological wastewater treatment technologies of N and P removal still present severe sustainability, technical and economical limitations, and such as complex operation such as multi-stage operation (aerobic, anoxic, anaerobic conditions), high energy requirements, poor effluent quality, and no phosphorus recovery process (Wang et al., 2015). Having single stage simultaneous C, N, and P removal systems in signal reactor is proven to be difficult because existence of all aerobic, anoxic, anaerobic zones in a single reactor is to be maintained. Recently, researchers tried to develop simultaneous C, N, P removal processes. However, some of researchers ended up with poor reactor performances (Cui et al., 2004; Mahmoud, et al., 2009; Tawfik et al., 2011). Lu et al., (2014) and Jiang et al. (2014) reported simultaneous C, N, and P removal reactors with higher removal efficiencies. However, their processes had more complex operations (Jiang et al., 2014; Lu et al., 2014) and/or longer start-up period (Jiang et al., 2014). Therefore, development of simple bioreactor to treat C, N, and P simultaneously is still challenging.

The objective of this study is to develop a simple bioreactor to treat C, N and P from synthetic wastewater simultaneously. To achieve this objective a simple lab scale continuous flow bioreactor was developed and operated to treat synthetic wastewater using sponge as the biomass retaining media. The purpose of introducing the sponges was to maintain anoxic/anaerobic local environments.

2. MATERIAL AND METHODS

2.1. Reactor Configuration and Operation

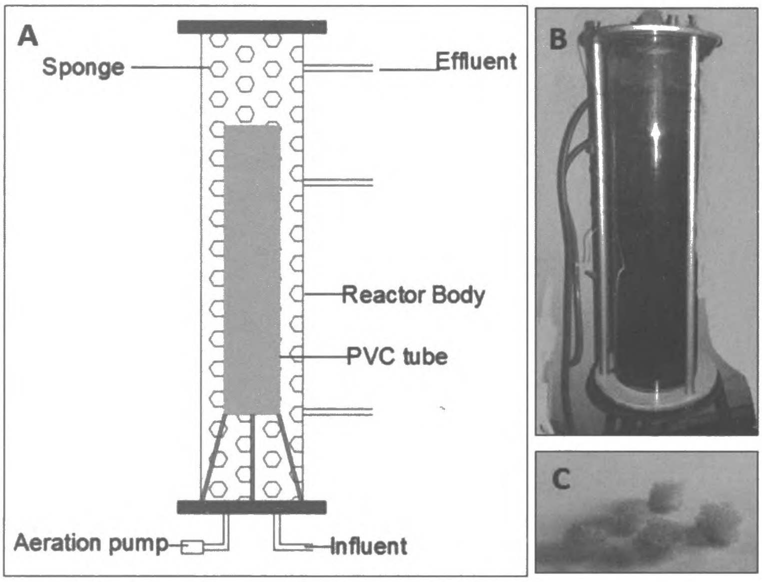

Figure 1 (A) A schematic diagram of the bioreactor; (B) Picture of the lab-scale bioreactor; and (C) A Picture of the sponges used in the reactor

A laboratory scale bioreactor was designed with a volume of 2.5 L (40cm height and 9cm diameter). The reactor was operated as a continuous flow reactor. The influent and air were fed at the bottom and the effluent was taken out from top. Air was supplied at a flow rate of 150 ml by a diaphragm pump. A ceramic air diffuser located at the bottom of the reactor was used for formation of small bubbles. As this bioreactor was operated under continuous flow, an overhead tank was provided to maintain a constant flow rate. Reactor consists of two aeration points at to have better circulation of flow as expected. A hollow PVC tube was introduced to the reactor system to enhance the flow circulation in the reactor. Two aeration points were located directly below the PVC tube. Therefore, flow direction in the PVC tube was upward while flow direction in the area between outer tube and PVC tube was downward. A net at the effluent outlet was provided to control the washout the materials with the effluent. DO and pH of the reactor was about 1.0 mg/L and 8.0, respectively. Reactor was inoculated with activated sludge (5 g-volatile suspended solid (VSS)/L) taken from

Dhaladhamaligawa wastewater treatment plant.

The reactor is filled with sponges (80 pieces of 6x6x6mm) as the attached media of the biomass. The reactor was fed with synthetic wastewater contained (Wasala et al., 2011); $NaHCO_3$ 3000 mg/L, $CaCl_2.2H_2O$ 13.5 mg/L, $MgSO_4.7H_2O$ 300 mg/L, K_2HPO_4 74 mg/L, Milk powder 50 mg/L, NaCl 28 mg/L, NH_4Cl 497 mg/L and Sugar 950 mg/L and trace elements contained: $FeCl_2.4H_2O$ 16 mg/L, $MnSO_4$ 0.2 mg/L, $CuCl_2.2H_2O$ 0.2 mg/L, $CoCl_2.6H_2O$ 0.2 mg/L, Ni 0.2 mg/L, and Zn 0.2 mg/L.

The lab scale bioreactor was operated for 10 weeks under two phases maintaining the HRT at 8 hours. The theoretical NH_4^+, organic C and phosphorus loading rates of the first phase (until end of 7th week) were 1.04 g-N/L/d, 1.2 g-COD/L/d and 0.004 g-P/L/d, respectively. In the phase two, the loading rates of NH_4^+, Organic C were doubled to investigate the feasibility of the reactor in treating N and C at higher leading rates.

2.2. Chemical Analysis

Removal efficiencies of N and P removal were monitored through measurement of N and P species in the influent and effluents from the reactor. Influent and effluent samples were collected from the reactor. The concentration of NO_3^- was measured using Cadmium reduction method with the HACH DR-2700 Spectrophotometer (HACH Corporation, Loveland, Colorado) (353 N, Nitrate MR P) and NitraVer 5 Nitrate Reagent Powder Pillows. NH_4^+ concentrations were measured using Salicylate with the apparatus HACH DR-2700 Spectrophotometer (HACH Corporation, Loveland, Colorado) (343 N, Ammonia HR TNT) and the High Range Test N Tube AmVer Nitrogen Ammonia Reagent. Total phosphate (TP) was measured by Molybdovanadate method is used with the apparatus and Reagent Powder Pillows.

C removal efficiency of the reactor was assessed through chemical oxidation demand (COD) and five day biological oxidation demand (BOD) measurements. All concentrations of total COD (T-COD) and the dissolved fraction of COD (D-COD) after passage through 0.45 µm membranes (Advantec Co., Ltd., Tokyo, Japan) were measured using the Hach Method using a HACH DR-2700 Spectrophotometer (HACH Corporation, Loveland, Colorado). BOD of the influent and effluent samples were measured using Alkali – Azide Winkler Method is used.

3. RESULTS AND DISCUSSIONS

3.1. C-removal Efficiency

COD and BOD in the influent and the effluent and their removal efficiencies shown in Figure 2A and Figure 2B, respectively. Average COD and BOD (± standard deviation) in the influent provided from a bio reactor in phase 1 were 336.0 ± 67.8 and 287.3 ± 19.7 mg/L, respectively. This corresponded to an average total COD and BOD loading rates of 0.966 ± 0.195 g-COD/L/d and of 0.826 ± 0.565 g-BOD/L/d, respectively. Average COD and BOD (± standard deviation) in the effluent provided from a bio reactor in phase 1 were 129.3 ± 102.6 mg/L and 45.8 ± 25.9 mg/L, respectively. The average COD removal rate and total COD removal efficiency in the phase 1 were 0.594 ± 0.192 g-COD/L/d and 65.0 ± 26.0 %, respectively while the average BOD removal rate and BOD removal efficiency were 0.725 ± 0.77 g-COD/L/d and 87.8 ± 6.0 %, respectively.

With the increasing of C loading rate in the phase 2 (Figure 2A), average COD (± standard deviation) in the influent and COD loading rate of the bio reactor was 1487 ± 67.8 mg/L and 4.28 ± 0.218 g-COD/L/d respectively. The average COD (± standard deviation) in the effluent was 964 ± 9.9 mg/L resulting an average COD removal rate and a COD removal efficiency of 1510 ± 240 g-COD/L/d and 35.1 ± 3.94 %, respectively. These results indicate that the C removal can be achieved by a single stage aerobic continuous flow simple bio reactor at low organic leading rate around of 1 g-COD/L/d. The preferable cause of degrading the reactor performances in phase 2 might be due the shock loading conditions to the microorganisms in the bio reactor.

Many researchers have extensively studied various types of complex bioreactors to treat C , N, and P simultaneously (Cui et al., 2004; Lu et al., 2015; Lv et al.,2016; Yilmaz et al., 2007; Jiang et al., 2016;Tawfik et al., 2012; Mahmoud et al., 2008). Even though they have used reactoers with complex reactor configurations and operations (Cui et al., 2004; Lu et al., 2015; Lv et al.,2016; Tawfik et al., 2012; Mahmoud et al., 2008), and long start-up periods (i.e granular systems) (Yilmaz et al., 2007; Jiang et al., 2016), their C removal efficiencies were comparable with the C removal efficiency of simple bioreactor this study.

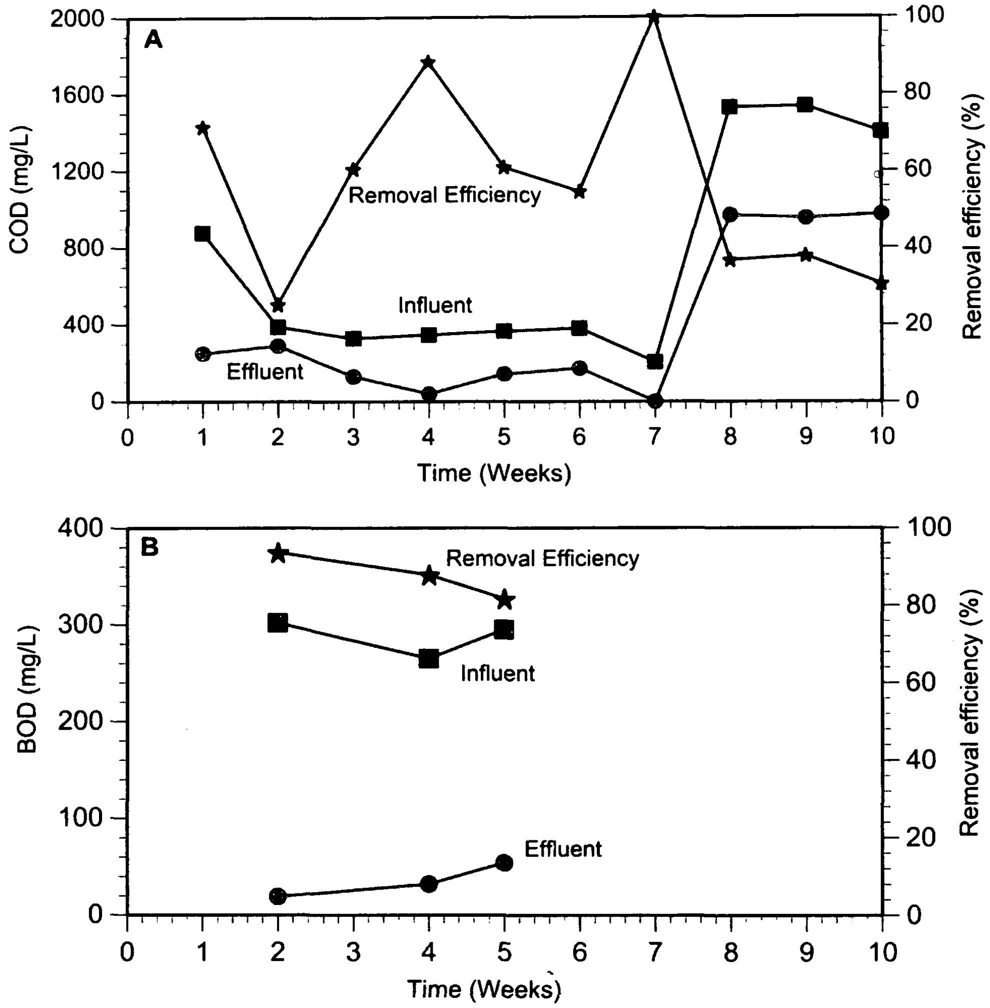

Figure 2 Performances of bioreactor. (A) COD removal performance; (B) BOD removal performance

3.2. N-removal Efficiency

NH_4^+, NO_2^-, and NO_3^- concentrations in the influent and the effluent and their removal efficiencies shown in Figure 3. Average NH_4^+ concentration (± standard deviation) in the influent provided to the bio reactor in phase 1 was 75.7 ± 43.9 mg-N/L. This corresponded to an average total N loading rates of 217.5 ± 126.2 mg-N/L/d. Average NH_4^+, NO_2^-, and NO_3^- concentrations (± standard deviation) in the effluent provided from a bio reactor in phase 1 were 31.0 ± 34.0 mg-N/L, 0.26 ± 0.18 mg-N/L and 6.67 ± 16.3 mg-N/L , respectively. The N mass balance during the steady operation period (From weeks 4 to week 7) revealed that total N removal rate and total N removal efficiency in the phase 1 during the steady operational period were 134.0 ± 92.6 mg-N/L/d and 86.8 ± 13.9 %, respectively. With the increasing of N loading rate in the phase 2, average NH_4^+ concentration (± standard deviation) in the influent provided to the bio reactor in phase 2 was 182.0 ± 46.7 mg-N/L resulting an average N loading rate of 523.2 ± 134.1 mg-N/L/d. Average NH_4^+, NO_2^-, and NO_3^- concentrations (± standard deviation) in the effluent provided from a bio reactor in phase 2 were 99.5 ± 26.2 mg-N/L, 0.45 ± 0.35 mg-N/L, 0 mg-N/L , respectively. Therefore, the resulted total N removal rate and total N removal efficiency in the phase 2 during the steady operational period were 235.9 ± 57.9 mg-N/L/d and 45.1 ± 0.50 %, respectively. Sudden increase in loading load in reactors cause more stresses and toxicity on microorganisms in biomass and therefore the N removal efficiency degradation upon doubling the loading rate is reasonable. However, investigating the maximum loading rates with gradual increase in loading rates should be invested in the future studies.

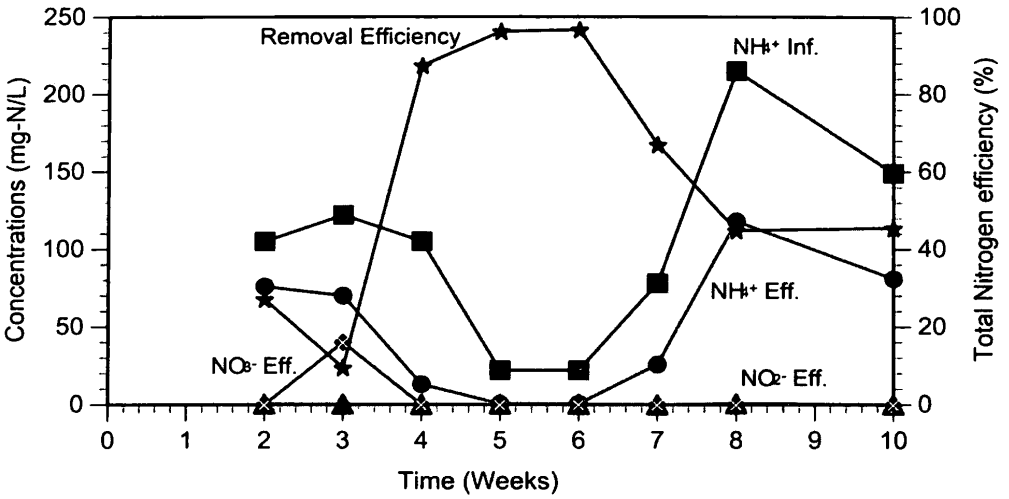

Figure 3 Nitrogen removal performances of the bioreactor

Decrease in NH_4^+ -N concentration from 75.7 ± 43.9 mg-N/L mg/L in the influent to 31.0 ± 34.0 mg-N/L L in the effluent was mainly due to the nitrification process by AOB and NOB. A fairly low NO_2^--N and NO_3^- -N concentration in the effluent of reactor is probably due to the heterotrophic denitrification. These results indicate that the environmental conditions in some anoxic environments were existed inside the reactor. Possible candidates for these anoxic environments are inner cores of activated sludge particles and inner part of the sponges. Li and Bishop (2004) measured in situ DO concentrations in activated sludge flocs using microsensors and they observed that inner part of the activated sludge flocs are mainly in the anoxic conditions. Moreover, we clearly observed that a thin biofilms were developed on the outer-surfaces of sponges. Okabe et al. (1999) concluded that oxygen penetrated down to few hundred micrometres in wastewater biofilms and therefore, it is more likely that inside of our sponges was in anoxic condition which promotes the denitrification. However, it should not be neglected that heterotrophic denitrication can be observed under the sub-oxic environments in biofilms (de Beer et al., 1997).

Several studies have reported N removal during the simultaneous C, N, and P removal processes(Cui et al., 2004; Lu et al., 2015; Lv et al.,2016; Yilmaz et al., 2007; Jiang et al., 2016;Tawfik et al., 2012; Mahmoud et al., 2008; Liu et al., 2015). Total N removal efficiency of our present study in phase 1 is same range with the literature reported by Liu et al. (2015) (85%) and Cui et al., (2004) (88%). However, Yimaz et al. (2007) have reported higher total N removal efficiency of 93% using a using granular sludge in a sequencing batch reactor though it is start-up time is significantly higher than that of our reactor. In contrast, total N removal efficiency of an anaerobic hybrid reactor and down flow hanging sponge treating C, N, P simultaneous was about 80% (Mahmould et al., 2008).

3.3. P-removal Efficiency

TP concentrations in the influent and the effluent and their removal efficiencies shown in Figure 4. Average TP concentration (± standard deviation) in the influent provided to the bio reactor in phase 1 during steady operation period (from week 4 to week 7) was 41.5 ± 15.7 mg-P/L. This corresponded to an average total P loading rates of 119.3 ± 45.1 mg-P/L/d. Average TP concentration (± standard deviation) in the effluent provided from a bio reactor in phase 1 during this operational period was 4.65 ± 3.0 mg-P/L. The P mass balance during the steady operation period revealed that total P removal rate and total P removal efficiency in the phase 1 during the steady operational period were 105.9 ±

38.1 mg-P/L/d and 89.7 ± 4.6 %, respectively. This higher removal efficiency of P was observed due to the presence of both oxic and anoxic environments within the bioreactor as described above in section 3.2. TP concentrations used in this study was about 50 mg-P/l and authors recommend investigating the feasibility of reactor for higher TP concentration in future studies.

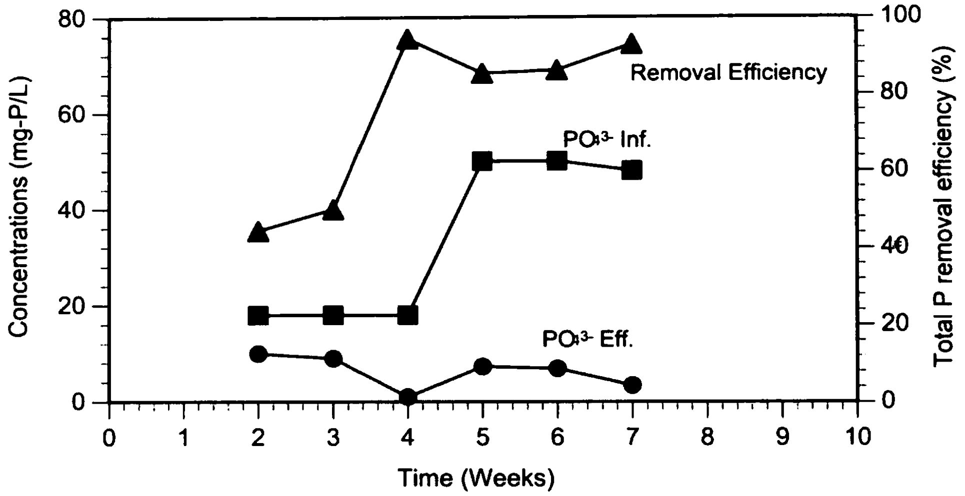

Figure 4 Phosphorus removal performances of the bioreactor

3.4. Effect of Loading Rates on Removal Efficiencies

The daily removal efficiencies of C, N, and P were plotted against their loading rates. The results are shown in Figure 5. C and N removal efficiencies were clearly decreases within increase in corresponding loading rates. According to the Figures 5A and 5B, maximum C loading rate for 100% of C removal was about 0.8 g-COD/L/D while optimum N loading rate for our particular reactor was laid below 0.1 g-N/L/d. P removal efficiencies were increased with increasing of P loading rates (Figure 5C) suggesting the optimum P loading rate was more than the maximum P loading rate applied during the reactor operational period

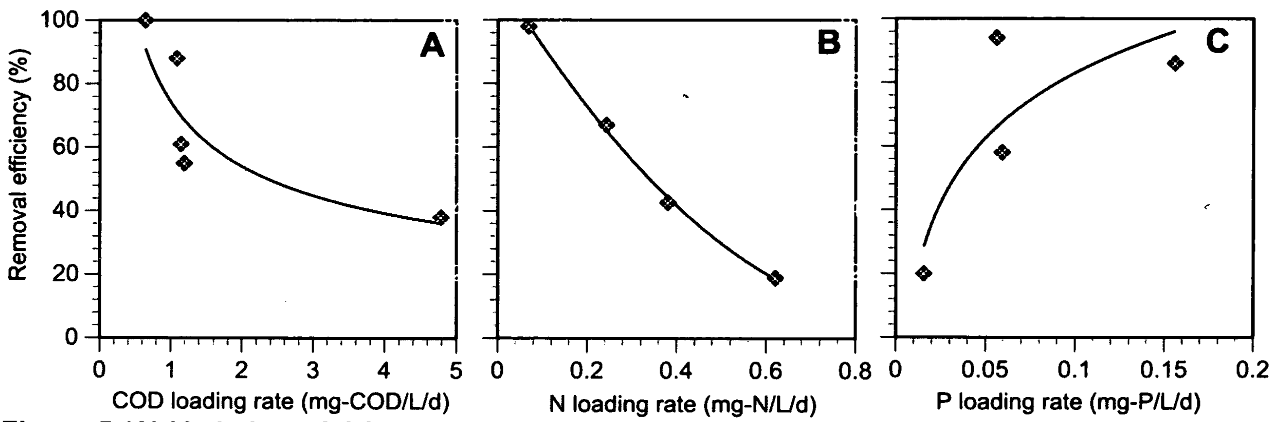

Figure 5 (A) Variation of COD removal efficiencies with the COD loading rate; (B) Variation of total nitrogen removal efficiency with the nitrogen loading rate; (C) Variation of phosphorus removal efficiencies with the phosphorus loading rate;

4. CONCLUSIONS

A simple bioreactor to treat C, N and P simultaneously from syntactic wastewater was operated. The reactor was capable of C, N, and P simultaneously at low substrate loading rate. The average COD removal and BOD removal efficiencies were 65.0 ± 26.0 % and 87.8 ± 6.0%, respectively while the total N removal efficiency and P removal efficiency were 86.8 ± 13.9 % and 89.7 ± 4.6%, respectively. Higher efficiency of N and P removal revealed that the significant space of the reactor has act as an anoxic environment during the operational period. The sudden increase in substrate loading rate to the reactor cause drastically decreases in reactor performances. Considering the simplicity and the performances of the reactor, the operation of the bioreactor explained in this study was successful.

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