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Nutrient Removal from Wastewater by Integrated Attached Growth Bioreactor

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ABSTRACT

Water body deterioration caused by natural and anthropogenic activities has been a source of concern for regulating agencies. Several problems such as eutrophication and blue baby syndrome are caused by the excessive presence of nutrients within the aquatic biota. This also presents danger to the benthic community. In this study, an integrated aerobic/anoxic attached growth bioreactor was evaluated for its performance in removing nutrients (ammonia and nitrate) from simulated synthetic low and medium strength domestic wastewater. The experiment was conducted in two stages. Stage 1 consists of ammonia concentration of about 16 mg L^{-1} whereas, stage 2 consists of ammonia concentration of about 26 mg L^{-1}. Bioreactor performance was also evaluated at two different Hydraulic Retention Time (HRT) of 7.2 and 12 days, respectively. Seed sludge was collected from a domestic sewage treatment plant. The performance of the bioreactor was monitored every two days with samples collected from aerobic compartment, anoxic compartment and net effluent. At HRT of 12 days, results at steady state condition shows that an effluent ammonia concentration of about 0.33 mg L^{-1} was obtained in stage 1. Steady state effluent ammonia concentration slightly increased to 0.65 mg L^{-1} in stage 2. Steady state effluent nitrate concentration (0.2 and 0.55 mg L^{-1}) was low in both stages 1 and 2. In both stages 1 and 2, ammonia removal reached 98%. The HRT was found to exert slight influence in nutrient removal using the integrated bioreactor. At HRT of 7.2 and 12 days, effluent ammonia concentration of 1.1 and 0.6 mg L^{-1} was obtained, whereas effluent nitrate was in the range of 0.55 and 0.22 mg L^{-1}, respectively. Chemical Oxygen Demand (COD) was also monitored at steady states and was found below 20 mg L^{-1} in both stages 1 and 2 at HRT of 12 days. This study, therefore demonstrates the capacity of an integrated bioreactor to mitigate the enormous challenge of water body deterioration and toxicity caused by indiscriminate discharge of polluted wastewater.

Key words: Attached growth, integrated bioreactor, ammonia, nitrate, eutrophication

INTRODUCTION

Over decades, water body deterioration caused by anthropogenic and natural activities has been a subject of concern (Ezechi *et al*., 2015a). Anthropogenic activities are primarily pollutions from industrial run-offs, agricultural run-offs, urban run-offs and sewage run-offs (Ezechi *et al*., 2015b).

Natural activities on the other hand are mainly from floods, earthquake and other natural disasters (Ezechi *et al*., 2014a). These two principle sources contribute high amounts of nutrients such as ammonia, nitrite and nitrate to water bodies. These nutrients make up a high percentage of global water body pollution and constitute about 25% of all water body impairments (EPA., 2007). Excessive enrichment of water bodies by nutrients can lead to a phenomenon known as eutrophication (Ghafari *et al*., 2008). This phenomenon presents unhealthy conditions for aquatic habitats and can lead to algae blooms. Another phenomenon associated with excessive enrichment of water bodies by nutrient is methaemoglobin. The reaction of excess nitrate with the hemoglobin of aquatic animals could cause shortage of oxygen and finally death (Camargo *et al*., 2005). The anaerobic reduction of nitrate to nitrite in the human intestines could lead to methaemoglobinaemia in infants (Nora'aini *et al*., 2005). Nitrite and nitrosamines have also been reported to play an etiologic role in advance pregnancy outcomes and chronic diseases such as cancer (Griesenbeck *et al*., 2009). As a consequence of severe water body contamination/pollution, primarily from sewage effluents, the Malaysia Department of Environment (DOE) in 2009 revised the discharge limit for ammonia-nitrogen in sewage effluents from 50-5 mg L^{-1} (DOE Malaysia, 2009). Thus, water body contamination by nutrients could present danger to both aquatic organisms and humans through consumption. Therefore, the elimination of nutrients from wastewater could supplement limited fresh water resources, especially in arid areas (Ezechi *et al*., 2012a).

Recently, it was reported in few datasets that several countries will become water stressed due to increased water scarcity (Seckler *et al*., 1998). Thus, several water scarce countries will employ the use of non-conventional water resources to mitigate the adverse effect of water scarcity (Qadir *et al*., 2007). To this end, investigation into cheap, sustainable, yet effective water treatment methods have intensified. Such methods include Fenton oxidation process (Meric *et al*., 2004), coagulation and flocculation (Guida *et al*., 2007), membrane processes (Chianese *et al*., 1999), electrochemical processes (Ezechi *et al*., 2012b, 2014b, 2015c; Isa *et al*., 2014), adsorption processes (Bansode *et al*., 2004; Ezechi *et al*., 2015d).

Biological wastewater treatment processes are proven effective processes for nutrient removal from wastewater. It is widely utilized in various configurations depending on the objective of such purpose. Conventional biological treatment processes are associated with large space requirement for installation due to its segregated compartments and hinders its optimal use in nuclear and compact environments. It is reported that about 60% of the total cost of conventional biological treatment process operation is invested in sludge disposal in landfills, land application or incineration (Wei *et al*., 2003). With the closure of many landfills due to maximum usage, rapid development of new landfills are hindered by its associated environmental consequences (Ezechi *et al*., 2011). Therefore, there is a need to modify the existing conventional biological treatment process to a smaller footprint, yet effective process.

The integration of the various biological wastewater treatment compartments into a single entity is a suitable alternative to mitigate the large space requirement associated with the conventional process. The aerobic compartment oxidizes ammonia while the anoxic compartment reduces nitrate. Both compartments are essential for thorough wastewater treatment. In the anoxic compartment, the role of the denitrifying bacteria is vital for denitrification in a wastewater treatment plant especially in a post-anoxic process (Ezechi *et al*., 2015e). The advantages of such process include smaller footprint, single degradation pathway, cost effective and easy operation (Chan *et al*., 2009). Additionally, integrated bioreactors could be significantly used in nuclear settlements, urban cities and mountainous areas. The knowledge and integration of aerobic/anoxic compartments for the elimination of nutrients (nitrification/denitrification) is still at infancy. Subsequently, the inclusion of an attached growth media into the integrated bioreactor further provides the microorganisms, additional surface area for growth. Its advantages include retention of higher biomass concentration and lower sensitivity to toxicity (Chang *et al*., 2010).

The objective of this study is to investigate the performance of an integrated bioreactor treating low and medium strength domestic wastewater at different ammonia concentrations and Hydraulic Retention Time (HRT), respectively.

MATERIALS AND METHODS

Bioreactor setup: The bioreactor was configured as a pre-nitrifying process. The aerobic compartment (10 L) was integrated into the anoxic compartment (20 L) and both chambers were integrated into the clarifier (150 L). The aerobic compartment has a cyclindrical openings at the edge of its bottom for the flow of wastewater into its coupled second compartment. The coupled second compartment is strongly fixed to the outer side of the aerobic compartment and serves as a semi-permeable filter that allows only the flow of liquid while retaining the aerobic microorganisms (Phase separation). The anoxic compartment consists of an oval opening at the bottom that allow the flow of wastewater into the clarifier. The clarifier is connected with the effluent outlet and a Mixed Liquor Recirculation (MLR) pipe at the bottom into the anoxic compartment. The integrated bioreactor has a total volume of 180 L and experiments were conducted from 2013-2014.

Wastewater preparation: The wastewater used in this study is synthetic preparation. A low and medium strength domestic wastewater was simulated using purina alpo substrate and ammonium chloride. The wastewater was formulated by dissolving appropriate amount of purina alpo substrate and ammonia chloride to yield ammonia concentrations of 16 and 26 mg L^{-1} for stages 1 and 2, respectively. The COD concentration was in the range of 250 and 500 mg L^{-1}, respectively. The influent wastewater characteristics are summarized in Table 1.

Attached growth media: The attached growth media used for both the aerobic and anoxic compartments were fabricated using Perspex. In the aerobic compartment, the media has an octagonal shape with a height of 20 cm and width of 17 cm. The media was designed with 10 layers consisting of an even amount of hollow pores for the easy flow of wastewater and oxygen. In the anoxic compartment, the circular media also consists of 10 layers fixed together with a connector pole and have even number of hollow pores for easy flow of wastewater.

Table 1: Average influent wastewater characteristics

Parameters	Stage 1	Stage 2
pH	7.3	7.5
NH_3 (mg L^{-1})	16.0	26.0
NO_3 (mg L^{-1})	1.4	2.0
TSS (mg L^{-1})	301.0	456.0
COD (mg L^{-1})	250.0	500.0
BOD (mg L^{-1})	104.0	255.0

COD: Chemical oxygen demand, BOD: Biochemical oxygen demand, TSS: Total soluble solids

Bioreactor operation: The influent wastewater was applied to the aerobic compartment using a Masterflex Peristaltic pump at two different HRT of 7.2 and 12 days. Seed sludge was collected from a domestic sewage treatment plant with a mixed liquor suspended solids of about 3000 mg L^{-1}. The bioreactor was allowed to acclimate with the biomass for a period of 20 days. Ceramic air diffusers were placed at the bottom of the aerobic compartment to provide the microorganisms with oxygen. The wastewater flows into the aerobic compartment in a downward process. Wastewater then flows into the coupled aerobic second compartment through the openings of the aerobic compartment. Subsequently, the coupled aerobic second compartment allows the flow of wastewater into the anoxic compartment from its semi-permiable filter in an upward process while retaining the aerobic microorganisms. The wastewater then flows out of the anoxic compartment through the opening at its bottoms into the clarifier for settling. The effluent wastewater flows out from the clarifier. The mixed liquor recirculation was conducted once every 90 min into the anoxic compartment. The wastewater was prepared daily. Samples from the influent, aerobic compartment, anoxic compartment and net effluent were collected every two days for analysis. The bioreactor was considered in steady state when all its compartments achieve a near constant removal rate over a period of 6-8 days. All analysis were triplicated.

Analytical procedure: Ammonia and Nitrate were analysed by Nessler and cadmium reduction method, respectively. The COD concentration was measured using spectrophotometric method. Mixed Liquor Volatile Suspended Solids (MLVSS) were analyzed according to the 21st edition of standard methods for the examination of water and wastewater (APHA, AWWA and WEF., 2005). pH, DO and temperature were daily monitored concurrently using a pH meter (Sension™), DO meter (YSI 550 A) and a thermometer (Thermolyne P/N MEX-147 IMM 76 MM MCT).

RESULTS AND DISCUSSION

Figure 1 represents the performance of the bioreactor for ammonia removal in the aerobic, anoxic and effluent compartments respectively at HRT of 12 days. In both stages 1 and 2 as shown in Fig. 1, oxidation of ammonia was prominent in the aerobic compartment. Ammonia concentration of about 0.33 and 1.45 mg L^{-1} was obtained in stages 1 and 2, respectively. An increase in the effluent ammonia concentration of the aerobic compartment was observed when initial ammonia concentration was elevated. The aerobic compartment was prominent in the removal of ammonia in the bioreactor, leaving the anoxic compartment with little ammonia concentration. Recall that nitrification is the autotrophic oxidation of ammonia to nitrite, then to nitrate. This usually takes place in the presence of oxygen as electron source and could be represented by the energy yielding two step oxidation reaction expressed below.

Nitroso-bacteria:

$$2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 4H^+ + 2H_2O$$

Nitro-bacteria:

$$2NO_2^- + O_2 \rightarrow 2NO_3^-$$

Total oxidation reaction:

$$NH_4^+ + 2O_2 \rightarrow NO_3^- + 2H^+ + H_2O$$

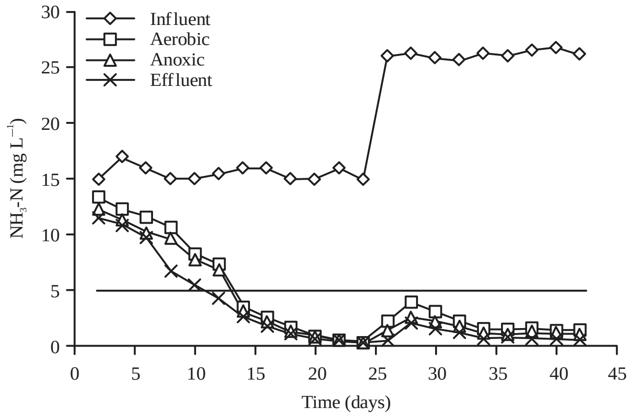

Fig. 1: Ammonia removal from aerobic, anoxic and effluent compartments at HRT of 12 days

The anoxic compartment received the unconsumed substrates from the aerobic compartment which have only a small concentration of ammonia left. The ammonia concentration of the anoxic compartment at steady state conditions were 0.3 and 1.2 mg L^{-1} for stages 1 and 2, respectively (Fig. 1). The anoxic compartment consists mainly of denitrifying bacteria which have low ammonia removal capacity. The slight ammonia removal in the anoxic compartment could be attributed to uptake in microorganism growth and nitrification in low DO concentration of about 0.2-0.3 mg L^{-1}. This is in agreement with the report of Ying *et al*. (2005).

In the effluent compartment (Fig. 1), residual ammonia concentrations of 0.3 and 0.6 mg L^{-1} was obtained in stages 1 and 2 at steady state conditions. In all three compartments (aerobic, anoxic and effluent), a tilt increase of residual ammonia concentration was observed when the initial concentration was raised. After a few days of acclimation, the bioreactor stabilized with the new influent concentration and attained steady state. Increasing ammonia concentration resulted to increase of effluent concentration in all three compartments respectively. Similar observation is reported elsewhere (Ramos *et al*., 2007).

Due to the tilt increase of residual ammonia concentration (Fig. 1, day 26) at the start of phase two, two distinct mechanisms could be responsible for ammonia removal in the bioreactor. In phase 1, carbonaceous substrate removal, ammonia assimilation and endogenous respiration could have played a significant role in ammonia removal whereas in stage 2, biomass re-stabilization, nitrification and exogenous substrate consumption was prominent. This observation is in accordance with reports elsewhere (Hamoda *et al*., 1996). The rapid and efficient oxidation of ammonia in the aerobic compartment indicates good oxygen and mass transfer capacity of the bioreactor.

The wastewater has a low influent nitrate concentration according to the experimental plan. In the initial phase (stage 1) of the experiment, nitrate accumulated in the aerobic compartment (Fig. 2, Day 0-8) and was higher than the influent nitrate concentration due to ammonia oxidation. This observation also reflects the good ammonia oxidation capacity of the bioreactor. From day 9 onwards, nitrate concentration gradually decreased through the process of mass transfer into the anoxic compartment. A steady state nitrate concentration of about 0.4 mg L^{-1} was obtained in stage 1 (HRT 12 days). A similar observation was made in stage 2 in the aerobic compartment, with nitrate concentration accumulating in the initial stage upto about 4.9 mg L^{-1} from day 26-30. However, mass transfer of nitrate into the anoxic compartment resulted in a decrease of nitrate

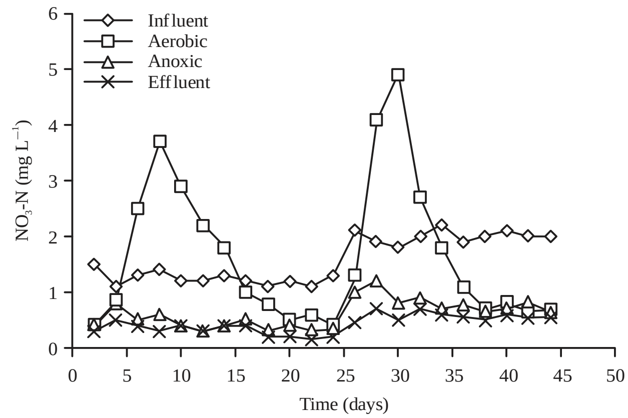

Fig. 2: Nitrate removal from the aerobic, anoxic and effluent compartments at HRT of 12 days

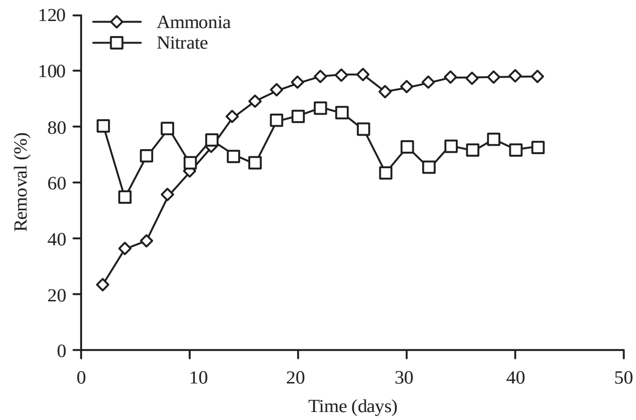

Fig. 3: Ammonia and nitrate removal efficiency (HRT 12 days)

concentration in the aerobic compartment from day 32-44. At steady state conditions, a nitrate concentration of about 0.7 mg L^{-1} was obtained. The carbon source for the anoxic compartment dependent on mass transfer of residual carbon from the coupled aerobic second compartment. Consequently, nitrate was transferred to the anoxic compartment for utilization by denitrifying bacteria. In the anoxic compartment (Fig. 2), nitrate was rapidly used up by denitrifying bacteria, resulting to a low nitrate concentration (0.33 and 0.65 mg L^{-1}) at steady state conditions of stages 1 and 2, respectively. The effluent nitrate concentration was also observed to be low (0.2 and 0.55 mg L^{-1}) in stages 1 and 2 (Fig. 2), respectively.

In Fig. 3, the removal efficiency of ammonia and nitrate by the bioreactor is presented. In both stages 1 and 2, ammonia removal efficiency was in the range of 98%, whereas, nitrate removal efficiency was about 84.6% in stage 1 and 72.5% in stage 2. The decrease of nitrate removal efficiency in stage 2 could be due to higher nitrate mass transfer from the bio-oxidation of ammonia in the aerobic compartment.

It is noteworthy that the integration of this process could be either pre-nitrification in which case the aerobic compartment receives the influent feed or post-nitrification where the influent feed is applied first to the anoxic compartment. In the later, an external carbon source is required and effluent nitrate concentration is a subject of concern. In the former, the unconsumed carbon from the aerobic compartment could be utilized for system balance. In the case of the removal capacity of the various comapartments of the bioreactor (aerobic and anoxic), it was observed that aerobic compartment was responsible for higher organic matter removal. Del Pozo and Diez (2005) observed that the aerobic oxidation accounted for about 96% COD removal while the anoxic removal stood for only about 2.6% in their study of slaughterhouse wastewater treatment using anaerobic-aerobic fixed film reactor. The distinguishing feature of the principle bioreactor compartments (aerobic and anoxic compartments) in this study is that the aerobic compartment receives the raw wastewater containing high substrate whereas the anoxic compartment receives unconsumed substrates from the preceding stages. Ra *et al*. (2000) investigated the biological nutrient removal with an internal organic carbon source for the treatment of piggery wastewater using two real time control technologies known as Sludge Separation Strategy (SSS) and Sludge Addition Strategy (SAS). In both technologies (SSS and SAS), the authors observed a 97.8 and 98.6% ammonia removal efficiencies, respectively. In the case of nitrate, SAS was reported to enhance denitrification, which resulted in low nitrate concentration more than SSS. Won and Ra (2011) observed a near 100% ammonia removal from a post-aerobic sequential batch reactor system treating piggery wastewater. However, effluent nitrate concentration was high (0-83 mg L^{-1}) during the experiment due to the operational sequence (feeding, anoxic, anaerobic, aerobic, settle, discharge). In a pre-denitrifying process, effluent nitrate is mainly controlled by regular Mixed Liquor Recirculation (MLR) into the anoxic compartment or the inclusion of a final anoxic compartment in the treatment process. However, in a pre-nitrifying process, nitrate as the end product of nitrification could accumulate in the aerobic compartment and slowly move into the anoxic compartment through mass transfer. Obaja *et al*. (2003) observed that nitrate increased constantly when all ammonium was nitrified to nitrate in their nitrification and denitrification study using a sequencing batch reactor. However, ammonia and nitrate removal efficiency reached 99.7 and 99.9%, respectively. A similar observation was made by Hamoda *et al*. (1996) in their study of biological nitrification kinetics in a fixed film bioreactor. Muhammadi *et al*. (2012) compared the performance of an Extended Aeration Activated Sludge (EAAS) with Submerged Membrane Bioreactor (SMBR) treating high strength wastewater. The authors observed a 88 - 99% ammonia removal for SMBR and 71-98% for EAAS. However, more nitrate was removed from the SMBR. Ramos *et al*. (2007) reported a nitrogen removal efficiency of about 41% for tests with an influent flowrate of 15 L day^{-1} and 300% and a maximum of 77% for test with an influent flowrate of 12 L day^{-1} and 600% in their study of biological nitrogen and phenol removal from saline wastewater by submerged fixed film reactor. The underlying principle for effective nutrient removal in this study could be attributed to the operational sequence and the inclusion of attached media in both the aerobic and anoxic compartments.

Influence of HRT on the bioreactor performance was evaluated by varying two HRT (7.2 and 12 days) at initial ammonia concentration of 26 mg L^{-1}. The results show that increase in HRT resulted to increase of the effluent ammonia concentration.

At HRT of 7.2 days (Fig. 4), effluent ammonia concentration was about 1.1 mg L^{-1} but decreased to 0.6 mg L^{-1} when HRT was increased to 12 days. In the case of nitrate (Fig. 4), effluent nitrate concentration of 0.55 mg L^{-1} was obtained at HRT of 7.2 days but decreased to 0.22 mg L^{-1} when HRT was increased to 12 days. It is obvious that longer HRT provides the microorganisms ample time to utilize the substrate. Influence of HRT was investigated by Hong *et al*. (2012) who reported that increasing HRT led to an increase in nitrogen removal for a submerged membrane bioreactor operation. Four different HRT (4, 2, 1.3 and 1 h) was varied. Trans-membrane pressure was more

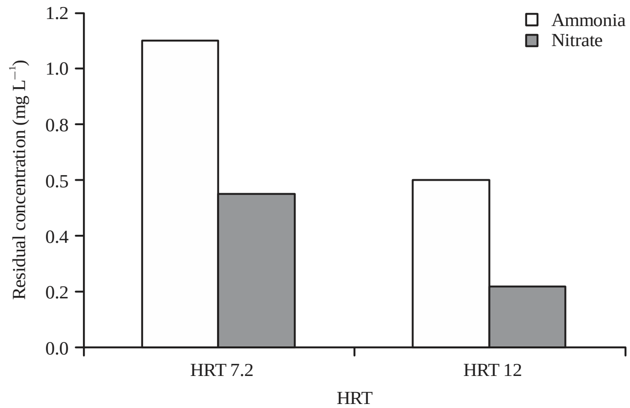

Fig. 4: Influence of HRT on the bioreactor

Table 2: Steady state results for all compartments (HRT 12 days)

	Steady state stage 1				Steady state stage 2			
Sample	pH	NH_3 (mg L^{-1})	NO_3 (mg L^{-1})	COD (mg L^{-1})	pH	NH_3 (mg L^{-1})	NO_3 (mg L^{-1})	COD (mg L^{-1})
Aerobic compartment	7.4	0.33	0.40	9	7.3	1.45	0.70	33
Anoxic compartment	7.7	0.30	0.33	7	7.6	1.12	0.65	24
Effluent compartment	7.8	0.30	0.20	3	7.4	0.60	0.55	14

significant on the lowest HRT (1 h). Nitrogen removal of 74.6, 67, 64.5 and 56.7% were obtained for HRT of 4, 2, 1.3 and 1 h, respectively. Similar observation is documented elsewhere (Mann and Stephenson, 1997).

The COD was monitored at steady state conditions of stage 1 and 2. A COD concentration of 9, 7 and 3 mg L^{-1} was obtained in stage 1 for the aerobic, anoxic and effluent compartments respectively as shown in Table 2. In stage 2, COD concentration in the aerobic, anoxic and effluent compartments increased to 33, 24 and 14 mg L^{-1}, respectively (Table 2). Ying *et al*. (2005) observed a mean COD removal efficiency of 96% for a membrane bioreactor treating food processing wastewater. pH of the various compartments was found to be in the optimum zone of nitrification/denitrification process.

CONCLUSION

An integrated attached growth bioreactor was successfully used for the elimination of nutrients (ammonia and nitrate) from simulated synthetic domestic wastewater. It was observed that bioreactor have the capacity to remove ammonia and nitrate from wastewater. An ammonia removal efficiency of about 98% was obtained at two different ammonia influent concentrations of 16 mg L^{-1} and 26 mg L^{-1}, respectively. In the case of nitrate, the removal efficiency dropped from 84.6-72.5% when influent concentration was elevated. Influence of HRT on the bioreactor performance was evaluated. It was found that increasing HRT from 7.2-12 days results to increase in removal efficiency of both ammonia and nitrate. The COD, MLVSS and pH were all monitored. Effluent COD was found to be very low (3 mg L^{-1}) in stage 1 and also in stage 2 (14 mg L^{-1}). Biomass concentration was quantified in the aerobic and anoxic compartments at steady state. Microbial growth was observed to be more significant in the aerobic compartment. This study, therefore demonstrates that an Integrated Bioreactor (IB) could be used to mitigate and ameliorate the deteriorating and toxic conditions of water bodies through effective treatment process before discharge.

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