Available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/watres

Mixed carbon sources for nitrate reduction in activated sludge-identification of bacteria and process activity studies

M. Hagman[a,*], J.L. Nielsen[b], P.H. Nielsen[b], J. la C. Jansen[a]

[a]Water and Environmental Engineering, Department of Chemical Engineering, Lund University, P.O. Box 124, SE-221 00 Lund, Sweden
[b]Department of Biotechnology, Chemistry and Environmental Engineering, Aalborg University, Sohngaardsholmsvej 57, DK-9000 Aalborg, Denmark

ARTICLE INFO

Article history:
Received 13 August 2007
Received in revised form
23 October 2007
Accepted 24 October 2007
Available online 7 November 2007

Keywords:
Denitrification
Carbon source
Microautoradiography
Fluorescence in situ hybridisation
Nitrate uptake rate
Activated sludge

ABSTRACT

Mixtures of methanol and acetate as carbon source were investigated in order to determine their capacity to enhance denitrification and for analysis of the microbial composition and carbon degradation activity in activated sludge from wastewater treatment plants. Laboratory batch reactors at 20 °C were used for nitrate uptake rate (NUR) measurements in order to investigate the anoxic activity, while single and mixed carbon substrates were added to activated sludge. Microautoradiography (MAR) in combination with fluorescence in situ hybridisation (FISH) were applied for microbial analysis during exposure to different carbon sources. The NUR increased with additions of a mixture of acetate and methanol compared with additions of a single carbon source. MAR–FISH measurements demonstrated that the probe-defined group of *Azoarcus* was the main group of bacteria utilising acetate and the only active group utilising methanol under anoxic conditions. The present study indicated an improved denitrification potential by additions of a mixed carbon source compared with commonly used single-carbon additions. It is also established that *Azoarcus* bacteria are involved in the degradation of both acetate and methanol in the anoxic activated sludge.

1. Introduction

The denitrification process involves the conversion of nitrate or nitrite to nitrous oxide or molecular nitrogen. Heterotrophic denitrification requires the presence of a biodegradable carbon source, which often are present in the incoming wastewater to a municipal wastewater treatment plant (WWTP). However, occasionally addition of an external carbon source is needed for satisfactory nitrogen removal. Since this increases the running costs it is of great interest to reduce these additions as much as possible by optimising the efficiency of the substrate towards the actual microbial consortia. As a result, a more efficient process will lead to increased potential for extended capacity in the treatment plant, also during periods with peak loadings.

The population of heterotrophic biomass in activated sludge is influenced by many factors. Most important are the composition of the electron acceptor and donor in the wastewater, the design and the operation of the treatment plant. The impact on the microbial functional groups might be especially significant when the operation includes additions of an external carbon source since less variable substrate composition may lead to less complex microbial diversity.

During the last 20 years many different carbon sources have been tested for denitrification (e.g. Grabinska-Loniewska et al., 1985; Werner and Kayser, 1991; Christensson et al.,

*Corresponding author. Tel.: +46 46 2228998; fax: +46 46 2224526.
E-mail address: marinette.hagman@chemeng.lth.se (M. Hagman).
0043-1354/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.watres.2007.10.034

1994; Eioroa et al., 2004) and most often only single sources are applied. At WWTPs, especially methanol and ethanol are commonly used since they often turn out to be financially favourable. Few studies with addition of mixed industrial wastewater have been performed (Sage et al., 2006) but the detailed understanding of the microbiology and the potential increased process efficiency is unknown.

Denitrification ability is known from many bacterial species of distinct phylogenetic origin, but little is known about the identity of abundant denitrifiers in activated sludge. Several studies have been dealing with enrichment, isolation and cultivation of denitrifiers from activated sludge, but these isolates do not necessarily represent the dominant and important denitrifiers actually present in activated sludge. Culture-independent techniques have shown that the dominant denitrifying populations in activated sludge belong to the genera of *Azoarcus*, *Thaurea*, *Aquaspirillum*, *Zoogloea*, *Rhodocyclus*, and possibly also *Dechloromonas*, *Rhodobacter* (Osaka et al., 2006; Thomsen et al., 2004, 2007).

In the present study, the nitrate uptake rates (NURs) for different single and mixed carbon sources were measured and compared with microautoradiography (MAR) in combination with fluorescence in situ hybridisation (FISH) measurements. The purpose was to identify the biomass involved in the substrate degradation in anoxic environment. By combined use of MAR and FISH it is possible to detect cells that take up and utilise a single radioactively labelled substrate, and subsequently determine cell identity using a wide range of already published fluorescently labelled oligonucleotide probes. Substrate preferences by the involved microbial consortia can be found, and thereby allow prediction of the potential of addition of a single or a mixed carbon source to increase the denitrification capabilities.

2. Material and method

2.1. Activated sludge sampling

Activated sludge was collected from three full-scale nitrogen-removing WWTPs in Sweden: Källby WWTP in Lund; Sjölunda WWTP in Malmö and Ryaverket WWTP in Gothenburg. At Källby WWTP and at Ryaverket WWTP the sludge was collected from the aeration basin and at Sjölunda WWTP the sludge was collected from the flotation tank, which is located after the denitrifying Kaldnes Moving Bed^{TM} reactor. At Källby, no external carbon is used. At Ryaverket, nitrogen removal is performed in a pre-denitrifying system with denitrification in a high-loaded activated sludge plant and nitrification in a trickling filter. Ethanol is added in the activated sludge system in order to compensate for insufficient carbon in the raw wastewater. At Sjölunda methanol is added for post-denitrification in a Kaldnes Moving Bed^{TM} reactor.

2.2. Nitrate uptake rate measurements

NUR measurements were in general performed as described in Kristensen et al. (1992). The experiments were performed in 1 l batch reactors at 20 °C; nitrate was added to a start concentration in the sludge between 25 and 40 mg nitrate-N/l. In order to avoid limitations of essential nutrients, $(NH_4)_2SO_4$ (0.0472 g/l) and K_2HPO_4 (0.0283 g/l) were added. In each case, the carbon source (200 mg/l) was added after about 30 min. Nitrogen gas was supplied to the reactor in order to keep anoxic conditions in the activated sludge during the entire procedure. Samples were taken out (10 ml) just after carbon addition and further on every 10 min. Nitrate was measured spectrophotometrically (Dr. Lange Lasa 100 with LCK 339 ampoules). The maximum NUR presented in this study is based on the nitrate reduction in activated sludge with carbon and nitrate in excess, which further on is related to the volatile suspended solid (VSS). Measurements of suspended solid (SS) and VSS were performed according to the Swedish and European Standard (SS-EN 872). SS is obtained by measuring the difference in weight between a filtered sample (glass microfibre filters (GF/C)) before and after it has been dried at 105 °C for at least 1 h. VSS is established by the difference in dried weight before and after 1 h combustion at 550 °C.

2.3. Oxygen uptake rate measurements

The oxygen uptake rate (OUR) followed in general the method described in Kristensen et al. (1992). Activated sludge was tested in 1 l batch reactors at 20 °C. Nutrients ($(NH_4)_2SO_4$ 0.0472 g/l and K_2HPO_4 0.0283 g/l) were added. Allylthiourea 12 mg/l was used to inhibit nitrification during the experiment. The oxygen level in the reactor was followed with a WTW Oxymeter using Oxi 196 and Oxi 197 probes. The OUR was obtained by measuring the decrease of oxygen during at least 5 min with the oxygen concentration given every 30 s (while no oxygen was supplied to the system); the OUR was further found from the linear regression of the slope of that curve. By intermittently turning the oxygen on and off, the OUR was followed during a longer period. The total duration of each OUR test was about 5 h in total and the carbon source was added after approximately 1 h. By taking the VSS into account, the specific OUR for a particular activated sludge was obtained. The maximum specific OUR presented in this paper includes the endogenous respiration and represents the highest oxygen consumption rate when a carbon source was added in excess to the activated sludge.

2.4. FISH analyses of activated sludge communities

Biomass samples were fixed at 4 °C for 2 h. For Gram-negative bacteria, 4% paraformaldehyde freshly prepared in phosphate-buffered saline (PBS), was used and for Gram-positive bacteria, 50% ethanol in PBS was used. FISH analyses were made as described by Amann (1995). The probes used, and references, in this study can be seen in Table 2. Details on probe sequences and hybridisation conditions can be found at probeBase (Loy et al., 2003). Probes were labelled with Cy3 or FLUOS (5(6)-carboxyfluorescein-*N*-hydroxy-succinimide ester) fluorochromes and purchased by Thermohybaid, Germany. CitiFluor (CitiFluor Ltd., Leicester, UK) were used to mount the samples prior to visualisation by a Zeiss LSM 510 Meta confocal laser scanning microscope. The FISH-defined populations (Cy3-labelled) were quantified by image analysis