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Microbial Culture Dynamics and Performance of an Anammox Sequencing Batch Reactor System

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In this study, a 5L sequencing batch reactor was successfully operated for the treatment of simulated wastewater using anammox process. The reactor was inoculated with a pre enriched anammox biomass obtained from a wastewater treatment plant. The reactor was operated in two stages differentiated by different nitrogen loading rate (NLR) i.e. 5 $g.N{\bullet}L^{-1}{\bullet}d^{-1}$ and 6.3 $gN{\bullet}L^{-1}{\bullet}d^{-1}$. The suspension culture was highly active. The highest removal efficiency for total nitrogen achieved by the reactor was 93% at the highest nitrogen loading rate of 6.3 $gN{\bullet}L^{-1}{\bullet}d^{-1}$. Furthermore, a modified Stover Kincannon model was used to evaluate the performance of the reactor. A maximum substrate removal rate of 34 $gN.L^{-1}.d^{-1}$ was predicted. The modified Stover-Kincannon was more suitable for the description of nitrogen removal in the reactor, with the regression coefficient of R^2 =0.9739.

1. Introduction

Biological treatment processes are recommended for the treatment of wastewater effluents before discharging into receiving water bodies due to lower operation cost and energy saving . Nitrogen removal is one of the most crucial wastewater treatments required due to its contribution to eutrophication of the water bodies (Daims et al., 2006). Various innovative biological nitrogen removal processes such as Single reactor High activity Ammonia Removal Over Nitrite (SHARON), completely autotrophic nitrogen removal over nitrite (Canon) process, De-ammonification and oxygen-limited autotrophic nitrification-denitrification (Oland) process, have been developed (Verstraete and Philips., 1998). However, anaerobic ammonium oxidation (anammox) process is the latest technique for improved nitrogenous compounds removal from wastewater. This process is carried out by autotrophic bacteria of the type Planctomycetes called anammox bacteria which combine ammonium and nitrite to produce nitrogen gas and a small amount of nitrate in anoxic conditions. The anammox process is considered economical and low energy alternative to the conventional biological nitrogen removal, which is generally accomplished through successive aerobic autotrophic nitrification and anoxic heterotrophic denitrification (Cho et al., 2010). The application of anammox process has been developed for wastewater treatment using different reactors. The types of commonly used microbial cultures for the anammox process are granule-based and attached growth cultures. However, a gap still exists on the use of suspension cultures for anammox process. Therefore the aim of this study was to establish the anammox process with a sequencing batch reactor (SBR) inoculated with suspension microbial culture. Emphasis was set on performance of the reactor and substrate removal kinetics.

2. Kinetic model

The Stover Kincannon model is a mostly used mathematical model for determining the substrate removal rate as a function of substrate loading rate. The model was initially used for the attached growth biomass performance in a rotating biological contactor (Stover and Kincannon., 1982) using the following equation:

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$$\frac{dS}{dt} = \frac{Q(S_i - S_e)}{A} = \frac{U_{\max} \frac{QS_i}{A}}{K_B + \frac{QS_i}{A}} \tag{1}$$

Where dS/dt is the substrate removal rate ($mg.L^{-1}.d^{-1}$), Q is the flow rate (L/d), Si is the influent substrate concentration, Se is the effluent substrate concentration (mg/L), A is the total disc surface area on which biomass concentration is immobilized (m^2). K_B represents the saturation value constant ($g/d.m^2$) whereas U_{max} is the maximum substrate removal rate constant ($g.L^{-1}.d^{-1}$).

The original model was later modified and used to predict the bioreactor performance (Yu et al., 1998). In this approach, the suspended biomass concentration was compared with the attached biomass. When the surface area (A) is replaced by reactor volume (V), the Stover Kincannon model is modified as follows:

$$\frac{dS}{dt} = \frac{U_{\max} \frac{QS_i}{V}}{K_B + \left(\frac{QS_i}{V}\right)} \tag{2}$$

For this equation, units of K_B change to $g.L^{-1}.d^{-1}$

Equation 2 can be linearized as follows

$$\frac{1}{dS/dt} = \frac{V}{Q(S_i - S_e)} = \frac{K_B}{U_{\max}} \cdot \frac{V}{QS_i} + \frac{1}{U_{\max}} \tag{3}$$

If 1/(dS⁄dt) is deduced as V/(Q(Si-Se)), the inverse of the removal rate and is plotted against V/QSi, the inverse of the loading rate, a straight line plot is obtained. From this plot, the slope gives K_B/U_{max} and the intercept of the straight line gives $1/U_{max}$.

The substrate balance for the reactor can be expressed as follows:

$$Q.S_i = \frac{dS}{dt}.V + QS_e \tag{4}$$

Replacing equation 2 in the above equation gives

$$Q.S_i = \frac{U_{\max} \frac{QS_i}{V}}{K_B + \left(\frac{QS_i}{V}\right)}.V + QS_e \tag{5}$$

This equation can be solved for effluent substrate concentration by introducing the values of U_{max} and K_B values using the following equation

$$S_e = S_i - \frac{U_{\max} S_i}{K_B + \left(Q.{}^{S_i}\!/_{V}\right)} \tag{6}$$

3. Materials and methods

3.1 Reactor description

The SBR with a working volume of 5L was used. The pH was maintained between 7.5 and 8 without specific control. The medium was homogenized by a magnetic stirrer. A set of two peristaltic pumps was used to introduce the feeding solution and to discharge the effluent. Timers controlled the actuations of the pumps and valves and regulated the different periods of the operational cycle. The reactor was flushed continuously Argon to maintain anaerobic conditions. All the tubing was norprene tubes, to prevent the diffusion of oxygen inside the system

3.2 Operational conditions

The SBR was operated in 12 h cycles distributed as follows during operation: 600 min of feeding and mixing, 45 min of settling, 15 min of effluent withdrawal. The feeding supplied to the reactor was prepared using the mineral salt medium. The reactor was operated in 2 different stages depending on the nitrogen loading rate. The Hydraulic Retention Time (HRT) was maintained at 0.4 days.

3.3 Culture media and inoculum

Synthetic medium was supplemented with ammonium and nitrite (at required concentrations) in the form of $(NH_4)_2SO_4$ and $NaNO_2$, respectively. The composition of the synthetic medium was (per litre deionized water) 1.25 g of $KHCO_3$, 0.05 g of NaH_2PO_4, 0.2 g of $MgSO_4 \cdot 7H_2O$, 0.3 g of $CaCl_2 \cdot 2H_2O$, 0.006 g of $FeSO_4$, 0.006 g of EDTA and 1.25 ml trace elements solution ((Jetten et al. , 2005). The trace elements solution contained (per litre deionized water) 0.4 g of $ZnSO_4 \cdot 7H_2O$, 0.04 g of $CuSO_4.5H_2O$, 0.1 g of KI, 0.2 g of $FeCl_3.6H_2O$, 0.4 g of $MnSO_4 \cdot H_2O$, 0.2 g of $Na_2MoO_4.H_2O$. 0.4 of $ZnSO_4.7H_2O$, 1 g of NaCl, 0.1 g of $CoSO_4$, 0.1g of $CaCl_2$, 0.01g of $AlK(SO_4)_2.12H_2O$ and 0.05 g of H_3BO_3. The reactor was inoculated with a suspension culture of anammox biomass.

3.4 Analysis

Ammonium, nitrite and nitrate were analysed calorimetrically according to the following methods:
•Nitrate analysis – add 10µl saturated sulfumic acid and 40µl reactor effluent together. To the mixture add a total of 0.2 ml reagent containing 5% salicylic acid in 98% sulphuric acid and 2ml 4M NaOH (4ºC). This solution is analysed in a spectrophotometer at 420nm after a 30 minutes reaction.
•Ammonium analysis – add 760 µl of a solution containing 0.54% ortho-pthalaldehyde, 0.05% β-mercaptoethanol and 10% ethanol in 400mM potassium phosphate buffer (pH 7.3) to a 40 µl reactor effluent sample. This solution is analysed in a spectrophotometer at 420nm after a 30 minutes reaction.
•Nitrite analysis – add 950 µl of a reagent containing 1% sulfanilic acid and 0.05% N- naphthylethylenediamine in 1 M H_3PO_4 to 50µl of reactor effluent. This is followed by a spectrophotometric analysis at 540nm after 5 minutes reaction.

4. Results and Discussion

4.1 Performance of the reactor

The SBR for an anammox process was started-up with nitrogen loading rate (NLR) of 5 g.N•L-1•d-1. The reactor was operated for about 120 days. The reactor was operated in two stages. In the second stage, the initial NLR was increased from 5 g.N•L^{-1}•d^{-1} to 6.3 gN•L^{-1}•d^{-1} by increasing the concentrations of nitrogen compounds. However, only NH_4^+ concentrations were increased. Nitrite concentrations remained the same as in the first to avoid the inhibitory effect of nitrite in the system. Figure 1 illustrates the performance of the reactor. Over the period of 120 days the nitrogen removal efficiency was about 93% (Table 1) with nitrite almost completely consumed (>97%) (Figure 1).
When NLR was increased to 6.3 gN•L^{-1}•d^{-1} concentrations of NH_4^+were slightly increased in the effluent and later they started to decrease gradually.

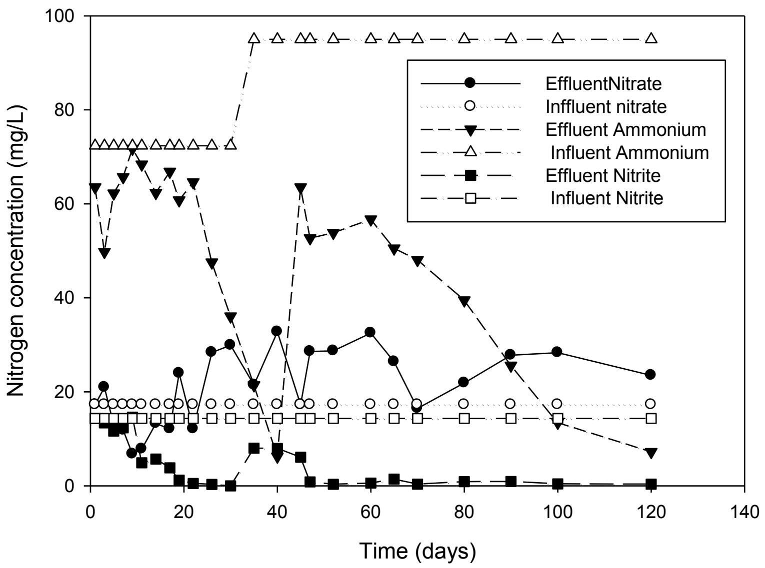

Figure 1: Performance of anammox suspension in SBR Influent: NH_4^+ Δ, NO_2^-□, NO_3^-○, Effluent NH_4^+▼, NO_2^- ■, NO_3^-●

In an anammox process nitrogen gas has to be produced. In order to reach the conclusion that anammox was taking place in the reactor, gas production was also monitored. The production of the gas in the reactor was confirmed by a gradual increase in readings of the gas metre that was used. In addition, calculations of nitrogen balance were made and it was found that 1.22 moles of nitrite were consumed and 0.2 moles of nitrate were produced per mole of ammonium consumed. Though these values are not exactly the same as those of the anammox process proposed by Strous et al., (1998) they are too close and are comparable to the theoretical stoichiometry of anammox. Therefore it can be concluded that anammox process definitely took place in the reactor and was able to remove up to 93% of total nitrogen in the system.

Table 1: Nitrogen removal rate and efficiency of the anammox reactor

Time (days)	Substrate removal rate (gN/L.d)			Substrate removal efficiency %		
	NH4-N	NO2- N	Total nitrogen	NH4-N	NO2- N	Total nitrogen
1	0.5085	0	0.5085	12.2054	0	10.1875
14	0.576	0.498	1.074	13.8234	60.3432	21.5145
19	0.6668	0.7561	1.4229	16.0042	91.6192	28.5056
22	0.447	0.7963	1.2433	10.7281	96.4841	24.9061
26	1.4274	0.8078	2.2352	34.2595	97.8743	44.777
30	2.0898	0.8253	2.9151	50.1583	100	58.3986
35	4.239	0.3637	4.6028	77.4742	44.0715	73.0963
40	5.1125	0.3674	5.4799	93.4378	44.5217	87.0266
100	4.6992	0.8017	5.5009	85.8845	97.138	87.3595
120	5.0568	0.8054	5.8622	92.42	97.5881	93.0974

4.2 Substrate removal kinetics

The nitrogen removal kinetics of the anammox reactor was determined using modified Stover-Kincannon model. Figure 2 shows the plot of V/[Q(Si – Se)], the reciprocal of substrate removal rate against V/(QSi), the reciprocal of substrate loading rate. Saturation value constant KB and maximum substrate removal rate Umax were calculated from the slope and intercept of the line plotted in Figure 2 and determined to be 35.8 and

34gN·L^{-1}.d^{-1} respectively. This implied that the anammox reactor had a maximum total nitrogen removal rate of 34 g N•L^{-1}•d^{-1}. These values were higher than those obtained in other studies (Gong et al. , 2008, Jin and Zheng. , 2009, Ni et al. , 2010). The maximum total nitrogen removal rate from the experimental data was 5.8622 g.N.$L^{-1}d^{-1}$. This value was much less than that of predicted values and only accounted for 17% of Umax indicating the nitrogen removal full capacity of the reactor has not been reached yet. The plot also gave correlation coefficient of R^2=0.7061 thus supporting the appropriateness of the modified Stover-Kincannon model.
The effluent nitrogen concentration was calculated using equation 6 after introducing the K_B and U_{max} values in equation 3. The calculated values were compared with experimental data in order to validate the model. Figure 3 illustrates the comparison between predicted and experimental effluent concentrations. The linear relationship was obtained from the comparison. The linear relationship represented a good agreement between experimental and predicted total nitrogen effluent concentrations and gave a high correlation coefficient (R^2=0.9739). This indicated that Stover-Kincannon model was suitable for nitrogen removal kinetics in sequencing batch reactor.

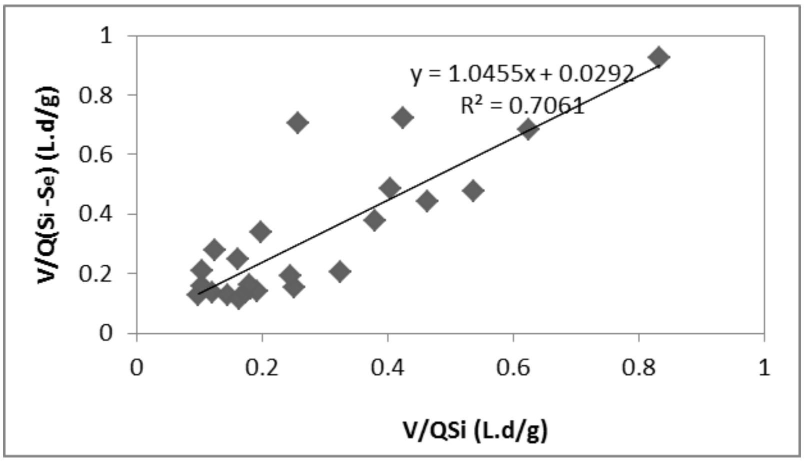

Figure 2: Nitrogen removal model plot– modified Stover-Kincannon model

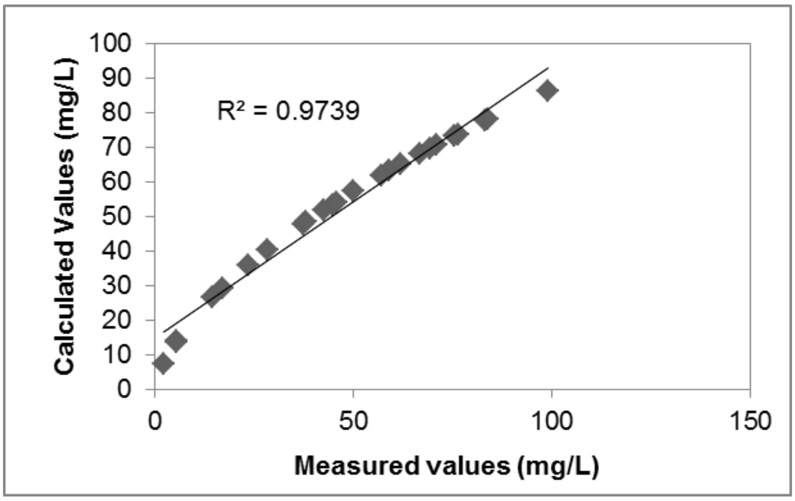

Figure 3: Validation of the modified Stover-Kincannon model

5. Conclusions

This study presents the achievability of successful start-up of the anammox process in a laboratory-scale sequencing batch reactor. The start-up duration of an anammox reactor with pre-enriched non-granular sludge was significantly reduced compared to the ones reported in literature. The study showed that anammox non-granular culture had a great activity. A maximum substrate removal rate of 34 gN.$L^{-1}d^{-1}$ was predicted. The model simulation matched the experimental data very well, proving the Stover-Kincannon model to be appropriate for nitrogen removal kinetics for the anammox process.

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