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IMPACT OF ULTRASOUND TREATMENT ON THE BIOFILM STRUCTURE AND PERFORMANCE OF A MEMBRANE BIOFILM REACTOR FOR TERTIARY HYDROGEN DRIVEN DENITRIFICATION OF MUNICIPAL WASTEWATER

ZASTOSOWANIE ULTRADŹWIĘKÓW DO KONTROLI STRUKTURY BIOFILMU I POPRAWY DZIAŁANIA REAKTORA DO DENITRYFIKACJI ŚCIEKÓW KOMUNALNYCH Z WYKORZYSTANIEM AUTOTROFICZNYCH BAKTERII UTLENIAJĄCYCH WODÓR

This study aimed to investigate the impact of the ultrasound treatment on autotrophic denitrification of low organic carbon content wastewater with a biofilm growing on a hydrogen diffusing membrane.Ultrasound treatment significantly affected the thickness of the biofilm. When no mixing or ultrasound treatment was applied to the system, biofilm thickness averaged 813+/-254 µm due to accumulation of solids on the biofilm surface. Ultrasound treatment resulted in the reduction of biofilm thickness to 335+/-104 µm. In contrast, the biofilm density, expressed as total solids per liter of biofilm volume exhibited an increasing trend when ultrasound treatment was introduced. No significant changes in carbohydrates and EPS content within biofilm was observed, however overall cell viability dropped significantly from 31+/-1% to only 15+/-1% with ultrasound treatment, suggesting that the biofilm population is susceptible to ultrasound treatment. Ultrasound treatment proved to be successful in improving denitrification efficiency. Rates for operation without any shear force were on the average 60% lower than those obtained with ultrasound treatment, indicating the importance of limitation in diffusion caused by increased biofilm thickness and laminar flow conditions. Effluent solids at steady state conditions stabilized at levels consistently below 20 mg/l, which is lower than widely adopted discharge limit of 30 mg/l. The COD values measured in the effluent during steady state conditions were in the range of 60-102 mg COD/l which is below local discharge requirements of 150 mg COD/l. Proper ultrasound treatment is thus able to improve MBfR performance without risk of COD breakthrough.

Celem eksperymentu bylo zbadanie wplywu ultradzwiekow na proces autotroficznej denitrifikacji w systemie opartym na biofilmie pokrywajacym membrane zastosowanym do oczyszczania sciekow zawierajacych niskie stezenia wegla organicznego. Ultradzwieki umozliwily znaczace zmniejszenie grubosci biofilmu. Rezim nie zawierajacy zastosowania ultradzwiekow umozliwial akumulacje zawiesin i zaowocowal utworzeniem biofilmu o grubosci 813+/-254 µm. Ultradzwieki umozliwily redukcje grubosci biofilmu do 335+/-104 µm i spowodowaly jednoczesny wzrost w gestosci biofilmu. Nie zaobserwowano znaczych zmian w zawartosci weglowodanow i polymerow w biofilmie, niemniej pomiary wykazaly spadek w zywotnosci bakterii z 30+/-1% do 15+/-1% po zastosowaniu ultradzwiekow. Zastosowanie ultradzwiekow spowodowalo 60% wzrost wskaznikow redukcji azotanow na skutek zmniejszenia grubosci biofilmu i poprawionej dyfuzji skladnikow odzywczych. Jednoczesne pomiary zawiesiny i ChZT w sciekach wykazaly stale wartosci ponizej 20mg zawiesiny/l i 120 mg ChZT /l. Wlasciwe zastosowanie ultradzwiekow umozliwilo polepszenie wydajnosci systemu bez ryzyka naglych wzrostow stezenia ChZT w odplywie.

1. Introduction

The feasibility of membrane biofilm reactors application for autotrophic nitrate removal in wastewater treatment has been demonstrated in previous researches ([1],[21],[22],[6]). Membrane biofilm reactors for hydrogen driven denitrification combine all the advantages of the hydrogen driven process and address concerns of inefficient gas delivery and the necessity for long solid retention times (SRT) required by autotrophic bacteria. However the applicability of the fiber – membrane biofilm reactor for tertiary wastewater treatment depends also on stability of operation. Previous experiments showed that denitrification efficiency in MBfR can be strongly repressed by limitation in hydrogen ([5]) or nitrate diffusion in the thick biofilm ([3],[7]). This may lead to dual diffusive limitations, which was described by Terada et al. (2006) who observed the deterioration of denitrification efficiency as biofilm structure changed. One of the main concerns is thus excess biofilm development, which may lead to lower efficiency, biofilm sloughing and brings the risk of breakthrough of total and volatile solids as well as COD into the effluent ([14]). Controlling biofilm structure i.e. its thickness, density and composition is then a key factor which affects membrane biofilm reactors efficiency.

Ultrasound is able to deagglomerate bacteria clusters or inactivate bacteria through a number of physical, mechanical and chemical effects arising from acoustic cavitations. On collapse, cavitiation bubbles produce enough energy to mechanically weaken or disrupt bacteria or biological cells via number of processes such as shear forces induced by microstreaming within bacterial cells, resonance of bacterial cells or chemical attack due to the formation of radicals ([17]).

So far ultrasound has been applied to membrane system as the treatment minimizing membrane fouling. Tests identified two main mechanisms which were responsible for membrane cleaning. The created shear effect resulted from collision of micro particles with membrane surface and chemical reactions between membrane and hydroxyl radicals produced during acoustic cavitations ([24]). The ultrasound was proved to be efficient in improving membrane permeability and mitigate membrane fouling ([4],[12]).

Thus ultrasound treatment could be effective in minimizing the biofilm thickness and controlling membrane biofilm reactor performance.

The ultrasound can also provide powerful disinfection (percent of kill bacteria). The tests showed that increasing duration of exposure and intensity of ultrasound in the low kilo- hertz range (20kHz and 40kHz) increase the percent of killed bacteria. Low intensity ultrasound (higher frequencies) results in declumping, low kill rate and no significant decrease in bacterial cell numbers ([17],[11]). Contrary to high frequency ultrasound, the low frequency treatment characterizes with good penetration through polluted stream, which suggest its better applicability for large volume tanks ([17]). This might be especially viable for biofilm treatment as application of ultrasound treatment for biofilm removal showed high resistance of the attached bacteria clusters ([19]). Peterson and Pitt (2000) found that ultrasound treatment was not significantly detrimental to biofilm viability which was attributed to protection barrier created from extracellular polymeric substances (EPS) matrix and resulted only in de-clumping effect. They also observed and described with mathematical model that transport of substances within pores of the biofilm increases during ultrasound treatment with lower intensities and decreases for higher frequencies ([20]). Application of lower frequencies ultrasound could be then useful tool for controlling biofilm thickness, improve mass transport conditions with no significant negative impact on biofilm viability.

It should be noted that application of the ultrasound can cause release of organic matter into solution and thus lead to increase in the chemical oxygen demand of the treated stream. The release of the substances can be a result of rapture of the bacterial cells or the extraction of EPS ([15]). On the other hand, with sonication, H_2O is known to decompose in collapsing cavitation bubbles to yield OH radicals ([13],[24]). These radicals diffuse into the bulk liquid and increase the radical concentration in the solution thus enhancing the decomposition rate of organic matter ([10]).Properly selecting the ultrasound intensity and working time could be effective ways of controlling the biofilm thickness, structure and MBfR performance.

This study aimed to investigate autotrophic denitrification of low organic carbon content wastewater within a biofilm growing on a hydrogen diffusing membrane. The objective of the experiment was to evaluate the possibility of controlling the process rates, as well as biofilm parameters by applying ultrasound treatment. Specific objectives of the carried experiment were to evaluate the impact of ultrasound on the biofilm structure (i.e. biofilm thickness, density, and composition), biofilm viability, denitrification rates and effluent quality (total and volatile solids concentration, chemical oxygen demand).

2. Materials and Methods

2.1. Reactor Operation

The experimental set-up involved the laboratory-scale biofilm reactor with volume of 3 L. The reactor was operated in continuous flow mode with hydraulic retention time (HRT) 4 hours. The low operational HRT was chosen in order evaluate the system under high loading while make it practical in a laboratory setting. The bioreactor was seeded with a population of autotrophic denitrifiers at the first day of the operation. Hydrogen necessary for the process was delivered through the submerged fibre membrane module (GE Water & Process Technologies - ZENON Membrane Solutions). Constant hydrogen supply at the pressure of ~2.5 psi was maintained throughout the experiment which assured that hydrogen was not a limiting component.

The reactor was placed in the ultrasound bath (FS220 Ultrasound Cleaner, power 250W and frequency 44KHz +/-6%) (Fig. 1) and exposed to the ultrasound treatment (2 min, twice a day). The actual testing period, which lasted 75 days, followed the period when no external shear force was applied to the system (control conditions).

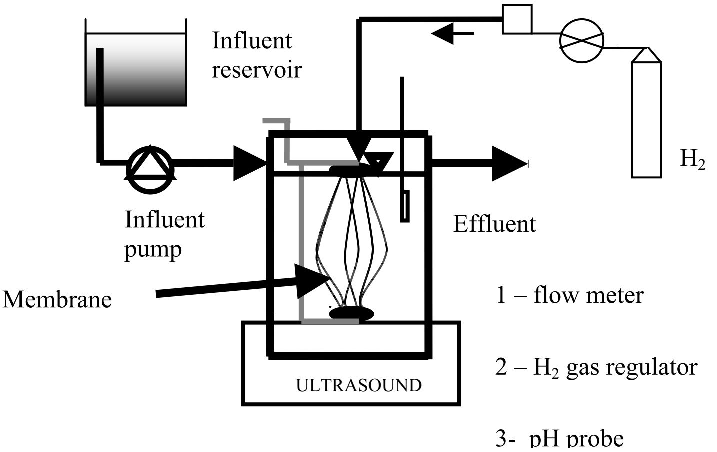

Fig. 1 Membrane biofilm reactor set – up

Rys. 1 Schemat reaktora

The reactor was fed with un-disinfected final effluent collected from the City of Winnipeg-North End secondary wastewater treatment plant. Nitrates were added to the feed in the form of $NaNO_3$. The main influent wastewater parameters are presented in Table 1. No pH control was implemented.

Tab. 1 Feed wastewater characteristics

Tab. 1 Parametry ścieków użytych podczas eksperymentu

Parameter	Units	Value
SCOD	mg l-1	53+/-11
alkalinity	mg $CaCO_3$ l^{-1}	236+/-48
temperature	C	21+/-0.6
DO	mg l-1	2+/-1.1
pH	-	7.2+/-0.3

2.2. Analytical Methods

Samples of influent and effluent for NO_3, NO_2, TSS and VSS analysis were taken each day of reactor operation and stored at ~ 4ºC. NO_3, NO_2 concentrations in samples taken from influent and effluent were determined colorimetrically using automated nitrogen analyzer (QuikChem 8500 Lachat Instruments) following Standard Methods 4500-NO3-F ([2]). The measurements were taken in room temperature and all presented denitrification rates were calculated for 20ºC.

Mixed liquor suspended solids (TSS and VSS) were analyzed following Standard Methods 2540D and 2540E ([2]).Total chemical oxygen demand (TCOD) and soluble chemical oxygen demand (SCOD) of samples taken from influent and effluent were measured using the Hach Digestion Vials and Hach Spectrophotomer (Hach, USA) before and after filtration through the 0.45 μm membrane filters. All the determined values were based on results from duplicates. The dissolved oxygen (DO) concentration and pH was determined using HACH Sension 378 DOmeter and ORION 91-05 pH electrode, respectively.

Biofilm samples for thickness, density, composition (carbohydrates and proteins content) and EPS content were collected from the membrane surface. The membrane with biofilm was removed from reactor for 15 min and placed in vertical position to allow excess water to drain. The biomass was removed from the fibres of known length and number and put into 5ml plastic syringe, partially filled with deionized water. Biofilm thickness was calculated basing on the liquid volume displaced by the biomass and the area scrapped from the module.

Biofilm volumetric density was obtained by determining total and volatile solids of the sample ([8]). The carbohydrates and protein content within biofilm was determined using the anthrone ([23]) and modified Bradford method with glucose and BSA as standards respectively.

The characterization of the extracellular polymeric substances (EPS) bound within the biofilm were carried out by measuring the dry weight content (at 105 ºC), volatile dry weight content (at 550 ºC) of extracted EPS. The EPS were extracted in physical process, which consisted of three steps: (1) addition of DOWEX MARATHON C cation exchange resin and extraction (2 h at 20ºC), (2) centrifugation and (3) acetone and ethanol precipitation (24 h at 4ºC).

The viability of cells was determined with LIVE/DEAD BacLight Bacterial Viability Kit (L7012) through quantitative assays with RF-1501 SHIMADZU Spectorofluorophotomer (P/N 206-62901) with PC-1501 Personal Fluorescence Software. The tests were carried out according to procedure suggested by kit provider (Molecular Probes Invitrogen detection technologies).

3. Results and discussion

3.1. Impact of ultrasound on biofilm thickness and density

The biofilm structure i.e. its thickness and density was a function of levels of shearing stress applied to the biofilm. Fluctuations in biofilm structure (i.e. its thickness and density) during the testing periods are presented in Figure 2. Different operating shearing stress regimes resulted in different thickness of biofilm covering the membrane. Lack of any shear force (control conditions) resulted in increase of biofilm thickness in reactor. When no mixing or ultrasound treatment was applied to the system biofilm thickness averaged 813+/-256 µm due to accumulation of solids on the biofilm surface. Ultrasound treatment resulted in reduction of biofilm thickness to 335+/-104 µm. Relatively low standard deviation suggests that this type of treatment allows to maintain similar biofilm thickness along the membrane fibers. The T test comparing phases without shear force and with ultrasound treatment confirm significant change with p value equal to p= 0.0017 suggesting that ultrasound treatment can be viable method of minimizing biofilm accumulation.

The results of simultaneous measurements of biofilm density are shown also on Fig. 2. Total solids content within the biofilm increased when ultrasound treatment was introduced to operational mode. The total solids concentration was equal to 71+/-6 g TS l^{-1} and 101 +/-25 g TS l^{-1} in reactor operated without any shear force and exposed to ultrasound treatment respectively. The volatile solids content within the biofilm remained statistically unchanged regardless changes in the applied level or type of shear force. Volatile solids measured were equal to 45+/-4 g VS l^{-1} and 57+/-13 g VS l^{-1} in reactor operated without any shear force and exposed to ultrasound treatment respectively. As the result of changes in biofilm density observed VS/TS ratio decreased from 0.63+/-0.01 for control conditions to 0.56+/-0.01 in biofilm exposed to ultrasound. Previously obtained results ([6]) indicate that biofilms carrying out hydrogenotrophic denitrification can contain high content of inert solids and that a VS/TS ratio higher than 0.3 needs to be maintained to assure stable biofilm operation and prevent biofilm sloughing.

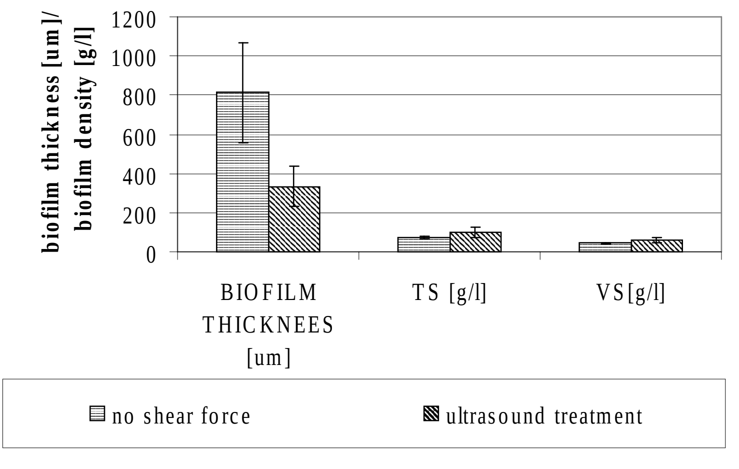

Fig. 2 Fluctuations in biofilm thickness and density in MBfR exposed to no shear force and ultrasound treatment

Rys. 2 Zmiany w grubości i gęstości biofilmu w reaktorze bez i z ultradźwiekami

3.2. Impact of ultrasound on EPS accumulation and biofilm composition

Data presented in Fig. 3 show the changes in EPS, protein and carbohydrates content within the biofilm for different operating conditions. The measurements showed that EPS content remained similar in reactors with no shear force (63+/-5 mg EPSgVS^{-1}) and with ultrasound treatment (57+/-26 mg EPSgVS^{-1}). These results suggest that while hydrodynamic shear force is known to stimulate EPS production ([18]), ultrasound treatment does not affect EPS accumulation within a biofilm.

Although carbohydrates have often been regarded as the most important extracellular components, proteins were found at relatively higher levels than carbohydrates in this study. Protein concentrations varied from 51+/- 26 mg proteins/g VS to 84+/-6 mg proteins/ g VS, while concentrations of carbohydrates varied from 25+/-9 mg carbohydrates / g VS to 31+/-11 mg carbohydrates / g VS. This observation is consistent with work by Jahn and Nielsen (1998)[9] who also observed that proteins and humic substances were the main components of biofilms. The calculated carbohydrates to proteins ratio (c/p ratio) varied from 0.2 to 0.6, which is also comparable to the results obtained by Jahn and Nielsen who analyzed sewer biofilms and observed c/p between 0.25-0.6.

However contrary to previous research, where higher shear force led to overproduction of carbohydrates, the results of this study did not show any significant correlation carbohydrates content (c) and shear force caused by ultrasound treatment. The results presented on figure 3 show that carbohydrates content remained unchanged regardless of changes in applied shear stress. It was equal to 25+/-10 mg g VS^{-1} and 31+/- 11 mg g VS^{-1} in reactor operated without any shear force and reactor exposed to ultrasound treatment respectively.

The content of proteins was however affected by ultrasound treatment. The protein content decrease from 84+/-6 mg g VS^{-1} when no shear force was applied to 51+/- 25 mg g VS^{-1} reactor exposed to ultrasound treatment. This resulted in change in calculated carbohydrates to protein ratio (c/p). Fig. 4 represents the changes in c/p ratio for different operating regimes. The results suggest that ultrasound treatment may lead to increase in c/p ratio. It increased from 0.30+/-0.09 when no shear stress was applied to 0.75+/-0.42 in reactor exposed to ultrasound treatment. It is believed that proteins provide most of the binding sites within a biofilm. Thus increased c/p ratio caused by ultrasound might lead to risk of biomass sloughing, VSS and COD breakthrough in the effluent.

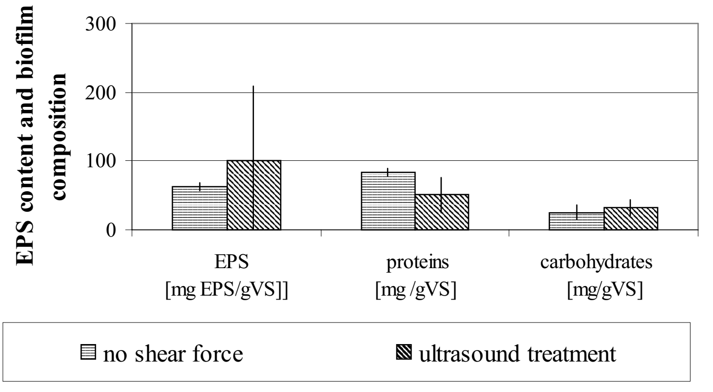

Fig. 3 *Fluctuations in EPS, proteins and carbohydrates content in MBfR exposed to no shear force and ultrasound treatment*

Rys. 3 *Zmiany w zawartości EPS, protein i wodorowęglanów w reaktorze bez i z ultradźiekami*

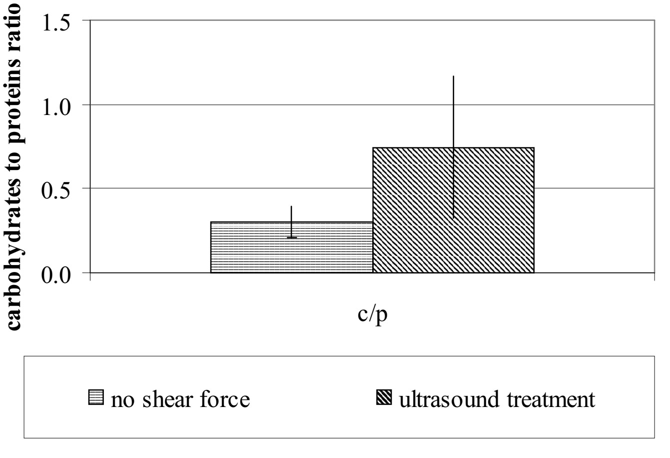

Fig. 4 *Fluctuations in c/p ratio in MBfR exposed to no shear force and ultrasound treatment*

Rys. 4 *Zmiany w wartośi c/p w reaktorze bez i z ultradźiekami*

3.3. Impact of ultrasound on bacteria viability

Data presented on the Fig. 5 show changes in bacteria viability within the biofilm for different operating conditions. The determined viability of bacteria in MBfR varied from 15% to 30 % depending on applied shear force. The results suggest that applying ultrasound treatment for minimizing biofilm accumulation deteriorated bacteria viability. The percent of live bacteria decreased significantly from 31+/-1% to only 15+/-1% suggesting that biofilm population is susceptible to ultrasound treatment. Careful choice of proper dosage has to be done in order to prevent killing of bacteria and thus possible diminishing systems efficiency.

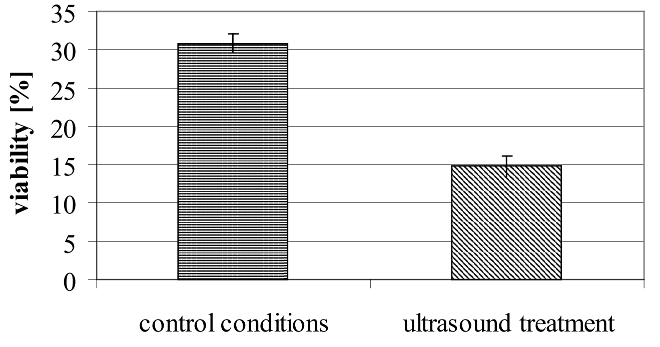

Fig. 5 Impact of ultrasound on bacteria viability

Rys. 5 Wpłw ultradźieków na żwotność bakterii w biofilmie

3.4. Impact of ultrasound on denitrification rates

Observed changes in biofilm thickness, composition and bacteria viability affected obtained denitrification rates. Denitrification rates obtained for different shearing stress regimes are presented in Fig. 6.

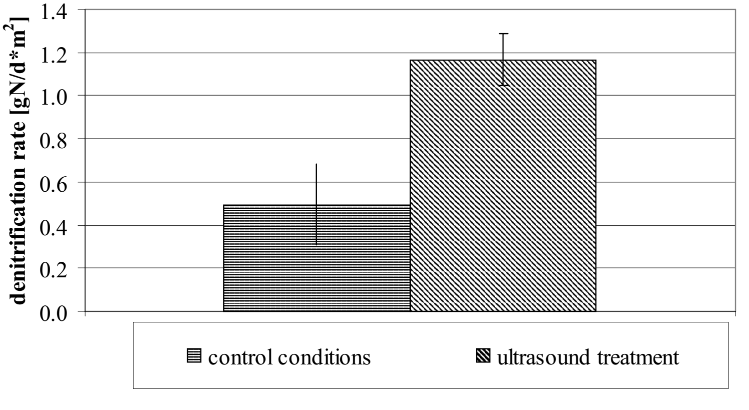

Fig. 6 Denitrification rates obtained in MBfR exposed to no shear force and ultrasound treatment

Rys. 6 Wskaźnik redukcji azotanów w reaktorze bez i z ultradźwiekami

Introduction of ultrasound allowed minimizing biofilm thickness and thus improving denitrification rates. Rate obtained for no shear force operating mode was low and equal to only 0.49+/- 0.09 $gNd^{-1}*m^{-2}$ indicating the importance of limitation in diffusion caused by increased biofilm thickness (813+/-256 μm) and laminar flow conditions. Ultrasound treatment allowed to maintain lower biofilm thickness (335+/-104 μm) and despite observed decrease in bacteria viability resulted in much higher denitrification rate equal to 1.17+/- 0.11 $gNd^{-1}*m^{-2}$. A t-test conducted on the data shows significant differences in denitrification rates obtained for different operational conditions indicating that ultrasound can be used to control removal rates.

3.5. Impact of biofilm structure and composition on denitrification rate

The obtained results suggest that application of ultrasound allows changing the biofilm structure and thus affects the denitrification rates. Summary of the results is presented in Table 2. No significant impact of ultrasound treatment on carbohydrates and EPS content was observed. Biofilm thickness and protein content within biofilm was reduced during operational mode which included ultrasound treatment.
The inversely proportional correlation between biofilm thickness and denitrification rates is presented in Table 2. Observed reduction in biofilm thickness corresponded with significant increase in denitrification rate. It was speculated that ultrasound treatment and changes in biofilm structure stimulated bacteria activity. Despite decrease in viability calculated specific denitrification rates increased from 0.09 mg NO_3-N $*(h*g\ VS)^{-1}$ when no shear force was applied to 0.58 mg NO_3-N $*(h*g\ VS)^{-1}$ during ultrasound treatment. Specific active denitrification rate, calculated with determined alive part of the biomass were even higher. Those rates increased from 0.30 mg NO_3-N $*(h*g\ VS)^{-1}$ to 3.38 mg NO_3-N $*(h*g\ VS)^{-1}$ for no shear stress mode and ultrasound treatment respectively. This suggests that despite decrease in bacteria viability actual activity of biomass increases which leads to higher observed denitrification rates.

Tab. 2 Summary of biofilm parameters and obtained denitrification rates

Tab. 2 Podsumowanie uzyskanych wskaźników usuwania azotanów i parametrów biofilmu

Parameter	Unit	Control conditions	Ultrasound treatment
Thickness	um	813+/-254	335+/-104
Density based in TS	$g\ l^{-1}$	71+/-6	101+/-25
Density based in VS	$g\ l^{-1}$	45+/-4	58+/-14
Viability	[%]	31+/-1	15+/-1
Denitrification rate	gNO_3- $N/d*m^2$	0.49+/-0.19	1.17+/-0.12
Specific denitrification rate	mg NO_3-N/h *gVSS	0.09	0.58
Specific active denitrification rate	mg NO_3-N/h *gVSS	0.30	3.38

3.6. Solids and COD in influent

Successful application of the tested membrane biofilm reactor for polishing final effluent depends also on the obtained COD, TSS and VSS concentrations in the effluent, particularly if this is the last treatment step and there is no post-filtration.

After initial detachment of biomass due to the sudden change of hydrodynamic conditions (between testing phases) effluent solids stabilized at levels consistently below 20 mg l^{-1}, which is lower then widely adopted discharge limit of 30 mg TS l^{-1} from wastewater treatment plants. The results presented in the Table 3 show that the average effluent concentrations of total solids are equal to 16+/-10 mg l^{-1} and 12+/-0.3 mg l^{-1} in reactor with no shear force and ultrasound application respectively. The volatile solids concentrations in effluent were equal to 13+/- 8 mg l^{-1} and 7+/-2 mg l^{-1} for two tested conditions.

Tab. 3 Influent and effluent total and volatile solids concentrations

Tab. 3 Zawiesina w dopływie i odpływie z/do reaktora

		no shear force		ultrasound treatment	
	unit	influent	effluent	influent	effluent
TSS	mg l^{-1}	7+/-2	16+/-10	16+/-13	12+/-0.3
VSS	mg l^{-1}	5+/-2	13+/-8	7+/-2	7+/-2

Total and soluble COD can be affected by VSS concentration and the excreted soluble EPS thus COD breakthrough is one of the main concerns for membrane biofilm reactors. The averaged values of COD influent and effluent from reactor for no shear force mode and for operation with ultrasound treatment are presented on Fig. 7. When no shear force was applied significant fluctuations in total COD were detected. Due to biomass sloughing the total effluent COD increased occasionally up to 80+/-1.5 mg COD l^{-1} .However no significant changes in the average influent and effluent soluble COD during steady state conditions were observed. Soluble influent and effluent COD remained statistically unchanged and were on the average equal to 51+/- 3 mg COD l^{-1} and 54+/-3 mg COD l^{-1}.

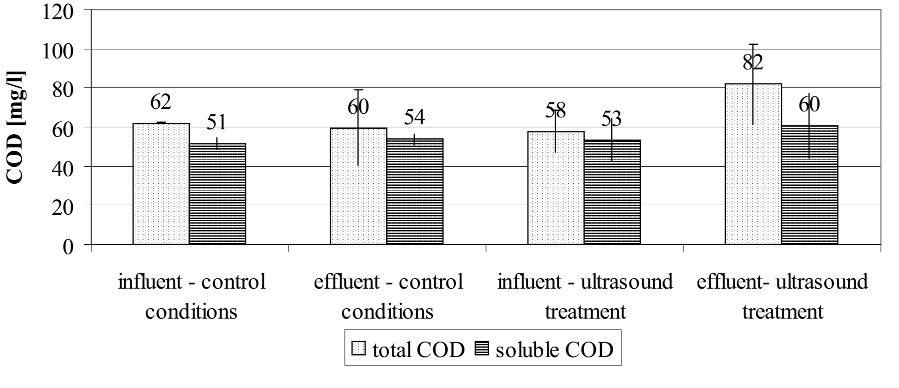

Fig. 7 Changes in total and soluble COD

Rys. 7 Zmiany w wartościach ChZT w dopływie i odpływie

Ultrasound treatment allowed to minimize the biofilm thickness due to declumping of biofilm and thus resulted in increased total COD in the effluent (Figure 7).

The total COD measured in the effluent was equal to 82+/-20 mg COD l^{-1} while values determined for influent were equal to 58+/-10 mg COD l^{-1}. No significant changes in soluble COD were detected. The influent and effluent soluble COD was equal to 53+/-10 mg COD l^{-1} and 60+/-16 mg COD l^{-1} respectively. The values measured in the effluent during steady state conditions were in the range of 60-102 mg COD l^{-1} which is below local discharge requirements of 150 mg l^{-1}. These results suggest that no intensive cell rapture or EPS extraction occurred for the chosen dosage of ultrasound. Proper ultrasound treatment is thus able to improve MBfR performance without risk of COD breakthrough.

4. Conclusions

1. Ultrasound treatment improved denitrification rates by reducing biofilm thickness.
2. Operation without any shear force resulted in biofilm thickness of around ~ 800 µm, while ultrasound treatment decreased the thickness to ~ 300 µm.
3. The highest average and maximum denitrification rates, equal to 1.17 gN (d $*m^2)^{-1}$ and 1.34 gN (d $*m^2)^{-1}$ respectively, were obtained for ultrasound treatment.
4. The specific active denitrification rates increased from 0.30 mg NO_3-N $(h*gVSS)^{-1}$ when no shear force was applied to 3.38 mg NO_3-N $(h*gVSS)^{-1}$ for ultrasound treatment suggesting that ultrasound can be viable tool increasing MBfR performance.
5. Ultrasound treatment has no significant impact on volatile solids, EPS and carbohydrates content.
6. Ultrasound treatment minimizes biofilm thickness, protein content and bacteria viability which results in higher bacteria activity and high observed specific denitrification rates.
7. No significant sloughing of biomass and COD breakthrough was observed during steady state conditions.

5. Acknowledgment

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