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Nitrate removal efficiency of bacterial consortium (*Pseudomonas* sp. KW1 and *Bacillus* sp. YW4) in synthetic nitrate-rich water

Sundaram Rajakumar[a], Pudukadu Munusamy Ayyasamy[b,*], Kuppusamy Shanthi[c], Palanisami Thavamani[d], Palanivel Velmurugan[e], Young Chae Song[a], Perumalsamy Lakshmanaperumalsamy[e]

[a] *Division of Civil and Environmental Engineering, Korea Maritime University, Busan 606 791, South Korea*
[b] *Department of Environmental Engineering, The Catholic University of Korea, Bucheon 420 743, South Korea*
[c] *Department of Environmental Sciences, P.S.G. College of Arts and Science, Coimbatore 641014, India*
[d] *CERAR-CRC CARE, University of South Australia, Adelaide 5095, Australia*
[e] *Department of Environmental Sciences, Bharathiar University, Coimbatore 641046, India*

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Abstract

The efficiency of bacterial isolates to reduce nitrate from synthetic nitrate-rich water was tested using a batch scale process. Two efficient nitrate reducing bacterial species were isolated from water samples collected from Kodaikanal and Yercaud lakes. Bacterial analysis of the samples revealed the presence of nitrate reducing bacteria belonging to the genera *Pseudomonas*, *Bacillus*, *Micrococcus* and *Alcaligenes*. Among the isolates, the consortium of *Pseudomonas* sp. KW1 and *Bacillus* sp. YW4 was found to be efficient in nitrate reduction. Influences of various carbon sources, incubation temperature and pH on nitrate reduction from synthetic wastewater were also studied. The results showed a rapid and efficient process of nitrate removal (99.4%) from synthetic wastewater supplemented with starch (1%), inoculated by bacterial consortium (*Pseudomonas* sp. KW1 and *Bacillus* sp. YW4) at incubation temperature of 30 °C at pH 7. This observation has led to the conclusion that the bacterial consortium was responsible for nitrate removal from synthetic nitrate-rich wastewater.

Keywords: Kodaikanal; Yercaud; Synthetic wastewater; Bacterial consortium

1. Introduction

Nitrate contamination of both surface and ground water has been a topical issue throughout the world since the 1970s and has become an increasingly serious environmental problem. Nitrogen-containing compounds released into environment can create serious problems, such as eutrophication of rivers [1–4]. Deterioration of water quality and potential hazard to human health, because nitrate in the gastrointestinal tract can be reduced to nitrite ions [5–7]. In addition, nitrate and nitrite have the potential to form N-nitrous compounds, which are potent carcinogens [8]. Anthropogenic sources such as nitrogenous fertilizers, animal wastes and septic systems and other nitrate sources related to urban development can increase nitrate concentrations in surface and ground water [9–12]. Biological removal of nitrate is widely used in the treatment of domestic and complex industrial wastewaters [13–18].

It can cause health problems in infants and animals, as well as eutrophication of water bodies [19]. The international drinking water quality standards decreased <50 mg/L as a "recommended" level and 50–100 mg/L as "acceptable" level for nitrate (NO_3^-), which equates to 11.3–22.6 mg/L when expressed as NO_3–N [20].

Despite denitrification being considered to be anoxic process, nitrate respiration has been observed under aerobic conditions in a number of bacteria, including *Escherichia coli* [21], *Paracoccus denitrificans* GB17, *Pseudomonas aeruginosa* [22], *Comomonas* sp. strain SGLY2 [23], Zoogloea [24], *P. den-*

* Corresponding author. Tel.: +82 2 2164 4889; fax: + 82 2 2164 4111.
E-mail address: pmayyasamy@gmail.com (P.M. Ayyasamy).

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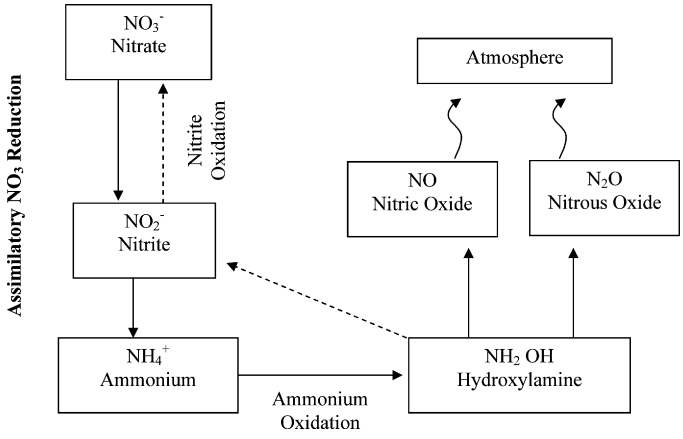
itrificans DSM 2944 [25] and ε-caprolactam [26]. Aerobic nitrate-respiring bacteria may be particularly important in environments with minimal or fluctuating O_2 availability. Under controlled conditions, they could decrease the costs for an anoxic tank, or at least decrease its size [27]. Investigation of aerobic metabolism of *Pseudomonas* and *Bacillus* species has revealed many interesting features of assimilatory nitrate reduction to ammonia [28,29]. Tiedje [30] reported that *Bacillus* species could carry out anaerobic dissimilatory reduction of nitrate to ammonia via nitrite. This anaerobic process has long been considered to be a way of dissipating electrons under anaerobic conditions. However, *Bacillus* species is capable of using nitrate and nitrite as the alternative electron acceptors.

Ayyasamy et al. [31] attained assimilatory denitrification process using aerobic *Pseudomonas* sp. (RS 7) in the ground water system. The assimilatory nitrate reduction under aerobic state was shown in the following schematic diagram [32,33].



The organisms like *Pseudomonas* sp. KW1 and *Bacillus* sp. YW4 were isolated from Kodaikanal and Yercaud lake water and sediment were used individually and consortium for the removal of nitrate from synthetic wastewater. Consortium proved better efficiency for nitrate removal. Hence this paper mainly focused consortium on the removal of nitrate. In this study we have not used the lake water for the removal. Instead we prepared the synthetic medium to study the efficiency of nitrate removal of the above isolates. The aim of the present paper was to investigate the applicability of the aerobic mixed bacterial cultures (KW1 + YW4) isolated from lake water and sediment for high rate nitrate reduction from synthetic wastewater. Attempts were made to optimize the carbon source, temperature, pH values, inoculum concentration to achieve as rapid nitrate removal as possible, and to improve economical effectiveness of the process.

2. Materials and methods

The bacterial species *Pseudomonas* sp. (KW1) and *Bacillus* sp. (YW4) were isolated from the water samples of the Kodaikanal and Yercaud lakes. The selected isolates were screened for the nitrate reduction efficiency by nitrate reduction test using potassium nitrate broth (5 g of peptone per liter, 3 g beef extract per liter, 5 g of sodium chloride per liter, 5 g of potassium nitrate per liter in the pH 7.0).

2.1. Effect of various carbon substrates on nitrate removal in synthetic medium

The mineral salt medium containing 100 mg/L of nitrate and 1% concentrations of different carbon substrates such as glucose, starch, cellulose, sucrose and acetic acid were prepared. One mL of inoculum containing 10^4 CFU/mL of bacterial cells of consortium (KW1 + YW4), the highly efficient consortium among tested, was inoculated and kept in a shaker (120 rpm) at 30 °C for 48 h. The samples were drawn aseptically at regular intervals (6, 12, 18, 24, 30, 36, 42 and 48 h) and growth (pour plate technique), nitrate (phenol disulphonic acid method), nitrite (NEDA method) and ammonium (Nessler's reagent) were estimated [34].

2.2. Effect of various starch concentrations on nitrate removal in synthetic medium

Starch was found to be ideal carbon source in nitrate removal compared to the other carbon sources such as glucose, cellulose, sucrose and acetic acid. Hence, starch at different concentrations was used to find out the optimum concentration. In this experiment, mineral salt medium (MSM) was prepared with 100 mg/L of NO_3^- and supplemented with various concentrations (0.2, 0.4, 0.6, 0.8, 1, 1.2 and 1.4%) of filter sterilised—0.2 μ starch. To this, 1 mL of inoculum containing 10^4 CFU/mL of bacterial consortium (KW1 + YW4) was aseptically inoculated and kept in a shaker (120 rpm) at 30 °C for 48 h. The sterilised synthetic medium without any carbon source was maintained as control to compare the efficiency of carbon source on nitrate removal by bacterial species. The samples were drawn at regular intervals (6, 12, 18, 24, 30, 36, 42 and 48 h) and growth (pour plate technique), nitrate (phenol disulphonic acid method), nitrite (NEDA method) and ammonium (Nessler's reagent) were estimated [34].

2.3. Effect of various temperatures on nitrate removal in synthetic medium

Mineral salt medium (MSM) with 100 mg/L of NO_3^- and supplemented with 1% starch was prepared and sterilised. Cells of 10^4 CFU/mL were inoculated to the medium and kept in a shaker (120 rpm) at 25, 30, 35, 40 and 45 °C for 48 h. At every 6 h, the bacterial growth, nitrate, nitrite and ammonium contents in the medium were determined.

2.4. Effect of various pH on nitrate removal in synthetic medium

Mineral salt medium (MSM) with 100 mg/L of NO_3^- at various pH (6, 7, 8 and 9) and supplemented with 1% starch was prepared and sterilised. Starch at 1% was found to be effective concentration in nitrate removal compared to the other concentrations. Hence, 1% starch was used as a carbon source for further study. About 10^4 CFU/mL of the cells were inoculated to the medium and kept in a shaker (120 rpm) at 30 °C for 48 h.

The samples were drawn aseptically at regular intervals (0, 6, 12, 18, 24, 30, 36, 42 and 48 h). The bacterial growth, concentrations of nitrate, nitrite and ammonium in the medium were determined.

2.5. Effect of various cell concentration of bacterial inoculum (KW1 + YW4) on nitrate removal in synthetic medium

Different inoculum dosage of 1 OD (10^4 CFU/mL) (1, 2, 3, 4 and 5%) of bacterial consortium was inoculated in sterilized mineral salt medium (MSM) with 100 mg/L of NO_3^- supplemented with 1% starch and kept in a shaker (120 rpm) at 30 °C for 48 h. At every 6 h, the bacterial growth, concentrations of nitrate, nitrite and ammonium in the medium were determined. Since 1% bacterial inoculum showed about 95% nitrate reduction at 18 h, this dosage was selected for pilot scale study.

2.6. Analysis of variance for effect of different carbon source, starch dosage, pH, temperature and inoculum dosage on nitrate reduction

Analysis of variance of the effect of different concentration of carbon sources, starch, pH, temperature and cell number of the consortium on nitrate reduction was carried out using IRRISTAT Version 3/93.

3. Results

3.1. Effect of various carbon sources on the growth of bacterial species, nitrate removal, nitrite and ammonium formation

Effect of various carbon sources (glucose, starch, cellulose, sucrose and acetic acid) on the growth of bacterial

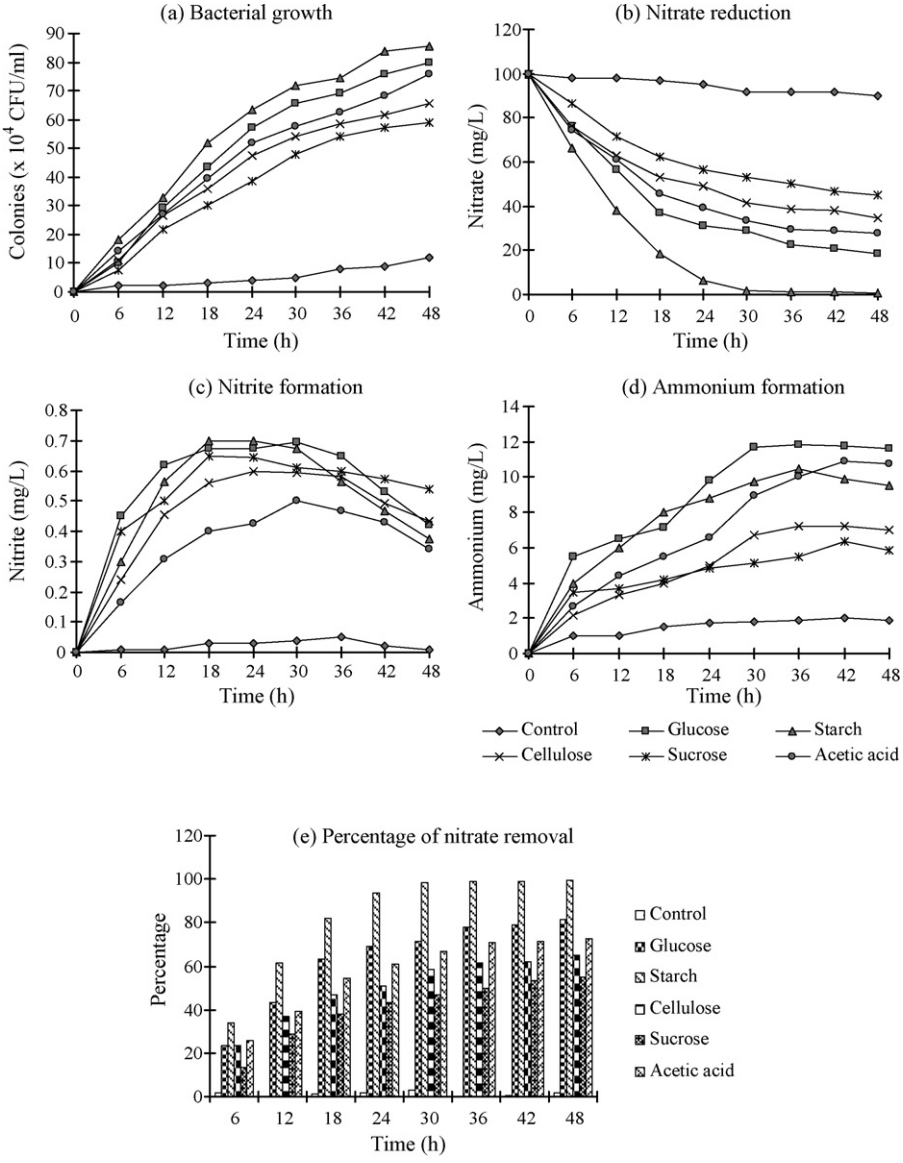


Fig. 1. Effect of various carbon sources on nitrate removal by bacterial consortium KW1 + YW4 in synthetic medium with 100 mg/L of nitrate.

species were found to be maximum (86×10^4 CFU/mL) in synthetic medium supplemented with starch followed by glucose (80×10^4 CFU/mL) at 48 h. The bacterial species reduced maximum amount of nitrate from 100 to 0.6 mg/L (99.4%) in synthetic medium supplemented with starch as a carbon source followed by glucose (from 100 to 18.35 mg/L). In acetic acid, cellulose and sucrose, the nitrate reduction was from 100 to 27.7 mg/L (72.3%), 100 to 34.8 mg/L (65.2%) and 100 to 44.9 mg/L (55.1%), respectively. The nitrate reduction was very negligible and constant in the medium without carbon sources. Among the five carbon sources, starch exhibited the highest nitrate reduction rate. This indicates that our denitrifier (KW1 + YW4) utilizes starch as a carbon source more than the other carbon sources such as glucose, cellulose, sucrose and acetic acid.

The formation of nitrite was found to be maximum (0.8 mg/L) in synthetic medium supplemented with starch. In glucose, cellulose and sucrose, the maximum level of nitrite formation was recorded at 24 h which was 0.68, 0.65 and 0.6 mg/L. The formation of ammonium was higher in synthetic medium amended with glucose at 30 h and there was no further significant change in ammonium formation till 48 h. The ammonium formation was less in other carbon sources such as starch, cellulose, sucrose and acetic acid when compared to glucose (Fig. 1).

3.2. *Effect of starch on the growth of bacterial species, nitrate removal, nitrite and ammonium formation*

The maximum growth of 85×10^4 CFU/mL was observed at 1% concentration of starch and minimum (56×10^4 CFU/mL) in 0.2% starch at 48 h. Whereas in the synthetic medium containing starch at 0.4, 0.6 and 0.8%, the growth was 70×10^4, 80×10^4 and 84×10^4 CFU/mL, respectively. Similarly, higher concentration at 1.2 and 1.4% of starch, the growth

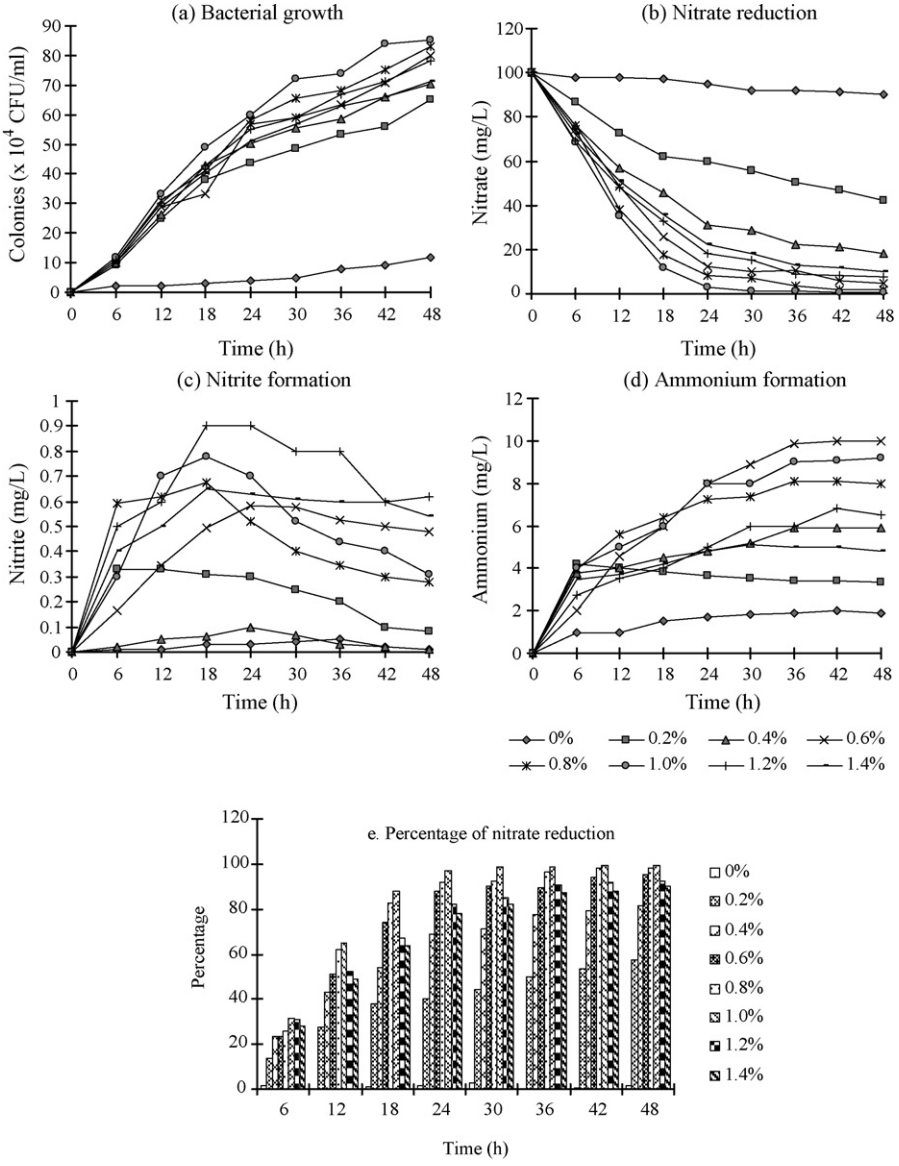


Fig. 2. Effect of various starch concentrations on nitrate removal by bacterial consortium (KW1 + YW4) in synthetic medium with 100 mg/L of nitrate.

was found to decrease and showed only about 78×10^4 and 71×10^4 CFU/mL, respectively. The consortium KW1 + YW4 reduced maximum level of nitrate (99.4%) in synthetic medium with 1% starch after incubation for 48 h. Whereas in the concentration of 0.2, 0.4, 0.6 and 0.8% starch amended synthetic medium, the nitrate reduction was 81.65, 95.3 and 98.4%, respectively. The maximum reduction of 99.4% was noticed at 30 h. At 24 h it was 95%. The nitrite formation was found to be maximum (0.9 mg/L) in synthetic medium supplemented with 1.2% starch at 18 h and then decreased thereafter. The maximum level of ammonium (9.96 mg/L) was also formed in synthetic medium supplemented with 0.6% starch at 36 h. After 36 h the ammonium formation was more or less constant in all the concentration of starch (Fig. 2).

The above results showed that 1% of starch as carbon source was found to be optimum for nitrate reduction by the bacterial consortium [KW1 + YW4]. Hence, starch at 1% concentration was used as carbon source for further experiments of removal of nitrate. The concentration of dissolved oxygen (DO) was also estimated before and after the treatment. There is no signification reduction of DO in the medium amended with various concentration of starch.

3.3. Effect of various temperatures on the growth of bacterial consortium, nitrate removal, nitrite and ammonium formation

The maximum growth of 84×10^4 CFU/mL was observed at 30 °C at 48 h followed by 35 °C (78×10^4 CFU/mL). Whereas the growth decreased when the experiment was carried out at 40 °C (70×10^4 CFU/mL) and 45 °C (63×10^4 CFU/mL). At lower temperature (25 °C) the growth was only 43×10^4 CFU/mL at 48 h. The bacterial consortium reduced nitrate from 100 to 0.6 mg/L (99.4%) in synthetic medium amended with 1% starch at 30 °C incubation at 48 h. At the temperatures of 25, 35, 40 and 45 °C, the nitrate reduction

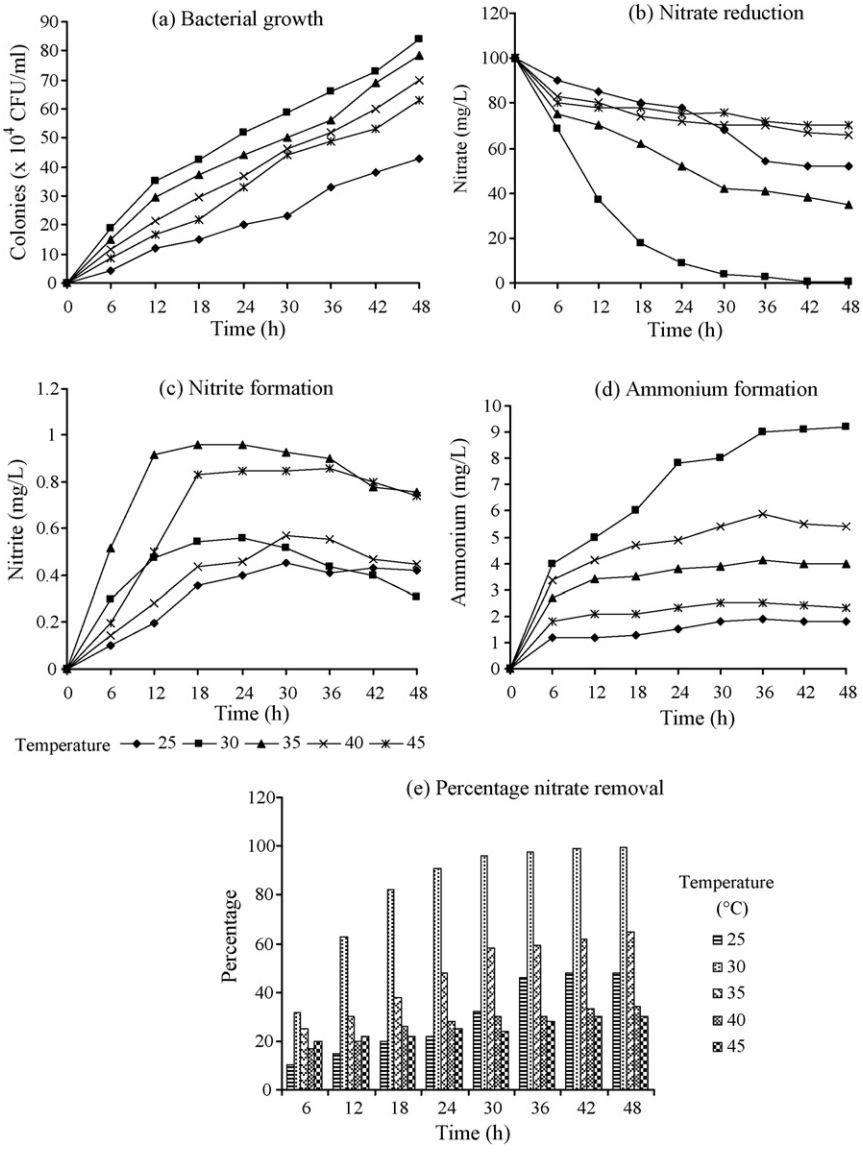


Fig. 3. Effect of various temperatures on nitrate removal by bacterial consortium (KW1 + YW4) in synthetic medium with 100 mg/L of nitrate.

was 48.2, 65, 34 and 30%, respectively. At 30 °C, about 90% of reduction was noticed at 24 h and attained 99.4% at 48 h. The maximum nitrite (0.96 mg/L) formation was observed at 35 °C followed by 45, 30, 40 and 25 °C. The maximum amount of ammonium (9.2 mg/L) was formed during nitrate utilization at 30 °C after 48 h. In the case of 25 °C, the ammonium formation was very less than other temperatures (30, 35, 40 and 45 °C) (Fig. 3).

3.4. Effect of pH on the growth of bacterial consortium, nitrate removal, nitrite and ammonium formation

The growth of bacterial consortium in synthetic medium containing 100 mg/L with 1% starch is given in Fig. 4. The maximum growth of 84.5×10^4 CFU/mL was observed in pH 7 at 48 h followed by pH 8 (71×10^4 CFU/mL). In the case of pH 6 and pH 9 the growth was less. The maximum of nitrate was reduced from 100 to 0.61 mg/L (99.4%) in synthetic medium amended with 1% starch in pH 7 at 30 °C for 48 h. At pH 6, 8 and 9 the reduction of nitrate was 16, 50 and 25%, respectively. The maximum nitrite (0.81 g/L) formation was observed in pH 7 followed by 9, 6 and 8. The maximum amount of ammonium (11.5 mg/L) was formed during nitrate utilization at pH 8 at 48 h (Fig. 4d). In the case of pH 6, 7 and 9 the ammonium formation was very less.

3.5. Effect of various cell concentration on the growth of bacterial consortium, nitrate removal, nitrite and ammonium formation

The growth of bacterial consortium KW1 and YW4 at 5% was maximum (105×10^4 CFU/mL) in synthetic medium containing nitrate followed by 4% (103×10^4 CFU/mL) at 48 h. In the case 1% the growth was 85×10^4 CFU/mL. The bacterial inoculum at 5% reduced maximum level of nitrate from 100 to 0.2 mg/L (99.8%) in synthetic medium followed by 4% (99.7%) at 48 h of incubation. About 99.3% of nitrate was reduced in synthetic medium inoculated with 1% of inoculum. There was

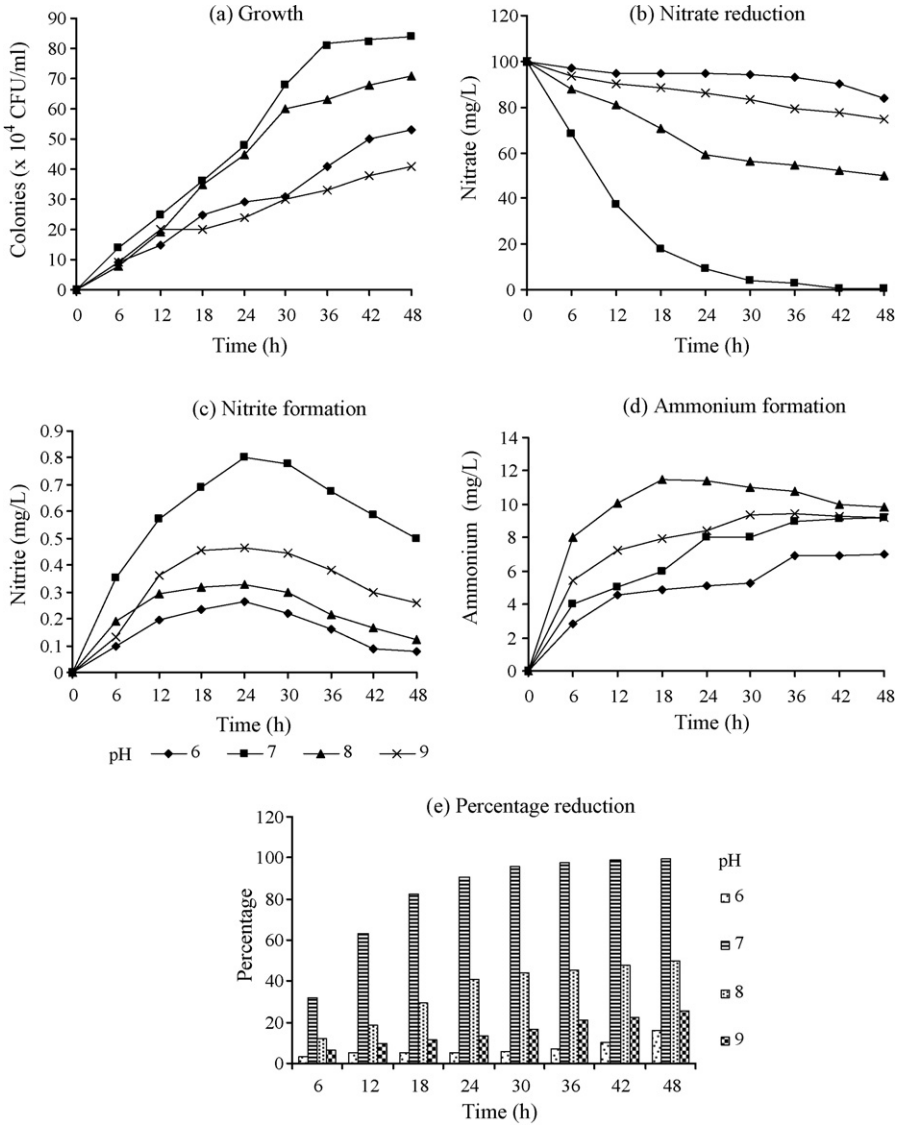


Fig. 4. Effect of various pH on nitrate reduction by bacterial consortium (KW1 + YW4) in synthetic medium with 100 mg/L of nitrate.

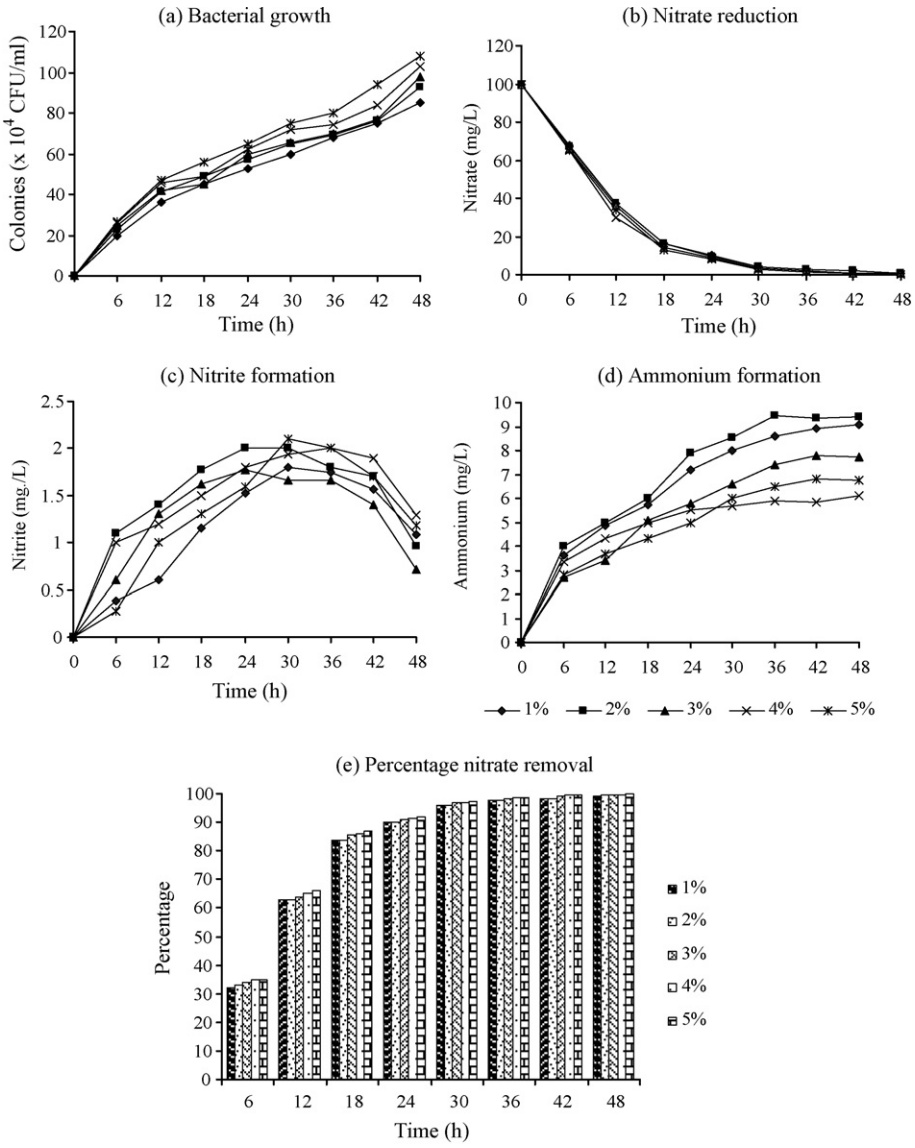


Fig. 5. Effect of various cell concentrations of bacterial inoculum (KW1 + YW4) on nitrate removal in synthetic medium with 100 mg/L of nitrate.

no significant reduction of nitrate at 5% when compared to 1% inoculum. The nitrite formation during nitrate reduction was high in synthetic medium with 5% inoculum at 30 h. After 30 h the formation of nitrite was found to be less. The amount of ammonium formed during nitrate reduction varied in all the inoculum dosage. The amount of ammonium formed was maximum (9.44 mg/L) in synthetic medium at 2% dosage (Fig. 5).

3.6. Analysis of variance for effect of different carbon source, starch dosage, pH, temperature and inoculum dosage on nitrate reduction

The analysis of variance for effect of different carbon source, starch dosage, pH, temperature and inoculum dosage on nitrate reduction is given in Table 1 and the different carbon sources, effects of starch concentration, temperature, pH and different dosage of bacterial inoculum were found to be significant at 1% level.

4. Discussion

4.1. Removal of nitrate from synthetic medium by bacterial consortia

The bacterial consortium KW1 + YW4 reduced maximum amount of nitrate which was about 99.4% in synthetic medium supplemented with starch as a carbon source followed by glucose (81.7%). In acetic acid, cellulose and sucrose, the nitrate reduction was 72.3, 65.2 and 55.1%, respectively. From the results of the above study conducted with various carbon sources (glucose, starch, acetic acid, cellulose and sucrose) inoculated with KW1 and YW4, the maximum concentration of nitrate was removed in synthetic medium supplemented with 1% starch as carbon source when compared to other carbon sources. Methanol, ethanol and acetic acid were commonly used as organic substrates to provide the reducing power for nitrate elimination [35–38] have studied nitrate removal at higher concentrations

Table 1
Analysis of variance for effect of different carbon substrates, starch dosage, pH, temperature and consortium dosage on nitrate reduction

Source	Degree of freedom	Sum of squares	Mean squares	*F*-ratio
Effect of carbon substrates				
Treatment	39	59263.06	1519.57	143660.20**
Carbon source (CS)	4	24062.23	6015.56	568712.65**
Duration (*T*)	7	33430.15	4775.74	451499.44**
CS × *T*	28	1770.68	63.24	5978.61**
Error	80	0.846	0.011	
Total	119	59263.91		
Effect of starch dosage				
Treatment	55	117592.26	2138.04	46785.49**
Starch dosage (SD)	6	39613.87	6602.31	144474.49**
Duration (*T*)	7	74532.77	10647.54	232993.80**
SD × *T*	42	3445.62	82.04	1795.20**
Error	112	5.12	0.046	
Total	167	117597.38		
Effect of pH				
Treatment	31	99697.07	3216.03	76.52**
Different pH (pH)	3	81784.38	27261.46	648.60**
Duration (*T*)	7	11322.31	1617.47	38.48**
pH × *T*	21	6590.38	313.83	7.47**
Error	64	2689.98	42.03	
Total	95	102387.05		
Effect of temperature				
Treatment	39	77309.52	1982.29	99.01**
Different temperature (DT)	4	54422.02	13605.50	679.52**
Duration (*T*)	7	15895.25	2270.75	113.41**
DT × *T*	28	6992.26	249.72	12.47**
Error	80	1601.77	20.02	
Total	119	78911.30		
Effect of consortium dosage				
Treatment	39	2715.61	69.63	6925.73**
Consortium dosage (CD)	4	110.74	27.68	2753.64**
Duration (*T*)	7	2444.48	349.21	34733.75**
CD × *T*	28	160.39	5.73	569.73**
Error	80	0.804	0.010	
Total	119	2716.14		

** Significant at 1% level.

from industrial wastewater supplemented with double nutrient source such as sucrose and glycerol by *Klebsiella oxytoca*. Nitrate removal in synthetic wastewater with 50 mg/L of nitrate amended with various carbon sources like glucose, sucrose, cellulose and acetic acid by microbial consortium under aerobic and anaerobic conditions and found that acetic acid was the suitable carbon sources for bacterial consortium (*Alcaligenes* sp. W-4 (LRS 1), *Alcaligenes* sp. S-8 (LRS 2) and *Micrococcus* sp. S-11 (LRS 3)) that reduced about 99.2% of nitrate in synthetic wastewater [40]. About 89.08% nitrate reduction from ground water supplemented with 1% starch as a sole carbon source by *Pseudomonas* sp. (RS 7) [31]. Our results, are in good agreement with the results of another study [39] where the use of starch as a carbon source in the on-site biological treatment of nitrate in ground water was successful with a nitrogen removal efficiency of 99.5% and a C/N ratio of 2.58 corresponding to 4.3 g of soluble starch per 1 g of nitrate nitrogen. The reason why starch exhibited the highest nitrate reduction rate can be explained by the fact that our bacterial culture is amylolytic (starch degraders) and is capable of utilizing starch as a carbon source. The aerobic condition during denitrification was maintained throughout the biological treatment [40]. In another study [41] have also noted no changes on the rate of dissolved oxygen, oxygen uptake and carbon dioxide evolution during the aerobic degradation of starch.

The temperature effect on the denitrification rate is another important feature in the design of a denitrification process [42,43]. Thermophilic and psychrophilic denitrifying bacteria are known to have different temperature optima than the mesophiles. The bacterial consortium KW1 and YW4 reduced maximum percentage of nitrate (99.4%) in synthetic medium amended with 1% starch at 30 °C incubation at 48 h. At the tem-

peratures of 25, 35, 40 and 45 °C, the nitrate reduction was only about 48.2, 65, 34 and 30%, respectively. Maximum of 89.08% of nitrate was reduced at 30 °C [31]. In our study higher percentage removal of nitrate was observed (99.4%) at 30 °C. The lower and higher temperature might have affected the bacterial growth in synthetic medium containing nitrate.

The effect of the different pH (6, 7, 8 and 9) on nitrate removal by bacterial consortium KW1 and YW4 was carried out. During the study, the maximum level 99.4% of nitrate was reduced in synthetic medium amended with 1% starch in pH 7 at 30 °C at 48 h. At pH 6, 8 and 9 the reduction of nitrate was 16, 50 and 25%, respectively. The nature of alkaline and acidic conditions may interfere in nitrate reduction. Hydrogenotrophic denitrification study was carried out using a fluidized bed sand reactor [44]. The results revealed that the optimum pH for nitrate removal was 7.5. The consortium functions to its maximum at neutral pH and the drinking water is normally near neutral pH and hence the consortium may be suitable. Our pervious study also confirmed that the nitrate removal was about 99.3% with various cell concentrations, of 1% inoculum. In the medium at 5% inoculum the nitrate removal was found to be 99.8%. There was no significant variation noted from 1 to 5% inoculum. However, the input concentration of nitrate was relatively low (100 mg/L). The rapid nitrate removal between 1 and 5% inoculum was attributed to the active bacterial growth using nitrate as a nutrient source since lower concentration of nitrate was the only inorganic substrate in synthetic medium.

4.2. Formation of nitrite and ammonium in synthetic medium

During nitrate reduction, accumulation of significant amount of nitrite and ammonium was observed in synthetic media with different carbon sources and environmental factors such as temperature and pH. The accumulation of nitrite in bacterial culture may be in principle due to either assimilatory or dissimilatory nitrate reduction or due to heterotrophic nitrification. However it is unlikely that nitrite accumulation results from nitrate assimilation since nitrate and nitrite reduction should highly coupled during assimilation. The nitrite so generated can be further reduced either to ammonia, or to nitric oxide, nitrous oxide, and dinitrogen [45]. The nitrite reduction rate under aerobic conditions was very low and results from previous work with *Alcaligenes faecalis* [46] indicated that the observed N_2O production may not be due to reduction of NO_2^-, but may be a by product of heterotrophic nitrification. Low NO_3^- supply and a non-limiting supply of fermentable substrate favours rapid reduction to NH_4^+, high NO_3^- and limited energy source appear to permit reduction only to NO_2^-. The nitrite was accumulated in the cultures of *Pseudomonas denitrificans*, ATCC 13867 when nitrate was present at higher concentrations [47].

Several authors [47–49] have observed that nitrite can inhibit denitrification, especially at high concentration. Accumulation of extracellular nitrite has been reported during denitrification in pure cultures implying that, under some conditions, denitrifying bacteria transport the nitrite intermediate out of the cell and later take the extracellular nitrite back in to the cell for complete denitrification.

In aerobic condition the predominant fate of NO_3^- reduction is generally believed to be reduction to N_2O and N_2 by bacterial respiration (denitrification). In our experiments nitrate reduction has lead to the formation of nitrite and ammonium which means the bacterial genera might have catalyzed an alternative reduction pathway, dissimilatory reduction of nitrate to NH_4^+ [50,51]. There is no fundamental argument why denitrification cannot occur under oxic condition. However, only during the past few years this activity received some attention [25,51–54].

5. Conclusion

In this study, the genera of *Pseudomonas, Bacillus, Micrococcus* and *Alcaligenes* were isolated from water samples collected from two different lakes and were found to be nitrate reducers. Among them, *Pseudomonas* sp. KW1 and *Bacillus* sp. YW4 were found to be the most efficient in terms of nitrate reduction. From the above results, it could be concluded that nitrate reduction by KW1 and YW4 were influenced by various carbon sources, temperature and pH. The rate of bacterial growth and nitrate reduction were high in synthetic wastewater supplemented with 1% starch as the sole carbon source compared with those in the synthetic wastewater supplemented with glucose, cellulose, sucrose and acetic acid under aerobic conditions at an optimum temperature of 30 °C and pH 7. Hence, 1% starch could be used as the best carbon source and concentration for nitrate removal in synthetic wastewater for the bacteria used in this study. Similarly, the temperature at 30 °C and neutral pH were optimum in the synthetic wastewater amended with 1% starch. According to the World Health Organization the permissible limit of nitrate in drinking water is 45 mg/L. In this study using bacterial consortium, nitrate could be reduced below the permissible limit within 12 h. Thus, starch is a successive nutrient source for the bacterial growth and could be useful to remediate wastewater containing nitrate.

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