Impact of single and complex external carbon sources on denitrifying microbial community in sidestream treatment systems

Aneta Luczkiewicz[1], Przemyslaw Kowal[2], Joanna Majtacz[1], Katarzyna Jankowska[1], Slawomir Ciesielski[2], Krzysztof Czerwionka[1], Krishna R. Pagilla[3], Jacek Makinia[1],

1 Gdansk University of Technology, Gdansk, Poland

2 University of Warmia and Mazury in Olsztyn, Olsztyn, Poland

3 Illinois Institute of Technology, Chicago, USA

Presenting Author: Przemyslaw Kowal

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Introduction

Support of denitrification with external carbon source is needed to meet low total nitrogen limits in treated effluents. Historically, several organic carbon sources have been used to augment the rate of the denitrification process (methanol, ethanol, acetate, molasses, and brewery wastes), however, methanol is reported as the most widely used owing to its low cost and low sludge production rate. Since the cost of methanol experienced significant variability, other available and relatively inexpensive carbon sources, such as fusel oil (by-product of ethanol production) should be considered. In this study dinitrification of high nitrogen loaded sludge digester liquor (sidestream) was investigated in two parallel sequencing batch reactors (SBRs) using ethanol and fusel oil. To make a comprehensive model for process design, the impact of these single and complex carbon sources on microbial community structure was analyzed using denaturing gradient gel electrophoresis (DGGE) and fluorescent in situ hybridization (FISH).

Material and Methods

SBR experiments were carried out in 2 parallel reactors (4 dm^3), first - for 4 weeks and second - for 5 weeks. Each cycle started with nitrification phase of feed sludge digester liquor, then the external carbon source was added to support denitrification phase, and followed by settling and decantation phase. Fusel oil, used in this study as a complex carbon source, containing mainly: 2-methyl-1-butanol (32%), ethanol (11%), 3-methyl-1-butanol (7%), 2-methyl-1-propanol (7%), while the remaining part consisted of other amyl alcohols, acids, esters and aldehydes. Basic physical and chemical parameters of mixed liquor were controlled by in situ pH, dissolved oxygen and redox potential. Analysis of N_{tot}, $N\text{-}NH_4$, $N\text{-}NO_3$, $N\text{-}NO_2$, P_{tot}, $P\text{-}PO_4$ and COD was also performed. The results were additionally supported by conventional nitrate uptake rate (NUR) measurements.

The change in microbial community structure was analyzed using 16S rDNA PCR-DGGE fingerprints. Gels were stained with ethidium bromide and visualized under transillumination. Fluorescent in situ hybridization (FISH) was also performed to estimate the diversity and

abundance of bacteria involved in denitrification. The 16S rRNA oligonucleotide probes were used according to Nielsen et al. (2009). The relative abundance of each probe-defined bacterial group was analyzed as a ratio of the probe fluorescing area to the area fluorescing with the EUBmix probe.

Results and Discussion

The addition of fusel oil and ethanol in two parallel SBRs resulted in a continuous increase of NUR during the first 30 days (Fig. 1.1a). It indirectly confirms that fusel oil and ethanol can be metabolised by mixed microbial community of denitrifiers. The obtained DGGE profiles of activated sludge samples tested with ethanol and fusel oil sources also showed relatively stable microbial structure with high species diversity (Fig. 1.1b). It is noteworthy, however, that both carbon sources caused slight shift in the microbial community in the 5th week of the second experiment (Fig. 1.1b). According to FISH analysis the bacteria from the beta-subclass of *Proteobacteria* were mainly detected. *Azoarcus* cluster, *Thauera* cluster and *Acidovorax*-like denitrifiers were the most commonly found, while *Curvibacter* less frequently. The observations suggest that in the anoxic phase similar bacteria are involved in the degradation of both fusel oil and ethanol.

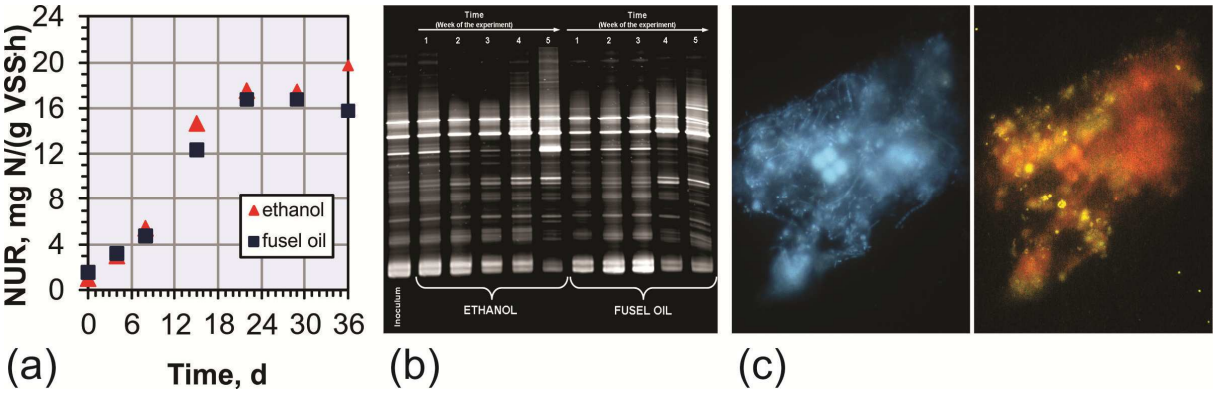

Figure 1.1 The parallel NUR measurements (a), DGGE profiles for activated sludge samples collected over five-week period (b), examples of FISH analysis (c).

Conclusions

The addition of fusel oil and ethanol resulted in a significant enhancement of the denitrification efficiency of sludge digester liquor. NUR data were supported by microbiological analysis, which indicated that in anoxic phase both fusel oil and ethanol can be metabolized by mixed microbial community.

References

Nielsen H., Daims H. and Lemmer H., editors (2009). FISH Handbook for Biological Wastewater Treatment: Identification and Quantification of Microorganisms in Activated Sludge and Biofilms. 1 ed. London : *IWA Publishing Company*.