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An anaerobic-aerobic sequential batch process with simultaneous methanogenesis and short-cut denitrification for the treatment of marine biofoulings

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ABSTRACT

Although combination of denitrification and methanogenesis for wastewater treatment has been widely investigated, an application of this technology to solid waste treatment has been rarely studied. This study investigated an anaerobic-aerobic batch system with simultaneous denitrification-methanogenesis as an effective treatment for marine biofoulings, which is a major source of intermittently discharged organic solid wastes. Preliminary NO_2^- -exposed sludge was inoculated to achieve stable methanogenesis process without NO_2^- inhibition. Both high NH_4^+ -N removal of 99.5% and high NO_2^- -N accumulation of 96.4% were achieved on average during the nitritation step. Sufficient CH_4 recovery of 101 L- CH_4 kg-COD $^{-1}$ was achieved, indicating that the use of NO_2^- -exposed sludge is effective to avoid NO_2^- inhibition on methanogenesis. Methanogenesis was the main COD utilization pathway when the substrate solubilization occurred actively, while denitrification was the main when solubilization was limited because of substrate shortage. The results showed a high COD removal efficiency of 96.0% and a relatively low nitrogen removal efficiency of 64.4%. Fitting equations were developed to optimize the effluent exchange ratio. The estimated results showed that the increase of effluent exchange ratio during the active solubilization period increased the nitrogen removal efficiency but decreased CH_4 content in biogas. An appropriate effluent exchange ratio with high anaerobic effluent quality below approximately 120 mg-N L $^{-1}$ as well as sufficient CH_4 gas quality which can be used as fuel for gas engine generator was achieved by daily effluent exchange of 80% during the first week and 5% during the subsequent 8 days.

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1. Introduction

The amount of intermittently discharged organic wastes has increased in recent years and the establishment of an appropriate treatment method is becoming a worldwide social problem. For instance, recent global warming has increased the spread of animal carcass waste (Patz et al., 2005; Ayres et al., 2009). The amount of suddenly beached seaweed at seashores has also increased and almost all of these are disposed of as organic waste during beach clearing (Smetacek and Zingone, 2013). Marine biofouling organisms such as mussels and barnacles, which colonize coastal marine structures (i.e. ship hulls and water intakes of coastal nuclear/thermal power plants), are also intermittently discharged wastes (Fitridge et al., 2012). Intermittently discharged organic wastes

are commonly treated by transient treatment methods including direct incineration and/or landfilling because rapid management is prioritized (Kikuchi and Furuta, 2001; Ye et al., 2011). However, these treatments often cause a high environmental burden; therefore, development of an alternative treatment method is required.

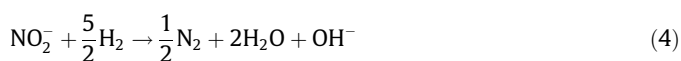
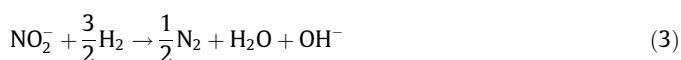
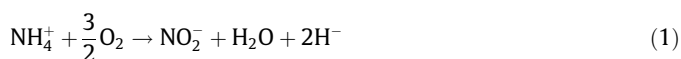
Anaerobic digestion is considered an appropriate technology to treat intermittently discharge wastes because of its relatively low energy consumption and its potential for producing calorific biogas as a form of CH_4 (Mata-Alvarez et al., 2000). The effluent from anaerobic digesters contains high nitrogen content (mainly NH_4^+) and requires additional treatments such as biological nitrification-denitrification processes (Penetra et al., 1999). As a matter of fact, these post-treatments often cancel out the benefits of anaerobic digestion because nitrification needs mechanical aeration and denitrification requires organic additives such as methanol to conduct their reactions (Takata et al., 2013). In particular, such multi-step treatments are not suitable for intermittently discharged wastes because the facility does not work continually because of the nature of the batch wastes discharge. Thus, more

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compact and simplified batch treatment method should be developed.

During the last two decades, simultaneous denitrification and methanogenesis in a single anaerobic reactor has been studied by different researchers as a simplified biological process (Akunna et al., 1992; Bernet et al., 2000; Andalib et al., 2011). This process has the following advantages over the conventional separated biological processes: (1) reduction of initial facility cost because a multifunctioning single reactor is required and (2) reduction of operational cost because organic carbon from target wastes can be used as an electron donor for denitrification instead of a costly organic chemical. Recently, the combined process of simultaneous denitrification and methanogenesis with nitrification has been introduced to remove nitrogen via NO_2^- (Sun et al., 2015). Compared with the common nitrogen removal pathway via NO_3^- , this process can theoretically save up to 25% of the oxygen consumption for oxidizing NH_4^+ as well as 40% of the carbon source for denitrifying nitrogen oxide (Peng and Zhu, 2006; Pambrun et al., 2008). The following equations showed the stoichiometric reaction of NH_4^+ oxidation to NO_3^- (Eqs. (1) and (2)) and nitrogen oxides (NO_2^- and NO_3^-) reductions to N_2 gas (Eqs. (3) and (4)):



Although several studies showed the occurrence of denitrification and methanogenesis successfully in continuous feeding process (Akunna et al., 1992; Sun et al., 2015), toxic effects of NO_2^- to many bacterial activities, including denitrifying bacteria itself and methanogens, have been reported by researches concerning batch feeding experiments (Zumft, 1993; Tugtas and Pavlostathis, 2007). Many literatures showed NO_2^- has a stronger inhibitory effect especially on methanogenesis activity than NO_3^- (Clarens et al., 1998; Kluber and Conrad, 1998; Banihani et al., 2009). For instance, acetoclastic methanogenesis by *Methanosarcina mazei* was severely inhibited by only 2.5 mg L^{-1} NO_2^- -N while only partially inhibited by $200\text{--}1000 \text{ mg L}^{-1}$ NO_3^- -N (Clarens et al., 1998). Banihani et al. (2009) reported that the inhibition imparted by NO_3^- was not directly due to NO_3^- itself, but instead due to intermediates such as NO_2^- produced during the denitrification process. As a result, the methanogenesis was severely inhibited by the presence of low concentration of NO_2^- -N ranging from 7.6 to 10.2 mg L^{-1} . A previous study demonstrated that the use of preliminarily NO_2^- -exposed anaerobic sludge enhanced NO_2^- tolerance of methanogenesis and stable methanogenesis was achieved under the relatively high NO_2^- -N concentration of 1000 mg-N L^{-1} (Akizuki et al., 2015). This study was conducted in batch assays using a synthetic nitrogen source; thus, further study should be conducted in anaerobic-aerobic sequential reactors to clarify the actual applicability for treating intermittently discharged organic wastes.

The aim of this study was to evaluate the carbon and nitrogen removal performance of combining denitrification and methanogenesis in single anaerobic reactor with nitrification reactor under sequential batch operation by inoculating NO_2^- -exposed sludge to an anaerobic reactor. In addition, fitting equations were developed using experimental results to demonstrate an appropriate water effluent exchange ratio that can achieve high effluent quality which meets the national effluent standards applied in Japan as

well as high biogas quality which can be used as fuel for gas engine generator.

2. Materials and methods

2.1. Substrate and seed sludges

Blue mussels ranging in size range of 1–8 cm were collected from marine structures such as the concrete walls of piers in Otsuchi Bay of Iwate Prefecture, Japan. The shells of the blue mussels were cleaned and transported to the laboratory in the same way as Akizuki et al. (2013). The blue mussels with their shells were used as the sole substrate. The total solid (TS), total volatile solid (TVS), total chemical oxygen demand (TCOD) and organic nitrogen contents of the shell-fish meats were 155, 137, 248 and $15.2 \text{ g kg-wet}^{-1}$, respectively.

Mesophilic anaerobic sludge was collected from the full-scale sewage sludge treatment plant of Hokubu Sludge Treatment Center, Kanagawa prefecture in Japan. The denitrifying and nitrifying sludges were collected from the full-scale anaerobic-anoxic-oxic (A_2O) treatment plant of Holubudaini Wastewater Treatment Center, Kanagawa prefecture in Japan. The anaerobic and denitrifying sludges were preliminarily exposed to NO_2^- according to Akizuki et al. (2015) and the exposed sludges were used as seed sludge for the anaerobic reactor.

Nitrification can be achieved through the reduction of the activity of nitrite oxidizing bacteria (NOB) without affecting ammonia oxidizing bacteria (AOB). The nitrifying sludge was acclimated to for approximately one month under following conditions: $\text{DO} = 1.0 \pm 0.1 \text{ mg L}^{-1}$, $\text{pH} = 7.8 \pm 0.1$, temperature = $37 \pm 1 \text{ }^\circ\text{C}$ and NH_4^+ -N loading rate = $1.0 \text{ g-N L}^{-1} \text{ day}^{-1}$, according to the procedure reported by Ruiz et al. (2003) and Jianlong and Ning (2004). At the end of the acclimation period, high NO_2^- -N accumulation efficiencies of almost 100% were achieved. The acclimated nitrifying sludge was used as seed sludge for the aerobic reactor.

2.2. Experimental unit

An anaerobic-aerobic sequential batch system was used, as described in Akizuki et al. (2016): (1) an anaerobic reactor with an effective volume of 15 L; (2) an aerobic reactor with an effective volume of 4.5 L; (3) a centrifugal separator (Model-6000, Kubota) for solid-liquid separation; and (4) a process controller (EPC-2000, Eyela) connected to a PC (Fig. 1A). The anaerobic reactor had an oxygen redox potential (ORP) sensor and the ORP value was monitored continuously by the process controller. The aerobic reactor had pH and DO sensors and the values were controlled by the process controller; maintaining the pH at 7.8 ± 0.1 by adding 1 M NaHCO_3 and maintaining the DO at $1.0 \pm 0.1 \text{ mg L}^{-1}$ using an air pump.

2.3. Experimental set-up and operating conditions

The experimental set-up consisted of: (i) addition of 3.0 kg of wet blue mussels with their shells without grinding into the anaerobic reactor, leading to initial total COD (TCOD) and total nitrogen (TN) concentrations of 6800 mg L^{-1} and 1020 mg-N L^{-1} , respectively, and an initial COD/N ratio of 6.67; (ii) addition of 7.8 L of NO_2^- -exposed anaerobic sludge into the anaerobic reactor; (iii) addition of 3.0 L of acclimated nitrifying sludge with high nitrification performance into the aerobic reactor; (iv) addition of deionized water into the reactor (4.5 L for the anaerobic reactor and 1.5 L for the aerobic reactor); and (v) displacement of the anaerobic reactor's headspace air (head space volume: 3.1 L) with Ar gas to create anaerobic conditions. Next, an anaerobic-aerobic sequential

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