Investigating the Process Kinetics and Nitrogen Gas Production in Anammox Hybrid Reactor with Special Emphasis on the Role of Filter Media

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Abstract—Anammox is a novel and promising technology that has changed the traditional concept of biological nitrogen removal. The process facilitates direct oxidation of ammonical nitrogen under anaerobic conditions with nitrite as an electron acceptor without addition of external carbon sources. The present study investigated the feasibility of Anammox Hybrid Reactor (AHR) combining the dual advantages of suspended and attached growth media for biodegradation of ammonical nitrogen in wastewater. Experimental unit consisted of 4 nos. of 5L capacity AHR inoculated with mixed seed culture containing anoxic and activated sludge (1:1). The process was established by feeding the reactors with synthetic wastewater containing NH_4-H and NO_2-N in the ratio 1:1 at HRT (hydraulic retention time) of 1 day. The reactors were gradually acclimated to higher ammonium concentration till it attained pseudo steady state removal at a total nitrogen concentration of 1200 mg/l. During this period, the performance of the AHR was monitored at twelve different HRTs varying from 0.25-3.0 d with increasing NLR from 0.4 to 4.8 kg N/m^3d. AHR demonstrated significantly higher nitrogen removal (95.1%) at optimal HRT of 1 day. Filter media in AHR contributed an additional 27.2% ammonium removal in addition to 72% reduction in the sludge washout rate. This may be attributed to the functional mechanism of filter media which acts as a mechanical sieve and reduces the sludge washout rate many folds. This enhances the biomass retention capacity of the reactor by 25%, which is the key parameter for successful operation of high rate bioreactors. The effluent nitrate concentration, which is one of the bottlenecks of anammox process was also minimised significantly (42.3-52.3 mg/L). Process kinetics was evaluated using first order and Grau-second order models. The first-order substrate removal rate constant was found as 13.0 d^{-1}. Model validation revealed that Grau second order model was more precise and predicted effluent nitrogen concentration with least error (1.84±10%). A new mathematical model based on mass balance was developed to predict N_2 gas in AHR. The mass balance model derived from total nitrogen dictated significantly higher correlation (R^2=0.986) and predicted N_2 gas with least error of precision (0.12±8.49%). SEM study of biomass indicated the presence of heterogeneous population of cocci and rod shaped bacteria of average diameter varying from 1.2-1.5 mm. Owing to enhanced NRE coupled with meagre production of effluent nitrate and its ability to retain high biomass, AHR proved to be the most competitive reactor configuration for dealing with nitrogen laden wastewater.

Keywords—Anammox, filter media, kinetics, nitrogen removal.

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I. INTRODUCTION

NITROGENOUS compounds like ammonium are predominantly found in the wastewater generated from various industries i.e. slaughter house, pharmaceuticals, coke oven, tanneries and fertilizer effluents. The concentration of NH_4-N in these industrial effluents ranges from 220 to 2600 mg/l [1]-[6]. The conventional nitrogen removal process is uneconomical and characterized by high energy demand, operating cost and global N_2O emissions (3%) [7], thus contributing significantly to climate change. Anammox is one such novel, promising and cost effective alternative to conventional treatment systems that facilitates direct oxidation of ammonium nitrogen under anaerobic conditions with nitrite as an electron acceptor. Significant reduction in aeration costs, exogenous electron donor saving and low sludge production makes the process techno-economically feasible and competitive over the existing conventional treatment technologies. While, the newly discovered anammox process opens up the new possibilities for nitrogen removal from wastewater, the major constraint for field-scale application is the slow growth rate of anammox bacteria ranging to several months or more [8], [9] and low biomass yield (0.11-0.13g/g) [10]-[16]. To overcome these constraints and limitations, various reactor configurations such as upflow anaerobic sludge blanket (UASB), circular stirred tank reactor (CSTR), anaerobic membrane bioreactor (AnMBR), sequential batch reactor (SBR), membrane sequential batch reactor (MSBR), fluidized bed reactor (FBR) and rotating biological contactor (RBC) [10], [17], [18] were investigated. Among these, the hybrid reactor configuration which combines the dual advantages of attached and suspended growth, has demonstrated excellent potential in terms of rapid startup, high sludge retention and good granulation [19]-[21]. However, the process kinetics and performance of this reactor configuration has not been studied in detail.

A sound knowledge of process kinetics and mathematical models is extremely essential for optimizing the reactor performance in order to exercise better control on process design and its operation. A number of mathematical models e.g., First-order substrate removal model [22], Stover and Kincannon model [23], Grau second-order [24] and Monod model [25] are available in literature to evaluate the substrate removal kinetics in anammox reactors. Abbas et al. [26] investigated the process kinetics of pilot scale internal loop airlift bio-particle (ILAB) reactor using synthetic wastewater and established higher correlation coefficients of 97.5% and

94.2% for Grau second order and Stover Kincannon model, respectively. Ni et al. [27] investigated the performance of upflow anaerobic sludge blanket (UASB) reactor and revealed that both Grau-second order and Modified Stover-Kincannon model produced the best fit and precisely predicted the substrate removal kinetics in granular anammox reactor. Although kinetics of substrate removal has been investigated in detail by several researchers [28]-[30], limited attention has been paid on the nitrogen gas production kinetics. In the above context, the major objective of the research was to evaluate nitrogen gas production kinetics using a new mathematical model based on the concept of mass balance of nitrogen in anammox process. In addition, the role of filter media in AHR was also assessed towards additional nitrogen removal and reduction in sludge washout rate.

II. KINETIC MODELS

The first-order and Grau second-order model was used to evaluate the substrate removal kinetics in AHR. While, the kinetics of N_2 gas production was investigated by mass balance model.

A. First-Order Substrate Removal Model

The general equation for first-order substrate removal model can be expressed by (1):

$$\frac{S_i - S_e}{\theta} = k_1 S_e \tag{1}$$

where S_i and S_e are influent and effluent total nitrogen concentrations (g/l), k_1 is the first order substrate removal constant (1/d), θ is the hydraulic retention time (day). The value of k_1 can be derived from the slope of the line by plotting $(S_i - S_e)/\theta$ versus S_e in the above equation.

B. Grau Second-Order Substrate Removal Model

The generalised equation for Grau second-order substrate removal model can be expressed by (2):

$$\frac{HRT}{E} = a + bHRT \tag{2}$$

where HRT is the hydraulic retention time (d), E represents substrate removal efficiency (%), a and b are the kinetic constants.

C. Mass Balance Model

The general equation for mass balance can be expressed by (3):

$$Input = Output + Accumulation \tag{3}$$

The model is developed based on the conservation of mass considering that total mass of nitrogen entering into the bioreactor is equal to the sum of the mass of nitrogen leaving from the reactor (N_2 gas + Effluent nitrogen) and the amount of nitrogen being accumulated as synthesized biomass. Mathematically, this can be expressed by (4):

$$N_i = N_{eff} + N_{gas} + N_{syn} \tag{4}$$

where N_i = Influent nitrogen (sum of NH_4-N and NO_2-N), mg/l; N_{eff} = Effluent nitrogen (sum of NH_4-N, NO_2-N and NO_3-N), mg/l; N_{gas} = Equivalent N produced as N_2 gas, mg/l; N_{syn} = Nitrogen being accumulated as synthesized biomass, mg/l. The equivalent nitrogen which is converted into N_2 gas can be determined by (5):

$$N_{gas} = N_i - (N_{eff} + N_{syn}) \tag{5}$$

In the above equation, if the sum of the nitrogen remaining in the effluent and nitrogen accumulated in the form of synthesized biomass is substituted by Ne, the equation can be reduced to (6):

$$N_{gas} = N_i - N_e \tag{6}$$

where N_e = Equivalent nitrogen (mg/l). Thus, in a continuous system, N_2 production is proportional to flow rate (Q), influent nitrogen concentration (Ni), equivalent nitrogen concentration (Ne) which can be expressed by (7):

$$N_g \propto Q(N_i - N_e) \tag{7}$$

In the above equation, if k_g is the proportionality constant then the equation for generation of N_2 in anammox process can be expressed as (8):

$$N_g = k_g Q(N_i - N_e) \tag{8}$$

where k_g is the proportionality constant referred as gas constant (ml/mg), which can be determined from the straight line plot of N_g versus $Q(N_i-N_e)$ as per (8).

III. MATERIALS AND METHODS

A. Experimental Setup of AHR

The experimental setup of AHR is shown in Fig. 1. The reactor was fabricated of transparent acrylic plastic with an internal diameter of 10 cm and height 65 cm. The total working volume was 5 litres. Corrugated polyvinyl chloride (PVC) pipes of length 2.25 cm and diameter 2.25 cm were used as filter media. Total 55 nos. of PVC carriers were added to the reactor to constitute an AGM. The sludge blanket in the lower half of the reactor constitutes suspended growth system while filter media in the upper part provides attached growth for the microorganisms. To assess the effect of AGM, two outlets (Outlet I and II), were provided, one above and the other below the filter media, for facilitating the collection of samples (Fig. 1 (a)). The reactor was also completely covered with black cloth to avoid the growth of phototrophic organisms and oxygen production [31].

B. Origin of Inoculum Sludge

Mixed seed culture of anoxic and activated sludge, 1:1 (v/v) was used as inoculum for AHR. Anoxic sludge was collected from the bottom of waste stabilization pond treating municipal sewage. The activated sludge was collected from Durgapur Coke Oven Effluent Treatment Plant. The mixed inoculum

sludge was greyish black in color having volatile suspended solids (VSS) content 1.68 g/l. The reactor was fed with the synthetic wastewater using a peristaltic pump to maintain a constant flow rate. The composition of synthetic wastewater used in the study was adopted from Van de Graaf et al. [17].
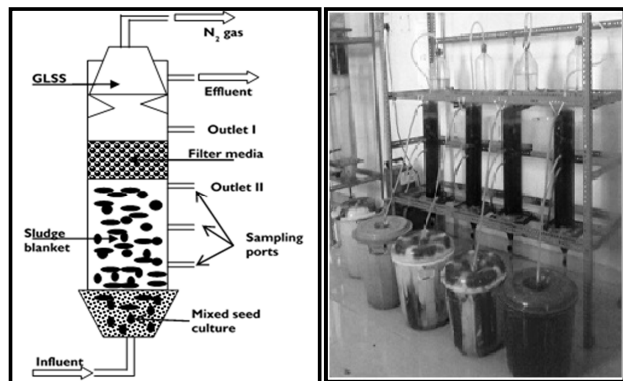

Fig. 1 (a) Schematic diagram of AHR (b) Experimental set-up of AHR

C. Strategy of Operation

The reactors were started with initial influent ammonium and nitrite concentrations of 100 mg/l each to maintain the optimal nitrite-ammonium ratio of 1:1. After establishment of pseudo-steady state condition, ammonium and nitrite concentrations were gradually increased to 600 mg/l each, and maintained constant thereafter. The performance of AHR was monitored at different HRTs, varying from 3.0–0.25 d, to arrive at the optimal HRT for smooth functioning of the reactor. The data from HRT study was also used to calculate kinetic coefficients of different mathematical models so as to investigate both substrate removal and N_2 gas production kinetics. The effect and contribution of the FM toward nitrogen removal and biomass retention was assessed by analyzing the effluent samples from outlets I and II at an HRT of 0.25 d. The study was performed for a minimum period of three weeks (260–281 days) and the average contribution of the FM towards nitrogen removal and reduction in the sludge washout rate was worked out.

D. Analytical Methods

Effluent samples were collected and analysed on daily basis for NH_4-N, NO_2-N and NO_3-N as per the standard methods [32]. The volume of gas liberated was measured by water displacement method while its composition was analysed through Gas Chromatograph (GC) equipped with thermal conductivity detector (TCD) using DPK Spherocarp column. The GC was operated at injector and detector temperatures of 120ºC and 150ºC, respectively. H_2 @ 20 ml/min was used as a carrier gas. SEM (Scanning electron microscopy) was adopted as a tool to study the morphology of granules formed in anammox process.

E. Quality Control/Quality Assurance Procedure (QA/QC)

The laboratory reagent blanks were prepared and analyzed to determine if any interference was present in the effluent samples. The precision of the measurements were estimated using triplicate sample analysis. The relative percentage difference (RPD) between two parallel samples was calculated and cross verified. In case the RPD exceeded > 5%, the samples were recollected and analyzed. The average of the triplicate readings was reported as the final value. Continuous calibration checks were performed during GC analysis after injection of every 10 samples. If the RPD between the response of the initial calibration and the calibration check standard was > 10%, the instrument was considered as out of calibration, and was recalibrated. The high precision gas standards procured from Chemtron Science Laboratories Pvt. Ltd., Mumbai were used for calibration.

IV. RESULTS AND DISCUSSION

A. Effect of HRT on the Performance of AHR

The performance of AHR under varying HRTs and NLRs is depicted in Fig. 2. Analysis of data indicated that the performance of the reactor in terms of nitrogen removal efficiency increased with increase in HRT. The maximum nitrogen removal (97.5%) was obtained at an HRT of 2.25 day corresponding to NLR of 0.53 kg N/m³d. However, the decrease in the NRE beyond at HRT of 1 day was marginal. Hence, the HRT of 1.0 day was considered optimal to achieve substantial nitrogen removal of 95.1%. When the HRT was further reduced from 0.75 d to 0.25 d, the NRE dropped considerably from 89.4% to 78.6% respectively. This may be due to the increased NLR in AHR. A similar profile was observed for ammonium removal. Suneethi and Joseph [33] investigated the performance of AnMBR under varying NLRs and HRTs and reported ammonium removal efficiency (ARE) of 81% at an optimal HRT of 1.5 d. Ni et al. [28] also reported decrease in total nitrogen (TN) removal efficiency from 89.9% to the lowest 80.7% in a anaerobic non-woven membrane bioreactor, with decrease in HRT from 2.9 days to < 1 day. The higher NRE observed at lower HRT in our study might be attributed to the hybrid configuration of bioreactor and the use of mixed inoculum sludge.

Nitrate production, which is considered as a bottleneck in field-scale application of anammox, did not vary significantly and was found comparatively lower (37.2 to 52.3 mg/l) than reported in the literature [30], [34]. This might be attributed to significantly high nitrite removal efficiency, which could have minimized the chances of nitrate formation in AHR. Strous et al. [35] reported that the excess nitrite in the system led to the formation of nitrate and hydroxylamine in this process. As nitrite removal was consistently higher, the formation of excess nitrate was not observed in our study.
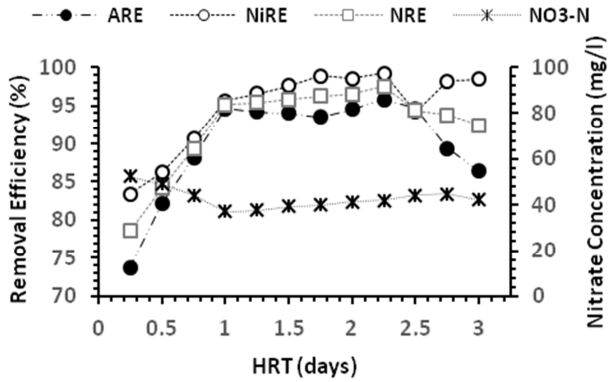
Fig. 2 Nitrogen removal profile of AHR at different HRTs

B. Contribution of Attached Growth System towards Nitrogen Removal and Percent VSS Reduction

ARE and NiRE of AHR at outlets I and II are depicted in Fig. 3. Average ARE of the reactor at outlets I and II were 74.5% and 47.2%, respectively. Similar profile was observed for nitrite removal at outlets I and II. Analysis of data indicated that AGM in AHR contributed an additional 27.2% ammonium removal. The sludge washout rate which plays an important role in governing the biomass retention was also estimated at outlets I and II. The average sludge washout rate at outlets I was considerably reduced (1.04 g/d) than outlet II (3.74 g/d) respectively. This may be attributed to the presence of AGM which acts as a mechanical sieve and thus reduces the biomass washout. This is evident from additional 72% reduction in the VSS profile between outlets I and II (Fig. 4). Duan et al. [19] also reported that the use of non-woven carrier as AGM effectively increased the biomass retention by 2.8% and overall NRR by 8.1 % in the hybrid reactor. The study revealed that the AGM in AHR not only enhances the nitrogen removal but also considerably reduces the sludge washout from the reactor. This feature eliminates the major constraints of anammox process and thus offers comparatively enhanced biomass retention and higher biomass yield in addition to increased substrate removal efficiency.
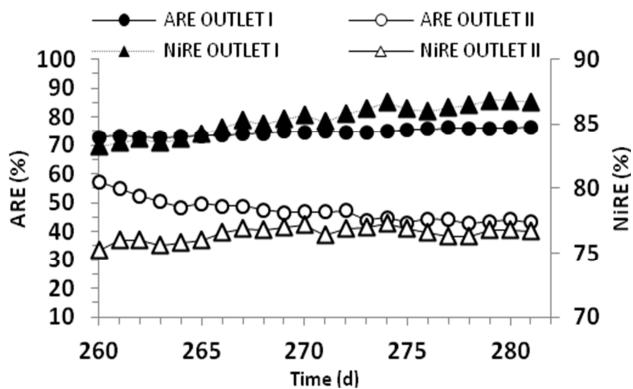

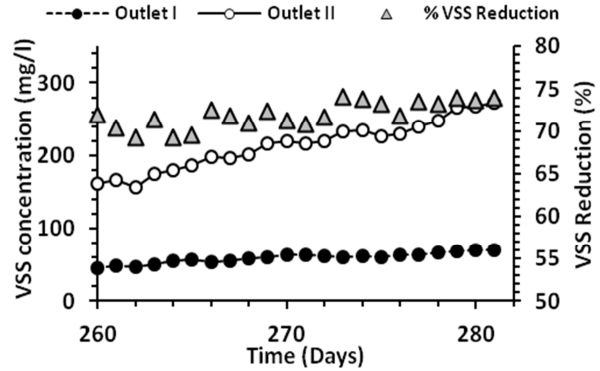Fig. 3 Contribution of attached growth system towards ammonium and nitrite removal

Fig. 4 Contribution of attached growth system towards percent VSS reduction

C. Determination of Nitrogen Removal Kinetics

1. First-Order Substrate Removal Model

The first order model plot is depicted in Fig. 5. The value of first order kinetic constant, k_1 was calculated as 13 d^{-1} (Table I) from the slope of the straight line, when $(S_i - S_e)/\theta$ was plotted against S_e as per (1). The higher degree of correlation coefficient ($R^2 = 0.837$) suggests that the model can further be validated to evaluate substrate removal kinetics in AHR using (9) as:

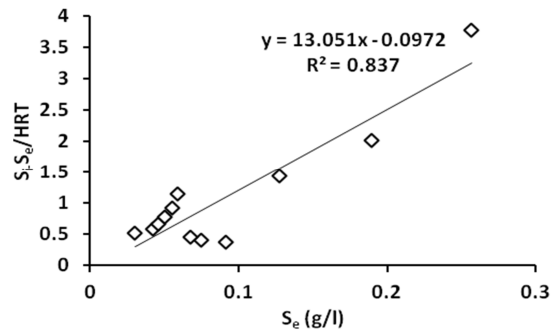$$S_e = \frac{S_i}{k\theta + 1} \qquad (9)$$

Fig. 5 First order kinetic model plot

2. Grau Second-Order Model

To assess the applicability of Grau second-order model, Θ/E was plotted against Θ as per (2). The values of second-order constants a and b were 0.0267 and 1.045 respectively (Table I). Higher value of correlation coefficient ($R^2 = 0.998$) clearly indicates that Grau second-order model is most appropriate for describing anammox process kinetics in AHR (Fig. 6). Ni et al. [28] stated that this model is also capable of predicting substrate removal at any loading conditions, irrespective of the order of reaction kinetics. Abbas et al. [26] also investigated the process kinetics and reported higher correlation coefficients (97.5%) for Grau second-order model in internal loop airlift bio-particle (ILAB) reactor. Accordingly, for testing the validity of the model, following (10) was used for prediction of effluent substrate concentration:
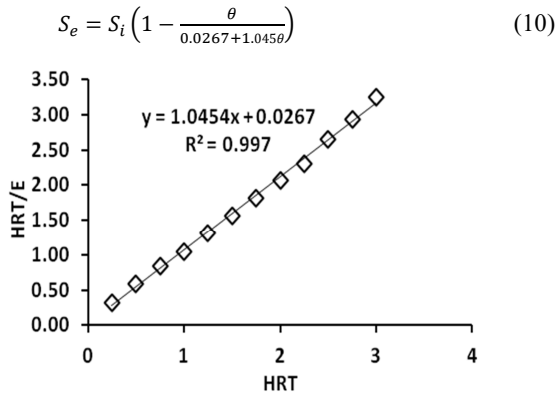
$$S_e = S_i \left(1 - \frac{\theta}{0.0267 + 1.045\theta}\right) \qquad (10)$$

Fig. 6 Grau second-order model plot for substrate removal kinetics

D. Determination of Kinetics of N₂ Production

1. Mass Balance Model

The mass balance model plot showed significantly higher correlation co-efficient (R^2=0.996) (Fig. 7). Higher correlation coefficient of the model plot signifies that this may accurately be applied for prediction of N_2 gas in AHR. This may be attributed to the fact that unlike the other models, this model not only considers the part of influent nitrogen utilised in biomass synthesis, but also takes into account nitrate produced in the treated effluent. Thus, gives more accurate estimation of of N_2 gas in AHR and eliminates the errors associated with the other models. For testing the validity of the model, the N_2 gas production can be predicted by (11) as:
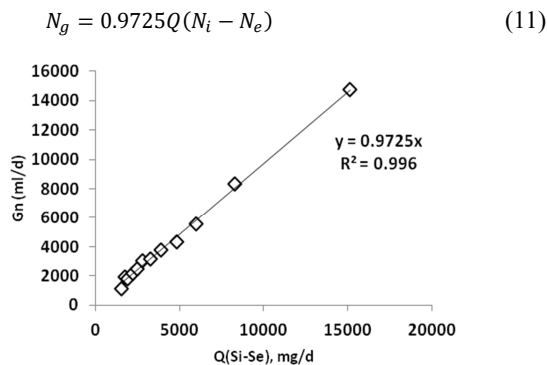
$$N_g = 0.9725 Q(N_i - N_e) \qquad (11)$$

Fig. 7 Mass balance model plot for nitrogen gas production kinetics

TABLE I
SUMMARY OF KINETIC CONSTANTS CALCULATED FROM VARIOUS MODELS

Kinetic Models	Kinetic Const.	Observed Value	Observed R^2	Rptd Value	References
First order	k_1 (1/d)	13.0	0.837	5.30-11.64	[27], [28]
Grau second-order	a (1/d)	0.026	0.998	0.055-1.397	[27]-[30]
	b	1.045	0.998	0.964-1.136	
Mass balance	K_{sg}(ml/mg)	0.972	0.996	-	Our study

E. Validation of Models

For testing the validity of the models, new set of observed values were compared with predicted values calculated from the respective models (Fig. 8). In case of substrate removal kinetics, a good linear relationship was obtained between observed and predicted effluent substrate concentrations. However, the percentage error between the observed and predicted values from Grau second-order model (1.84±10.1%) was significantly lower than first order model (11.0±39.1%). This clearly indicates that Grau second-order model is most appropriate and can precisely predict substrate removal kinetics in AHR. Similarly, nitrogen gas could successfully be predicted (R^2=0.986) using mass balance model (Fig. 9) with a standard error of 0.12±8.4%. The value of t_{cal} was substantially lower than t_{crit} indicating that the models are unbiased and can suitably be applied.
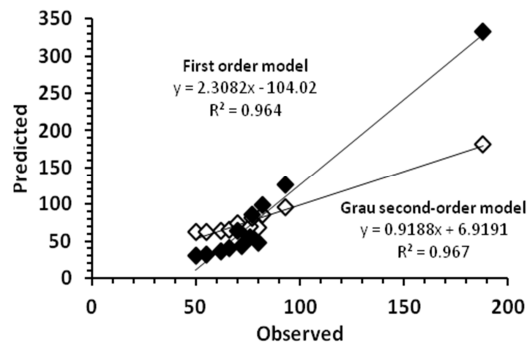

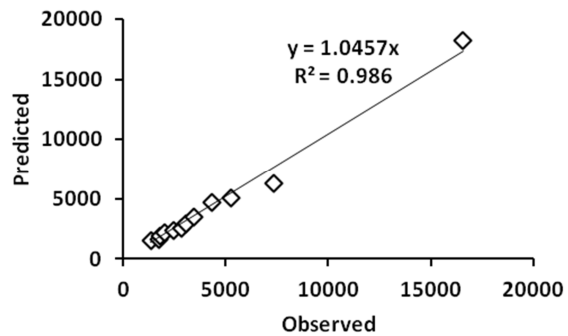Fig. 8 Comparison of predicted and observed values from substrate removal models

Fig. 9 Comparison of predicted and observed values from mass balance model

F. Morphological Study of Anammox Granules

Enhanced biomass retention and good granulation are the key factors which govern the process performance and stability of bioreactor. The morphology of the granules demonstrated presence of heterogeneous bacterial population consisting of both cocci and rod shape along with few clusters of filamentous microorganism (Fig. 10 (a)). Tang et al. [36] also demonstrated similar morphology of anammox bacteria in UASB reactor. A few stayed separate, while most tended to grow in cluster as reported by other researchers [13], [37]. The surface morphology showed heterogeneous and rough surface with irregular projections. SEM images of granular sludge depicted that the granules were almost spherical in shape with an average size of 1.5-2.0 mm. The size distribution of the granules observed in our study is comparable with the values

reported (0.01-2.00 mm) in literature [19], [38]. Interstitial void/cavities, which are usually found embedded in the mesh connecting several microbial cell clusters, were also seen on the surface. These interstitial void/cavities serve as channels for transport of substrate, metals and gases through the granules.
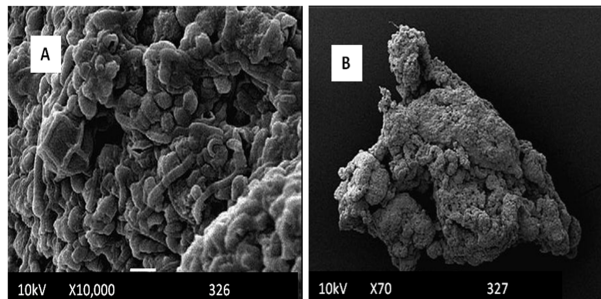

Fig. 10 (a) Cluster of cocci and rod shaped bacteria in anammox granular sludge (b) SEM photos of granular sludge in AHR

V. Conclusion

AHR demonstrated excellent nitrogen removal of 95.1% at an optimal HRT of 1 day corresponding to NLR of 1.2 kgN/m^3.d. Filter media in AHR contributes 27.2% additional ammonium removal and reduces the sludge washout rate by 72%. Effluent nitrate concentration, which is one of the bottlenecks of anammox process is also significantly reduced in hybrid reactor configuration. Kinetic modelling of AHR revealed that Grau second-order model is more appropriate and can precisely predict effluent nitrogen concentration with least error of precision. A newly developed mathematical model based on the concept of mass balance predicted nitrogen gas precisely with percentage error of 0.12±8.4%. SEM study indicated the presence of heterogeneous population of both cocci and rod shaped anammox bacteria with average granule size of 1.5-2.0 mm. Owing to the enhanced biomass retention, significant reduction in sludge washout rate coupled with meagre nitrate production in the treated effluent, AHR proved to be the most promising technology to treat nitrogen laden wastewater.

Acknowledgement

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