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Nitrate removal and extracellular polymeric substances of autohydrogenotrophic bacteria under various pH and hydrogen flow rates

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ABSTRACT

In recent years there has been an increasing interest in the use of autohydrogenotrophic bacteria to treat nitrate from wastewater. However, our knowledge about the characteristics of extracellular polymeric substances (EPS) releasing by these activities is not yet very advanced. This study aimed to investigate the change in EPS compositions under various pH values and hydrogen flow rates, taking into consideration nitrogen removal. Results showed that pH 7.5 and a hydrogen flow rate of 90 mL/min were the optimal operating conditions, resulting in 100% nitrogen removal after 6 hr of operation. Soluble and bound polysaccharides decreased, while bound proteins increased with increasing pH. Polysaccharides increased with increasing hydrogen flow rate. No significant change of bound proteins was observed at various hydrogen flow rates.

Introduction

Nitrate released by human activities, such as agriculture and industry enters groundwater through untreated discharge. Water containing high levels of nitrate adversely influences human health and other life forms. Specifically, methemoglobinemia in infants (Islam and Suidan, 1998) and gastric cancer in adults (Sunger and Bose, 2009) are considered to be caused by excessive doses of nitrate in drinking water. Furthermore, a residual amount of nitrate is capable of inducing eutrophication phenomenon which directly affects water quality and landscape of the natural water bodies. The removal of nitrate from water and wastewater is thus necessary to avoid its undesirable effects.

The reduction of nitrate into harmless nitrogen gas (*i.e.*, denitrification) by biological processes has become the most popular technology, compared to others such as coagulation, filtration and disinfection (Ghafari et al., 2008). Heterotrophic bacteria are mostly used in conventional biological systems in which these types of bacteria utilize organic matter to gain electrons for their metabolism. Nonetheless, heterotrophic bacteria do not show advantages when wastewater, mostly from industry, containing low concentration of organic matter is treated. Moreover, when using heterotrophic bacteria, the addition of an appropriate amount of organic carbon increases the operational cost of wastewater treatment plant (WWTP). To date then, a question of great interest in wastewater treatment is the presence of high concentrations of nitrate and low organic carbon in the influent of a WWTP. Autotrophic bacteria can alternatively be used in treatment of these types of wastewater. In some previous studies, autotrophic bacteria have been demonstrated to effectively remove nitrate from

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wastewater by using sulfur-limestone (Zhou et al., 2011) or hydrogen gas (Mousavi et al., 2014; Xia et al., 2010; Visvanathan et al., 2008) as an electron donor, and inorganic carbon such as CO_2 or HCO_3^- as carbon sources. Autohydrogenotrophic bacteria, a type of autotrophic bacteria which utilizes hydrogen gas as an electron donor, have been widely used to treat nitrate from ground water and wastewater in recent years. In mixed cultures, there are many types of bacteria which play different roles in sludge suspension. Ghafari et al. (2009a) showed that the denitrification process was carried out by "nitrate respiring" bacteria for the reduction of nitrate to nitrite and by "true denitrifying" bacteria for the reduction of nitrite to nitrogen gas. However, Chang et al. (1999) proposed that nitrate and nitrite reductase of *Alcaligenes eutrophus* were responsible for the reduction of nitrate to nitrite and nitrite to nitrogen gas. The denitrification reactions are given as below (Kurt et al., 1987):

$$NO_3^- + H_2 \rightarrow NO_2^- + H_2O \tag{1}$$

$$NO_2^- + 1.5H_2 + H^+ \rightarrow 0.5N_2 + 2H_2O \tag{2}$$

The overall reaction is:

$$NO_3^- + 2.5H_2 + H^+ \rightarrow 0.5N_2 + 3H_2O \tag{3}$$

In order to culture autohydrogenotrophic bacteria, various types of reactor has been proposed, such as a microporous membrane bioreactor (Mansell and Schroeder, 2002; Visvanathan et al., 2008), a sequencing batch reactor (Mousavi et al., 2014; Rezania et al., 2005), and a biofilm-electrode reactor (Chen et al., 2014). High nitrate removal rate (up to 100%) has been reported by many researchers (Ghafari et al., 2009b; Mansell and Schroeder, 2002; Vasiliadou et al., 2006). From another perspective, operational parameters, such as pH (Ghafari et al., 2010; Lee and Rittmann, 2003), temperature (Chen et al., 2014; Rezania et al., 2005), hydrogen flow rate (Khanitchaidecha and Kazama, 2012), or inorganic carbon source (Ghafari et al., 2009a) have gained much attention because of their direct effect on the performance of autohydrogenotrophic bacteria. Among them, pH and hydrogen flow rate are few of the important factors which directly affect nitrogen removal and nitrite accumulation. Lee and Rittmann (2003) showed that the optimal range of pH for autohydrogenotrophic bacteria was between 7.7 and 8.6, in which the maximum removal rate of nitrate was observed at pH 8.4. The accumulation of nitrite was dramatically increased at pH higher than 8.6. Rezania et al. (2005) reported that the optimum pH for nitrate and nitrite reduction was dependent on temperature (pH 9.5 at 25 ± 1°C and pH 8.5 at 12 ± 1°C). Another author suggested that appropriate pH values fell in range 6.0–7.0 (Chen et al., 2014). Glass and Silverstein (1998) found that no significant change of nitrate concentration was observed at pH values lower than 7.0 when carrying out experiments with heterotrophic denitrifying bacteria. Hydrogen gas is essential in controlling autohydrogenotrophic bacteria; its low solubility in aqueous solution (1.6 mg/L at 20°C), however, prevents the widespread use of this type of bacteria. Chang et al. (1999) found that nitrate reductase of *Alcaligenes eutrophus* was inhibited under low dissolve hydrogen concentration (<0.1 mg/L), while it was below 0.2 mg/L for nitrite reductase. Thus, an appropriate hydrogen flow rate and pH value must of necessity be considered in the system.

The bacteria in a sludge suspension are present not only in free form, but also as aggregates which are formed by many different types of microbes and materials such as extracellular polymeric substances (EPS). EPS are metabolic products excreted from microbial activities which play an important role in forming microbial aggregates. An EPS matrix has a significant influence on the morphology, structure, physicochemical and biological properties, as well as on the adhesion and cohesion phenomenon of microbial aggregates (Tsuneda et al., 2003). EPS may also protect cells from harsh living conditions, and even supply organic carbon as food for heterotrophic bacteria under starvation conditions (Liu and Fang, 2002; Zhang and Bishop, 2003). Furthermore, EPS is found to be an important factor in the flocculation, settling and dewatering in sludge suspensions (Liu and Fang, 2003). The main components of EPS are proteins and polysaccharides, which contribute up to 89% of total EPS compositions (Tsuneda et al., 2003). Hou et al. (2015) found that the concentration of polysaccharides in loosely bound extracellular polymeric substances increased to reduce the effect of CuO nanoparticles on bacteria. Although much research has been done on the nitrate treatment capability of autohydrogenotrophic bacteria, the effect of pH and hydrogen flow rates on the releasing of EPS from the activities of this type of bacteria has not been investigated to any extent. This study set out to investigate the change in compositions of EPS and to observe nitrate removal, nitrite accumulation and nitrogen removal under various pH values and hydrogen flow rates.

1. Materials and methods

1.1. *Experimental setup*

Activated sludge containing denitrification bacteria was collected from a sequencing batch reactor in Hsinchu, Taiwan. It was pre-treated to remove large particles and then inoculated in a specially designed 5-L reactor for culturing autohydrogenotrophic bacteria as can be seen from Appendix A Fig. S1. Herein, a continuous flow bioreactor equipped with a clarifier was set up. Synthetic wastewater was prepared with an initial nitrate concentration of 87.14 ± 6.07 mg N/L as sole electron acceptor, sodium bicarbonate as carbon source with C/N 0.5, $Na_2HPO_4{\cdot}12H_2O$ with N/P 5 and 1 mL/L trace solution with the following compositions: $MgSO_4{\cdot}7H_2O$ 10 g; $ZnSO_4{\cdot}7H_2O$ 2.2 g; $CaCl_2{\cdot}2H_2O$ 7.3 g; $MnCl_2{\cdot}4H_2O$ 2.5 g; $FeSO_4{\cdot}H_2O$ 5 g; $CuSO_4{\cdot}5H_2O$ 0.2 g; $CoCl_2{\cdot}6H_2O$ 0.5 g; KI 0.166 g; H_3BO_3 0.124 g; $Na_2MoO_4{\cdot}2H_2O$ 0.08 g, in 1 L deionized water. The reactor was continuously operated by a peristaltic pump (MasterFlex L/S, model 7518-10) with a flow rate of 5 mL/min and was mixed by a magnetic stirrer (Corning PC-310). The speed of the magnetic stirrer was adjusted so as to completely mix the sludge suspension. The concentration of mixed liquor volatile suspended solid (MLVSS) was 2372 ± 703 mg/L. Hydrogen gas was supplied as electron donor through a bubble stone with a flow rate of 50 mL/min. The pH was controlled by automatically adding phosphoric acid to keep at desired values. Throughout the experiment, dissolved oxygen (DO) was kept at ≤0.5 mg/L to ensure anoxic conditions. Hydraulic retention time was