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Short Note

Microbial Denitrification of Ground Water – Batch Study

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Abstract: Nitrate pollution of ground water is increasing alarmingly in various countries. Biological denitrification has been found as the most inexpensive and effective technique for nitrate removal. The present work involves batch studies for heterotrophic biological denitrification using cotton as the carbon source and *Pseudomonas fluorescens* NCIM 5059 and *Pseudomonas stutzeri* NCIM 5136 as the microbial strains. A maximum removal of 91.0 mg/l at 30 ºC for the strain *Pseudomonas fluorescens* and 89.2 mg/l at 40 ºC for the strain *Pseudomonas stutzeri* was observed for an initial nitrate concentration of 100 mg/l. Both the organisms followed Monods Growth Kinetics with μ_{max} values of 5.88 $days^{-1}$ for *Pseudomonas fluorescens* and 5 $days^{-1}$ for *Pseudomonas stutzeri*, respectively.

Key words: Nitrate removal, biological denitrification, ratio of cotton consumed to nitrogen in nitrate removed.

Introduction

Agricultural practices, soil characteristics and meteorological conditions are responsible for eventual nitrate accumulation in ground water. In India, nitrate in groundwater was found to be in the range 0.1–870 mg/l with an average of 65 mg/l and above in Gujarat, Rajasthan, Karnataka, Punjab, Haryana and West Bengal Karnataka Rural Water Supply and Sanitation Agency (KRWSSA Report 2000). Possible health consequences of nitrate ingestion include Methenoglobinemia, blue baby syndrome in infants under six months of age (Ward et al., 2005) and the possible formation of n-nitroso compounds in the gastric systems which are known to be carcinogens in the digestive system (Janos et al., 2001). The World Health Organization (WHO) has set a safe limit of 45 mg NO_3^-mg/l to regulate nitrate concentration in drinking water. Several methods with different performance and cost levels are available in treating drinking water to reach WHO standards (Kapoor and Viraraghava, 1997; Ovez et al*.,* 2006; Ovez, 2006; Oskar et al., 2007; Willie et al*.,* 2007; Mary and Brennan et al., 2009; Prashant et al*.,* 2010) but the most economical process is biological denitrification. Present work deals with batch denitrification using two different strains of *Pseudomonas* namely *Pseudomonas fluorescens* and *Pseudomonas stutzeri* with carbon source as unprocessed cotton (*Gossypium hirsutum*).

Materials and Methods

Batch tests were carried out using a sample of ground water supplemented with KNO_3 and phosphate (as K_2HPO_4) to make nitrate concentration of 100 mg/l. The Erylmyer flasks containing 500 ml of above prepared water sample were supplemented with cotton as the carbon source. The amount of cotton added for nitrate concentration of 100 mg/l varied from 60 mg to 150 mg (Soares, 2000; Xu et al*.,* 2009). These flasks were innoculated with the strains of *Pseudomonas fluorescens* NCIM 5059 *and Pseudomonas stutzeri* NCIM 5136 at 30°C and 40°C, respectively. The nitrate concentration and dry cell mass were determined each day using standard methods. The microbial strains used were procured from National Chemical Laboratory, Pune.

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Results and Discussion

The results revealed that after the 10th day, denitrification almost stopped in the ground water samples. The amount of cotton consumed during the period of 12 days was found to vary from 25.3 to 31 mg for both the strains. It was seen that the initial chosen mass of cotton does not have significant effect as long as it is greater than the cotton consumed as shown in Figures 1 and 2. The rate of denitrification achieved for both the strains of *Pseudomonas* showed that the maximum denitrification occurred for the ratio of mass of cotton consumed to that of mass of nitrogen in NO_3^- removed equal to 3.02.

The experiments were then carried out for nitrate concentration of 100 mg/l and cotton 60 mg (corresponding to the ratio of mass of cotton consumed to that of mass of nitrogen in NO_3^- removed equal to 3.02) for both the strains of *Pseudomonas fluorescens* NCIM 5059 *and Pseudomonas stutzeri* NCIM 5136 at 30 °C and 40 °C, respectively. It was seen that the nitrate concentration in the water gradually dropped as the time progressed, however, after nearly 10 days the

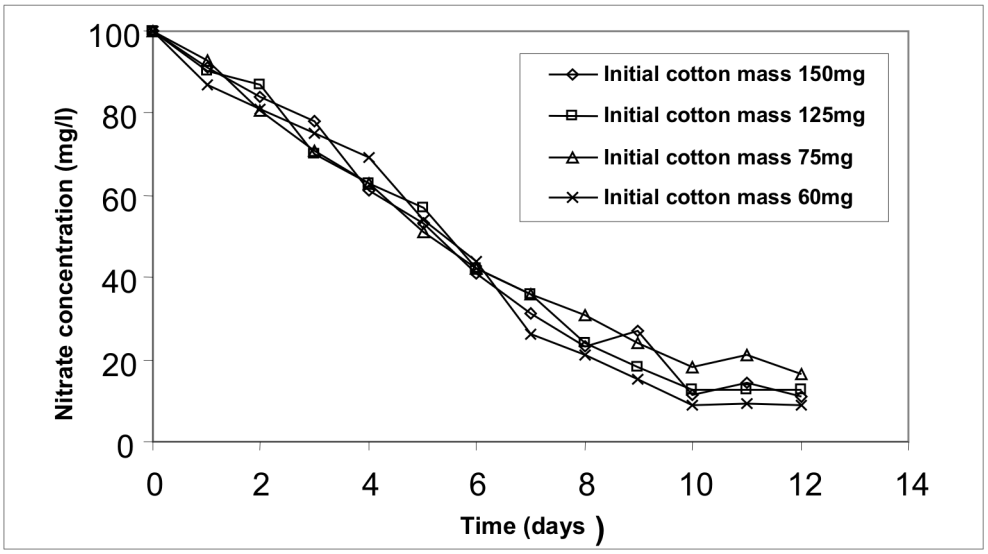

Figure 1: Variation of nitrate concentration at different cotton weights using *Pseudomonas fluorescens* NCIM 5059 at 30 °C for an initial nitrate concentration of 100 mg/l.

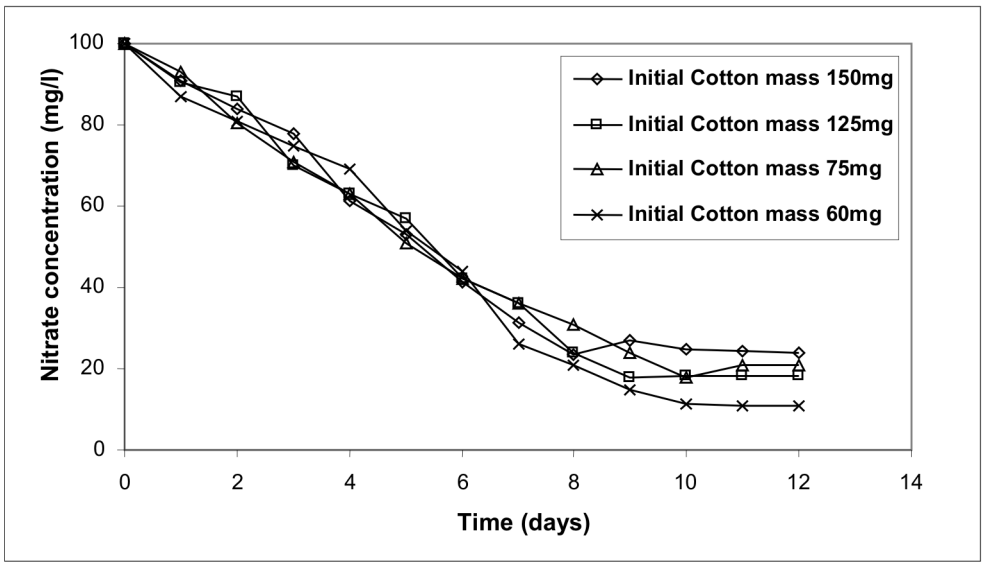

Figure 2: Variation of nitrate concentration at different cotton weights using *Pseudomonas stutzeri* NCIM 5136 at 40 °C for an initial nitrate concentration of 100 mg/l.

concentration of nitrate became almost constant. The Dry Cell Mass of the microorganism kept on increasing as the time progressed. After 10 days, the Dry Cell Mass of microorganism hardly showed any variation which indicates that the stationary phase was reached after the 9th day for both the strains. For an initial nitrate concentration of 100 mg/l the Dry Cell Mass was observed to reach a maximum (constant) value of 1.2 g DCM/l for *Pseudomonas fluorescens* NCIM 5059 and 1.1g DCM/l for *Pseudomonas stutzeri* NCIM 5136 as shown in Figures 3 and 4. This was on account of unavailability of sufficient nitrate; hence the micro-organism was unable to show any growth even in the presence of sufficient quantity of cotton. The amount of nitrate removed by the strain *Pseudomonas fluorescens* NCIM 5059 (91 mg/l) was slightly greater than that of *Pseudomonas stutzeri* NCIM 5136 (89.1 mg/l).

It was assumed that the process followed Monod growth kinetics in which cell mass characterized the biophase.

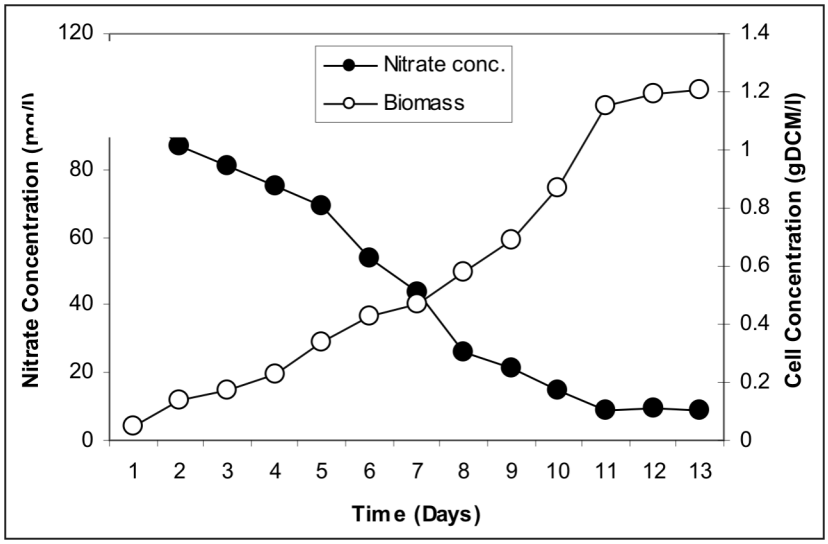

Figure 3: Variation of cell mass *Pseudomonas fluorescens* NCIM 5059 and nitrate concentration with time, at 30°C for an initial nitrate concentration of 100 mg/l.

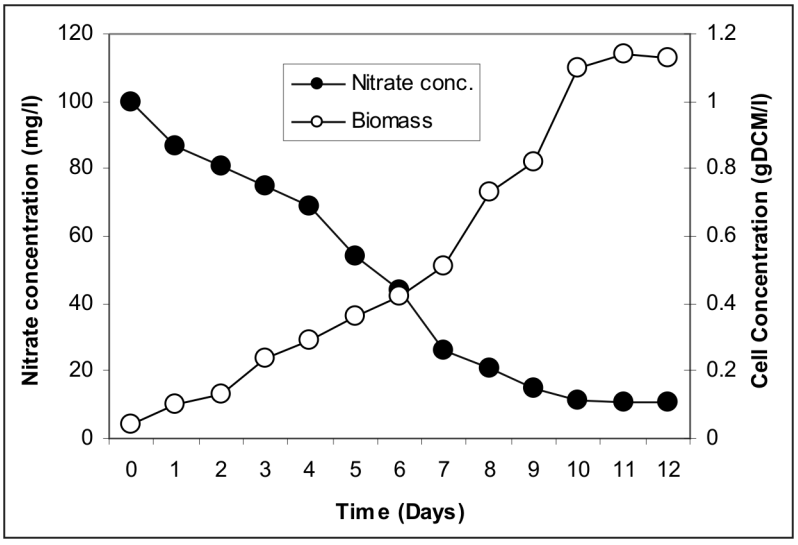

Figure 4: Variation of cell mass *Pseudomonas stutzeri* NCIM 5136 and nitrate concentration with time, at 40°C for an initial nitrate concentration of 100 mg/l.

The experimental data was generated (as shown in Tables 1 and 2) for both the strains from the growth rate curve and the Monod kinetic parameters were determined by plotting the specific growth rate versus nitrate concentration for both the strains as shown in Figures 5 and 6.

The Monods parameters as determined for the strain *Pseudomonas fluorescens* NCIM 5059 were μ_{max}= 5.88 days^{-1} K_s = 5.25 mg/l and that for the strain *Pseudomonas stutzeri* NCIM 5136 were μ_{max} = 5 days^{-1} K_s = 4.1125 mg/l, respectively.

This data was simulated to check the suitability of Monods model for the process of denitrification using regression analysis (Statistica 6.0 software). It was seen that the model predicted the actual response to a satisfactory extent. The predicted specific growth rate values are as shown in Tables 1 and 2. The model had a coefficient of multiple determination equal to 0.835 for strain *Pseudomonas fluorescens* NCIM 5059 and 0.718 for the strain *Pseudomonas stutzeri* NCIM 5136. The results obtained from the regression analysis were then compared with the experimental data as shown in Figures 7 and 8.

Table 1: Experimental and predicted values of specific growth rate of cell mass for the strain *Pseudomonas fluorescens* NCIM 5059 at 30 °C for an initial nitrate concentration of 100 mg/l

Time (days)	*X (DCM/l)*	*S (mg/l)*	*1/x*	*1/s*	*μ (day^{-1})*	*$1/\mu_{experimental}$ (day)*	*$1/\mu_{predicted}$ (day)*
0	0.050	100.000	20.000	0.010	1.000	1.000	3.087
1	0.140	87.000	7.143	0.011	0.357	2.800	3.208
2	0.170	81.000	5.882	0.012	0.382	2.615	3.278
3	0.230	75.000	4.348	0.013	0.304	3.286	3.358
4	0.340	69.000	2.941	0.014	0.235	4.250	3.453
5	0.430	54.000	2.326	0.019	0.163	6.143	3.781
6	0.470	44.000	2.128	0.023	0.160	6.267	4.124
7	0.580	26.000	1.724	0.038	0.181	5.524	5.406
8	0.690	21.000	1.449	0.048	0.174	5.750	6.152
9	0.870	15.000	1.149	0.067	0.224	4.462	7.704
10	1.150	9.000	0.870	0.111	0.078	12.778	13.623
11	1.190	9.300	0.840	0.108	0.017	59.500	60.009
12	1.210	9.000	0.826	0.111	0.000	-	-

Table 2: Experimental and predicted values of specific growth rate of cell mass for the strain *Pseudomonas Stutzeri* NCIM 5136 at 40 °C for an initial nitrate concentration of 100 mg/l

Time (days)	*X (DCM/l)*	*S (mg/l)*	*1/x*	*1/s*	*μ (day^{-1})*	*$1/\mu_{experimental}$ (day)*	*$1/\mu_{predicted}$ (day)*
0	0.04	100	25.000	0.010	1.550	0.645	2.244
1	0.1	87	10.000	0.011	0.700	1.429	2.406
2	0.13	81	7.692	0.012	0.538	1.857	2.498
3	0.24	75	4.167	0.013	0.229	4.364	2.605
4	0.29	69	3.448	0.014	0.207	4.833	2.731
5	0.36	54	2.778	0.019	0.194	5.143	3.168
6	0.42	44	2.381	0.023	0.179	5.600	3.625
7	0.51	26	1.961	0.038	0.255	3.923	5.332
8	0.73	21	1.370	0.048	0.288	3.476	6.326
9	0.82	15	1.220	0.067	0.305	3.280	8.393
10	1.1	11.1	0.909	0.090	0.064	15.714	10.935
11	1.14	10.8	0.877	0.093	0.026	38.000	36.312
12	1.13	10.8	0.885	0.093	0.009	113.000	-

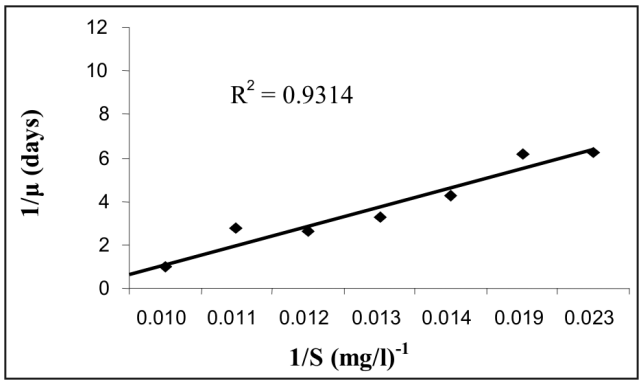

Figure 5: Double reciprocal plot of specific growth rate versus nitrate concentration for *Pseudomonas fluorescens* NCIM 5059 strain at 30 °C for an initial nitrate concentration of 100 mg/l.

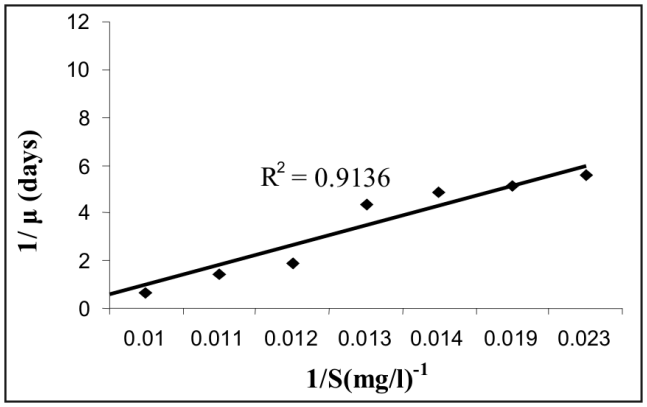

Figure 6: Double reciprocal plot of specific growth rate versus nitrate concentration for *Pseudomonas stutzeri* NCIM 5136 strain at 40 °C for an initial nitrate concentration of 100 mg/l.

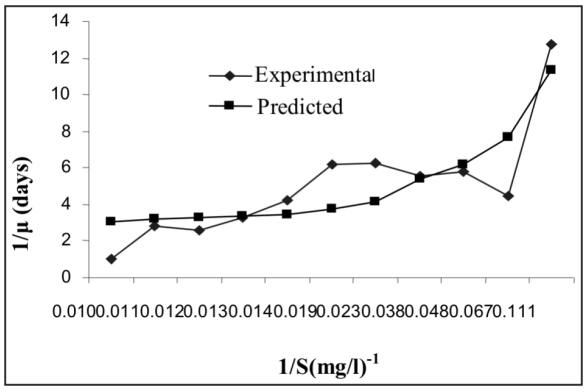

Figure 7: Comparison of 1/μ Vs 1/S for both experimental and predicted values using regression analysis for the strain *Pseudomonas fluorescens* NCIM 5059 at 30 °C for an initial nitrate concentration of 100 mg/l.

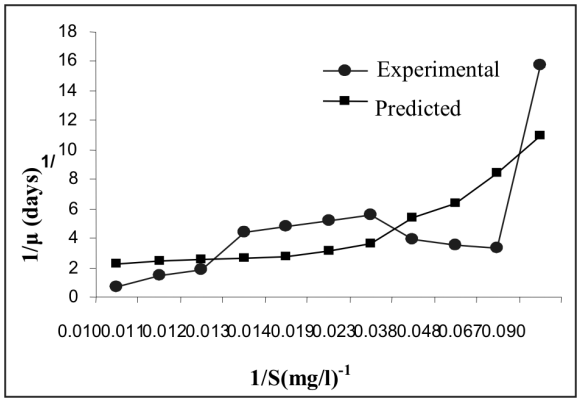

Figure 8: Comparison of 1/μ Vs 1/S for both experimental and predicted values using regression analysis *Pseudomonas stutzeri* NCIM 5136 strain at 40 °C for an initial nitrate concentration of 100 mg/l.

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