IMPACT OF A COMPLEX EXTERNAL CARBON SOURCE ON THE MICROBIAL COMMUNITY AND DENITRIFICATION EFFICIENCY IN ACTIVATED SLUDGE SYSTEMS

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INTRODUCTION

It has been demonstrated that fusel oil, a distillery by-product containing over 50 different compounds, could be used as an alternative carbon source for denitrification. In this study, changes in the microbial community and denitrification efficiency were investigated during long-term studies (2-5 months) at two Johannesburg-type (JHB) activated sludge systems: a pilot plant and full-scale plant (Fig. 1).

MATERIAL AND METHODS

Effect of biomass acclimation to fusel oil was conducted in a pilot scale Johannesburg (JHB) system of the total volume of 30.0 dm^3 (Fig 2.). The volumetric ratios of main zones (anaerobic: anoxic : aerobic = 4:9:14 dm^3) were similar to the corresponding ratios in the full-scale bioreactor. A small anoxic tank (V = 3 dm^3) was used in the RAS lane. The mixed liquor in the anaerobic and anoxic tanks was maintained homogenous by mechanical stirrers. Both systems were fed with the actual settled wastewater. The influent flow rate was constant (= 1.20 dm^3/h) and proportional to the average actual influent flow rate to the full-scale bioreactor. The RAS and internal (mixed liquor - MLR) recirculations were set at 1.20 and 3.60 dm^3/h, respectively. A solution of fusel oil was added to the main anoxic zone.

The biological step of study full-scale WWTP consists of four JHB bioreactors and two circular secondary clarifiers. The total volume of each bioreactor is 18,512 m^3 and contain anoxic zone on the RAS line (514 m^3), anaerobic zone (2672 m^3), anoxic zone (4210 m^3), aerobic zone (9825 m^3) and deoxygenation zone on the MLR line (1291 m^3). The RAS and MLR recirculations were 110% and 600% of influent flow rate, respectively.

The performance of the pilot scale JHB systems over the experiment period was evaluated by regular analyses (once a week) of NO_3-N and PO_4-P in the anaerobic and anoxic effluent, MLSS and MLVSS in the aerobic zone, and COD, NO_3-N, NH_4-N, PO_4-P in the final effluent. During the acclimation process, the "*conventional*" NURs with the process biomass (withdrawn from the aerobic zone) were measured on a regular basis (every 3-7 days).

In each experiment, fusel oil was applied to the bioreactor and the selective pressure caused on activated sludge community was analyzed using 16S rDNA PCR-DGGE fingerprints along with fluorescent „in situ" hybridization (FISH). To better explore biological processes RT-PCR-based approach was used to investigate the expression of two denitrification genes (*nir*S and *nir*K).

RESULTS AND DISCUSSION

In the lab-scale and full-scale plants, the analyses were carried out for the activated sludge biomass in the experimental line (with the addition of fusel oil) and in reference line (without addition of fusel oil)(Tab.1). It was observed that the addition of fusel oil resulted in NURs approximately threefold higher than in reference line (Fig 3), while the DGGE profiles showed a slow shift in the microbial community with maintaining high species diversity (Fig. 4).

Table 1. Schedule of sludge sampling for microbiological testing.

number of samples	day	addition of fusel oil
T2/R1/R2/0	inoculum	-
T2/R1/1	14	+
T2/R1/2	21	+
T2/R1/3	42	+
T2/R1/4	63	+
T2/R2/1	14	-
T2/R2/2	21	-
T2/R2/3	42	-
T2/R2/4	63	-

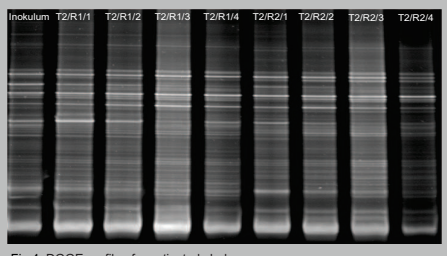

Fig.4. DGGE profiles for activated sludge

Based on the FISH analysis, the bacteria from *Curvibacter*, *Azoarcus* and *Thauera* clusters were mainly detected in denitrifying community, while *Acidovorax*-like denitrifiers were found less frequently (Fig 5). Meanwhile, in studied microbial community, the presence of both nitrite reductase genes was confirmed by PCR amplification from extracted DNA, but only transcription of *nir*S was detected.

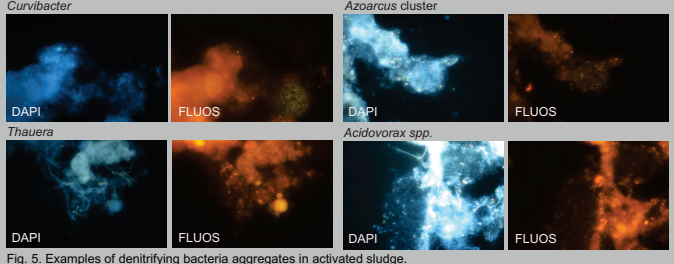

Fig. 5. Examples of denitrifying bacteria aggregates in activated sludge.

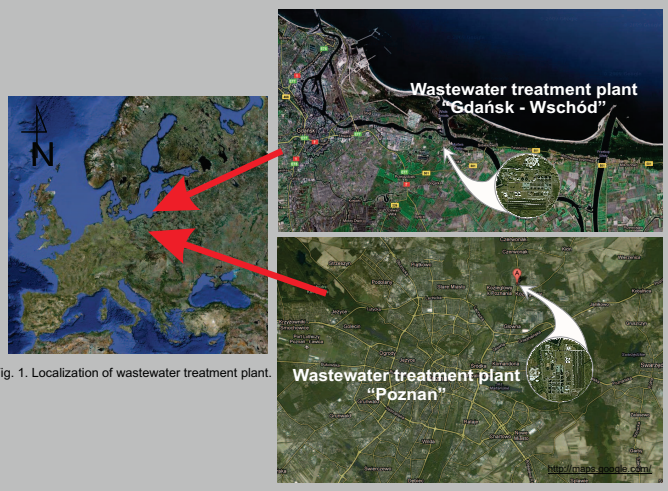

Fig. 1. Localization of wastewater treatment plant.

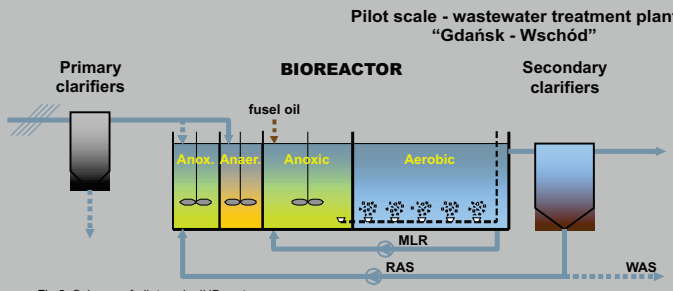

Fig.2. Scheme of pilot scale JHB system.

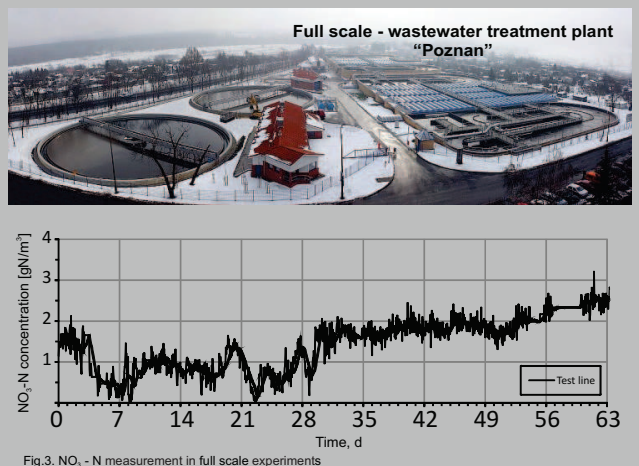

Fig.3. NO_3 - N measurement in full scale experiments

CONCLUSION

Introduction of the novel external carbon sources to enhance nitrogen removal from the wastewater requires better understanding of it influence on physiological structure of microbial consortia. This study reported that fusel oil did not affect significantly the structure of microorganisms populations and at the same time ensured higher efficiency of denitrification.

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