ASSESSMENT OF POTENTIAL REMOVAL OF NITRATE FROM SYNTHETIC MEDIUM BY SELECTED HEROTROPHIC BACTERIA – AN APPROACH FOR REDUCING NITRATE IN GROUNDWATER OF JAFFNA PENINSULA

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Introduction

The removal of nitrate in groundwater is of great interest because excessive nitrate in groundwater and surface water is a growing problem worldwide including Sri Lanka, mainly due to excessive application of fertilizers. The World Health Organization has set a limit of 11 mg/l NO_3^- - N for human consumption. Consumption of excess nitrates can have several detrimental health effects such as methahemoglobinemia, stomach cancer and adverse reproductive outcomes [2]. Groundwater is the major natural water resource in the Jaffna Peninsula, Sri Lanka, where, population is entirely dependent on the groundwater resources for all the purposes [3]. The water samples in wells where there is agricultural activity have NO_3^- - N levels between 20 to 50 mg/l [4]. Though nitrate contamination of ground water has been reported in Sri Lanka, there has been little research reported on remediation of such pollution except few phytoremediation studies [5]. Thus it is necessary to remove nitrate from groundwater resources to reduce its harm to the environment. Nitrate from the contaminated water can be removed by ion exchange, reverse osmosis, electro dialysis and some other chemical treatments. The most promising and versatile approach being studied is biological denitrification. Biological denitrification is highly selective and efficiency of the process is very high and can reach nearly 100%, which is not matched by any other methods available for nitrate reduction. Since most of the nitrate reducing bacteria are heterotrophs, source of organic carbon is an important component of the denitrification process. Usually, dissolved carbon sources, such as ethanol, methanol, acetate or glucose, are used as electron donor for nitrate reduction. The aim of the present study is to identify the applicability of five selected bacterial strains for nitrate removal in synthetic medium. The best five strains were selected based on the efficiency of nitrate removal (>50%) from nutrient broth. They were assessed for potential of reducing nitrate content in synthetic medium with glucose as a carbon source and control.

Materials and Methods

Sample collection, Isolation and cultivation

Environmental samples such as; compost, swine manure, soil sample from municipal solid waste dumping place, paddy soil and poultry manure were collected for isolation of denitrifying bacteria from Jaffna District. Cultures were cultivated aerobically on specific medium with glucose as sole carbon source [6].

Screening of denitrifiers

Different screening criteria were used to categorize the isolated cultures and obtain the desired strains. In initial categorization cultures with dissimilar (morphology) cultural characteristics were selected and screened using their natural capabilities to degrade nitrate on BTB (Bromothymol Blue) medium with nitrate [6]. For quantification studies nutrient broth with nitrate was used.

Nitrate reduction in synthetic medium

Nitrate removal of the five selected isolates were evaluated in modified mineral salt medium (potassium dihydrogen phosphate, 0.1 gl^{-1}; dipotassium hydrogen phosphate, 1gl^{-1}; calcium chloride, 0.005 gl^{-1}; magnesium sulphate, 0.1 gl^{-1}; pH 7) with 0.5% of glucose as carbon source. In briefly, fresh culture suspensions of strains were prepared in sterile distilled water with equal density. 0.5 ml of an inoculum was inoculated to 50 ml of mineral salt medium containing 150 mg/l of KNO_3 and incubated for four days at 30°C at 120 rpm in mechanical shaker for 96 hours. The control was also kept with the same concentration of nitrate but without carbon source and bacterial inoculum.

Analytical methods

Liquid samples were drawn at 24 hours interval in aseptic condition and centrifuged at 10000 rpm for 20 minutes. The, supernatant was used for NO_3^--N analysis by using colorimetric method [1].

Tentative identification of strains

Gram staining, catalase test, motility test, glucose acid test and starch hydrolysis test were performed for tentative identification of strains.

Data analysis

Statistical analysis was done by using Minitab.

Results and Discussion

Isolation and culturing of bacteria

In the present work, glucose was used as external carbon source for biological nitrate reduction by heterotrophic bacteria in synthetic medium. Selected five strains A2, A13, A15, A19 and GB (unidentified) were isolated from municipal compost, swine manure, municipal solid waste dumping place, paddy soil and poultry manure respectively, from Jaffna district.

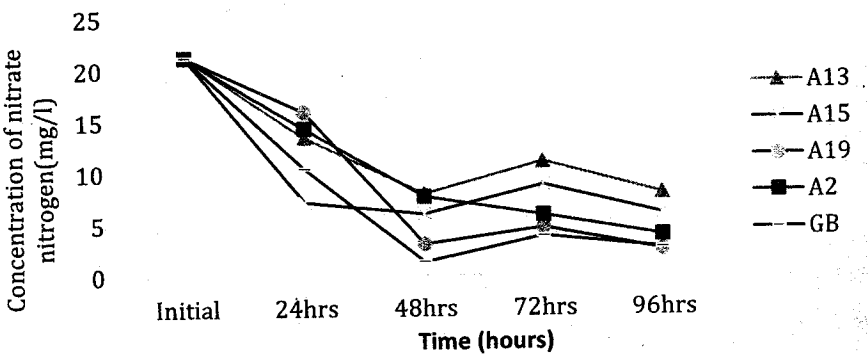

Figure 1: Concentration of nitrate nitrogen in synthetic medium inoculated with five bacterial strains in four days of incubation

Nitrate removal in synthetic medium

The concentration of nitrate nitrogen in the medium contains glucose treated with different strains are shown in Figure 1. A similar pattern of reduction was observed for most of the strains. The significant difference was observed in all treatments (bacteria strains) with control (21.175 mg/L) after four days of treatment. At the end of the day no significant difference was observed between the strains A19 and GB. All the strains

reduced nitrate nitrogen below permissible level (11 mg/l). Highest reduction was observed by strain GB and A19 after four days. Strain A2 showed continuous reduction of nitrate in four days of incubation. Strain GB had highest nitrate reduction (93.7%) in 48 hours of incubation. All the strains reduced the nitrate nitrogen below permissible level (11 mg/L) at 48 hours of incubation. After 48 hours of incubation, except A2 strains, concentration of NO_3^- - N started to fluctuate. This might be due to different mechanisms for strains to perform heterotrophic nitrification and caused by the oxidation of intermediates due to brief exposure to air during sampling for analysis [7]. Tentative identification of selected five strains are summarized in Table 1 below. All five strains were observed as catalase positive, motile and gave positive result for glucose acid test.

Table 1. Tentative identification of strains

Descriptor	A2	A13	A15	A19	GB
Gram staining	(+)	(-)	(+)	(-)	(-)
Catalase test	(+)	(+)	(+)	(+)	(+)
Motility test	(+)	(+)	(+)	(+)	(+)
Glucose acid test	(+)	(+)	(+)	(+)	(+)

Conclusion

The experimental results showed that all the strains tested, educed the nitrate nitrogen below 11 mg/L (WHO limit) (from 21.175 mg/L of NO_3^- - N) in four days of incubation with glucose as external carbon source. Among those strain GB had highest NO_3^- - N reduction (93.7%) in 48 hours of incubation. Strains A2, A19 and GB reduced NO_3^- - N more than 80% in four days of incubation. Strains A13 and A15 were found to have 62.7% and 72% of nitrate reduction in four days of incubation. Possibly almost all the strains A2, A13, A15, A19 and GB could be potentially used for bioremediation of nitrate contaminated groundwater of Jaffna district.

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