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Kinetics with optimization studies of nitrogen and organic elimination from wastewater via heterotrophic biomass conversion process

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ABSTRACT

Heterotrophic biomass conversion (HBC) research was carried out for the removal of N-NH_3 and organic carbon from synthetic wastewater. Ammonium nitrate and glucose were used as the nitrogen and organic carbon source, respectively. In this study, N-NH_3 and organic nutrient concentrations were varied, keeping the biomass concentration invariable. The kinetics followed dual rates, i.e. faster initial rate followed by a slower one. The consumption of N-NH_3 and COD followed first-order kinetics. Kinetic model such as Monod was studied. The pH during the HBC process showed an increasing trend which may be due to heterotrophic nitrification (HN). Parameters like N-NO_3^-, N_2O, N-NO_2^-, time, and dissolved oxygen were studied. A part of N-NH_3 utilized for the emission of N_2O may be due to HN. Analyses of variance were carried out for better interpretation of results. Optimization studies were carried out to minimize N_2O emission and maximize N-NH_3 along with COD removal.

Keywords: HBC; Kinetics; Monod; Diffusion; Optimization; Statistics

1. Introduction

Water plays an important role in supporting biotic system. The water quality gets changed day by day due to rapid growth in population, industrialization, and agricultural activities. The nitrogenous (inorganic and organic) and organic pollutants released due to various anthropogenic activities increases the pollution load of different water bodies. This gradual contamination creates a nutrient enrichment condition, which leads to eutrophication and increase in biochemical oxygen demand (BOD), thereby decreasing the quality of water bodies.

Nitrogen compounds are highly soluble in water, due to which its removal through chemical precipitation is not feasible. Nitrogenous pollutants present in wastewater are usually treated by both nitrifying and denitrifying bacteria [1]. Throughout wastewater treatment process N_2O emission is observed [2–4], a major greenhouse gas (GHG), [5,6] which causes global temperature increase as well as ozone exhaustion [7,8]. Both denitrifying and nitrifying bacteria are reducing and oxidizing in nature. Denitrifiers reduce NO_3^- to N_2, whereas nitrifiers oxidize NH_3 to NO_3^- [4,9]. Consequently, the total modification requires two steps, i.e. anaerobic and aerobic [10]. In order to beat the problem, few other methods have been developed

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such as anaerobic ammonium oxidation (ANAMOX) and heterotrophic biomass conversion (HBC). During ANAMOX method, NH_3 is transformed to N_2 and the remarkable intermediate product obtained is N_2O, while HBC directly converts NH_3 to biomass [11], thus retaining the nitrogen values. The HBC process requires organic carbon source to convert NH_3 to biomass [11]. HBC method was mostly carried out by the facultative heterotrophs under aerobic conditions and they may undergo denitrification under anaerobic conditions [12]. The reaction kinetics depends on various factors such as pH, alkalinity, temperature, oxygen, and NH_3 [13]. HBC method is an alkali-consuming, CO_2-releasing, and pH-reducing process [12,13]. Oxygen demand requirement is slightly more to carry out this process, which can be achieved through proper aeration [13]. HBC can be considered environment friendly because N_2O emission is not observed during the process. Normally in HBC process, microbial biomass production is 40 times more than the biomass generated in nitrification method [12,13], which could be used as a biofertilizer after harvest. The bacteria undergoing the HBC process result in a high growth rate [11]. Next to the HBC route, the transfer of NH_3 to NO_3^- by heterotrophic nitrification (HN) was reported to be carried out by heterotrophic bacteria under aerobic conditions releasing NO_3^- as the final product [4,14]. The intermediate products formed during HN process are N_2O, NH_2OH, and NO_2^- [15]. In HN course, the pH normally lowers below pH 7, which initiates higher N_2O emission [16]. It may be prevented by maintaining a nearly neutral pH. According to literature, the suitable pH range for nitrifiers is 6–8 [15]. Organic carbon is used as a source of energy by nitrifying bacteria. The substrates, intermediates, and products were same for both heterotrophic as well as autotrophic nitrifiers. HN produces a less N_2O, but under high dissolved oxygen (DO), low pH, and organic source will produce more N_2O [4]. The different species of bacteria that have the HN ability are *Pseudomonas, Alcaligenes faecalis*, and *Comamonas* sp. [15,17]. Still, abundant understanding of HN series based on the detailed physiological as well as parametric studies in batch and continuous cultures is preferred.

HBC method is very much complex; thus, an in-depth analysis is necessary to relate HBC along with various parameters. Considering few of above-mentioned parameters, some attempts have been taken to settle on the kinetics, establishment of rate equation, and statistical explanation of results. N_2O emission observed during the treatment process may be due to the heterotrophic nitrifiers, which are present partially in the consortium. Also, insufficient availability of literature in this area increases the extent of this problem. Accordingly, as a part of our systemic studies this communication exclusively aims to set up a mathematical co-relation between the parameters in order to minimize the response like N-N_2O and maximize N-NH_3, and COD removal through response surface modeling (RSM). RSM technique is used to optimize the response of a multivariate system by exploring the relationships between various variables and their combined effects on response variables [18,19]. Batch experiment studies were conducted to study the effect of variables such as nitrogen and organic carbon source along with days.

2. Material and methods

2.1. Sampling and enrichment

Soil sample was collected from a paddy field in Tangibanta, a rural location 20 km away from Bhubaneswar, India at a depth of 0–15 cm using an auger [20]. The heterotrophic bacteria were isolated from the agricultural soil using MSN (mineral, salt, and nutrient) liquid media (Composition: CH_3CO-ONa-7.86 g/L, KH_2PO_4-0.2 g/L, $(NH_4)_2SO_4$-0.5 g/L, $MgSO_4.7H_2O$-0.04 g/L, $Ca(NO_3)_2$-0.04 g/L). The isolated strains were enriched on a regular basis by re-inoculating into freshly prepared MSN media to enrich the biomass and increase their activity. The consortium mainly contained species like *Pseudomonas aeruginosa*, *Proteiniphilum acetatigenes*, and *Alcaligenes faecalis*. The species identification was done by 16S rDNA-based method technique at the Indian Institute of Technology, Roorkee.

2.2. Experimental setup

As the consortium is facultative in nature, incubation studies were carried out under aerobic conditions, which are favorable to carry out HBC and HN processes. Two variables were varied such as N-NH_3 and organic carbon source to study the HBC kinetics. Ammonium nitrate (NH_4NO_3) and glucose ($C_6H_{12}O_6$) were used as nitrogen and organic carbon source, respectively. The incubation experiments were carried out by 100 mL of solution (synthetic wastewater) containing 90 mL MSN media excluding nitrogen and organic carbon source, 9 mL consortium, and 1 mL of various concentrations of nitrogen source using incubation bottles (Borosil, 250 mL). The pH was adjusted initially to 7, to provide a favorable growth condition for heterotrophic bacteria using Na_2CO_3. The nitrogen and organic carbon source concentrations ranged from 50 to 250 mg/L N-NH_3 and from 0.5–5 g/L,

respectively. To maintain the aerobic conditions, the incubation bottles along with samples were kept in Julabo SW-22 shaking incubators at 35˚C. The gas and liquid samples were drawn at regular intervals (12 h) using a hypodermic syringe for N_2O analysis along with various water parameter studies. For gas samples, the bottles were covered with airtight rubber caps for one hour. In incubation studies, 70 mg inoculums were used for each bottle initially. The entire experiments were carried out for 3 days.

2.3. Gas sampling and analysis

Air samples were drawn through a disposable syringe every 12 h. Gas chromatograph (GC), Shimadzu AA30 with electron capture detector, was used to analyze N_2O concentration. The GC is equipped with auto gas samplers, semi-micro columns, and appropriate software to process the acquired data. The GC was regularly standardized using NIST primary standard gases. N_2O in solution was determined by drawing a known amount of solution by a hypodermic needle and introduced to a reactor under vacuum. After 5 min, the N_2O stripped was drawn and analyzed in GC as explained previously. The dissolved N_2O in solution was estimated using Bunsen absorption coefficient using Eq. (1) [21].

$$Y = x \times \alpha \qquad \text{(Solution volume/head space volume)} \tag{1}$$

where $\alpha = 0.485$, x is the mass in head space, y is the mass in solution.

Each experiment was carried out in duplicate and an average value was taken for interpretation of results. The variation of duplicate rate was within a range of ± 5%.

2.4. Water sample analysis

Parameters such as nitrate nitrogen ($N\text{-}NO_3^-$), nitrite nitrogen ($N\text{-}NO_2^-$), ammonia nitrogen ($N\text{-}NH_3$), DO, and COD were analyzed by following the standard methods [22]. pH of the samples was measured using EUTECH-pH 1,500 meter. Analysis of total volatile suspended solids (TVSS) was performed by following the guidelines given by the standard methods [22]. NH_2OH was measured using a spectrophotometer (UV-1200; Shimadzu, Kyoto) [11].

2.5. Kinetic study

Different approaches like evaluation of rate equation and Monod were carried out to determine the reaction kinetics for different variables like variation of NH_4NO_3 and $C_6H_{12}O_6$ using Microsoft Excel 2007 program.

2.6. Statistical analysis

2.6.1. Analysis of variance

Two-way analysis of variance (ANOVA) was used to evaluate the different variables like time, NH_4NO_3, and glucose. Using the null hypothesis technique, the significance of the various parameters was determined. ANOVA was carried out using Microsoft Excel 2007.

2.6.2. Multivariate statistical analyses

The incubation data were subjected to multivariate statistical analysis to evaluate the effect of various incubation parameters on the nutrients' removal rates. Multiple linear regression analysis (MLRA) has previously been utilized [23] to determine the significance of specific parameters among data-sets. MLRA were conducted using the stepwise onward integration method. MLRA were carried out using SPSS-10.

2.6.3. Principal component analysis

The incubation data were subjected to Principal Component Analysis (PCA) for evaluating the influence of various incubation parameters on the HBC and HN rates. PCA was conducted using SPSS-10 previously to determine the significance of various parameters [23]. In PCA, eigenvalues were used to establish the percentage and cumulative percentage of variances. A varimax rotation of different varifactors with factor loading was calculated using eigenvalues >1 and sorted by the results having value >0.5 to have $p < 0.01$.

2.6.4. RSM (optimization)

The incubation studies were carried out by statistically designed experiments [18,19]. The principal steps are determination of response variables, factors and factor level, choice of experimental design, and statistical analysis of data. For this purpose, experiments were carried out by using NH_4NO_3 and $C_6H_{12}O_6$ as nitrogen and organic carbon source, respectively. For this work, Design Expert 8 was used.

All experiments were carried out in quadruplicate and average was used for interpreting the results. The variation was within ± 5%.

3. Results and discussion

3.1. Variation of NH_4NO_3 concentrations

A series of incubation experiments were conducted by varying the NH_4NO_3 concentration from 50 to 250 mg/L N-NH_3 keeping the organic carbon source, and glucose concentration constant at 5 g/L. Therefore, NH_4NO_3 substrate can be considered as the limiting one. The depletion of NH_4NO_3 during the incubation experiments can be either through HBC or HN or both. The reactions for HBC and HN are as follows (Eqs. (1) and (2)) [13,15].

$$NH_4^+ + 1.18C_6H_{12}O_6 + HCO_3^- + 2.06O_2 \rightarrow C_5H_7O_2N + 6.06H_2O + 3.07CO_2 \quad (2)$$

$$NH_3 \rightarrow NH_2OH \rightarrow NO_2^- \rightarrow NO_3^- \quad (3)$$

In HBC, the nitrogen is directly taken up by the biomass in the presence of organic carbon source. On the contrary, HN proceeds through various steps finally oxidizing NH_3 to NO_3^- [15]. N_2O is produced during the formation of intermediates like NH_2OH and NO_2^- [4]. In our system, the degradation of NH_4NO_3 takes place through HBC as well as HN; therefore, an in-depth analysis was carried out. The results are shown in Fig. 1 for the initial concentration of NH_4NO_3 as 50 mg/L N-NH_3. Similar results were obtained for other initial NH_4NO_3 concentrations. The concentration of NH_2OH was very marginal and, therefore, was not shown. The N-NH_4 and COD concentrations decreased progressively with incubation time, which suggests the activities of the micro-organisms. In all the cases, the depletion of N-NH_4 followed dual rates, i.e. faster initial rate followed by a slower one. The faster initial rate continued up to 40 h of incubation and thereafter the degradation rate decreased considerably. After 60 h, the rates of degradation were marginal; therefore, in all experiments the incubation time was restricted to 60 h. The biomass concentration, on the other hand, increased with time. The N-NO_2^- and N-N_2O initially increased and decreased as time progressed. The emission of N_2O (in liquid as well as gas) increased with the increase of NO_2^- concentrations and similar observations were reported earlier [24]. The N-N_2O concentration in aqueous state was more than that in gaseous phase. The N-NO_2^- and N-N_2O rates increased with the increase of initial NH_4NO_3 concentrations. The pH showed slight increase which may be due to HN reactions [15]. The DO in all cases was more than six, indicating good aerobic conditions maintained during the incubation studies.

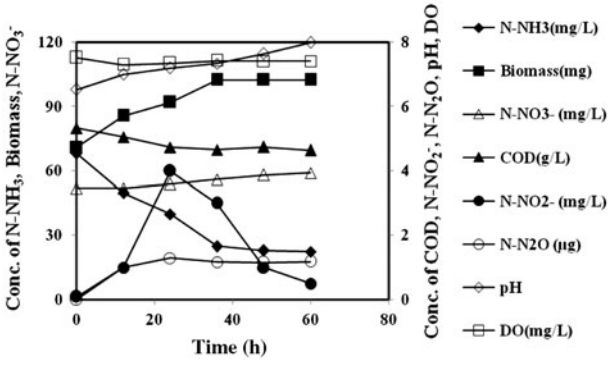

Fig. 1. Behavior of variables such as N-NH_3, biomass, pH, DO, COD, N-NO_2^-, N-NO_3^-, N-N_2O, and time during incubation studies. (Initial conditions: (NH_4NO_3)–50 mg/L N-NH_3, $C_6H_{12}O_6$-5 g/L, temp-35˚C, and pH-7).

The rates of biomass formation, degradation of N-NH_4, N-NO_2^-, COD, and N-N_2O are shown in Table 1. The rates of all these increased with the increase of initial NH_4NO_3 concentrations. Since the degradation of NH_4NO_3 assumed to follow both HBC and HN routes, therefore an attempt was made for nitrogen distribution in the overall incubation experiments. In these experiments, a majority amount (>85%) was reported in the biomass formation. The formation of N-N_2O gas in all the cases was less than 0.15%, which indicates low GHG load in the atmosphere in the entire process. Fig. 2 shows the nitrogen balance for different initial NH_4NO_3 concentrations. It was observed that the nitrogen conversion to biomass through HBC route decreased with the increase of NH_4NO_3 concentration, whereas a reverse trend was observed in the case of HN. The nitrogen uptake by biomass through HBC route decreased from 91.33 to 63.84% when the NH_4NO_3 concentration in the incubation experiments increased from 50 to 250 mg/L of N-NH_3. The emission of N-N_2O also increased with the increase of initial NH_4NO_3 concentrations during incubation experiments which may be due to an increase in the oxidation of N-NH_3 through the HN route.

3.2. Variation of organic source

A series of incubation experiments were carried out by varying the glucose concentration from 500 to 1,500 mg/L keeping the initial concentration of NH_4NO_3 constant at 100 mg/L of N-NH_3. Therefore, in the present set of experiments the limiting one is glucose (carbon source). The parameters such as COD, N-NH_3, N-NO_2^-, N-NO_3^-, N-N_2O, pH, DO, and TVSS were monitored at regular intervals. The depletion of N-NH_3 followed dual rates, i.e. faster initial rate

Table 1
Determination of rates and dependence factor

$N\text{-}NH_3$ (mg-N/L)	$N\text{-}N_2O$ (μg- N/h)	$N\text{-}NO_3^-$ (mg/L- N/h)	$N\text{-}NO_2^-$ (mg/L- N/h)	$N\text{-}NH_3$ (mg/L- N/h)	COD (mg/L/h)	Biomass (mg/h)	Dependence factor	
							R^2	$n1$
Rate-ammonium nitrate variation								
50	0.05	0.13	0.16	0.77	10.70	0.52	0.96	0.24
100	0.08	0.20	0.18	0.99	12.44	0.64		
150	0.10	0.24	0.20	1.05	14.19	0.69		
200	0.13	0.26	0.23	1.09	16.52	0.67		
250	0.15	0.27	0.27	1.16	17.64	0.72		
Rate-glucose variation								
$C_6H_{12}O_6$ (mg/L)	$N\text{-}N_2O$ (μg-N/h)	$N\text{-}NO_3^-$ (mg/L-N/h)	$N\text{-}NO_2^-$ (mg/L-N/h)	$N\text{-}NH_3$ (mg/L-N/h)	COD (mg/L/h)	Biomass (mg/h)	R^2	$n2$
500	0.03	0.08	0.04	0.48	7.24	0.32	0.83	0.59
750	0.04	0.09	0.06	0.55	8.38	0.37		
1,000	0.05	0.15	0.08	0.57	8.70	0.34		
1,250	0.06	0.22	0.12	0.72	10.92	0.66		
1,500	0.08	0.24	0.18	0.99	14.96	0.61		

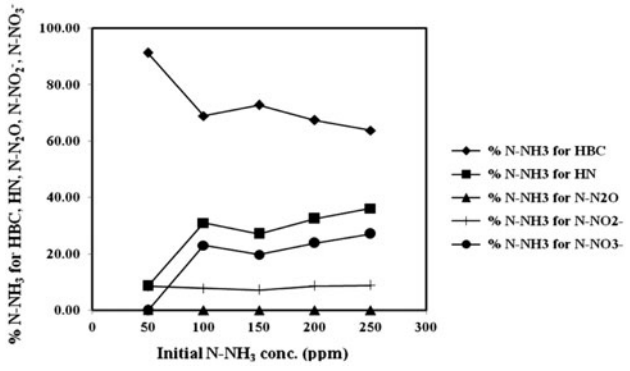

Fig. 2. The chart describes the nitrogen distribution for HBC, HN ($N\text{-}NO_2^-$, $N\text{-}NO_3^-$, and $N\text{-}N_2O$) in percent during NO_4NO_3 concentration variations. (Initial conditions: (NH_4NO_3): 50–250 mg/L $N\text{-}NH_3$, $C_6H_{12}O_6$-5 g/L, temp-35°C, and pH-7).

followed by a lower one as was observed in the earlier case. The rates of $N\text{-}NH_3$ depletion varied directly with COD and indirectly with the rates of $N\text{-}N_2O$, $N\text{-}NO_2^-$, biomass, and $N\text{-}NO_3^-$ as shown in Table 1. The DO values in all cases were more than five, thereby proving proper aeration during the incubation experiments. The pH in the present case showed slightly higher values compared to the initial one, which may be due to HN process [15]. During nitrogen balance, the same trend was observed for different initial glucose variations, i.e. nitrogen conversion to biomass through HBC route decreased with the increase of glucose concentration, whereas a reverse trend was observed in the case of HN (results not shown).

3.3. Kinetics consideration

3.3.1. Evaluation of rate equation

In the present case, two variables were considered such as NH_4NO_3 $C_6H_{12}O_6$. The kinetics can follow either the first or the second order. A reaction is said to be of the first order if log (Concentration) and time would follow linearity and the specific reaction rate can be calculated from the slope. For the second-order reaction, a plot of 1/concentration vs. time would give the linearity and the specific reaction rate can be calculated from the slope. From the coefficient of determination values, it can be concluded that the reaction follows first-order rate kinetics as shown in Table 1. As the reaction depends on the two variables such as NH_4NO_3 and $C_6H_{12}O_6$, the reaction can be termed as pseudo-first order. As the kinetics depends on two variables, the rate equation can be written as in (Eq. (4)).

$$\text{Rate} = -dc/dt = k(\text{ammonium nitrate})^{n1}(\text{glucose})^{n2} \tag{4}$$

where c = concentration of constituents determining the HBC and n is the order of reaction [25]. By converting the equation in logarithm form, the equation can be written as in (Eq. (5)).

$$\log(R) = \log k + n_1 \log(\text{ammonium nitrate}) + n_2 \log(\text{glucose}) \tag{5}$$

To determine the rate of equation with respect to each individual variable, the experimental data are arranged to fit Eq. (5). In order to get the final rate equations for the two variables, one parameter was varied at a time, keeping the other parameter constant. The individual "n" values were obtained from the slope. The n values for NH_4NO_3 and $C_6H_{12}O_6$ were calculated to be 0.24 and 0.59, respectively. By putting the n values, the rate equation can be written as in (Eq. (6)).

$$\text{Rate} = -dc/dt = k(\text{ammonium nitrate})^{0.24}(\text{glucose})^{0.59} \quad (6)$$

3.3.2. Evaluation of Monod model

The Monod model for single substrate limitation conditions can be written using Eq. (7).

$$dC_s/dt = k_4 C_x C_s/(K_s + C_s) \quad (7)$$

where dC_s/dt = substrate consumption rate (mg/L/h); K_s = half saturation constant (mg/L); C_s = concentration of substrate (mg/L); C_x = biomass concentration (mg/L); k_4 = maximum specific degradation rate (mg/L/h).

The two constants such as k_4 and K_s can be estimated from the slope and intercept of Lineweaver–Burk-type plot assuming the biomass concentration to be nearly constant. In the present case, the variation of biomass concentration varied in the narrow range; therefore, the assumption may be tenable. From ANOVA, the above assumption was found to be correct as discussed later. The slope and intercept values were calculated by plotting 1/rate of substrate (mg/L/h) vs. 1/concentration (mg/L). As discussed above, two different variables were considered such as NH_4NO_3 and $C_6H_{12}O_6$ and the results are shown in Fig. 3. The k_4 and K_s values were 1.3 and 33.99, respectively for NH_4NO_3 limiting case. Similarly for $C_6H_{12}O_6$, the k_4 and K_s values were 19.23 and 887.5, respectively. The data fit was good as the coefficient of determination values was high. The half saturation constants in both the cases were high [10,26], indicating that the substrate removal rate depends on the substrate concentration over a wide range.

3.4. Statistical interpretation

3.4.1. Analysis of variance

ANOVA was carried out to evaluate the different variables like duration, NH_4NO_3, and glucose. The results are shown in Table 2. For N-NH_4 variation, both time and N-NH_4 concentration played a significant role using the null hypothesis technique. This indicates that both the parameters played an important role in determining the kinetics of N-NH_3 removal. On the contrary, the biomass concentration for different initial N-NH_3 concentrations showed an insignificant role, thereby concluding that the biomass concentration changed with the change of N-NH_3 concentration (varied in a narrow range), which further supports the calculation of Monod's constant by assuming constant biomass concentration. Similar results were observed during COD variation. The COD and time played a significant role, whereas the biomass played an insignificant role with the variation of COD.

3.4.2. Multiple linear regression analyses

In this communication, a number of variables such as N-NH_3, COD, Biomass, pH, DO, N-NO_2^-, N-N_2O, and N-NO_2^- were considered. These variables can be correlated with each other by taking one parameter as the dependent and others as the independent variables. This correlation can be done with the help of MLRA. The primary objective in this study is to theoretically predict the dependent variable in relation to a set of independent variables. We have considered biomass as a dependent variable and N-NH_3, COD, and time as independent ones. Other variables were not considered as the values were either very small (N-NO_2^-, N-N_2O, and N-NO_3^-) or varied in a very small range (pH and DO), and thus may not play any significant role during the theoretical calculation of dependent variables. The coefficients' values for all the variables are shown in Table 3 along with all other statistical parameters such as correlation [23], standard error, F-change, and significant F-change. All these statistical parameters showed the validity of the

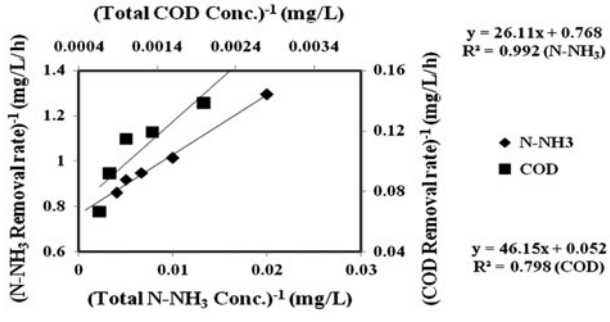

Fig. 3. The chart shows the plot between 1/rate (mg/L/H) of substrate vs. 1/conc. (mg/L) for both N-NH_3 and COD.

Table 2
Analysis of variance (ANOVA)

Variables	*F*-value	Probability (*p*)	*F*-critical	Remarks
N-NH_4NO_3				
Depletion of N-NH_3				
Time (h)	71.40	α = 0.05	2.71	Significant
N-NH_3	859.41	α = 0.05	2.87	Significant
Biomass production				
Time (h)	54.21	α = 0.05	2.71	Significant
Biomass	1.15	α = 0.05	2.87	Not significant
Glucose				
Depletion of N-NH_3				
Time (h)	25.78	α = 0.05	2.71	Significant
N-NH_3	45.09	α = 0.05	2.87	Significant
Biomass production				
Time (h)	19.58	α = 0.05	2.71	Significant
Biomass	1.64	α = 0.05	2.87	Not significant

equation in determining the theoretical value of dependent variables. Fig. 4 shows the experimental value and theoretical value using the coefficients values shown in Table 3. Fig. 4 shows good match between the theoretical as well as the experimental values, which further proves the validity of the equations established through MLRA technique.

3.4.3. Principal component analysis

PCA is another tool to determine the intra-correlation between all the variables, which on the other hand classifies the data into different compartments. The results are shown in Table 4. All the variables can be classified under four factors. Factor-I accounted for 33.25% of total variables with eigenvalues of 2.99. Factor-I contained two variables such as time and biomass and both were positively correlated, indicating that with time the biomass would increase. As Factor-I contained both time and biomass, the same can be termed as the "Biomass cycle." Factor-II had an eigenvalue of 2.78 with a cumulative variance of 64.20%. It contained three variables such as DO, N-NO_2^-, and N-N_2O. DO shows a negative correlation with the other two, which indicated that decrease of DO would increase the other two. The formation of N-N_2O and N-NO_2^- is due to the outcome of HN, which required oxygen for the oxidation of N-NH_3. In the nitrification process, N-NO_2^- and N-N_2O are formed as byproducts. The formation of N-N_2O increased with the increase in the concentration of N-NO_2^-. Factor-II can be termed as "Emission of Greenhouse Gas." As N-NO_2^- and N-N_2O are the products of nitrification, Factor-II can be termed as "Nitrification," Factor-III had an eigenvalue of 1.21 and accounted for 77.69% of the cumulative variance. It contained two variables such as COD and pH and both were negatively correlated. HBC reaction followed a acid-producing pathway; therefore, the decrease of pH would increase the COD depletion rate.

As Factor-III contained COD, it can be termed as "HBC." The last factor, Factor-IV, accounted for a

Table 3
Model summary

	R	Std. error	*F* change	Sig. *F* change
	0.91	5.49	4.18	0.05
Variable	Coefficients	Std. error	Beta	Sig.
Constant	71.58	2.35		1.45E-36
Time	0.55	0.04	0.87	2.76E-21
COD	1.59	0.35	0.26	2.58E-05
N-NH_3	−0.03	0.01	−0.12	0.045668

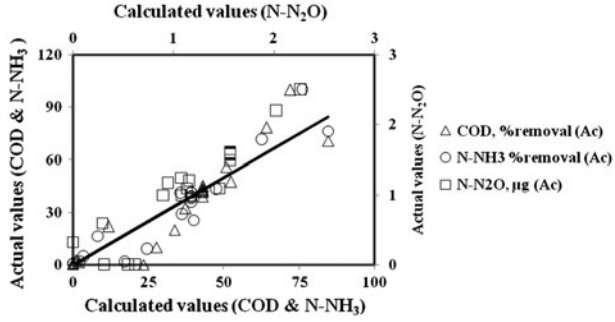

Fig. 4. Chart showing theoretical vs. experimental values of biomass obtained from MLRA.

cumulative variance of 90.46% with an eigenvalue of 1.15. It contained two variables such as N-NH_3 and N-NO_3^- and both are related to the nitrification reaction [15]. Factor-IV can be termed as "Nitrification."

3.4.4. Optimization studies using RSM

Optimization studies were carried out using Surface Response Model. Two parameters such as ammonium nitrate and glucose were varied to carry out the HBC process. Three variables such as incubation time, N-NH_3, and COD were considered. The responses were N-N_2O, COD, and N-NH_3. N-N_2O was considered as it is one of the GHG, and it therefore needs to be minimized. The concentrations of N-NH_3, N-N_2O, and COD were analyzed by collecting samples at regular intervals.

Coding was done to reduce the range of each factor to a common scale, regardless of the magnitude, the typical scheme being set to –1 as the lower level, +1 as the upper level, and 0 as the middle level using Eq. (8).

$$\text{Code} = \frac{\text{Actualvalue} - \text{Factormean}}{\text{Range of factorial value}/2} \tag{8}$$

Central composite design (CCD) with two labeled factorial points such as axial and center was done [27,28]. The axial point has all factors set to zero, except one factor which has the value $\pm\alpha$. Alpha represents the distance from the center of the designed space to an axial point. Since in this study the factors are less than five, the rotatable models were chosen, which corresponds to the alpha value of 1.68.

The factorial points such as N-NH_3, COD, and time were considered as the input value to analyze the responses such as N-NH_3, N-N_2O, and COD. The design summary and solutions obtained during optimization are shown in Table 5. The final equation in terms of coded factors, F values, Prob > F values, R^2 values, and adequate precision of the mentioned responses are shown in Table 5. The actual and calculated values for the responses are shown in Fig. 5 using the equation shown in Table 5. All the responses fit to the quadratic model. In the case of N-NH_3, the model F value was observed to be 7.44, which describes the model to be significant. The Prob > F less than 0.05 indicates the model terms to be significant.

Table 4
Principal component analysis

Correlation	Time	NH_3	Biomass	COD	pH	DO	NO_3	NO_2	N_2O		
Correlation matrix											
Time	1										
N-NH_3	−0.32	1									
Biomass	**0.88**	−0.32	1								
COD	−0.12	0.3	0.13	1							
pH	**0.51**	−0.5	0.29	−0.85	1						
DO	−0.2	0.08	−0.16	0.01	−0.17	1					
N-NO_3^-	0.07	**0.88**	0.1	0.33	−0.36	−0.04	1				
N-NO_2^-	0.03	0.25	0.21	0.35	−0.24	−0.39	0.36	1			
N-N_2O	0.37	−0.01	0.49	0.18	0.06	−0.44	0.24	**0.82**	1		
Rotated component matrix											
Component	Time	N-NH_3	Biomass	COD	pH	DO	N-NO_3^-	N-NO_2^-	N-N_2O	Eigen values	Cumulative variance
1	0.94		0.96							2.99	33.25
2						−0.77		0.84	0.84	2.78	64.2
3				0.96	−0.89					1.21	77.69
4		0.94					0.96			1.15	90.46

Table 5
Design summary

Study type	Response surface		Runs	20				
Design type	Central composite	Design model	Quadratic					
Factor	Name	Units	Minimum	Maximum	Mean	Std. dev.		
A	$N\text{-}NH_3$	mg/L	2.16	262.84	132.5	64.04		
B	COD	g/L	0.02	3.63	1.825	0.89		
C	Time	h	0.45	47.55	24	11.57		
Response	Name	Units	Minimum	Maximum	Mean	Std. dev.	Trans	Model
Y1	$N\text{-}N_2O$	μg	0	2.5	1.14	0.69	None	Quadratic
Y2	COD	% removal	0.18	100	35.01	28.22	None	Quadratic
Y3	$N\text{-}NH_3$	% removal	1.13	100	32.95	27.06	None	Quadratic
		Factor			Response			
Solutions	Number	$N\text{-}NH_3$ (mg/L)	COD (g/L)	Time (h)	$N\text{-}N_2O$ (μg)	COD (% Removal)	$N\text{-}NH_3$ (% Removal)	Desirability
	1	210	2.9	38	1.45	64.26	62.82	0.81
Best fit models								
Responses	Coded factors	Model F value	P value Prob > F	R^2	Adequate precision			
$N\text{-}N_2O$	$N\text{-}N_2O = 1.56 + 0.49A + 0.16B + 0.23C - 0.31AB + 0.07AC - 0.14BC - 0.04A^2 - 0.26B^2 - 0.31C^2$	5.18	0.0084	0.82	9.11			
COD	$COD = 42.95 + 14.57A - 14.48B + 18.19C + 1.26AB + 10.34AC + 3.06BC - 5.84A^2 + 1.68B^2 - 7.46C^2$	5.34	0.0075	0.83	8.28			
$N\text{-}NH_3$	$N\text{-}NH_3 = 39.40 - 10.68A + 11.69B + 18.06C + 1.55AB - 1.69AC + 13.96BC + 6.56A^2 - 8.21B^2 - 7.81C^2$	7.44	0.0021	0.87	10.26			

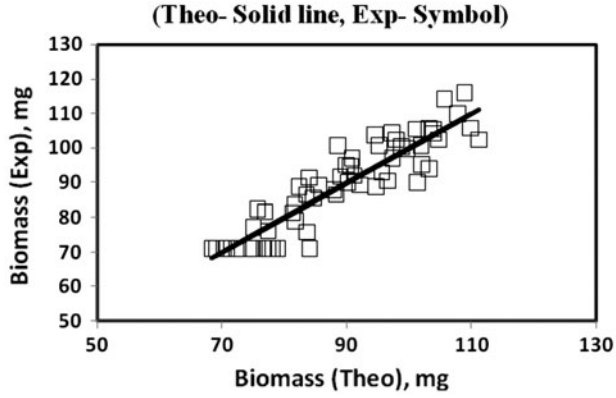

Fig. 5. Predicted vs. actual plot for all three responses like N-N_2O, COD and N-NH_3.

Adequate precision measures the signal to noise ratio. A ratio greater than four is desirable. All the statistical values are significant, which shows the validity of the model. Fig. 6(a)–(i) shows the surface and contour plots using the quadratic model, keeping one variable constant at a time.

Fig. 6(a) shows the plots for COD and N-NH_3 keeping the other variable such as time (38 h) constant. The N-NH_3 removal efficiency increased with the increase of COD concentration keeping N-NH_3 concentration low. For NH_4NO_3, 80% N-NH_3 removal was possible at COD and N-NH_3 concentration 2.4–2.9 g/L and 55–60 mg/L N-NH_3, respectively. All the optimized graphs were self-explanatory and hence were not explained.

4. Conclusions

The removal rates varied between 0.48 and 1.16 mg/L/h (N-NH_3) for NH_4NO_3 as well as $C_6H_{12}O_6$ concentrations. The N-N_2O emission was observed to be very less during nitrogen mass balance. ANOVA suggests the significance of incubation period, i.e. time as well as nutrient concentrations. Optimization studies were carried to minimize N_2O emission and simultaneous maximization of COD and N-NH_3 removal efficiency. The optimum values of the process time, initial N-NH_3, and COD concentration in the aqueous solution were found to be 38 h, 210 mg/L, and 2.9 g/L, respectively. During the optimal values

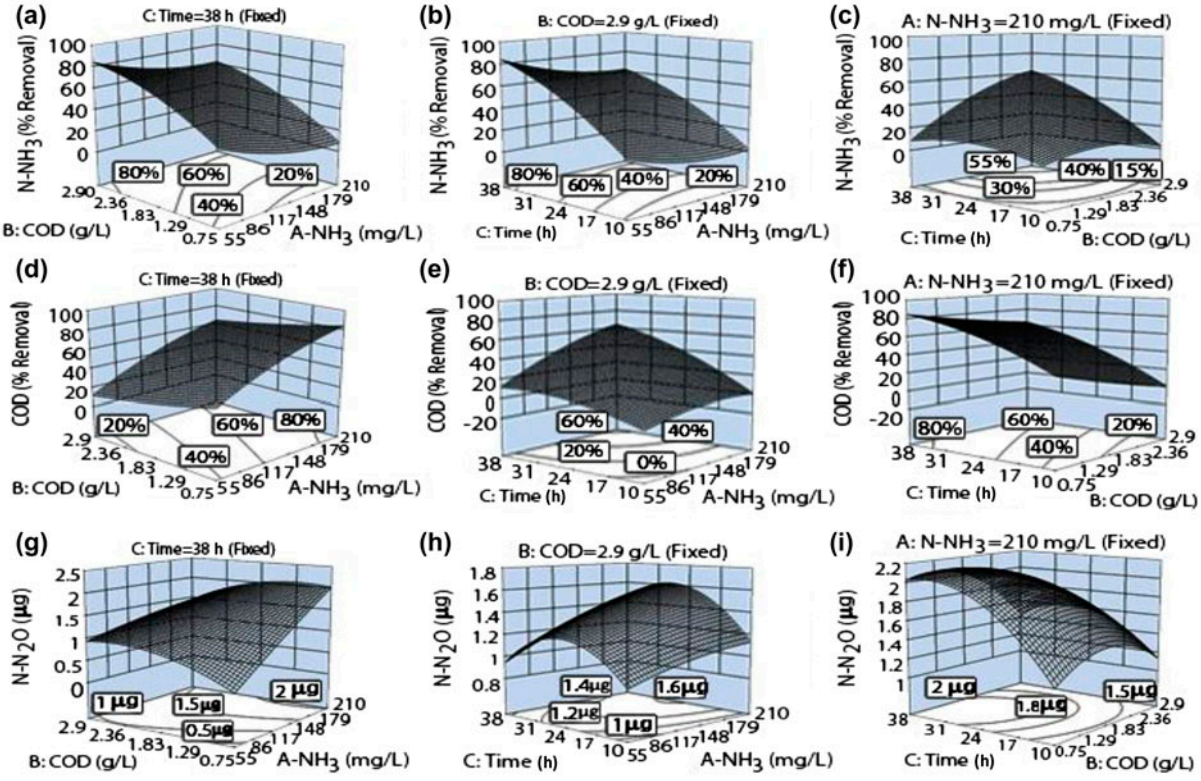

Fig. 6. Contour with 3D diagrams showing the optimization of three responses such as COD, N-N_2O, and N-NH_3 ((a)–(i)).

of the process parameters, a maximum N-NH_3 and COD removal of 62.82 and 64.26% was obtained, respectively. Also the N-N_2O formation was minimized to 1.45 μg.

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