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Spatial changes in carbon and nitrogen stable isotope ratios of sludge and associated organisms in a biological sewage treatment system

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ABSTRACT

Carbon and nitrogen stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) have been utilized as powerful tools for tracing energy or material flows within food webs in a range of environmental studies. However, the techniques have rarely been applied to the study of biological wastewater treatment technologies. We report on the spatial changes in $\delta^{13}C$ and $\delta^{15}N$ in sludge and its associated biotic community in a wastewater treatment system. This system consisted of an upflow anaerobic sludge blanket (UASB) and a down-flow hanging sponge (DHS) which is a novel type of trickling filter. The results showed clear spatial changes in the $\delta^{13}C$ and $\delta^{15}N$ of suspended solids (SS), retained sludge, and macrofauna (oligochaetes and fly larvae) in the system. The $\delta^{13}C$ and $\delta^{15}N$ was used as a natural tracer to determine the SS dynamic throughout the system. The results imply that SS in the DHS effluent was mainly eluted from the retained sludge in the lower section of the DHS reactor. The $\delta^{15}N$ of the retained sludge in the DHS reactor increased drastically from the inlet towards to the outlet, from −0.7‰ to 10.3‰. This phenomenon may be attributed to nitrogen conversion processes (i.e. nitrification and denitrification). The $\delta^{15}N$ of oligochaetes also increased from the inlet to the outlet, which corresponded well to that of the retained sludge. Thus, the $\delta^{15}N$ of the oligochaetes might simply mirror the $\delta^{15}N$ of the retained sludge. On the other hand, the $\delta^{13}C$ and $\delta^{15}N$ of sympatric fly larvae differed from those of the oligochaetes sampled, indicating dietary differences between the taxa. Therefore $\delta^{13}C$ and $\delta^{15}N$ reflected both treatment and dietary characteristics. We concluded that $\delta^{13}C$ and $\delta^{15}N$ values are potentially useful as alternative indicators for investigating microbial ecosystems and treatment characteristics of biological wastewater treatment systems.

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1. Introduction

Biological wastewater treatment is one of the most important processes worldwide for the treatment of municipal and industrial wastewater. The efficiency of the treatment depends critically upon the composition and activity of its microbial ecosystem, which includes a wide diversity of organisms such as bacteria, protozoa, and metazoa, across several trophic levels (Metcalf and Eddy, 2003). These organisms play an important role in the removal of organic substances and production/degradation of excess sludge within the food web. Therefore, understanding the ecological functional roles of organisms (e.g. predator–prey relationships) and mass or energy flows through the food web are fundamental to developing effective biological treatment processes.

The use of carbon and nitrogen stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) has proven to be a powerful tool for estimating the functional role of organisms and for tracing mass and energy flows through food webs (Post, 2002; Post et al., 2000; Woodward and Hildrew, 2002), and the number of studies using such methods has increased greatly in recent years (Boecklen et al., 2011; Post, 2002; Post et al., 2000; Woodward and Hildrew, 2002). Application of the technique relies on the empirical rule that the trophic enrichment of carbon isotopes is small, generally within 1‰ (DeNiro and Epstein, 1978). By contrast, the increase in $\delta^{15}N$ over one trophic level is usually positive and much greater, averaging 3.4‰ (Minagawa and Wada, 1984; Post, 2002). Therefore, $\delta^{13}C$ is an indicator of the ultimate source of organismal carbon, whereas $\delta^{15}N$ can be used to estimate trophic position (Boecklen et al., 2011; DeNiro et al., 1981; Minagawa and Wada, 1984; Peterson and Fry, 1987; Post, 2002). Moreover, stable isotope analysis has been applied to the identification of pollutant sources in aquatic food webs (Gaston and Suthers, 2004; Maksymowska et al., 2000) and in the inference of ecological processes (Granger et al., 2006; Mariotti et al., 1981).

Despite recognition of the method's use in research, there have been few applications to the study of naturally abundant stable isotope ratios in biological wastewater treatment technologies. Moreover, basic information such as the spatial and temporal distribution of stable isotope ratios within the suspended solids (SS), sludge, and macrofauna in biological wastewater treatment systems is limited. Kanazawa and Urushigawa (2007) determined the temporal distribution of $\delta^{13}C$ and $\delta^{15}N$ of the sludge in an oxidation ditch over a 3 year period. However, they focused on the relationship between nitrogen removal rate and the $\delta^{15}N$ in the solid phase sludge to develop a model that could be applied to natural systems. Stable isotope analysis was applied to the sewage sludge vermifiltration treatment system to estimate the trophic relationships of organisms such as earthworms within the food web (Xing et al., 2012). Within studies of biological wastewater treatment, stable isotope analysis has mainly been used to track the migration of selected substrates into various cellular components (Osaka et al., 2006).

Isotopic fractionation within a system is a prerequisite to allow the use of stable isotope ratios to investigate the functional role of organisms and mass flow in biological treatment processes. Since the isotopic fractionation of $\delta^{15}N$ of the substrate and products occurs during nitrogen conversion processes (i.e. nitrification and denitrification) (Granger et al., 2006; Koba et al., 1997; Mariotti et al., 1981, 1988), it may be a feature of biological treatment processes in which nitrogen conversion proceeds vigorously.

In this study, we used a wastewater treatment system consisting of an upflow anaerobic sludge blanket (UASB) as anaerobic treatment and a down-flow hanging sponge (DHS) as aerobic treatment. The DHS reactor is a novel trickling filter using a sponge media as the sludge carrier in which bacteria and macrofauna are present (Kubota et al., 2014; Onodera et al., 2013). The DHS reactor in combination with the UASB has been verified as a reliable and efficient system for industrial and municipal wastewater treatment for over a decade (Machdar et al., 2000; Onodera et al., 2014; Tandukar et al., 2007). Because of the plug flow regime, the characteristics of the retained sludge and microbial community structure vary throughout the reactor height (Kubota et al., 2014; Onodera et al., 2013), making the DHS reactor a suitable model for application of stable isotope analysis. The study aimed to reveal the spatial changes in stable isotope ratios within municipal sewage treatment systems. The SS in the effluent, retained sludge, and organisms were determined. The spatial gradient of the stable isotope ratios of retained sludge along the waterstream was observed. We also analyzed the relationship between the distribution of the stable isotope ratios and the treatment characteristics of the systems.

2. Materials and methods

2.1. *Configuration and operational conditions of the sewage treatment system*

A biological treatment system consisting of a UASB reactor pre-treatment unit and a DHS reactor post-treatment unit (UASB + DHS system), fed with municipal sewage, was operated stably over 2 years (start up: 3 May 2010) under ambient temperature conditions at a municipal sewage treatment plant at Nagaoka, Japan (Fig. 1). The volumes of the UASB and DHS reactors (sponge media) were 1148 L and 454 L, respectively. The DHS reactor consisted of 10 segments and was filled with sponge media. The sponge media consisted of polyurethane sponge cubes (33 mm each) and a cylindrical plastic net ring (φ33 mm × 33 mm). The sponge occupancy ratio was 53%. The void ratio was more than 97% and the average pore size of the sponge was 0.63 mm. A 32.5 L settler was used after treatment by the DHS reactor. The UASB reactor was fed with the settled sewage by a peristaltic pump. The UASB effluent was then fed to the DHS reactor and flowed down through the reactor under gravity. The hydraulic retention times (HRT) of the UASB and DHS reactors were 8.0 h and 3.2 h, respectively. The upflow velocity of the UASB reactor was 0.6 m/h. The DHS reactor was operated without sponge replacement or sludge washing throughout each experimental period. Details of the process configuration have been reported previously (Onodera et al., 2013; Takahashi et al., 2011).