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Enhanced biological nutrient removal by the alliance of a heterotrophic nitrifying strain with a nitrogen removing ecosystem

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Abstract

Nitrogen removal from synthetic wastewater was investigated in an airlift bioreactor (ALB), augmented with a novel heterotrophic nitrifier *Pseudonocardia ammonioxydans* H9^T under organic carbon to nitrogen ratios (C_{org}/N) ranging from 0 to 12. Effect of the inoculated strain was also determined on the settling properties and the removal of chemical oxygen demand (COD). Two laboratory scale reactors were set up to achieve a stable nitrifying state under the same physicochemical conditions of hydraulic retention time (HRT), temperature, pH and dissolved oxygen (DO), and operated under the sequencing batch mode. The level of DO was kept at 0.5–1.5 mg/L by periodic stirring and aeration. Each specific C_{org}/N ratio was continued for duration of 3 weeks. One of the reactors (BR2) was inoculated with *P. ammonioxydans* H9^T periodically at the start of each C_{org}/N ratio. Sludge volumetric index (SVI) improved with the increasing C_{org}/N ratio, but no significant difference was detected between the two reactors. BR2 showed higher levels of nitrogen removal with the increasing heterotrophic conditions, and the ammonia removal reached to the level of 82%–88%, up to10% higher than that in the control reactor (BR1) at C_{org}/N ratios higher than 6; however, the ammonia removal level in experimental reactor was up to 8% lower than that in control reactor at C_{org}/N ratios lower than 2. The COD removal efficiency progressively increased with the increasing C_{org}/N ratios in both of the reactors. The COD removal percentage up to peak values of 88%–94% in BR2, up to 11% higher than that in BR1 at C_{org}/N ratio higher than 4. The peak values of ammonia and COD removal almost coincided with the highest number (18%–27% to total bacterial number) of the exogenous bacterium in the BR2, detected as colony forming units (CFU). Furthermore, the removal of ammonia and COD in BR2 was closely related to the number of the inoculated strain with a coefficient index (R^2) up to 0.82 and 0.85 for ammonia and COD, respectively. These results suggest that it was more efficient for both the ammonia and carbon nutrient removals in a reactor inoculated with a heterotrophic nitrifier at high C_{org}/N ratio, inferring that the heterotrophic nitrifers would be practically more available in the treatment of wastewater with high level of ammonia and COD.

Key words: air-lift bioreactor (ALB); bioaugmentation; C_{org}/N ratio; COD and ammonia removal; sequencing batch mode; heterotrophic nitrifier; *Pseudonocardia ammonioxydans* H9^T

Introduction

Nitrogen (N) is one of the nutrients essential to living organisms. Microbial reactions drive the global nitrogen cycle and the process of nitrification is central to it. Human activities have drastically impacted the global nitrogen cycle. Excessive nitrogenous compounds released into the public water bodies not only result in direct toxicity to aquatic animals, but also increase the overgrowth of aquatic plants resulting eutrophication. Nitrogen pollution has major effects on both human health and the ecological functions of natural ecosystems.

Biotreatment is a cost-effective method for the wastewater before being discharged into the streams and rivers. The traditional sludge processes for N removal involve autotrophic nitrification and heterotrophic denitrification. Nitrification is achieved under aerobic conditions, while denitrification requires anaerobic conditions in the routine sludge practices (Arp *et al*., 2002). The two processes necessitate spatial or temporal separation. Nitrification is the key step in the removal of nitrogen and normally performed by autotrophic nitrifying bacteria, and the process requires low C_{org}/N ratio as it would be strongly inhibited if the ratio was higher than 0.25 or completely stopped at the ratios higher than 6 (Okabe *et al*., 1996; Osliso and Lewandowski, 1985). However, the usual C_{org}/N ratio in most wastewater is about 10 or even higher and subsequently results in poor nitrification performance (Okabe *et al*., 1996).

In some experiments, aerobic denitrifying strains have been used to improve the nitrification process. Patureau and Helloin (2001) achieved the combined phosphate and nitrogen removal in a sequencing batch reactor using the aerobic denitrifier, *Microvirgula aerodenitrificans*. Such practices are termed as bioaugmentation and involve the

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culturing of microorganisms with desired traits to a high level and subsequently returning them to the environment (Pieper and Reineke, 2000; Dua *et al*., 2002).

Bioaugmentation of sludge processes is a promising field regarding wastewater treatment. Recent research has suggested that no matter how effective bioaugmentation appears in a controlled laboratory environment, a bioagent will only perform its function if it is well adapted to its environment (Bouchez *et al*., 2000). Thompson *et al*. (2005) regarded the strain selection to be of utmost importance regarding the success of bioaugmentation. Beside the strain selection, manipulation of the physical parameters coupled with practices of bioaugmentation has shown a promising potential regarding wastewater treatment. Cattaneo *et al*. (2003) studied the performance of *Pseudomonas denitrificans* in a fluidized bed and a stirred tank reactor. They found out that the bacteria did well in the fluidized bed reactor regarding nitrogen removal. Similarly, Head and Oleszkiewicz (2005) have seeded a nitrifying sequencing batch reactor (SBR) with sludge acclimatized to different temperatures and found out that the system worked well when the sludge acclimatization temperature and the SBR temperature were the same. Andersson and Dalhammar (2006) used pure culture of *Comamonas denitrificans* ATCC 700936^{T} and recorded a rapid increase in denitrification activity within the initial 4 d. On wash out of the strain, augmentation with agar-embedded bacteria resulted in a minute restoration of denitrifying activity. Belia and Smith (1997) used *Acinetobacter lwoffii* to augment activated sludge in an SBR and observed that the sludge developed resilience to influent phosphate fluctuations, lower level of dissolved oxygen (DO) and biomass replacement. However, the COD and nitrogen removal capabilities of the sludge and its settling properties were not affected. Similarly, Howard and Yung-Tse (1995) concluded that bioaugmentation with addition of living microorganisms improves total organic carbon removal efficiency and reduces sludge production.

Bioaugmentation and seeding studies are nascent regarding the sludge processes and the relevant discussions are scanty in the published report. Moreover, the heterotrophic nitrification and the abilities of certain strains capable of simultaneous nitrification and denitrification need further study to evaluate the possibility of their use in the wastewater treatment processes and the contemplation of their role in the global nitrogen cycle. In their experiment with a heterotrophic nitrifier *Bacillus* strain MS30, Mevel and Prieur (2000) observed that the comparatively low nitrification rates of heterotrophic nitrifiers compared to the autotrophic nitrifiers are balanced by their ability to grow efficiently under diverse conditions.

In the case of activated sludge processes, Seixo *et al*. (2004) found out a linear relationship between the organic carbon, biomass and rate of nitrification. Peng and Zhu (2004) also established that biological nitrogen removal is strongly influenced by the influent C_{org}/N ratio. Xing *et al*. (2000) observed that the rate constants of organic oxidation, nitrification and denitrification were closely dependent on the C_{org}/N ratio values. Different levels of organic carbon provide a varied arena for the respiratory activity and diversity of microorganisms (Eiland *et al*., 2001). Verhagen *et al*. (1992) has shown that at higher C_{org}/N ratios, there is an inhibition of the nitrification process by a mixture of more competitive heterotrophic microorganisms. Bioaugmentation under different C_{org}/N ratios can be a novel experiment and of good value on the subject of the survival and nitrification performance of a particular strain and its contribution to the stability of the system.

In the present experiment, two airlift bioreactors (ALB) were used, one as a control (BR1) and the other one augmented with the strain (BR2). ALB was used since it gives better results in terms of granulation and floccular biomass formation (Freitas and Teixeira, 1999). Campos *et al*. (2003) has found better particle retention owing to a high density sludge, and efficient fluidization in an ALB without biofilm carrier. A series of different C_{org}/N ratios were applied to diversify the reactor's environment. In addition, by controlled aeration and stirring, the DO level was maintained in the range of 0.2 to 1.5 mg/L. In both the reactors, a submerged perforated polyethylene tube was used to provide an anoxic niche. To this diverse system under non-sterile conditions, a novel heterotrophic nitrifying strain *Pseudonocardia ammonioxydans* H9^{T} was used, which was recently isolated from the coastal sediments (Liu *et al*., 2006) and its survival and nutrient removing efficiency were observed. The symbiosis between activated sludge bacteria and an exogenous marine strain can be a new environmental technology under diverse physical and biological conditions.

1 Materials and Methods

1.1 Set up of reactors

Two identical laboratory scale airlift reactors (BR1 and BR2), each with a working volume of 3 L were installed (Fig.1). The parameters and running conditions for both reactors are the same and summarized in Table 1. Both the reactors were provided with submerged perforated polyethylene tubing encircling the peripheral part of the

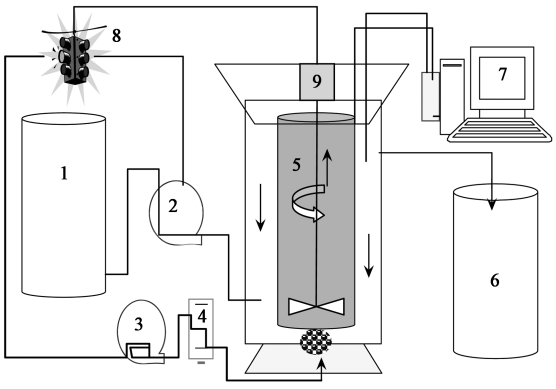

Fig. 1 Schematic diagram of the experimental set up of the nitrifying airlift reactor. (1) feed tank; (2) feed pump; (3) air pump; (4) air meter; (5) oxidation tank; (6) settler; (7) pH, DO, probes data Logger; (8) microprocessors for controlling the cycles; (9) stirrer.

Table 1 Running conditions and parameters of the two reactors

Parameter	Value
Working volume (L)	3
Temperature (°C)	25–30
DO (mg/L)	0.5–1.5
Aeration/stirring time (h/h)	1/1
Sampling time	11:00
Settling time	11:10, 30 min
Effluent discharge time	11:40, 10 min
Feeding time	11:50, 10 min

reactor to provide the organisms an anoxic niche. The tube had a length of 8.5 m, external and internal diameters of 0.54 and 0.43 cm, respectively, provided a total surface area of 0.5 m^2, and had holes of 0.21 cm diameter at about 8 cm intervals. Both reactors were operated in sequenced batch mode. Nitrifying process in the reactors was initiated with 300 ml of activated sludge, obtained from a local wastewater treatment plant, used as a source of indigenous microorganisms. The raw sludge was sieved and washed before being inoculated into the reactors. A startup time of 45 d was applied to the reactors during which stable nitrification was attained in the two reactors. Both the reactors were run on the synthetic ammonia oxidizing medium ($C_{org}/N = 0$, Table 2) to facilitate the proliferation of ammonia-metabolizing bacteria. Hydraulic retention time (HRT) of 2 d was applied initially, which was gradually reduced to 1 d toward the end of startup period.

1.2 Synthetic wastewater composition

Synthetic wastewater with gradually increasing C_{org}/N ratio was supplied to the two reactors. Each specific C_{org}/N ratio was continued up to duration for 3 weeks. To prevent spoilage, the feed reservoir of wastewater was kept in a refrigerator at 4°C. The wastewater composition is presented in Table 2 (modified after Seixo *et al*., 2004).

1.3 Strain *Pseudonocardia ammonioxydans* H9^T

P. ammonioxydans H9^T is a novel heterotrophic nitrifier isolated from coastal sediment sample collected from Jiao-Dong peninsula near Tsingdao City, Shandong Province, China (Liu *et al*., 2006). The strain thrives well on a blend of ammonia oxidizing medium (AOM) and Luria-Bertani (LB) medium (85% AOM:15% LB (*V*/*V*), Liu *et al*., 2006). The strain is easily distinguished due to formation of colonies resembling white chalk specs. Its substrate mycelia are brownish while the aerial ones are white.

1.4 Inoculation strategy

The experimental reactor BR2, in addition to the activated sludge, was inoculated with cells centrifuged from 300 ml culture of *P. ammonioxydans* H9^T grown at 30°C and shaken at 60 r/min, to an optical density ($OD_{600\ nm}$) of 2 with the same C_{org}/N ratio as that of the reactors. The cells were harvested and washed twice with sterile phosphate buffer before inoculation to avoid the introduction of any extra nutrients into the receiving reactor, as noted by Schwartz *et al*. (2000) stating that what appears to be successful bioaugmentation can be attributed to stimulation of biodegradation by nutrients added with the inoculum, rather than the inoculum itself. Subsequently, the BR2 was repeatedly inoculated with cells centrifuged from 300 ml of culture of $OD_{600\ nm}$ of 2, at day 5 of the start of every new C_{org}/N ratio. Each specific C_{org}/N ratio, as a whole, was continued for 3 weeks.

1.5 Colony forming units (CFU) determination

The sludge flocs were dispersed by purging a 1-ml sample five times through a sterile syringe of 1 ml. Bacterial number of the inoculated strain and the mixed sludge bacteria was determined as CFU on the nutrient (LB/AOM = 25:75, Liu *et al*., 2006) agar. The inoculated strain was easily distinguished from the endogenous sludge bacteria by their unique morphological features of colonies, which appear as whitish specs of chalk due to vertical mycelia and spores, while the base of the colony appears as dark brown due to horizontal filaments. Serial dilutions of 10^{-1} to 10^{-8} of 1 ml sample were prepared. Dilutions (0.1 ml) were spread out on plates to get a viable cell count. The plates were incubated at 30°C for 72 h.

1.6 Online measurement and data acquisition

DO, pH and temperature were routinely measured with a Clark type Oxygen Electrode (WTW oxi 232, Germany). Biomass activity and nitrification assay of the reactor were determined as oxygen uptake rate (OUR). OUR, according to Archibald *et al*. (2001), is the most rapid and simple method for measuring the sludge activity. The samples 100 ml (washed biomass in phosphate buffer) were transferred to the vessel and saturated with oxygen by bubbling air with a pump. Once oxygen saturation (7–8 mg O_2/L) was

Table 2 Synthetic wastewater composition

Constituent	Quantity	Constituent	Quantity
$C_6H_{12}O_6$ (mg/L)	0.00 ($C_{org}/N = 0$)	NH_4Cl (mgN/L)	120
	125.00 ($C_{org}/N = 0.5$)	NaCl (g/L)	10
	250.00 ($C_{org}/N = 1$)	$FeSO_4$ (mg/L)	55.00
	500.00 ($C_{org}/N = 2$)	K_2HPO_4 (mg/L)	140.00
	1,000.00 ($C_{org}/N = 4$)	$CaCO_3$ (g/L)	2.00
	1,500.00 ($C_{org}/N = 6$)	Trace metal solution* (ml/L)	2
	2,000.00 ($C_{org}/N = 8$)	Yeast extract (mg/L)	10
	2,500.00 ($C_{org}/N = 10$)	pH	7.8
	3,000.00 ($C_{org}/N = 12$)		

* Trace metal solution including (in g/L): 0.5 $MgSO_4 \cdot 7H_2O$; 0.6 $FeCl_2 \cdot 4H_2O$; 0.88 $CoCl_2$; 0.1 H_3BO_3; 0.1 $ZnSO_4 \cdot 7H_2O$; 0.05 $CuSO_4 \cdot 5H_2O$; 1.0 $NiSO_4$; 5 $MnCl_2$; 0.64 $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$; 5.0 $CaCl_2 \cdot 2H_2O$.

reached, the aeration was ceased and the oxygen electrode was placed in such a way that the opening of the vessel was barely closed. Samples were mixed using a magnetic stirrer during measurements. DO was recorded every 60 s, calculating the activity from the constant slope of oxygen concentration over time. The method consisting of an oxygen electrode connected to computer data logger was developed after Pernetti *et al*. (2002), Ciudad *et al*. (2005) and Schuchardt *et al*. (2005). This assay was used both for measuring the base line respiration, as well as the ammonia based oxygen depletion to assess nitrification. Specific oxygen uptake rate (SOUR) was calculated by the ratio of oxygen uptake rate to the biomass concentration.

$$\text{SOUR} = \frac{\text{OURn} - \text{OURc}}{\text{Biomass}}$$

The two main steps of the above-mentioned assay are: OURc of the cells to assess the endogenous respiration and OURn in response to substrate NH_4Cl supplied at initial concentration of 10 mgN/L.

1.7 Offline measurements

Biomass, COD, total nitrogen, ammonia, nitrites and nitrates were measured according to standard methods (APHA, 1998) for the examination of water and wastewater. Sludge residence time (SRT) was not maintained constantly, because biomass concentration, measured as mixed liquor suspended solids (MLSS), remained in the range of 3–6 g/L. All the parameters mentioned here were measured in triplicate, and the sampling time for above mentioned tests was day 7, 14 and 21 of the successive C_{org}/N ratios applied to the bioreactors. The sludge volumetric index (SVI) was calculated based on the equation:

$$\text{SVI} = \frac{\text{SV}_{30}}{\text{SS}}$$

where, SV_{30} (ml/L) is the volume of sludge obtained by allowing 1 L of wastewater to settle for 30 min. SS (g/L) is suspended solids concentration.

2 Results and discussion

2.1 Biomass and SVI of the two reactors and survival of strain H9^T in BR2

Reactor biomass concentration is plotted in Fig.2 as a function of time. During the startup, the biomass in both the reactors was about 2.5 ± 0.5 g/L. Bacterial biomass, measured as MLSS, increased linearly with the increasing C_{org}/N ratio, indicating the limiting nature of carbon supply in both the reactors.

Monitoring of the activated sludge settling properties, as well as the structural properties and the dynamics of microbial populations can provide insights into the relation between the microbial community change and process performance (Govoreanu *et al*., 2003). In this experiment, SVI improved gradually in both the reactors with increasing carbon supply. However, there was no significant difference between the two reactors, regarding the settling characteristics of the sludge. In certain other studies, for example the one by Ivanov and Wang (2006) using *Pseudomonas veronii* strain F, got improved SVI and granule formation in the activated sludge process due to the higher self and co-aggregation ability of the added cells. Since the *P. ammonioxydians* H9^T filaments on shaking are fragmented into rods (Liu *et al*., 2006), which may be the reason that they could not contribute significantly to the granule formation and in turn to the improvement of the SVI.

Organic carbon supply is known to influence the community structure (Eiler *et al*., 2003), and thus can be pivotal in the differential survival of different strains. In the present experiment, the inoculated strain H9^T showed fluctuations in CFU number during the system operation (Fig.3), its number was about 7×10^9 CFU/L at C_{org}/N ratios of 0 and 0.5, and then increased with the increasing of C_{org}/N ratio until to the peak survival, about 7.7×10^{10} CFU/L at C_{org}/N of 8, ten times of that at C_{org}/N ratios of 0 and 0.5. According to McLaughlin *et al*. (2006), initial survival problems of the introduced strain, the inoculum size or time needed for the inoculants to grow affects the process of acclimation. In addition, the abundance of the inoculated strain H9^T ranged from 8%–27% to the total bacteria with the increase of C_{org}/N ratio (Fig.3), showing

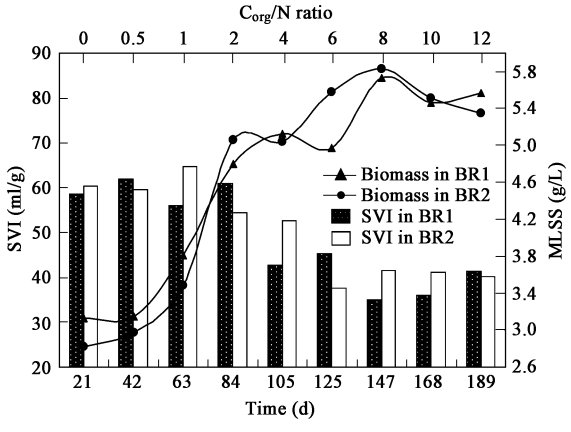

Fig. 2 Biomass and SVI profile of the two bioreactors.

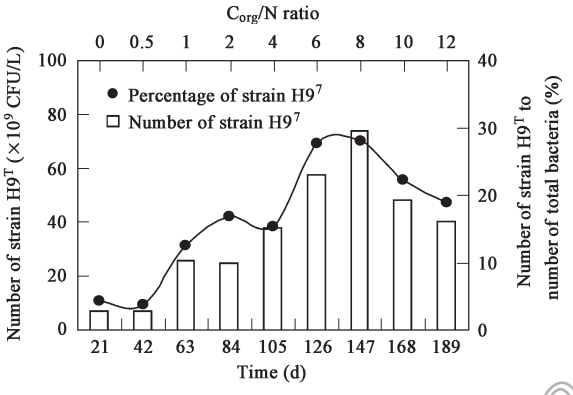

Fig. 3 Number (CFU) and percentage of inoculated strain H9^T to total bacterial number in BR2.

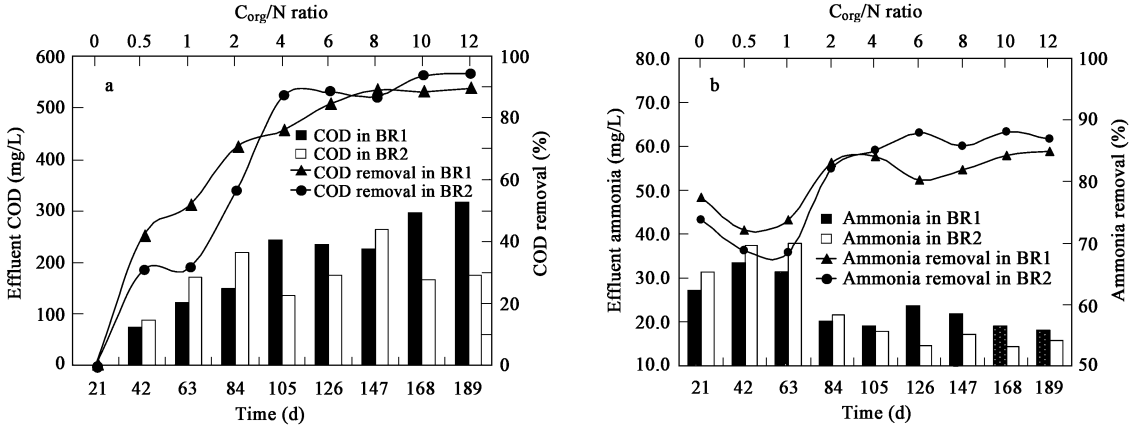

Fig. 4 Effluent COD and ammonia levels and their percentage of removals in the two bioreactors.

that cells of strain H9^T became more and more dominant in the microbial community of the seeded reactor.

2.2 Removal of COD and ammonia

Figure 4a illustrates the effluent COD concentrations and COD removal of the two reactors. The percentage of COD removal increased in both the reactors with time. The highest values of above 85%–90% were noted at C_{org}/N ratios of 4, 6, 8, 10, 12 in both the reactors. Compared to the control one, the effluent COD concentrations of the seeded reactor were higher at C_{org}/N ratios lower than 2, in contrast, they were lower at C_{org}/N ratios higher than 4. This might be due to the variation of the cell number of inoculated strain H9^T (Fig.3), and the peak values of COD removal almost coincided with the highest levels of CFU number of the introduced strain in BR2.

Variation of average effluent NH_4^+-N concentrations and percentage of removal at different C_{org}/N ratios are shown in Fig.4b. The effluent NH_4^+-N concentration decreased and the percentage of removal increased from day 21 to day 189. However, the removal percentage in case of the reactor inoculated with the *P. ammonioxydans* H9^T was observed to be greater at the higher carbon supply in the influent. From C_{org}/N ratio of 0 to 2, these values were higher in the control reactor. Regarding the effect of organic carbon on the ammonia removal by a specific strain, Joo *et al*. (2005) used *Alcaligenes faecalis* No. 4 and found out that the strain was more efficient at a C_{org}/N ratio of 10 and 20, but at C_{org}/N ratio of 5, the consumption of NH_4^+-N stopped at 120 mg/L, mainly due to exhaustion of carbon. In this experiment, the bioaugmented reactor became more efficient regarding NH_4^+-N removal at higher C_{org}/N ratios. The peak values for both the dominance (expressed as the percentage to total bacterial number) of the inoculated strain, and the ammonia and COD removal (%) were observed between day 105 to day 189. The percentage of ammonia removal was 10%–15% higher, while the COD removal was 15%–20% higher in the BR2 than those in control reactor (BR1), respectively, in heterotrophic phase of the present experiment. Certain other results lie close to those obtained with *P. ammonioxydans* H9^T. Kargi and Uygur (2005), in their bioaugmentation experiment, studied NH_4^+-N removals in two SBRs, the one was supplemented with *Halobactor halobium* ATCC 43214^T and the other as a control. At 5% salt concentration in both the reactors, they found the NH_4^+-N removal to be 15% higher in the bioaugmented reactor. In this experiment, the difference between the two reactors with respect to ammonia oxidation is not so significant. However, the bioaugmented reactor shows moderately improved efficiency regarding the total nitrogen removal (Fig.5). Generally, the positive correlation between ammonia and COD removal and the number of the inoculated strain (expressed as percentage of total bacterial number) at higher C_{org}/N ratios (Fig.3) highlight its preference for the heterotrophic conditions in the bioreactor. This differential survival and activity in the bioaugmentation experiment shows that diversification of the culture environment in terms of nutrient supply or physical conditions may result in an increase in the chances of survival for the added strain.

2.3 Formation of nitrite and nitrate

Nitrite and nitrate profiles are shown in Fig.6. Nitrite accumulation is much higher at the stages with lower carbon supply, apparently due to the dominance of the autotrophic bacteria. With the evolution of the heterotrophic bacteria toward the end of the experiment at higher C_{org}/N ratios, not only the nitrite has reduced but the nitrate has also

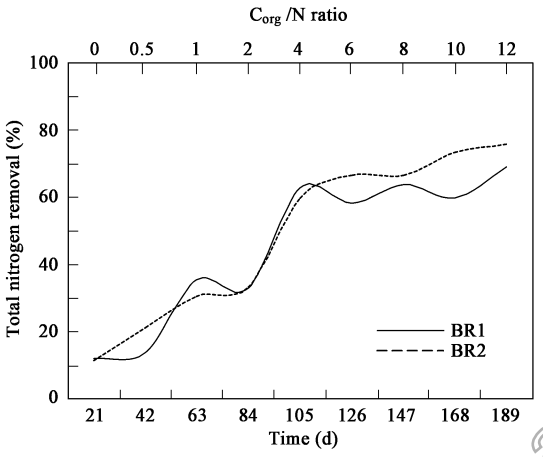

Fig. 5 Total nitrogen removal in the two bioreactors.

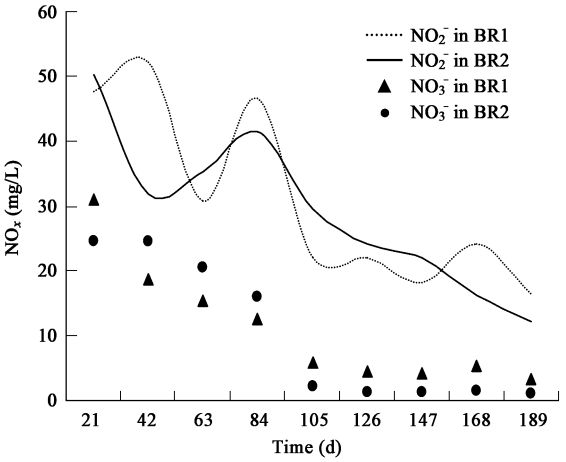

Fig. 6 NO_x profile of the two bioreactors.

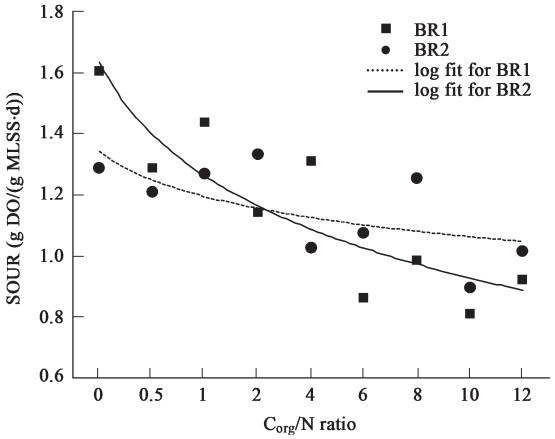

Fig. 7 SOUR in response to NH_4Cl with an initial concentration of 10 mg/L.

been reduced to the minimum values. The lowest values both for nitrite (11.6 mg/L), and nitrate (1.01 mg/L), were recorded at the highest C_{org}/N ratio of 12 applied to both the reactors. However, the removal percentage of nitrates was higher in the augmented bioreactor from day 105 to day 189. In addition, the ratio of NO_3^- to NO_2^- calculated during this experiment is lower in case of BR2, especially at higher C_{org}/N ratios (Table 3), which is an indicator of the higher denitrification rate. Similarly, Patureau *et al*. (2001) in their experiment found that, the higher quantity of aerobic denitrifiers and the carbon source is accompanied by the smaller percentage of apparent nitrification but higher percentage of denitrification.

Table 3 Ratio of NO_3^- to NO_2^- in the two bioreactors (%)

C_{org}/N ratio	NO_3^-/NO_2^- ratio in BR1	NO_3^-/NO_2^- ratio in BR2
0	56.53	48.24
0.5	37.15	37.16
1	24.50	20.15
2	31.26	36.87
4	11.88	2.94
6	12.94	4.01
8	18.52	3.48
10	16.21	4.50
12	14.10	3.57

2.4 Ammonia dependent oxygen consumption of the biomass from the reactors

Figure 7 shows the results of the respirometric assays with NH_4Cl as the substrate to observe the activity of nitrifying bacteria, using it as source of energy. The NH_4Cl based on SOUR was observed to decline with increasing C_{org}/N ratio in the reactors. The results in Fig.7 indicate a decrease in the nitrification activity of biomass. The slope of decline, however, is steeper in case of the control reactor, while the reactor seeded with the *P. ammonioxydans* $H9^T$ shows greater adaptability to the growing heterotrophic conditions. BR2 had higher preference for oxygen consumption in the heterotrophic, while the BR1 consumption was greater during the autotrophic state of the activated sludge.

2.5 Relationship between the inoculated *P. ammonioxydans* $H9^T$ and the removal of ammonia and COD

Figure 8 depicts the trends of the inoculated biomass measured as percentage of total bacterial number to COD and ammonia removal. The COD and ammonia removal exhibit a positive correlation to CFU percentage of *P. ammonioxydans* $H9^T$ with a coefficient index (R^2) up to 0.82 for ammonia removal, and a coefficient index (R^2) up to 0.85 for COD removal, despite the decline of the CFU percentage of inoculated strain at C_{org}/N ratios higher

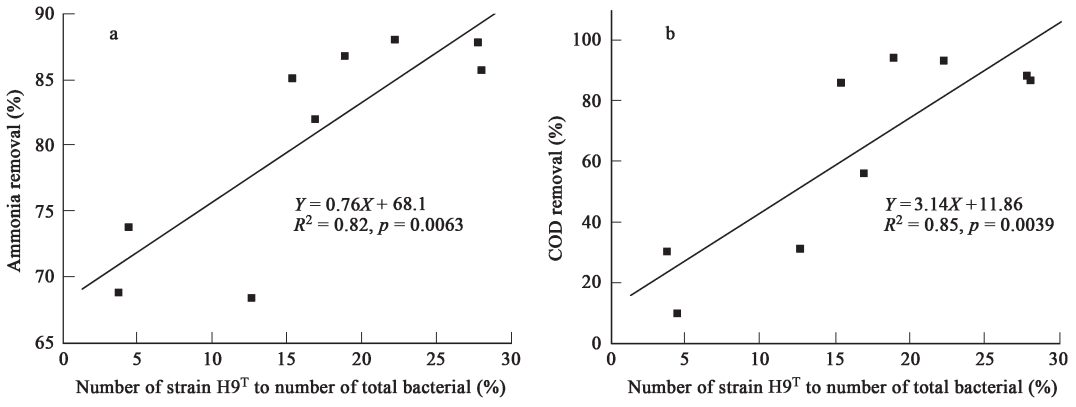

Fig. 8 Correlation between the cell number of inoculated strain $H9^T$ and the removal of ammonia (a), and COD (b) in BR2.

than 10. Similarly, the total nitrogen removal level of 60% was achieved by both the reactors at C_{org}/N of 4. Above this, the bioaugmented reactor showed significantly higher efficiency regarding the total nitrogen removal (Fig.5).

3 Conclusions

Nutrient removal in an ALB bioaugmented with *P. ammonioxydans* $H9^T$ was investigated. In this study, SVI decreased with the maturation of both the control and bioaugmented systems. SVI and biomass showed the same trend in both the reactors, and the added strain had no significant impact on the settling properties, but the addition of the strain enhanced the nutrient removal in this experiment. Applying a range of diverse physical conditions ensured the greater survival of the exogenous strain. The bioaugmented reactor showed better performance regarding the nitrogen and COD removal, especially at higher C_{org}/N ratios. Performance of the inoculated strain with respect to COD removal and de/nitrification illustrate that it can be a useful subject regarding further studies in the field of heterotrophic nitrification and enhanced biological nutrient removal (BNR) phenomena.

Acknowledgements

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