Experimental Study on Denitrification Using Coated Electrode of Immobilized Denitrifying Bacteria[1]

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Objective   To develop a coated electrode of immobilized denitrificants and to evaluate the performance of a bioelectrochemical reactor to enhance and control denitrification.   **Methods**   Denitrifying bacteria were developed by batch incubation and immobilized with polyvinyl alcohol (PVA) on the surface of activated carbon fiber (ACF) to make a coated electrode. Then the coated electrode (cathode) and graphite electrode (anode) were transferred to the reactor to reduce nitrate. **Results**   After acclimated to the mixtrophic and autotrophic denitrification stages, the denitrifying bacteria could use hydrogen as an electron donor to reduce nitrate. When the initial nitrate concentration was 30.2 mg NO_3^--N/L, the denitrification efficiency was 57.3% at an applied electric current of 15 mA and a hydraulic retention time (HRT) of 12 hours. Correspondingly, the current density was 0.083 mA / cm^2. The nitrate removal rate of the reactor was 34.4 g NO_3^--N / m^3·d, and the surface area loading was 1.34 g NO_3^--N / m^2·d.   **Conclusion**   The coated electrode may keep high quantity of biomass, thus achieving a high denitrification rate. Denitrification efficiencies are related to HRT, current density, oxidation reduction potential (ORP), dissolved oxygen (DO), pH value, and temperature.

Key words: Bio-electrochemical reactor; Immobilized microorganism; Electrolysis; Coated electrode; Denitrification

INTRODUCTION

The public health standard for nitrate in drinking water is set at 10 mg NO_3^--N/L (0.7 mmol/L) or less due to its toxicity and health effects[1-2]. However, contamination above the standard in surface water and groundwater has been observed in many countries[3-4].

Biological denitrification is a mechanism by which denitrifying bacteria use nitrate as a terminal electron acceptor, leading to the production of nitrogen gas at the presence of suitable carbon and energy sources which may be organic or inorganic chemicals. Hydrogen is an ideal energy source for denitrifying bacteria[5]. A few of denitrification systems have been developed to treat water using hydrogen.

Dries *et al.*[6] tested a two-column system to reduce nitrate using polyurethane as a supporting medium and have obtained the denitrification rate of 0.5 kg N / m^3·d at 20℃. Sakakibara *et al.*[7] proposed a batch system with two interconnected reactors, cathodal and anodic. H_2 could be produced in the denitrification reactor by electrolyzing water to be treated. When the electrode is transferred to the reactor and connected to the power supply, the microorganisms immediately take up hydrogen generated at the cathode. In a follow-up study, Sakakibara *et al.*[8] used a single-reactor continuously consisting of a tubular cathode with a concentric carbon rod (the anode), and found that the biomass is immobilized with sodium alginate on the surface of the cathode. Approximately 10 mg N/L is removed at an applied electric current of 2.5 mA and a hydraulic retention time (HRT) of 9 hours. More recently, Kiss *et al.*[9] carried out a preliminary laboratory

comparison of two configurations of a bio-electrochemical continuous denitrification process, and found that a single reactor where both the generation of hydrogen and denitrification take place and a two-reactor system where water is enriched with hydrogen in an electrolysis cell (EC) prior to entering a packed-bed bioreactor (BR).

The bio-electrochemical denitrification reactions proposed to date are the electrolysis of water coupled with biological or enzymatic denitrification[10-14]. Most studies demonstrate the simplicity of operation and maintenance, i.e. an application and control of electric current to perform treatments. However, when the current density is higher than 0.03 mA/cm^2, the denitrification rates decrease. This is due to the fact that in the process of electrolysis excessive production of hydrogen might inhibit the activation of denitrifying bacteria, the so called "hydrogen inhibition"[7]. We suppose that increasing the specific surface area of cathode, or enriching the biomass of denitrifying bacteria on the surface cathode may be a solution of "hydrogen inhibition".

This study was to investigate the feasibility of a novel denitrifican coated electrode system, which can be applied to treatments of dilute solutions such as groundwater or surface water. The results in this study have proved that some key factors, such as the applied current density, HRT, oxidation-reduction potential (ORP) and temperature all influence the bio-electrochemical denitrification.

MATERIALS AND METHODS

Experimental apparatuses were fabricated and operated at room temperature of 20℃. The basic reactor was an electrolysis cell (EC), in which heterotrophic denitrifying bacteria were incubated and acclimated. Dilute solution was fed batchwise into the reactor. Measurements were made for electrolyte concentrations, electric potential between electrodes, electric current, pH value and ORP at different operating conditions.

Reagent

Activated carbon fiber (ACF), graphite, polyvinyl alcohol (PVA) were purchased from Chinese markets.

Preparing of Denitrifican Coated Electrode

ACF was washed and baked to enhance its adsorption capacity, before used as a physical support of coated electrode. ACF and graphite were fixed and supported by a plexiglass frame with an efficient surface area of 8.0 cm × 11.0 cm.

Fig. 1 is a schematic of the bioelectrochemical reactor with an efficient volume of 700 mL. Graphite electrodes acting as anodes and two coated electrodes as cathodes were placed in parallel. A cation-permeable membrane was used to separate the electrodes preventing O$_2$ generated at the anode from reaching the water to be treated while allowing transfer of H$^+$.
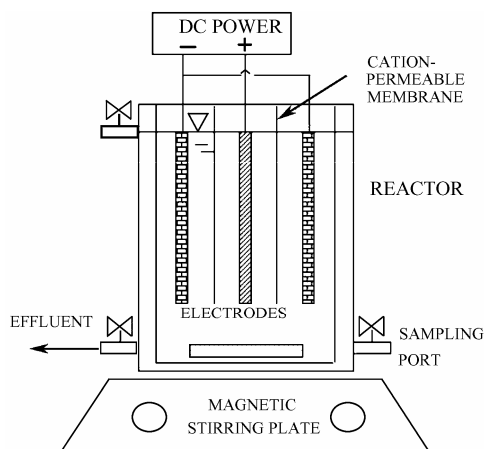

FIG. 1. Schematic of the coated electrode reactor.

After construction of the EC, the anode was removed from it. Biomass was collected from an upstream anaerobic sludge blanket[15] and inoculated into the EC to develop the biofilm on ACF. After seeding, the reactor was fed batch wise with a solution containing nitrate, phosphate, ethanol, nutrients, and vitamins to develop a denitrifying biofilm on the ACF surface. The development of denitrifying bacteria was monitored by measuring the decline of nitrate concentration in the reactor effluent and N$_2$ gas production, and by observing the development of a slime layer on the cathode surface. After several repetitions, a development of an ivory-colored biofilm became visible on the surface of the cathode.

A thin layer of denitrifican biomass (activated sludge) immobilized with polyvinyl alcohol (PVA) was then attached to the surface of cathode to make a coated electrode of denitrifying bacteria. Microorganism immobilization can enrich the quantity of denitrifying bacteria on the cathode significantly[16].

Acclimatation of Heterotrophic Denitrifying Bacteria

The electrodes were put in the EC. Coated electrodes were connected to the negative terminal of a DC power supply unit. Graphite electrode (anode) was placed between the two cathodes and connected to the positive terminal. The EC was fed

batch wise with a dilute solution of nitrate and buffer *etc*. The distilled water was purged with N_2 gas to strip out the dissolved oxygen prior to adding the chemicals.

As it could be assumed since most denitrifying bacteria on the cathodes were heterotrophs, which could neither utilize inorganic carbon nor hydrogen to reduce nitrate, they needed to be acclimatized before use. The acclimation could be divided into mixtrophic and autotrophic stages.

During the first stage, the feed solution was prepared by mixing 2.55 g $NaNO_3$ (substrate), 17.50 g KH_2PO_4, and 11.5 g Na_2HPO_4 (buffer) KNO_3, 0.24 g Na_2HPO_4, 0.36 g KH_2PO_4, 0.16 g methanol (0.1 mL), trace elements and distilled water (1000 mL). NaOH was added to prepare a feed solution having a pH of 7.0[17] (Fig. 2).
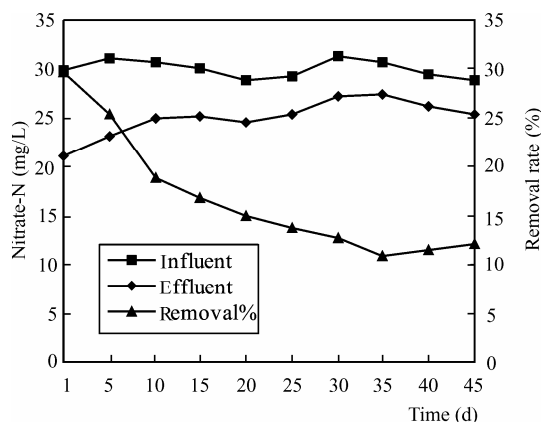

FIG. 2. The lst stage of acclimatation.

The area of the cathodes immersed in the solution of the EC was 176 cm^2. A current of 1 mA was passed for several days. Then the current was increased to 10 mA gradually. The low current level was used to prevent possible hydrogen inhibition in the electrochemical system. Samples (5 mL) were withdrawn from the reactor.

After the first stage of acclimation, a control test was run to confirm that denitrification caused by endogenous respiration did not occur. This control test was conducted under conditions similar to the first stage of acclimation but without passing a current. The second stage of acclimation was carried out after it was ascertained that denitrification due to endogenous respiration did not occur.

During the second stage, the feed solution was prepared by mixing 2.55 g $NaNO_3$ (substrate) and 2.25 g $NaHCO_3$ (buffer) in order to run the system without any nutrient. NaOH was added to prepare a feed solution having a pH of 8.0.

Optimum Denitrification Condition

To optimize the conditions of denitrification, a series of experiments were carried out in the presence of an initial nitrate concentration about 30 mg/L. The impacts of current density, dissolved oxygen (DO), oxidation-reduction potential (ORP), hydraulic retention time (HRT), and temperature on denitrification were observed.

Analysis Method

All the water quality indexes mentioned in the research were measured using Chinese national standard methods[18].

RESULTS

Process of Acclimatation

All the statistics of nitrate removal efficiencies could be categorized into 3 parts during the first stage of acclimation. Along with the reduction of carbon sources, denitrification efficiencies decreased within 10 days. From day 10 to day 35, the nitrate removal rates lessened, and bottomed out at 10.8% on day 35. During the rest 10 days of this stage, the nitrate removal rates leveled off.

Fig. 3 shows changes of nitrate concentration during the 2nd stage in the presence of current intensity of 10 mA. In this stage, the denitrification rates leaped within 50 days, and reached a plateau of about 37%.
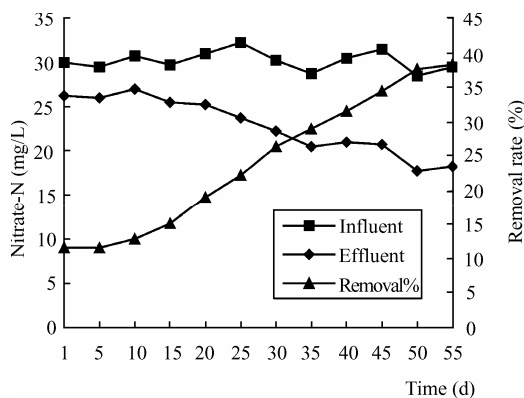

FIG. 3. The 2nd stage of acclimation.

Optimum Reaction Condition

When the current intensity changed from 3 mA to 30 mA, the denitrification efficiencies rose and fell sharply, reaching a peak of 57.3% at 15 mA (Fig. 4).

Correspondingly, about a threefold rise in the N_2 production and denitrification rate were observed. The initial nitrate concentration did not affect the rate of N_2. Under this condition, the current density was 0.0833 mA/cm^2，and the capacity loading and the surface area loading were 34.4 g NO$_3^-$-N/ m^3·d and 1.34 g NO$_3^-$-N/m^2·d, respectively.
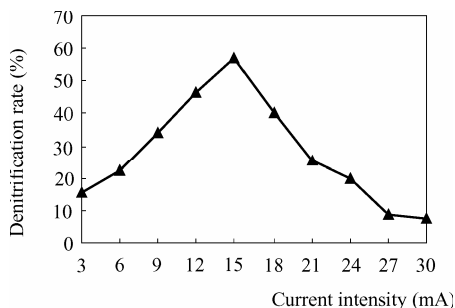

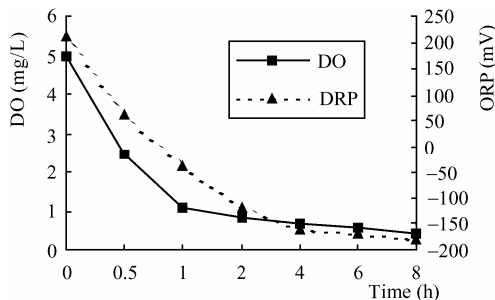FIG. 4. Impact of current intensity on denitrification.

FIG. 5. Changes of ORP and DO during denitrification at current intensity of 15 mA.
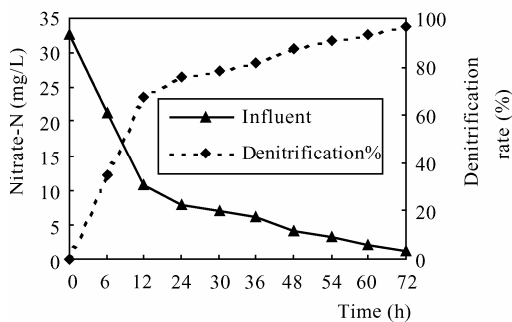

FIG. 6. Denitrification rate vs time.

HRT had a marked effect on the overall performance of the reactor. A study carried out at current intensity of 15 mA, and the initial nitrate concentration of 32.8 mg/L is presented in Fig. 6. The nitrate removal efficiencies rocketed during the first 12 hours. The reactor performed a very fast denitrification velocity of 1.83 mg NO$_3^-$-N/h, correspondingly, about 67.2% of the total nitrate was

reduced in this stage. There was a gentle increase of denitrification rates during the rest 60 hours.

Fig. 7 shows the changes of pH value vs time. The pH value of the reactor abated from 7.20 to 6.59 within 72 hours. A rising of the system temperature led to a medium growth of denitrification efficiencies correspondingly. When water temperature changed from 5℃ to 35℃, a threefold increase of actual denitrification rate was achieved (Fig. 8).
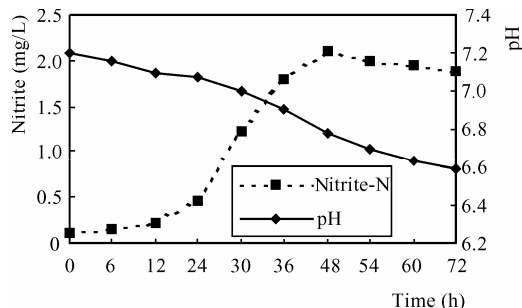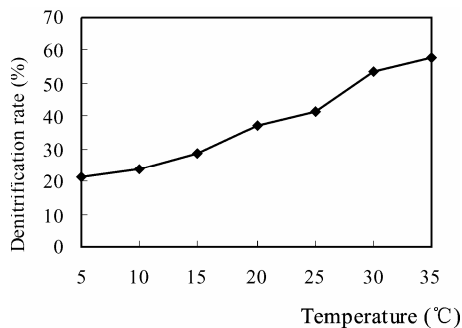

FIG. 7. Changes of nitrite and pH vs. time.

FIG. 8. Effect of temperature on denitification rate.

DISCUSSION

Process of Acclimatation

Heterotrophic denitrifying bacteria can not use hydrogen as an electron donor to reduce nitrate until they are acclimated. The process of acclimation can be divided into 2 stages, which takes about 100 days. Insufficient carbon sources may lead to changes of the metabolic properties of denitrifying bacteria, which are acclimated to use hydrogen as an electron donor to reduce nitrate gradually. The mechanism needs further study. Forty-five days later, denitrification rates rose moderately, suggesting an increasing number of denitrifying bacteria adapted to use hydrogen as an electron donor. From day 95 to day 100, a fairly stable removal efficiency of 38% was achieved, implying the end of acclimation.

Optimum Condition of Denitrification

Denitrification process is driven and controlled by electric current. It was reported that a current density of 0.03 mA/cm^2 is the threshold of "hydrogen inhibition" in bio-electrochemical denitrification reactor. In the present study, we observed the phenomenon of "hydrogen inhibition" with a much higher threshold of 0.0833 mA/cm^2. The surface area of coated electrode is about 176 cm^2, but the ACF might have a large specific surface area, about 1000 folds of its surface area, which enables the coated electrode to keep more denitrifying bacteria than conventional biofilm electrode. So it is reasonable to have a higher threshold of current density of hydrogen inhibition.

The presence of a large number of redox couples in anoxic environments makes it difficult to directly correlate the ORP to cellular metabolism. However, the ORP is an excellent indicator. In the coated electrode reactor system, DO and ORP decrease in 2 hours, suggesting that the system could offer an environment of anaerobic deoxidize. Hence, it is very easy to start denitrification in the reactor.

HRT affected the overall performance of the reactor. During the late 60 hours, the decreasing of average denitrification velocity might be due to the decrease of pH value and accumulation of nitrite, as shown in Fig. 7. Decreases of pH value might be due to excess H$^+$ produced by anode oxidation, which might inhibit the activation of nitrite reductase. So the reduction of NO$_2^-$ is a velocity-limiting step of denitrification.

Above all, the proposed bio-electrochemical reactor may perform an optimum efficiency of denitrification with a current intensity of 15 mA, HRT of 12 hours, and a relative higher temperature ranged from 5℃ to 35℃.

Advantages of Coated Electrode

Immobilization of bacteria has become a sophisticated technique for increasing the productivity of biochemical engineering process. PVA used for cell immobilization has been investigated intensively. The PVA-boric acid technique provides an easy and low-cost method for cell immobilization[19]. Activated sludge could be successfully immobilized without loss of biological activity due to the presence of extra cellular polymer in the sludge. In the present study, for the first time, PVA was used as a physical support for coated electrode, which enables the microorganisms to endure changes in cultivation conditions. A long-term experiment demonstrates that the biofilm is of high strength and durability.

Electrodes made of fibrous carbon or graphite have shown its advantages of better permeability to the treated solution over other electrode materials[20]. Besides, with the combination of great surface area of ACF and immobilization efficiency of PVA, the coated electrode can keep more denitrificants than average biofilm electrodes. Consequently, the coated electrode could overcome the "hydrogen inhibition" to a certain extent, and perform a high efficiency of denitrification.

CONCLUSION

The coated electrode reactor proposed here is very simple and considered feasible, especially for the treatment of low-strength nitrate solution. Total nitrate removal efficiency about 57% can be achieved at a current of 15 mA and HRT of 12 hours. This corresponds to a maximum nitrate removal rate of 1.34 g NO$_3^-$-N/m^2 of coated electrode surface area/day. Appreciable increases in the color of the effluent can be observed when the HRT is beyond 12 hours. The coated electrode could overcome the "hydrogen inhibition" to a certain extent, and perform a high efficiency of denitrification.

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