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BIOLOGICAL PURIFICATION OF GROUNDWATER POLLUTED WITH NITROGENOUS COMPOUNDS

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ABSTRACT

The maintainability of denitrifying granular sludge in upflow sludge blanket (USB) reactor was demonstrated through continuous treatment of soft groundwater supplemented with nitrate. Complete nitrate removal was achieved at volumetric loading rates (VLRs) up to 2.1 g N/L/d. The concentration of MLSS increased from 20 g/L at a VLR of 0.6 g N/L/d to 51 g/L at a VLR of 1.9 g N/L/d, above which it decreased. Settling velocities showed the same trend. However, granular size, calcium and magnesium contents of the granular sludge and protein, carbohydrate and nucleic acid contents of extracelluar polymers decreased steadily with an increase in VLR. These results suggest an optimum operational VLR in term of nitrate removal and sludge retention of about 2 g/L/d. Groundwater samples with nitrate concentration exceeding 10mg/L were successfully treated using an USB reactor with hydraulic retention times of 30 to 50 min.

KEYWORDS

Groundwater, nitrate, USB, denitrification, granulation, precipitation potential

INTRODUCTION

As a potable water resources, groundwater is an attractive option versus surface water due to comparatively high quality and reduced volume fluctuations. However, application of chemical fertilizers has resulted in increasing nitrate concentration in aquifers. The regulatory limit for nitrate in drinking water of 10 mg/L is commonly exceeded in groundwaters of areas with intensive agricultural activities. As a remedial measure for nitrate pollution, physicochemical methods offer some advantage in operational controllability and capacity to be automated. However, with these methods nitrate is only transferred to another phase. Conversely, biological denitrification has the potential of converting soluble nitrate to gaseous forms.

Retention of biomass is an important factor for the efficient operation of a denitrifying process. Upflow sludge blanket reactors (USB) have demonstrated effectiveness the formation of retainable granular sludge [(1)]. The principal of effective sludge granulation in USB process is based on calcium precipitation. Tarre *et al.* [(2)] showed that high mineral content in a water to be treated is an important factor in the development of manageable granular sludge. We recently demonstrated that denitrifying granular sludge can be maintained using water with low hardness (less than 120 mg $CaCO_3$/L) [(3)]. In that work, high pH of about 9.0 was a significant factor in enhancing mineral precipitation.

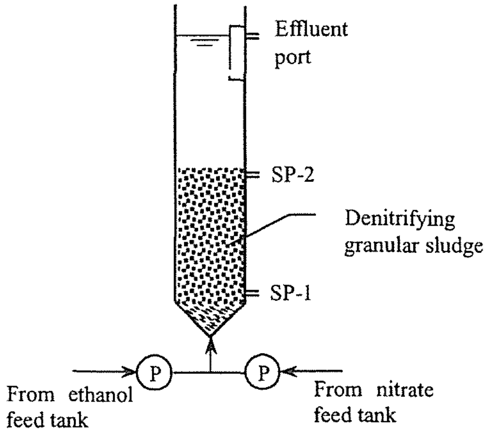
The objective of this research were:

a) To ascertain the maximum acceptable nitrate loading capacity of granular denitrifying sludge,
b) To investigate the physical, chemical, and biological characteristics of the sludge as they pertain to granule formation and retention,
c) To determine the kinetic constants for USB granular, and
d) To evaluate the applicability of USB process for nitrate removal of a polluted groundwater in Kumamoto Prefecture.

MATERIALS AND METHODS

Loading rate studies

Reactor and substrate; The USB reactor used in this research was made of glass and had a 2.5 L working volume with a 9.0 cm diameter and a cone shaped influent port (Fig. 1). The reactor was operated at a temperature of 25 °C. A mechanical stirrer with bent wire shaft was rotated at 20 rpm with a 5 minute on, 15 minute off cycle to enhance sludge-gas separation. The influent carrier was a tap water of groundwater origins and was supplemented with KH_2PO_4 (to 1.0 mg PO_4^{3-}/ L), $KHCO_3$ (to a total alkalinity of 160 mg $CaCO_3$/ L) and varying concentration of $NaNO_3$. Ethanol was fed from a separate influent tank at 0.1 L/hr at varying concentration to maintain an influent carbon to nitrogen ratios(C: N) of 1.1. 10N NaOH was used to adjust the influent pH to 9.0 to elevate the precipitation potential.



(SP: sampling port)

Fig. 1. Schematic diagram of the laboratory-scale USB reactor.

Operational conditions; The reactor operated continuously at a flow rate of 3.0 L/h for a corresponding hydraulic retention time (HRT) of 50 min and a vertical flow velocity of 0.013 cm/s. Influent nitrate concentrations were increased stepwise from 20 to 140 mg/L, and at each step the reactor was operated for about 7 days. Sludge was periodically wasted to maintain a sludge bed of about 2/3 the liquid volume.

Batch denitrifying study: Batch experiments were carried out at 25 °C in 250 mL of Erlenmeyer flasks equipped with gas exchangers. 25 mL of granular sludge sample was added to 175 mL of the influent used in continuous treatment loading rate studies. After headspace was flushed with nitrogen gas, batch tests were started under gentle mixing. Mixed liquor samples were withdrawn every 10 min and then the supernatants obtained by centrifugation (3,000 g for 10 min) were analyzed for water quality. Additionally, denitrifying sludge that had been cultivated with synthetic medium using methanol as the H-donor was used after being acclimated to ethanol for about 10 days. This suspended denitrifying sludge was used for comparative purposes with the granular sludge.

Tests with polluted ground water samples: Two polluted groundwater samples were collected from areas of intensive agricultural practice in Kumamoto Prefecture. One source was monitoring well, which was constructed in a town by Kumamoto Prefectural Office. The depth of this monitoring well is 40m. Second groundwater sample was taken from the personal well in town B. The depth of this well is 40m. Ethanol was added to these groundwater samples for denitrification to an influent C: N ratio of 1.1.The influent pH was adjusted to 9.0 by 10N NaOH additions to elevate the precipitation potential. Experiments were conducted at 25 °C under HRTs of 50 and 30 min.

Analytical methods

Bed total sludge and biomass concentrations were estimated by MLSS and MLVSS, respectively. Nitrate was determined by the UV spectrophotometric screening method, nitrite by the colorimetric method, hardness and calcium by the EDTA titrametric method, and alkalinity by the titrametric method with end-point at pH 5.0. Magnesium was measured with an atomic absorption flame spectrophotometer (Z-6100, Hitachi) and total organic carbon (TOC) with carbon analyzer (TOC-5050A, Shimadzu). Precipitation potential was determined graphically [4]. Extracelluar polymers were extracted from granular sludge by autoclaving for 30 min followed by settling and then centrifuging the supernatant at 3000 g for 30 min. Protein content was determined by Lowery method, carbohydrates by the phenol-glucose method and nucleic acids by the UV absorption method [5].

RESULTS

Nitrate removal capacity

At the start of the study, the influent nitrate concentration was 20 mg/L. The VRL was increased stepwise and nitrate removal efficiencies were determined (Fig. 2). Complete denitrification occurred at influent nitrate concentration up to 75 mg/L (VLR, 2.1 g N/L/d) after which nitrate and nitrite started appearing in the effluent. The nitrate removal efficiency of the reactor then dropped to 73% and 45% at VLRs of 3.2 g N/L/d and 4.2 g N/L/d, respectively. The relationship between nitrate VRL and volumetric removal rate (VRL) is illustrated in Fig.3. This figure suggests VLR limits of 2.0 g-N/L/d for complete denitrification.

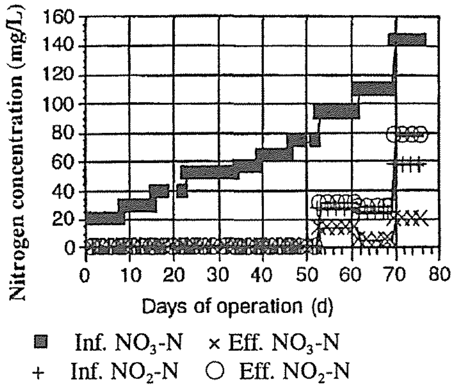


Fig. 2. Time courses of influent and effluent N during the study periods. (HRT=0.8h).

Physical characteristics of granular sludge

The granular sludge at the beginning of the study period was light cream in color with a yellowish tint and the average diameter was 2.7 mm. As the loading rate increased, the size of the granules decreased gradually down to 0.3 mm in diameter at a VLR of 2.7 g N/L/d and the color became whitish. Above a VLR of 2.7 g N/L/d, the granular sludge turned into a flocculent sludge with an increasing tendency to washout. The relationship between granule size and settling velocity, and the VLR are shown in Fig.4. As shown, granule size decreased with an increase in VLR whereas the settling velocity showed a parabolic trend.

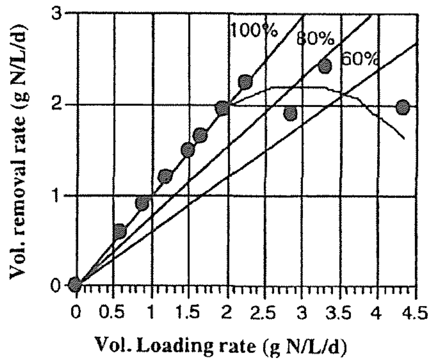


Fig.3. Relationship between volumetric loading and removal rates.

The settling velocity increased to a maximum of 2.1 cm/s at a VLR of 1.6 g N/L/d and then showed a slightly decreasing trend reaching 1.7 cm/s at 2.1 g N/L/d, after which it decreased sharply to 0.3 cm/s at 2.7 g N/L/d

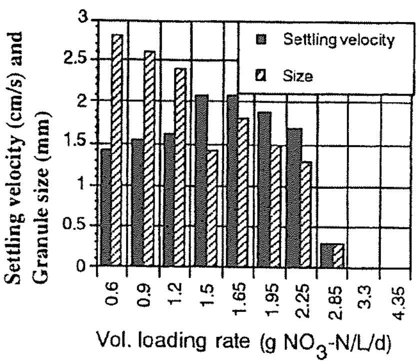


Fig. 4. Relationship between VLR to settling velocity and size of the granules.

Chemical characteristics of granular sludge

Fig. 5 shows the contents of calcium and magnesium of the sludge and the carbohydrate, protein and nucleic acid content of the extracellular polymers with respect to VLR. The content of all these components showed a decreasing trend with an increase in VLR. Calcium, magnesium, carbohydrate, protein and nucleic acid contents decreased from 24.2 % to 12.05 %, 0.7 to 0.22 %, 4.23 to 0.34 %, 3.2 to 0.09 % and 0.34 to 0.005 % of the MLSS, respectively.

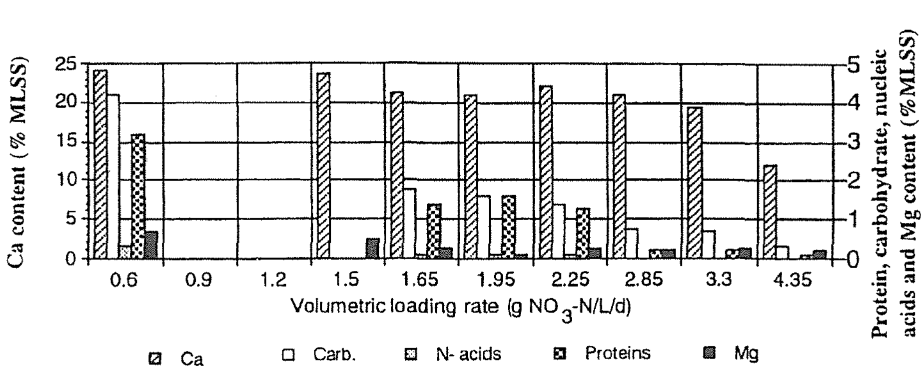


Fig. 5. Changes in Ca, Mg, protein, carbohydrates and nucleic acids with VLR.

Reactor performance

MLSS, VSS and the ash content of the granular sludge showed a parabolic trend with respect to VLR as shown in Figure 6. MLSS concentration increased from 19.6 g/L to a maximum of 50.2 g/L, VSS from 11.2 to 25 g/L, and ash content from 43 to 63 g/L. Results shown in Fig. 6 suggest a wide VLR range of 1.1 to 2.1 g N/L/d that would ensure high sludge MLSS, VSS and ash content. The effluent from the reactor was very clean with SS of less than 10 mg/L up to a VLR of 2.7 g N/L/d, above which the effluent SS increased to about 50 mg/L. At a VLR of 2.1 g N/L/d or lower when complete nitrate removal was realized, the average sludge yield (Y_{obs}) was 0.66 g VSS/g N removed.

Kinetic analysis of ethanol denitrification

Fig. 7 shows the relationship between nitrate concentration and specific denitrification rate for USB granular sludge and the other suspended denitrifying sludge. It is clear that denitrification reactions here follow the Michaelis-Menten equation:

$$v = v_m S / (K_m + S)$$

Where, v, v_m, K_m and S denote specific denitrification rate (mgN/gVSS/min), maximum specific denitrification rate (mgN/gVSS/min), saturation constant (mgN/L) and nitrate concentration ($mgNO_3$-N/L), respectively. Kinetic constants for two kinds of denitrifying sludges were determined by Line weaver Burk plots. It is clear that the value of saturation constant was lower for granular sludge defining a higher affinity for nitrate compared to that of suspended sludge.

USB treatment of actual groundwater polluted with nitrate

In order to understand the applicability of the USB process for the treatment of actual groundwater samples polluted with nitrate, continuous treatment experiments were conducted. Table 1 shows the water qualities of groundwater samples used in these experiments. Town A is well known for the production of watermelon in greenhouses. Owing to the intensive agricultural activities in

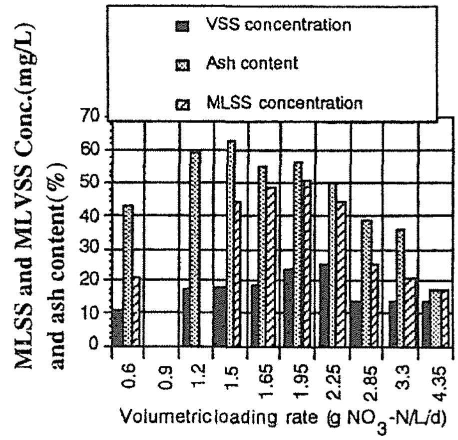


Fig. 6. Changes in MLSS, MLVSS and ash content with VLRs

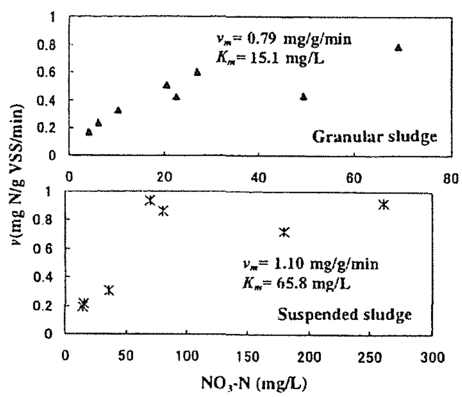


Fig.7. Relationship between nitrate concentration and specific denitrification rate

this town, serious groundwater pollution with nitrate occurred. Due to this problem, the Kumamoto Prefectural Office constructed a monitoring well in town A. The owner of the personal well in town B had been a pig farmer until 10 years ago. Owing to the inadequate disposal of pig manure, this well has continuously been polluted with nitrate. Nitrate concentration of these two well waters exceeds the drinking water quality standard of 10mg/L. Both Ca and alkalinity of these groundwater samples were higher than the average concentration for tap water in Kumamoto City. This might be due to the use of lime for neutralization of acidified agricultural soils. These water characteristics are well suited for the formation of denitrifying granular sludge in an USB treatment process.

Table 1. Water qualities of groundwater samples

Items	Town A	Town B
pH	7.23	6.92
NO_3-N (mg/L)	11.0	29.0
TOC (mg/L)	0.50	1.36
Alkalinity (mg/L)	63.0	102.0
Ca (mg/L)	24.0	44.1
Mg (mg/L)	3.39	1.58
K (mg/L)	9.24	8.96
Na (mg/L)	16.6	20.8
Fe (mg/L)	0.04	ND
PO_4^{3-} (mg/L)	0.06	0.10

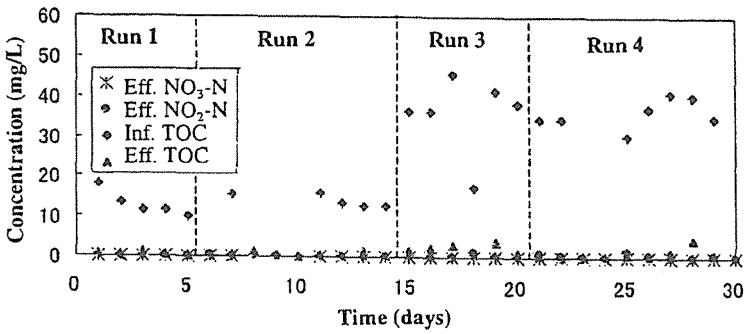


Fig. 8. Daily changes in water qualities during USB treatment.

Fig. 8 shows time course of continuous treatment of groundwater samples.

Table 2 shows the operational parameters for these experiments. Influent nitrate was completely removed by USB treatment even with a short HRT of 30 min. Owing to the high Ca and alkalinity, precipitation potential inside the USB reactor was sufficiently high without further addition of chemicals. The Ca content of granular sludge was increased during 14 days of continuous treatment and the granular sludge showed good settling properties. The nitrate concentration in town B was 2-times higher than that of town A. The high nitrate, though, was effectively removed by USB treatment even with a short HRT. Effluent TOC concentration was kept low throughout this phase of treatment due, in part, to the low influent C: N.

Table 2. Operational parameters for continuous experiments.

Items	Town A		Town B	
	Run 1	Run 2	Run 3	Run 4
HRT (min)	50	30	50	30
Inf. NO_3-N (mg/L)	11.0	11.0	29.0	29.0
Load. rate (g/L/d)	0.336	0.528	0.864	1.39
Inf. Ca (mg/L)	24.0	24.0	42.4	40.3
Eff. Ca (mg/L)	17.6	17.6	22.0	16.0
Inf. P.P	5.0	5.0	10.0	12.0
MLSS (g/L)	19.5	18.7	37.4	44.4
MLVSS (g/L)	8.1	8.0	14.3	17.2
Ash (%)	58.4	57.5	61.7	61.3
Bed height (cm)	22.0	25.5	23.2	24.0

P.P: precipitation potential

DISCUSSION

One object of this research work was to determine an optimal VLR in conjunction with sludge characteristics that would results in complete denitrifcation coupled with maximum sludge retention capability. Granular sludge demonstrated maximum retention capacity over a VLR range of 1.5 to 2.1 g N/L/d. Further, granules with a diameter of about 1.5 mm appear to have better retention characteristics than larger ones. From the comparison of treatment results, a VLR of about 2 g N/L/d appeared to be optimal under testing condition used here.

It appears that VLR is a major operational parameter in a USB reactor for selection of granule size, MLSS concentration and ash content of the sludge. The gradual reduction in calcium content of the sludge from 24.2 to 19.5% with increase in VLR from 0.6 to 3.2 g N/L/d does not appear to be causative factor for system failure. A pronounced drop in calcium content did not occur until well after system failure. However, the reduction of extracellular polymers in the sludge with an increase in VLR did appear to correlate with system failure.

The carbohydrate, protein, and nucleic acid contents of extracellular polymers were all decreased with an increase in VLR to 2.1 g N/L/d. The pronounced expression of extracellular polymers at low VLRs could be a response to environmental stress associated with low substrate levels giving some indication of the suitability of granular denitrifying sludge for groundwater treatment. The initial level of total extracellular polymers in the granular sludge was 7.8 %. This content appears to be lower than that of UASB sludge but compares well with that of activated sludge of municipal treatment plants.

These results for sludge retention correspond well with the optimal VLR of about 2 g-N/L/h determined for maximum nitrate removal. When the VLR was in excess of the suggested optimum, the granular sludge turned into a soft flock with a low bed MLSS concentration of about 16 g/L and had a high tendency to washout.

Groundwater samples tested in this study contained rather high concentration of Ca and alkalinity. Owing to this high concentration of Ca and alkalinity, granular denitrifying sludge with good settling properties was obtained. High denitrifying treatment performances were observed even a short HRT of 30 min. This indicates that the applicability of USB treatment process for nitrate removal of polluted groundwater in Kumamoto Prefecture.

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