EXTRACELLULAR POLYMERIC SUBSTANCES IN THE NITRIFYING ACTIVATED SLUDGE

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Abstract
The aim of the study was to find out the amount and composition of extracellular polymeric substances (EPS) produced by activated sludge bacteria during nitrification of high ammonia concentrations. Treatment of synthetic leachate-imitated and real landfill leachate wastewater was performed in seven lab-scale membrane bioreactors during approx. 9 months and at different sludge age. Proteins, saccharides, humic substances were measured in an activated sludge extract. The obtained results showed that proportions of analyzed constituents were approximate in synthetically loaded reactors: proteins 40-65%, saccharides 26-45% and humic substances 7-17%, while in real leachate loaded reactor the main constituent were saccharides (50-62%) and the rest was divided equally between proteins and humus. The total EPS production (sum of proteins, saccharides and humic substances) seems to be dependent mostly on ammonia load, but a stress originated from toxic wastewater constituents or short sludge age can also have influence on final EPS concentration.

Streszczenie
Celem pracy było określenie ilości i składu substancji zewnątrzkomórkowych (ang. extracellular polymeric substances, EPS) produkowanych przez bakterie osadu czynnego podczas nitryfikacji wysokich stężeń azotu amonowego. Proces oczyszczania ścieków syntetycznych oraz rzeczywistych imitujących odcieki wysypiskowe prowadzono w siedmiu bioreaktorach membranowych przez okres ok. 9 miesięcy metodą osadu czynnego przy różnych wiekach osadu. W ekstrakcie osadu czynnego oznaczano białka, cukry oraz substancje humusowe. W oparciu o uzyskane wyniki stwierdzono, że proporcje pomiędzy składnikami EPS są zbliżone dla reaktorów zasilanych ściekami syntetycznymi i wynosiły: białka 40-65%, cukry 26-45% i kwasy humusowe 7-17%, podczas gdy EPS osadu czynnego poddanego działaniu rzeczywistych odcieków wysypiskowych zawierało 50-62% cukrów a resztę w równych proporcjach stanowiły białka i kwasy humusowe. Całkowita ilość EPS (suma cukrów, białek i substancji humusowych) była zależna od obciążenia ładunkiem azotu amonowego, aczkolwiek stres wynikający z obecności substancji toksycznych zawartych w ściekach oraz krótki wiek osadu mogły również wpłynąć na ilość powstałego EPS.

Keywords: **Extracellular polymeric substances; Activated sludge; Nitrification.**

1. INTRODUCTION

Nitrification (biological oxidation of ammonia to nitrate) is a key process in the global cycle of nitrogen and an integral part of modern wastewater treatment plants. It is known that nitrifying bacteria have a longer growth rate than heterotrophic bacteria (Rittman and McCarthy, 2001) and they are also more vulnerable to toxins than heterotrophs. Beside number of xenobiotic substances, free ammonia and nitrous acid (which can occur in the environment/wastewater as a result of incomplete nitrification) are the main toxins for nitrifiers (Prosser et al., 1989).

In biological wastewater treatment, bacteria tend to aggregate by forming flocs and they are using extracellular polymers to glue together. The extracellular polymeric substances (EPS) are mainly responsible for the structural and functional integrity of biofilms/flocs and they are considered to be important for the physico-chemical properties of activated sludge and biofilms. The EPS can influence not only the flocculation process of activated sludge but an EPS layer surrounding bacterial cells can serve as a barrier protecting the cells from unfavorable agents of environment and retain the extracellular enzymes close to cell and preventing their dilution in a wastewater (Wingender et al., 1999). It was also noticed that different wastewater composition has influence on the EPS composition (Wilen et al., 2003). However, it is not known whether the composition is different due to wastewater composition or rather different biodiversity according to the type of wastewater and thus different metabolism abilities.

The aim of the study was to find out the intensity of EPS production during nitrification of high ammonia concentrations and its composition.

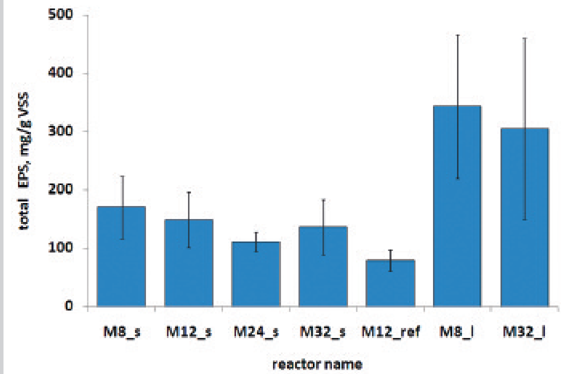

Figure 1.
Amount of EPS in nitrifying activated sludge

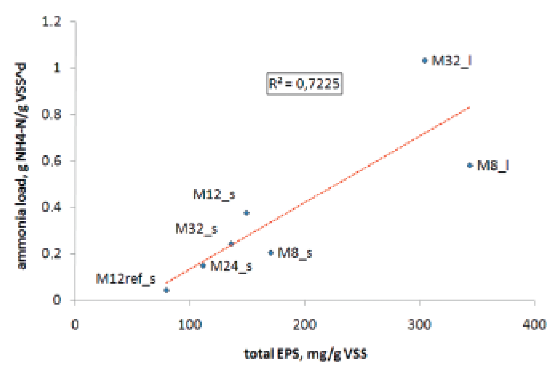

Figure 2.
Relation between ammonia load and EPS production

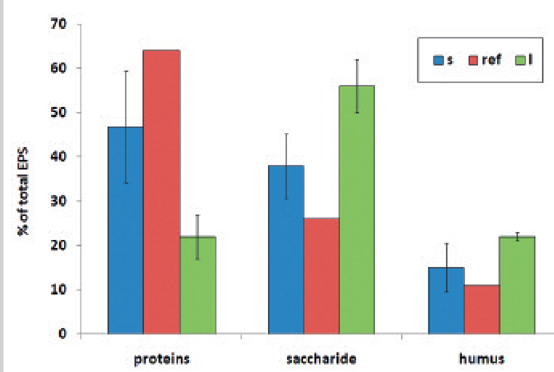

Figure 3.
Constituents of nitrifying activated sludge EPS

2. METHODS

2.1. Reactor system and operation

A nitrification process was performed in seven laboratory-scale bioreactors. Separation of wastewater from activated sludge was obtained thanks to membrane modules submerged in each bioreactor. Six investigated bioreactors were operated with high ammonia concentrations and one reactor was fed with low ammonia concentration. Reactors were fed with either synthetic wastewater (composed of NH_4Cl, Na_2HPO_4, bullion extract and CH_3COONa) or landfill leachate (see Tab. 1). The reactors differed in sludge age and type of wastewater. Tab. 1 shows parameters of the reactors.

The reactors were inoculated with activated sludge taken from the nitrification stage of the municipal wastewater treatment plant in Gliwice, Poland. As the experiment was not carried out for all the reactors in parallel the activated sludge was taken from WWTP in autumn 2004 (to seed and operate reactor

Table 1.
Parameters of membrane bioreactors

Reactor name	WW type	SRT d	OLR g/gVSSd	NLR g $N\text{-}NH_4^+$/gVSSd	C:N -
M8_s	S	8	0.079	0.207	0.4
M12_s	S	12	0.129	0.379	0.3
M24_s	S	24	0.052	0.151	0.3
M32_s	S	32	0.071	0.245	0.3
M12_ref	S	12	0.071	0.046	1.5
M8_l	L	8	0.975	0.584	1.2
M32_l	L	32	1.167	1.035	1.2

WW – wastewater, HRT – hydraulic retention time, SRT – sludge retention time (sludge age), OLR – organic loading rate, NLR – ammonia loading rate, VSS – volatile suspended solids, L – landfill leachate, S – synthetic wastewater

Table 2.
Organic substances removal and nitrification efficiency in the membrane reactors

	M8_s	**M12_s**	**M24_s**	**M32_s**	**M12_ref**	**M8_l**	**M32_l**
Organic compounds removal, %	64.3	89.2	76.9	93.1	54.5	not determined	not determined
Ammonia removal, %	98.8	99.6	99.6	99.8	99.8	47,8	86,3

M12_s, M32_s), spring 2005 (to seed and operate reactor M8_s, M24_s, M12_ref) and autumn 2005 (to seed and operate reactor M4_s, M8_l and M32_l). The oxygen concentration in the reactors was obtained with air aeration and kept at the level of 4 mg O_2/L. The pH level of 7-8 was adjusted on-line by $NaHCO_3$ addition. The temperature of the reactors was close to 20°C. Influent and effluent of the bioreactors were analyzed (ammonia, nitrite, nitrate and organic compounds) regularly to monitor the performance of organic compounds degradation and nitrification process. Following procedures were used: ammonia – determined colorimetrically with Nessler reagent (PN-C-04576-4:1994), nitrite – colorimetrically with alphanaphthyloamine reagent (Hermanowicz *et al*., 1999) and nitrate – colorimetrically with dimethylphenol reagent (ISO 7890-1). The VSS were calculated as a difference between mass of sample dried at 105°C for 1h and mass of its ash (burnt at 550°C).

Extracellular polymers analysis. Extraction of the polymeric substances was done thermally according to Karapanagiotis et al.,1989; Morgan et al., 1990; Chang and Lee, 1998; Kim et al., 2001. A sample of well mixed activated sludge was concentrated by low speed centrifugation (2000×g) for 20 minutes and the sludge pellet was resuspended in distilled water to the initial volume. The sample was then placed into water bath (80°C) for 1 hour. The sludge was separated from polymer extract by two-time centrifugation: first at 2000×g and then 4500×g in the duration of 20 minutes each time.

The extract was analyzed for proteins, humic substances, saccharide and TOC. The protein and humic substances concentration was made by means of the modification (Sharma and Krishnan, 1966) of Lowry' (Lowry et al., 1951) method with Folin-Ciocalteau reagent and calculated with application of correction coefficient due to humic substances occurrence (Frolund, 1995). Saccharides concentration was analyzed based on Dubois et al. (1956). Total organic carbon (TOC) concentration was achieved with the use of TOC Shimatzu analyzer. An amount of EPS was calculated as a sum of the particular EPS elements: proteins, humic substances and saccharides and calculated as mg per g VSS. The samples of activated sludge were taken every two weeks of the experiment.

3. RESULTS AND DISCUSSION

The experiment lasted in every reactor for about 300 days. First 40-200 days were covered by adaptation to particular sludge age and ammonia concentration. The adaptation step took around 5 times of particular sludge age.

Organic compounds degradation and nitrification removal efficiencies obtained after adaptation step are shown in Tab. 2.

The organic substances removal in the higher, synthetically ammonia loaded reactors was 64-93%, while in the reference reactor only 54.5%. Since the hydraulic organic load of the reactors was similar, the organic carbon:nitrogen (C:N) ratio was 0.3-0.4:1 for the higher synthetically ammonia loaded reactors and 1.3-1.5:1 for the reference reactor and highly leachate loaded reactors. The nitrification process was the most stable and it was running without disturbances only in the reference reactor. Huge nitrification fall, lasting approx. 50 days, was observed in reactor M12, M24 and M32. In reactor M32 it occurred during adaptation step (and thus it is not included in the results shown in the Tab. 2), while in reactor M12 and M24 after adaptation. In reactor M8 the fall of second stage of nitrification (conversion of nitrite to nitrate) appeared in the end of experiment and was caused by total decay of nitrite oxidizers bacteria (data not shown). Beyond the period of unstable nitrification, for the rest of experiment time the reactors showed high ammonia and nitrite oxidation. In the leachate loaded reactor with a sludge age equal 8 days only first phase of nitrification was observed during the whole the experiment time while in the reactor with sludge age of 32 in the first 60 days only nitritation (conversion of ammonia to nitrite) was observed and in consecutive 80 days full nitrification.

No clear relations between the amount of EPS and ammonia removal efficiency or inhibitors concentrations (free ammonia or nitrous acid appear while nitrification problems) were observed. However, in the reactors where the nitrification or nitratation collapse occurred, the EPS concentration variability was larger. In the reactors with small or no nitrification problems (independently from sludge age and influent composition), so for M24_s and M12ref_s, the variation coefficient of EPS concentration was up to 23%, while for the reactors with nitrification collapse incidents they were much higher – 30-50% for M12_s, M32_s, M32_l and M8_l.

Fig. 1 presents the average amount of total EPS reached during a period after adaptation to particular sludge age and ammonia concentration. As it can be seen in Fig. 1, the activated sludge from the reference reactor produced the fewest EPS, while in the activated sludge fed with real leachate (M8_l and M32_l) the EPS production was very high, from 3.8 to 4.3-times higher than in the reference reactor. The higher synthetically ammonia loaded reactors (M8_s, M12_s, M24_s and M32_s) showed average amount of EPS approx. 40-115% higher than in the reference reactor. However, the EPS amount in the leachate fed activated sludge was 80-200% and 280-330% higher than in the synthetically fed one and reference reactors, respectively.

In the nitrification systems the most dangerous compounds which can appear mainly while unstable nitrification is free ammonia and free nitrous acid. Hypothetically, the higher EPS concentration in the higher ammonia loaded systems can be explained to be a protection of activated sludge bacteria from that compounds. Comparison of nitrogen compounds concentration dynamics as well as EPS and its constituents dynamics showed none correlation between nitrification fall and the amount of EPS or its constituents. However, the amount of EPS was related to ammonia load, what is shown in Fig. 2. The higher ammonia load the higher amount of EPS.

In the synthetic fed reactors the influence of other than wastewater-originated compounds was excluded. The comparison of synthetically and real leachate loaded reactor shows that the amount of EPS in leachate loaded reactors was much higher than in the others. It is concluded than that not only free ammonia and nitrous acid but also other harmful substances contained in landfill leachate can influence the increase of the EPS production. It can also be seen that among synthetically and leachate loaded reactors always EPS from the youngest sludge age (8 days) has higher value and it may suggest that stress coming from short SRT can be also a parameter rising EPS production.

The amount of the EPS constituents is presented in Fig. 3. A trend proteins>saccharides>humic substances was obtained for all synthetically ammonia loaded reactors, however, the amount of proteins was higher in the reference reactor than in the higher loaded. Such relations between EPS constituents were also observed by other researchers (Frolund et al., 1994, Bura et al., 1998; Wilen et al., 2003).

It is not surprising that the amount of humus in landfill leachate loaded activated sludge was the highest as the wastewater itself is composed of humic substances. Wilen et al. (2003) also pointed huge part of EPS from activated sludge fed with leachate was covered by humic substances. In the leachate loaded reactors operated in the study (M8_l and M32_l) the main EPS constituent was saccharides.

4. CONCLUSIONS

Obtained results showed that the total EPS production (sum of proteins, saccharides and humic substances) is dependent mostly on ammonia load, however, a stress originated from toxic wastewater constituents or short sludge age can also have influence on the final EPS concentration. Proportions of analyzed constituents were similar in synthetically loaded reactors: proteins 40-65%, saccharides 26-45% and humic substances 7-17%, while in leachate loaded reactor the main constituent were saccharides (50-62%) and the rest was divided equally between proteins and humus.

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REFERENCES

[1] *Bura R., Cheung M., Liao B., Finlayson J., Lee B.C., Droppo I.G., Leppard G.G., Liss S.N.*; Composition of extracellular polymeric substances in the activated sludge floc matrix. Water Science and Technology, Vol.37, No.4-5, 1998, p.325-333

[2] *Chang I-C., Lee C-H.*; Membrane filtration characteristics in membrane-coupled activated sludge system – the effect of physiological states of activated sludge on membrane fouling. Desalination, Vol.120, 1998, p.221-233

[3] *Dubois M., Gilles K.A., Hamilton J.K., Reber P.A., Smith F.*; Colorimetric method for determination of sugars and related substances. Analyt. Chem., Vol.28, 1956, p.350-356

[4] *Frolund B., Keiding K., Nielsen P.H.*; A comparative study of biopolymers from a conventional and an advanced activated sludge treatment plant. Water Science and Technology, Vol.29, 1994, p.137-141

[5] *Frolund B., Griebe T., Nielsen P.H.*; Enzymatic activity in the activatedsludge floc matrix. Applied Microbiol. Biotechnol., Vol. 43, 1995, p. 755-761

[6] *Hermanowicz W., Dojlido J.*; Physical and chemical methods in water and wastewater analysis. Arkady, Warszawa, 1999

[7] *Karapanagiotis N.K., Rudd T., Sterritt R.M., Lester J.N.*; Extraction and characterisation of extracellular polymers in digested sewage sludge. J. Chem. Tech. Biotechnol., Vol.44, 1989, p.107-120

[8] *Kim J-S., Lee C-H., Chang I-C.*; Effect of pump shear on the performance of a crossflow membrane bioreactor. Water Research, Vol.35, No.9, 2001, p.2137-2144

[9] *Lowry O., Rosebrough N., Farr A.L, Randall R.*; Protein measurement with the Folin phenol reagent. J. Biol. Chem., Vol.193, 1951, p.265-275

[10] *Morgan J.W., Forster C.F., Evison L.*; A comparative study of the nature of biopolymers extracted from anaerobic and activated sludges. Water Research., Vol.24, No.6, 1990, p.743-750

[11] *Prosser J.I.*; Autotrophic nitrification in bacteria. Adv. Microb. Physiol., Vol.30, 1989, p.125-181

[12] *Rittmann B.E., McCarty P.L.*; Environmental Biotechnology: Principles and Applications. McGraw-Hill, New York, 2001

[13] *Sharma O.K., Krishnan P.S.*; Colorimetric estimation of humic acid with the phenol reagent of Folin and Ciocalteu. Analyt. Biochem., Vol.14, 1966, p.11-16

[14] *Wilen B.M., Jin B. Lant P.*; The influence of key chemical constituents in activated sludge on surface and flocculating properties. Water Research, Vol.37, 2003, p.2127-2139

[15] *Wingender J., Neu T.R., Flemming H.C.*; Microbial extracellular polymeric substances. Springer-Verlag, Berlin, Heidelberg, New York, p.1-53, 1999