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CHARACTERISTICS OF BIOFILM GROWING IN MOVING-BED BIOFILM SYSTEM

Biofilm was tested in the last phase of an experiment in a system with moving-bed biofilm carriers. The experiment was carried out in order to examine an integrated nitrogen and carbon removal. In every reactor of the system, the biofilms differed, both in their physical properties and microbiological composition. The biofilm thickness ranged from 50 to 300 μm. The most numerous bacteria fraction in the biomass were heterotrophs which dominated in all the reactors and constituted from 90.9% to 99.9% of active bacteria population. In the active bacteria population, there were at most 0.8% of nitrifiers.

1. INTRODUCTION

Biofilm is a microorganism population adhering to the surface of carrier, usually surrounded with extracellular polymer layer [5]. It forms a spatially heterogeneous, porous, three-dimensional structure consisting of microcolonies, around which there are channels filled with liquid. The channels have numerous branches forming a net which connects microcolonies with the liquid above the biofilm. They are important in substrate transport from liquid to microcolonies and in reaction product transport in the opposite direction. The extracellular mucilage is the habitat for organisms. It surrounds microcolonies and allows the transport of substrata from liquid to bacteria [2], [4], [5], [8], [10]. The structure of biofilm and its physical properties depend on hydrodynamic conditions in reactor, composition of wastewater fed to the reactor, biofilm age, and its species composition, which has been proved by investigations [4], [5], [8] and computer simulations [7]. The aim of this paper was to describe the biofilm growing in moving-bed biofilm reactor system.

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2. METHODS

The biofilm samples were taken from a system in which the process of nitrogen and carbon removal was investigated [3]. The system consisted of four reactors, in the first and the third ones (R1 and R3) denitrification took place, while in the second and the fourth ones (R2 and R4) – nitrification. In the system, biomass was grown on movable carriers made of polyethylene, whose density ranged from 0.92 to 0.96 kg/dm^3. The carriers were shaped as cylinders of the diameters of about 9 mm and the height of 7 mm, floating freely in wastewater.

In the biofilm samples, such physical properties as the biofilm thickness and specific surface area were determined. Biofilm thickness was measured under the microscope using a micrometric net. Specific surface area was determined by means of adsorption method using the Lissamine Scarlet 4R dye. Visual investigations of biofilm structure were carried out using a scanning electron microscope. The biofilm dry matter and volatile matter were also determined according to Polish norms (PN–78/C–04541). Additionally the carbonates were bonded by the water saturated with carbon dioxide. Biological properties of the biofilm were determined by microscopic analysis of its composition. The number of saprophytes was determined by means of the Koch plate method. The titre of ammonia and nitrite oxidizers were determined by inoculating them on the Winogradski mineral medium, and the titre of denitrifiers was determined on the Giltay medium.

3. RESULTS

The biofilm thickness in the system investigated varied from 5 to 300 μm. This was a result of biofilm detachment (in R1 and R3 reactors) and the differences in substrate loading. The thickest biofilm grew in the presence of oxygen which promoted simultaneous nitrification and oxidation of organic matter. At low loading with substrate and at high turbulence in the reactor, thin biofilm grew up. Its thickness did not exceed 50 μm. Essential differences in biofilm volatile matter per unit of carrier surface were due to the differences both in the biofilm thickness and its structure. A considerable porosity and fully developed specific surface of biofilm were observed in the nitrifying reactors (R2 and R4). In the denitrifying ones (R1 and R3), thicker biofilm of smaller porosity was formed. Similar observations were reported by other researchers [1], [9]. In all reactors, the biofilm growing on an internal surface of the carrier was thicker. The external surfaces were covered with very thin layer of biofilm, which filled the surfaces between carrier teeth. The layer of mineral sediment was formed on the surface of carrier. The thickest layer of the sediment was in the R1 reactor (to 280 μm), a slightly thinner – in the R3. The sediments detached themselves from the carriers in these reactors.

In the R1 reactor, a biofilm thickness varied from 20 μm to 160 μm, reaching on an average 65 μm (table). This resulted from the detachment of the sediments and the biofilm from a carrier. The biofilm adhered to a thick layer of mineral sediments of porous structure. The pores can be considered to be ventilating and transport channels. There were also filamentous forms of bacteria and dendritic structures protruding from the biofilm surface (figure 1). Highly specific surface area of biofilm per a unit of a carrier area testified to large accessible surface for mass exchange between the biofilm and the liquid. This biofilm was formed by the bacteria representing *Zoogleа* sp. Few free-living ciliates and colourless flagellates were found in the biofilm. Filamentous bacteria were rarely observed.

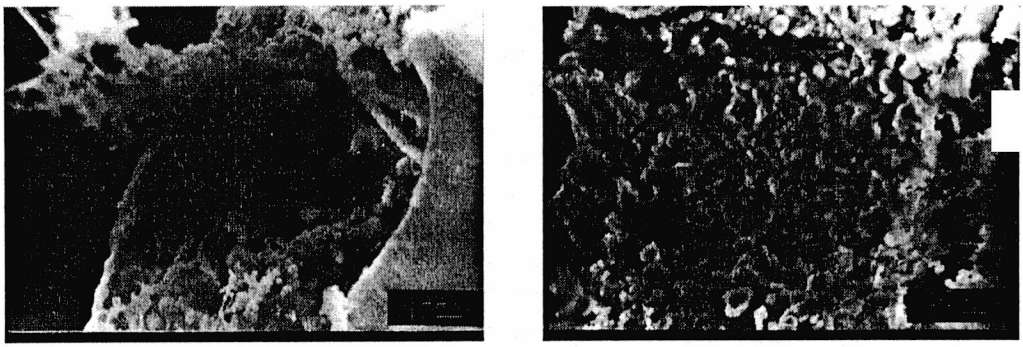

Fig. 1. Biofilm from reactor R1 (denitrification process) – transverse section

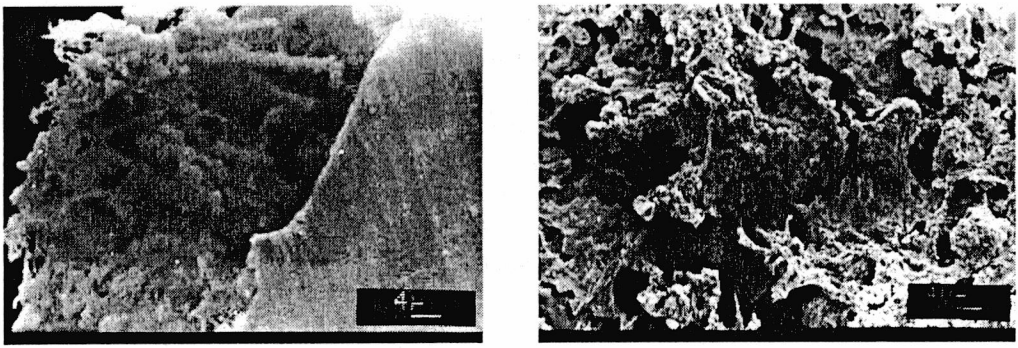

Fig. 2. The cross-section and the surace of biofilm from reactor R2

The biofilm was thickest in the R2 reactor and its thickness ranged from 142 to 312 μm. The specific surface area of a biofilm per a unit of a carrier area in the R2 was smaller than in the R1. This may be due to intense activity of large quantities of ciliates and rotifers which diminish the thickness of bacterial layer. The specific

surface area per a unit of the biofilm volatile solids (twice as great as that in reactors with denitrification) testified to extreme porosity of biofilm. Microscopic observations showed that in biofilm, numerous filamentous bacteria occurred. They were a good basis for growing colonies of the *Zooglea* sp. The biofilm comprised a great number of stalk ciliates and a small number of free-living ciliates, not very numerous rotifers, small numbers of nematodes. Biofilm composition testified to correct work of the reactor. The porous structure of the biofilm (figure 2) allows the contact between its surface and deeper layers and bidirectional flow of reactants and reaction products. The presence of filamentous and *Zooglea*-like bacteria can be noticed.

In the R3 reactor, the biofilm distribution on the carrier elements was clearly unequal. The biofilm thickness varied from 5 to 40 μm. The biofilm specific surface area per biofilm volatile mass unit in the R3 was similar to that in the R1. This proves that the biofilm porosity in these reactors was similar. Microscopic examinations showed that the biofilm had irregular surface with vents and spongy structures formed by the *Zooglea* sp. and filamentous bacteria. No metazoa were found in this reactor.

The changes in the biofilm thickness in the R4 reactor and in the R3 reactor were very similar, but the specific surface area per volatile mass unit of the former was very large. This testified to loose structure of this biofilm and numerous pores. Such a structure was formed in conditions when the biofilm growth was restrained by lack of ammonium nitrogen. Both this biofilm and the biofilm in the R2 reactor were formed by filamentous and *Zooglea*-like bacteria. In the R4 reactor, large number of stalk ciliate occurred, not very high number of free-living ciliate, rotifers, few nematodes. The presence of *Vorticella microstoma* and *Opercularia* sp. in the R2 and R4 proved that dissolved oxygen concentration dropped to 1 mg/dm^3 and even below this value in the liquid layer adjoining the biofilm. This means that this layer is important in the system tested.

The test carried out showed that heterotrophic bacteria were most plentiful and dominating in all reactors. Their percentage share in the population of active bacteria in biofilm ranged from 90.9 % to 99.9%. The greatest fraction of denitrifying bacteria was found in the R3 reactor, which proved that conditions for their growth were favourable. The number of denitrifying bacteria in the R1 was considerably lower. It may have resulted from biofilm suppression in this reactor and from stimulating the growth of heterotrophs by dissolved oxygen supplied by recirculating wastewater. A considerable number of denitrifying bacteria in the R2 testified to favourable conditions for denitrification. This was due to a relatively thick biofilm (over 200 μm) and high ammonium nitrogen loading. The smallest fraction of denitrifying bacteria was found in the R4. In this reactor, denitrification practically did not take place because of a thin and highly porous biofilm, which made it possible for the oxygen to penetrate all the biofilm layers.

Table

Results of biofilm testing

Parameter		Unit	Reactor R1	Reactor R2	Reactor R3	Reactor R4
Biofilm thickness	Average	μm	65	212	24	38
	Minimum	μm	20	142	5	5
	Maximum	μm	160	312	40	50
Biofilm mass	Average	mg v.s.s./1 element	8.448	2.809	7.017	1.563
		g v.s.s. /m^2	15.09	5.02	12.53	2.79
	Maximum	mg v.s.s. /1 element	10.54	4.51	9.81	2.32
		g v.s.s. /m^2	18.82	8.05	17.52	4.14
	Minimum	mg v.s.s. /1 element	5.48	1.02	3.33	0.42
		g v.s.s. /m^2	9.78	1.82	5.95	0.75
Specific surface area		m^2/m^2	1312.7	985.9	869.8	942.7
		m^2/g v.s.s.	87.0	196.5	69.4	337.7
Saprophytes		total bacteria number/cm^3 biofilm	7.7×10^5	1.2×10^6	1.5×10^9	7.5×10^6
		total bacteria number/g v.s.s.	3.3×10^6	5×10^7	2.9×10^9	1×10^8
Denitrifiers		bacteria number/cm^3 biofilm	1.3×10^4	4.0×10^4	1.5×10^8	5.6×10^3
		bacteria number/g v.s.s.	5.7×10^4	5.1×10^7	2.8×10^8	7.7×10^4
Ammonia oxidizers		bacteria number/cm^3 biofilm	$<10^2$	10^4	3.4×10^3	2.1×10^3
		bacteria number/g v.s.s.	$<1.2\times10^3$	4.3×10^5	6.6×10^3	2.9×10^4
Nitrites' oxidizers		bacteria number/cm^3 biofilm	$<10^2$	<10	3.4×10^3	2.1×10^3
		bacteria number/g v.s.s.	$<1.2\times10^3$	$<3.6\times10^3$	6.6×10^3	2.9×10^4
Bacteria fraction	Saprophytes	%	98.26	95.88	90.91	99.87
	Facultative heterotrophs	%	1.68	3.29	9.09	0.07
	Ammonia oxidizers	%	<0.03	0.82	0.0002	0.03
	Nitrites' oxidizers	%	<0.03	<0.007	0.0002	0.03

In active bacteria population, the share of nitrifiers approached at most 0.8%. Its greatest fraction was found in the R2 reactor. In this reactor, the autotrophs found advantageous conditions for development because the biofilm thickness was large enough to form a protecting heterotroph layer. This layer prevented the autotrophs from detaching from biofilm, which compensated for their low growth rate. Small fraction of nitrite oxidizers indicated that in this reactor short-range processes of nitrification and denitrification, without nitrites' oxidation, took place. The fraction of autotrophs in the R4 was considerably smaller and amounted to 0.06 % for ammonia and nitrite oxidizers. This may have resulted from little biofilm thickness (on an average 38 μm). Therefore, the nitrifiers were not prevented from detaching from the biofilm and were not able to form an internal layer in the biofilm. This may be due to a low concentration of substrates for nitrification.

The greatest number of heterotrophs was found in the R3 reactor. In other reactors, their number was lower by about two orders of magnitude. The values of our results are lower than these obtained by OKABE [6] and ZHANG [10]. They established that the total number of heterotrophs in heterotrophic and heterotrophic–autotrophic biofilm approached 10^9–10^{11} bacteria/cm^3, while in autotrophic biofilm, this figure ranged from 10^7 to 10^9 bacteria/cm^3. The number of denitrifying bacteria and autotrophs were also lower. Only the results for the R3 reactor, where the total number of nitrifiers reached 10^3 bacteria/cm^3 and that of denitrifying bacteria 10^8 bacteria/cm^3, were convergent with the literature data [10].

4. SUMMARY

In the moving-bed biofilm system, the biofilm thickness ranged from 50 to 300 μm and was considerably greater on the internal surfaces of carriers. The external surfaces were covered with a thin biofilm layer that filled the surfaces between the teeth of a carrier. Biofilms grown in all reactors tested differed both in physical properties and microbiological composition. The higher the organic loading of the reactor, the greater the biofilm thickness. Specific biofilm area in the reactors with nitrifiers was about twice as large as that in the reactors with denitrifiers. It was also observed that the biofilm porosity in these reactors was considerably higher. Heterotrophs were most numerous fraction of bacteria in biomass and constituted from 90 to 99.9% of active bacteria population, while autotrophs constituted at most 0.8% of active bacteria population.

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CHARAKTERYSTYKA BIOFILMU PORASTAJĄCEGO RUCHOMY NOŚNIK W PROCESIE ZINTEGROWANEGO USUWANIA AZOTU I WĘGLA

Badania biofilmu przeprowadzono w ostatniej fazie eksperymentu zintegrowanego usuwania azotu i węgla z ruchomymi nośnikami biomasy. Biofilm w każdym reaktorze układu był inny zarówno ze względu na własności fizyczne, jak i skład mikrobiologiczny. Jego grubość zmieniała się w granicach od 50 do 3000 μm. Najbardziej liczną grupą bakterii w biomasie były bakterie heterotroficzne, które dominowały we wszystkich reaktorach i stanowiły od 90,9% do 99,9% populacji bakterii aktywnych. Bakterie nitryfikacyjne stanowiły co najwyżej 0,8% populacji bakterii aktywnych.

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