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Lab scale process on the removal of nitrate in ground water enriched with denitrifying bacterium and starch as a sole carbon source

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ABSTRACT

In this study, an attempt was made to treat ground water nitrate using *Bacillus* sp. (SW-59) as a denitrifier and lime as a coagulant. The effect of various concentrations of starch on nitrate removal was investigated using mineral salt medium (MSM) containing 150 mg L^{-1} of nitrate. Starch at 0.5% was found to be ideal and removed 70.45% of nitrate within 96 h intervals. Thus, starch was selected as a potential carbon source for nitrate removal in groundwater. About 99.61% of nitrate was removed from the ground water containing 82 mg L^{-1} after 156 h with the formation of nitrite and ammonium. In another aspect, various concentrations of lime were applied since the bacterially treated ground water holds trace amount of nitrate, nitrite, ammonium and bacterial biomass. Lime at 150 mg L^{-1} exhibited the highest action on the elimination of nitrogenous components and bacterial biomass. Chlorination was employed with varying concentrations (0.2 to 0.5 mg L^{-1}) of commercial chlorine powder and 0.5 mg L^{-1} exhibits a maximum removal of bacterial counts from 122 to 9×10^4 CFU mL^{-1}. Since, the complete reduction of bacterial cells was not attained in this study, it is suggested that secondary disinfection can be furnished.

1. Introduction

Nitrate (NO_3^-) concentration in ground water has increased globally. The concentration of nitrate in ground water can be enhanced by shallow ground water table, manures, irrigation and abundant rainfall. Livestock feeding, barnyards, septic tanks, industrial effluents, animal and human contaminations are the other important sources contributing high amount of nitrate to the surface and ground water [1,2]. In and around areas of high urbanization, industrialization, municipal and industrial wastes may also contribute high levels of nitrate to ground water.

Excessive use of nitrogenous fertilizers in agriculture has been one of the primary sources of high nitrate in ground water [3–5]. There are numerous sources in the environment that contribute to the total nitrate content of natural system found in Hissar (1800 mg L^{-1}) and Mahendragarh (1620 mg L^{-1}) district of Haryana waters e.g. atmosphere, geological features, anthropogenic sources, atmospheric nitrogen fixation and soil nitrogen. TWAD Board, Chennai [6] reported that the nitrate contamination in ground water seems very high in some parts of Thiruvannamalai, Salem, Dharmapuri and Erode districts of Tamil Nadu, India and the concentration was virtually >100 mg L^{-1}.

Large amounts of nitrate in drinking water are a cause of disease called methemoglobinemia (blue baby disease), a blood disorder primarily affecting infants under six months of age [7]. High nitrate reduces assimilation of iodine by human body causing goiter and intake with drinking may lead to the birth of a malformed child. There are reports of other health disorders, namely hypertension, increased infant mortality, lymphatic cancer, non-Hodgkins lymphoma, gastric cancer, goiter, stomach cancer, thyroid disorder, cytogenetic effects and birth defects [8]. Due to several health hazards by nitrate contamination, removal of nitrate is essential from the contaminated water before being utilized.

Physical and chemical processes such as reverse osmosis, ion exchange, photocatalytic reduction, adsorption, electrodialysis and chemical denitrification have been developed for nitrate removal from water [9,10]. Although these techniques are effective in removing nitrate from contaminated water, they are very expensive for pilot scale operation with a limited potential application and generate secondary environmental contamination [11]. Owing to these limitations in the removal of nitrate from water and/or wastewater, the most versatile and widely used technology is biological denitrification [12].

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Fig. 1. Nitrate removal in ground water through a bioreactor designing.

Several authors have been studying the effects of carbon sources on the removal of nitrate from water and/or wastewater for biological processes. Gomez et al. [13] studied the effectiveness of three selected carbon sources, namely, sucrose, ethanol and methanol for the removal of nitrate from contaminated groundwater. They found that ethanol was the most suitable carbon source. Volokita et al. [14] succeeded significant levels of nitrate removal in the aqueous media with newspaper as a carbon source. Archna et al. [15] investigated a batch study for heterotrophic biological denitrification using cotton as the sole source of carbon. A maximum removal of 91.0 mg/L at 30 °C and 89.2 mg/L at 40 °C was observed for an initial nitrate concentration of 100 mg/L. Rashid and Schaefer [16] reported that among various carbon sources, glucose and cellulose induced a very high degree of nitrate removal in a soil under anaerobic condition. In another study conducted by Shanthi et al. [17], five different carbon sources, namely, glucose, glycerol, starch, methanol and acetic acid were investigated for their potential use in nitrate reduction from a synthetic solution with sewage and dairy sludge as inoculum. They found that there was almost no difference between these carbon sources, except for methanol, yielding 95 to 100% nitrate reduction after 96 h. Kim et al. [18] studied the denitrification of nitrate in contaminated groundwater incorporated with psychrophilic denitrifier and starch. In their study, the nitrogen removal efficiency of 99.5% at a hydraulic residence time of 1 h was obtained with a C/N ratio of 2.58, corresponding to 4.3 g of soluble starch per 1 g of nitrate. Thus, in our study, commercially available and cheaper carbon source, starch was selected as a potential candidate for nitrate removal from groundwater.

One of the major problems in biological methods used for nitrate removal from water is that secondary treatment is still required for bacterial removal. For the treatment of wastewater, coagulating agents have been widely applied to remove chemical ions and colloidal particles such as mineral colloids, microbial colloids and virus particles [19]. Lime acts as a precipitant for phosphates, many trace metals and bacteria, and was used as a coagulant for the removal of suspended and colloidal materials in municipal wastewater [20]. Although several authors used some coagulants for the removal of microorganisms, suspended and colloidal particles, a combined treatment using bacterial species and coagulants has not yet been conducted for the removal of nitrate. Hence this present study was done to find out the efficiency of the selected heterotrophic bacteria in the reduction of nitrate from synthetic and ground water. Removal of microbial biomass in the treated water also investigated through the optimized chlorination process.

2. Materials and methods

2.1. Collection of samples and analysis

Ground water contaminated with nitrate was collected under aseptic conditions in a pre-cleaned, acid washed 5 L plastic containers for the analysis of physico-chemical parameters from U. Soundapuram area of Rasipuram Taluk, Namakkal District, Tamil Nadu, India. Prior to collection of samples, the containers were thoroughly rinsed, 4–5 times with the water sample and then filled till the mouth avoiding air space. It was transported to the laboratory carefully in an icebox and stored at 4 °C for further study. The significant physico-chemical parameters such as pH, total solids (TS), total suspended solids (TSS), total dissolved solids (TDS), nitrate, nitrite and ammonium of the water sample was analysed by the standard methods [21–24].

2.2. Screening of carbon source on the growth of bacteria

Nitrate reducing organism, *Bacillus* sp. (SW-59) was obtained from Bioremediation laboratory, Department of Microbiology, Periyar University, Salem. The viability of the strain was checked by repeated streaking on nutrient agar and potential viable colony was stored in nutrient agar slants at 4 °C. For screening of carbon source on the growth of SW-59, the hydrolysis of starch, cellulose and glucose was studied using respective solid media at room temperature. Starch and cellulose agar was prepared, poured into sterile petridish and allowed for solidification. The test organism was streaked by spot inoculation on the medium and incubated at room temperature for 24 h. After incubation the degradability of the starch and cellulose was checked based on the diameter of the zones developed around the bacterial growth. Triple sugar iron (TSI) test was performed to find out the influence of glucose on the growth of SW-59 and its degradation ability. Starch has shown an effective bacterial growth and selected as a best carbon source for the nitrate removal studies.

2.3. Nitrate removal in synthetic medium under batch mode condition

Nitrate removal in mineral salts medium containing ($g\,L^{-1}$) 0.1 g of potassium dihydrogen phosphate, 1 g of dipotassium hydrogen phosphate, 0.005 g of calcium chloride, 0.1 g of magnesium sulphate, 0.05 g of sodium silicate and pH 7 was carried out using SW-59 under the batch mode condition at room temperature. For inoculums preparation, a loopful of culture was enriched in presterilized 100 mL nutrient broth. The flask was kept in a shaker at 120 rpm for 12 h at 30 °C. The culture broth was centrifuged at 4000 rpm for 20 min. Cell suspension was prepared using sterile distilled water and adjusted to 1.0 OD (10^4 CFU mL^{-1}) using UV Visible Spectrophotometer (Make: Cyberlab UV-100, USA). 1 mL containing 10^4 CFU mL^{-1} of the above suspension was used as inoculum for the nitrate reduction.

The mineral salts medium was prepared with 150 mg L^{-1} of NO_3^- and supplemented with 0.25, 0.5, 0.75, 1.0 and 1.25%