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BIOFILM IN GRAVITATIONAL SEWER SYSTEM AND ITS INFLUENCE ON WASTEWATER BIODEGRADATION

BŁONA BIOLOGICZNA W KANALIZACJI GRAWITACYJNEJ I JEJ WPŁYW NA BIODEGRADACJĘ ŚCIEKÓW

Abstract: The biofilm layer developed on the wall of sewer pipes is a common formation in the natural environment. The biofilm can be defined as a natural accumulation of microorganisms on the solid body surface, like the pecton covering the surface of solid in the river water or the devices of the biological sewage treatment plant. The proportions of particular species in biofilm composition are different in various WWTPs or rivers due to the environmental condition influence. In the sanitation a fresh sewage is characterized by high concentration of pollutants in a form of organic compounds with particles of various properties and dimensions. It forms the environment rich in nourishment substrates available both for the biofilm and the suspended biomass. The biofilm in sewers is spatially strongly heterogeneous. It consists of caverns, channels and pores filled with liquids or gases depending on actual environmental conditions. It can be said that it is a potently diversified system, important in the biodegration of sewage in gravitational sewerage. Basing on the field and the literature examinations the authors will present the most important sewer biofilm parameters and their influence on the sewage biodegradation, as well as a basic model of this process.

Keywords: sewer biofilm, gravitational sewer systems, sewage biodegradation in sewer conduits

The biofilm layer developed on the walls of sewer pipes is a common formation in the natural environment. The biofilm can be defined as a natural accumulation of microorganisms on the solid body surface [1, 2], like the pecton covering the surface of solid in the river water or covering the devices of the biological sewage treatment plant [2–6]. The proportion of particular species in the biofilm composition are different in various places of *wastewater treatment plants* (WWTPs) or rivers due to the environmental condition influence. In the sanitation, a fresh sewage characterized by high concentration of pollutants in a form of organic compounds with particles of

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various properties and dimensions, forms the environment rich in nourishment substrates available both for the biofilm and the suspended biomass.

Biofilm consists of microorganisms embedded in a glycocalyx that is predominately composed of microbially produced exopolysaccharide (EPS) [1, 7]. But in the sewer system, biofilm also contains a large fraction of inorganic material eg zeolite, sand, etc, organic, like fats and higher species of saprobe microfauna which can have a significant impact on the biofilm properties (Fig. 1) [2, 3, 8].



Fig. 1. Typical composition of biofilm in sewer system [2, 8]

The biofilm microorganisms develop the extracellular organic compounds enabling sedentary lifestyle. The mucus rich biofilm surface protects the microorganisms against washing away by the flowing sewage. It also stabilizes environmental conditions, prevents drying during the low filling of the conduits, and isolates from several chemical compounds and bacteriophages [2, 9–12]. The presence of biofilm considerably extends the mean residence time of heterotrophic biomass microorganisms inside the sanitation system. The development of significant populations of slower developing bacteria, such as sulfur reducing or executing methanogenesis is then possible [1, 2].

The population of bacteria existing inside the sanitation system is characterized by the ability to produce the innercellular spare materials – eg glycogen, which minimize the disparity in nutrients availability. It has a special significance inside the sanitation conduits of small diameter and high irregularity of sewage flow.

Biofilm structure

The fully developed biofilm is consisting of the characteristic layers [2, 13], determined by the availability of oxygen at the deeper located zones. The spatial variability of oxygenation results in the differences of structure properties, composition and transformation rate of chemical compounds inside the biofilm. The highly loaded biofilm contains, in the respective layers, the majority of microorganisms functional groups causing the pollutants biodegradation.

The spatial distribution of specific organisms groups defines the biological activity in the different zones of biofilm (Fig. 2). The research results show that the heterotrophic bacteria usually outnumber the autotrophic ones [2]. The heterotrophic bacteria spatial

| Zone | Predominant Bacteria | Metabolic Process | Limiting S | Reactants + Products |
|-----------------------|--------------------------------------|--------------------------------|------------|-------------------------|
| | | | | |
| Aerobic | Aerobic + nitrifying | Aerobic respiration | | CH2O |
| | neterotrophs | Heterotrophic nitrifacation | O2 CH2O | NO2 NO3 |
| | <i>Beggiatioa</i> –like filaments | Sulfur storage | | |
| Micro - aerophilic | Nitrate reducers | Nitrate reduction | | ♥ N2 |
| | Denitrifiers | Denitrification | O2 | |
| | Facultative anaerobes | Fermentation | | H ₂ S |
| Anaerobic | Sulfate reducers | Sulfate reduction | | |
| | Facultative anaerobes | Fermentation | | CO ₂ |
| Anaerobic | Methanogens | Methanogenesis | CH2O(?) | |
| | Facultative anaerobes | Fermantation | | |
| | | | ····· | - |

Fig. 2. The zones and processes in a typical stratified sewer biofilm [2]

distribution inside the biofilm is almost uniform, although the anaerobic facultative bacteria number increase from the level of 10^7-10^8 in deeper layers to 10^9-10^{10} CFU \cdot cm⁻³ (CFU – *colonies forming units*) in the surface layer. The population of nitrifying bacteria sustains inside the sanitation biofilm at the level of 10^4-10^6 MPN \cdot cm⁻³ (MPN – *most probably number*). It was proven that only about 25 % of all alive bacteria of surface layer is active metabolically in the real conditions, the rest remain inactive. Moreover, about 65 % of the whole biomass localized inside the biofilm in sanitation system forms the inert mass for biochemical transformations [2, 9].

The biofilm density increases along with the distance from the surface, from 14 kgTSS \cdot m⁻³ (TSS – *total suspended solids*) in the surface zone to 97 kgTSS \cdot m⁻³ in the deepest localized zone. The research shows that change in biofilm porosity also occurs, for the layers thicker than 500 µm, from 83–90 % for the surface layer to 67–64 % for the deepest layers [2]. The biofilm density also varies according to the location at wetted perimeter and increases, along with the height, from the sewage free surface to the bottom of the conduit.

The spatial distribution of prokaryotic microorganisms is also diversified by their rate of growth, because the faster developing groups usually cover the slower growing ones. Hence, the microorganisms of higher development rate accumulate near the surface of biofilm. According to the quantity of available nutrient substrate microorganisms inside the biofilm develop with different rate. Moreover, the prokaryotic organisms of biofilm are being permanently consumed by the eukaryotic heterotrophs.

The inner side of sanitation pipe walls is covered by the biofilm not only beneath but also above the wastewater free surface. This situation is connected to the frequent oscillation of sewage free surface level and also to the fact that nutrients may be transported by and consumed also directly from the humid air. It is clearly visible in locations of the turbulent flow – backdrop manholes or the other places of increased aerosols generation. Though, biofilm developed over the wastewater free surface is characterized by the noticeable lower oxygen activity than observed in case of the biofilm located beneath the surface. This difference is caused by the increased content of fat in the whole mass which, despite the decreased OUR activity (OUR – oxygen uptake rate), results in the increased resistance to erosion [2].

The biofilm developed inside the gravitational sanitation conduits reaches the thickness of, from a several, to over ten millimeters. The biofilm thickness changes and reaches its minimum at the high sewage flow velocity. The observed fibrous structures increase the process of oxygen consumption, thus reducing the oxygen uptake dependence from its concentration in the sewage [14, 15]. The numerous macro-pores, called "water channels" separated by the colonial organisms biomass are observed at biofilm surface of intensely irregular topography [13, 16]. The macro-pores facilitate mass transfer through biofilm then influencing the diffusion efficiency.

The exhaustive studies, describing the three dimensional processes of mass transport and biofilm development are not directly used in sewage biodegradation model. A number of expensive calibration measurements is required but the results quality may not clearly outperform the results of standard, less complicated models [13]. So the minimal number of input data, offering the satisfying results quality is required [1, 2, 11, 16]. This approach is illustrated in Fig. 3 containing the charts of real concentration changes of compounds dissolved in water, including the biofilm (left). The simplified form is also presented in Fig. 3 (right). Figure 3 presents also the boundary layer concentration for nitrates, influenced by nitrate usage in anoxic zone.



Fig. 3. A sketch of the boundary layer and the penetration depth, full model on the left and simplified one on the right [2, 8, 9]

Transformations of compounds containing biogenic substrates

The transformation processes of biogenic substrates occur inside the sanitation systems permanently or in the periodical pattern. But their influence on total pollutants load during the transport to WWTPs is limited.

The transformations of nitrogen may occur inside the biofilm layer – Fig. 4. However, the nitrifying bacteria quantity is usually about 1000 times smaller than the number of surrounding heterotrophic bacteria, hence nitrification inside the sanitation system plays only a minor role among the other biochemical processes. Denitrification inside biofilm occurs as along as nitrates originated from infiltration water are present. The nitrate presence in biofilm and wastewater does not affect the oxygen diffusion process in the biofilm [2]. Urea and protein hydrolysis are important sources of ammonium ion, thus considering the fact that these compounds are described by COD.



 \bigstar Reactions cataysed by procaryotes are marked with an asterisk

Fig. 4. Key reactions in the cycling of nitrogen species occurring in sewer system. The redox state of nitrogen is displayed on the left [2]

Figure 5 presents transformations of sulfur compounds in gravitational sanitation systems. The processes connected to the cycle of sulfur transformations result in pipes erosion and odour nuisance.



Fig. 5. Key reactions in the cycling of sulphuric species occurring in sewer system. The redox state of sulphur is displayed on the left, on the basis of [2, 8, 14]

Dynamic equilibrium of biofilm thickness

The biofilm thickness depends on the variety of different factors. The most important are: the growth of active cells biomass, accumulation of extracellular polymers and inert materials originated from the biomass dissolution as well as deposition of particles contained in sewage. The microorganisms biomass loss may be caused by the eukaryotic and prokaryotic organisms as well as the bacteriophages activity [2].

Erosion caused by the hydrodynamic effects of sewage flow results in a gradual but continuous biofilm mass loss. Abrasion and erosion causes the similar results. Shear stress at the level of $3-4 \text{ N} \cdot \text{m}^{-2}$ and above usually stops increase of biofilm depth on sanitation pipes walls. In case of pipes of diameter < 0.4 m, shear stress of given value appears at sewage flow velocity equal to about $1 \text{ m} \cdot \text{s}^{-1}$, for the pipes of larger diameter this velocity reaches the value of $1.2-1.4 \text{ m} \cdot \text{s}^{-1}$ [8].

The shear stress, after exceeding the limit value, causes periodical separation/erosion and transport of parts of biofilm downstream in a form of suspended biomass. This process may be supported by the internal transformations of the biofilm, resulting in decrease of strength of the layers of biofilm due to substrates or gases destroying the biofilm from inside [2].

All processes mentioned above together with the extreme events, like sudden uncontrolled flow with a high velocity or poisonous substances discharge, result in the reduction of the active biomass of biofilm. Atrophy of biofilm occurs also as a result of biomass extinction caused by deficiency of nutrients. As a final effect of these transformations, biodegradable substances immobilized inside biofilm are transformed to the soluble substrates by enzymes. The dissolved substrates may be used by biofilm organisms or diffuse outside the biofilm where they are transported by the stream of flowing sewage.

Biofilm dimensions, as it was reported in the literature [14], both, surface area and thickness depend on hydrodynamic conditions of flow inside sanitation pipes more than on biomass increase. The hydrodynamic conditions of flow are characterized by a high variability in time and length of the selected sanitation conduit. Moreover, flowing sewage stream generates shear stress of different values in different points of an active cross-section. The highest values are reached close to the pipe bottom and the lowest near the side walls close to the wastewater free surface. The abrasion forces caused by solid suspension drag are also very important in biofilm removal. Biofilm resistance to removal may be increased by fat settlement along the walls of sanitation pipe, it is especially important close to the free wastewater surface [2, 9, 10, 15, 17, 18].

Thus, the influence of velocity flow and shear stress on structure and properties of biofilm is evident [15]. It was proven during the laboratory studies at high values of shear stress when the eroded elements of biofilm caused biodegradation of available COD up to 80–90 % [14]. Up to 90 % of the whole biomass eroded by shear stress is being removed during the first 1–2 minutes of stress impact [2]. It was also observed that biofilm fragments separation declines after limiting the inflow of nutrients [2, 19]. The biofilm developed during the flow inducing shear stress of low value erodes significantly faster than biofilm developed in the environment of shear stress of high

value. The highest biofilm accumulation may be, in turn, achieved at shear stress value about 1.5 N \cdot m⁻². The rapid change of sewage flow velocity influence the mass transport and biofilm thickness while the long-lasting slow changes of the same amplitude result in biomass structure changes.

Modelling of biofilm influence on sewage biodegradation

The model presented below consists of two important processes: hydrolysis of suspended fractions and heterotrophic biomass growth in biofilm. Both are coupled with mass exchange terms. The research conducted *in situ* showed that biofilm is an important factor of transformation and biodegradation processes of pollutants in sanitation systems [2, 8, 20, 21]. In some specific cases its share in sewage biodegradation may be higher than the share of suspended biomass. This situation appears during the flow of low value shear stress and minor filling of the sanitation pipe. The internal biofilm processes application in a numerical model of sewage biodegradation allows to obtain the result comparable to the results obtained by models considering only the suspended biomass [4], with simultaneous possibility of decreasing the number of considered hydrolyzing fractions. The sewage temperature is a significant element of presented modeling description, it is important because it highly influences the enzymatic activity of microorganisms.

Hydrolysis

Considering the hydrolysis of *n* suspended fraction (each one hydrolyzes with different rate) in sewage, caused by enzymes of suspended biomass and biofilm in given temperature, the following equation describing the ρ intensity of this process may be written [8]:

$$\rho_{h,n} = k_{hn} \frac{X_{Sn} / X_{Bz}}{K_{Xn} + X_{Sn} / X_{Bz}} \frac{S_O}{K_O + S_O} \left(X_{Bz} + \varepsilon X_{Bf} \frac{A_{Bf}}{V} \right) \alpha^{(T-20)}$$
(1)

where: k_{hn} – hydrolysis rate constant for the *n*-th suspension fraction [d⁻¹],

- K_{Xn} saturation constant for the hydrolysis of *n*-th suspension fraction [gCOD \cdot gCOD⁻¹],
- X_{Sn} concentration of *n*-th hydrolysable fraction [gCOD \cdot m⁻³],
- X_{Bz} heterotrophic active biomass in form of suspension [gCOD \cdot m⁻³],
- X_{Bf} heterotrophic active biomass in biofilm [gCOD \cdot m⁻²],
- S_O dissolved oxygen concentration [gO₂ · m⁻³],
- K_O saturation constant for the dissolved oxygen [gO₂ · m⁻³],
- A_{Bf} / V ratio of wetted surface of biofilm to sewage volume = R_h^{-1} [m⁻¹], ε – constant of biomass efficiency [-],
 - α temperature coefficient for processes in sewage [-],
 - T temperature [°C].

Heterotrophic biomass growth in biofilm

In order to describe the growth rate of heterotrophic biofilm biomass in aerobic conditions the deterministic model based on information presented in [8, 22] may be used:

$$\rho_f = k_{1/2} S_O^{1/2} \frac{Y_{Hf}}{1 - Y_{Hf}} \frac{S_S}{K_{sf} + S_S} \frac{A_{Bf}}{V} \alpha_f^{(T-20)}$$
(2)

where: $k_{1/2}$ – reaction rate constant of 1/2 power for surface usage of dissolved oxygen by biofilm [gO₂^{1/2} · m^{-1/2} · d⁻¹],

- oxygen by biofilm $[gO_2^{1/2} \cdot m^{-1/2} \cdot d^{-1}],$ Y_{Hf} – efficiency rate for X_{Bf} (yield coefficient) $[gCOD \cdot gCOD^{-1}],$
- K_{Sf} saturation constant for biofilm dissolved biodegradable fraction [gCOD · m⁻³].

The knowledge of a minimal number of kinetics and stoichiometric factors is required to presented description of pollutant fraction hydrolysis rate and heterotrophic biofilm biomass development. But the presented model is not directly connected to the conduit hydraulics because the steady state of biofilm thickness is assumed.

Conclusions

The information presented above and the cited literature reports allow to draw the following conclusions concerning the composition, structure and biofilm preservation in different substrate load and variable shear stress:

- The fully developed, mature biofilm (older than 12 days) is more resistible to erosion than the younger biofilm.

- The amount of eroded biomass depends to the shear stress value.

- The amount of biomass removed from the biofilm by the increasing value of shear stress results from the conditions of biofilm development – the higher stress during the development phase causes more stable and resistible biofilm.

- The higher shear stress during the biofilm development results in its unequal spatial development.

- Sewer biofilm is a strongly diversified system playing an important role in sewage biodegradation process, but still not sufficiently recognized and described.

- The inner biofilm processes application in a numerical model of sewage biodegradation allows to obtain the results comparable with the results obtained by models considering only the suspended biomass, with simultaneous possibility of decreasing the number of considered hydrolyzing fractions.

- The significant element of presented modeling description is also the sewage temperature, important because it influences the enzymatic activity of saprobe micro-organisms.

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References

- [1] Bishop P.L.: Biofilm structure and kinetics. Water Sci. Technol. 1997, 36(1), 287-294.
- [2] Huisman J.L.: Transport and transformation process in combined sewers. IHW Schriftenreihe 2001, 10, 1–180.
- [3] Łagód G., Malicki J., Montusiewicz A. and Chomczyńska M.: Wykorzystanie mikrofauny saprobiontów do bioindykacji jakości ścieków w systemach kanalizacyjnych. Arch. Environ. Protect. 2004, 30(3), 3–12.
- [4] Łagód G., Sobczuk H. and Suchorab Z.: Modelling of transformation and biodegradation of pollutants in sewer system. Proc. ECOpole '05. Jamrozowa Polana–Hradec Kralove 2005, 159–165.
- [5] Łagód G. and Sobczuk H.: Transformation and biodegradation of pollutants in sewer systems as a processes leading to sewage self-purification. Ecol. Chem. Eng. 2006, 13(3–4), 247–254.
- [6] Łagód G., Malicki J., Chomczyńska M. and Montusiewicz A.: Interpretation of the results of wastewater quality biomonitoring using saprobes. Environ. Eng. Sci., 2007, 24(7), 873–879.
- [7] Raunkjær K., Hvitved-Jacobsen T. and Nielsen P.H.: Transformation of organic matter in a gravity sewer. Water. Environ. Res. 1995, **67**(2), 181–188.
- [8] Hvitved-Jacobsen T.: SEWER PROCESSES Microbial and Chemical Process Engineering of Sewer Networks. CRC PRESS, Boca Raton–London–New York–Washington 2002.
- [9] Bishop P.L., Tian C.Z. and Yun-Chang F.: *Effects of biofilm structure, microbial distributions and mass transport on biodegradation processes.* Water Sci. Technol. 1995, **31**(1), 143–152.
- [10] Æsoy A., Storfjell M., Mellgren L., Helness H., Thorvaldsen G., Odegaard H. and Bentzen G.: A comparison of biofilm growth and water quality changes in sewers with anoxic and anaerobic (septic) conditions. Water Sci. Technol. 1997, 36(1), 303–310.
- [11] Hermanowicz S.W.: Two-dimensional simulations of biofilm development: effects of external environmental conditions. Water Sci. Technol. 1999, 39(7), 107–114.
- [12] Lewandowski Z., Webb D., Hamilton M. and Harbin G.: *Quantifying biofilm structure*. Water Sci. Technol. 1999, **39**(7), 71–76.
- [13] Beyenal H. and Lewandowski Z.: Modeling mass transport and microbial activity in stratified biofilm. Chem. Eng. Sci. 2005, 60, 4337–4348.
- [14] Norsker N.H., Nielsen P.H. and Hvitved-Jacobsen T.: Influence of oxygen on biofilm growth and potential sulfate reduction in gravity sewer biofilm. Water Sci. Technol. 1995, **31**(7), 159–167.
- [15] Wilderer P.A., Cunningham A. and Schnidler U.: Hydrodynamic and shear stress: report from the discussion session. Water Sci. Technol. 1995, 32(8), 271–271.
- [16] DeBeer D., Stoodley P. and Lewandowski Z.: Liquid flow and mass transport in heterogeneous biofilms. Water Res. 1996, 30(11), 2761–2765.
- [17] Ahyerre M., Chebbo G. and Saad M.: Sources and erosion of organic solids in a combined sewer. Urban Water 2000, 2, 305–315.
- [18] Banasiak R., Verhoeven R., De Suttera R. and Tait S.: *The erosion behavior of biologically active sewer sediment deposits: Observations from a laboratory study.* Water Res. 2005, **39**, 5221–5231.
- [19] Tijhuis L., Hijman B., van Loosdrecht M.C.M. and Heijnen J.J.: Influence of detachment, substrate loading and reactor scale on the formation of biofilms in airlift reactors. Appl. Microbiol. Biotechnol. 1996, 45(1-2), 7–17.
- [20] Hvitved-Jacobsen T., Vollertsen J. and Nielsen P.H.: Koncepcja procesu i modelu dla przemian mikrobiologicznych zachodzących w ściekach w kanalizacjach grawitacyjnych. Materiały Międzynarodowej Konferencji Naukowo-Technicznej "Usuwanie związków biogennych ze ścieków", Kraków 1997, 227–239.
- [21] Hvitved-Jacobsen T., Vollertsen J. and Nielsen P.H.: A process and model concept for microbial wastewater transformations in gravity sewers. Water Sci. Technol. 1998, **37**(1), 233–241.
- [22] Almeida M.C., Butler D. and Davies J.W.: Modelling in-sewer changes in wastewater quality under aerobic conditions. Water Sci. Technol. 1999, 39(9), 63–71.

BŁONA BIOLOGICZNA W KANALIZACJI GRAWITACYJNEJ I JEJ WPŁYW NA BIODEGRADACJĘ ŚCIEKÓW

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Abstrakt: Warstwa błony biologicznej narastająca na ściankach przewodów kanalizacyjnych jest formacją pospolicie występującą w środowisku naturalnym. Szeroką definicję błony biologicznej stanowi stwierdzenie, że jest to naturalna akumulacja mikroorganizmów na powierzchni ciała stałego. Przykładem może być chociażby pekton pokrywający przedmioty zanurzone w wodzie rzecznej, czy też biofilm porastający zanurzone w ściekach części urządzeń biologicznej oczyszczalni ścieków. Jednakże proporcje udziału poszczególnych gatunków w składzie biofilmu kanalizacyjnego są inne niż w oczyszczalniach czy rzekach, ze względu na różne warunki środowiskowe. W systemach kanalizacyjnych świeże ścieki charakteryzują się dużą koncentracją zanieczyszczeń w postaci związków organicznych o różnorodnych właściwościach oraz rozmiarach cząstek. Tworzy to środowisko życia mikroorganizmów bogate w składniki odżywcze, dostępne zarówno dla błony biologicznej, jak i biomasy zawieszonej. Błona biologiczna ma silnie heterogeniczną strukturę jakościowo-przestrzenną z licznymi zagłębieniami i porami wypełnionymi cieczą bądź gazem, w zależności od aktualnych warunków środowiskowych. Można więc stwierdzić, że jest mocno zróżnicowa-nym systemem, odgrywającym znaczącą rolę w procesach biodegradacji ścieków w kanalizacji grawitacyjnej. Korzystając z badań terenowych oraz literaturowych, autorzy prezentują najważniejsze właściwości biofilmu kanalizacyjnego, jego wpływ na biodegradację ścieków oraz podstawowe modele opisujące ten proces.

Słowa kluczowe: błona biologiczna, kanalizacja grawitacyjna, biodegradacja ścieków w kanalizacji