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Research Article

Analysis of Biofilm in Anaerobic Wastewater Treatment Reactor

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ABSTRACT

The relevance of the research problem derives from the insufficient number of studies on the populations of the bacterial ecosystems in the anaerobic fluidized-bed bioreactors.

Current study was aimed at the examination of the formation of the anaerobic biofilm on the carrier surface during the treatment of liquid sludge from primary and secondary settling basins of the wastewater treatment plant. The experimental studies were conducted in the laboratory setting; optical, scanning and transmission electron microscopic examination was performed to determine the composition of the anaerobic populations in the steady state.

Biofilm development on the carrier surface was found to proceed in several stages, the biofilm was firmly attached to the load surface, having an average thickness of 1-3 mm. Microbial population of the mature biofilm had heterogeneous composition, with the prevalence of two morphological families – Methanothrix on the surface and Methanosarcina in the lower layers of the film, and Bacilli-, cocci-, spirilla- and sarcina- were present either singly or in fragments of flocculi. the populations were embedded in the exopolysaccharide matrix with the highest density within the layers adjacent to the carrier. An extensive network of channels, going through the whole volume of the matrix and facilitating emission of gases, formed during substrate decomposition, and transfer of nutrients to the lowest layers of the film was found. The content of this paper can be useful for the experts and scientists, specializing on the issues of the biological treatment of wastewater.

Keywords: biological wastewater treatment, fluidized bed, biofilm, bioreactor, biological populations.

INTRODUCTION

Wastewater produced during human life and industrial development has large negative impact on the environment. Wastewater treatment plants in settlements and industrial plants constitute the major safety barrier, protecting water bodies from polluted wastewater. Biochemical treatment plants extensively implement biological methods, based on the activity of aerobic and anaerobic microorganisms.

At aeration plants anaerobic fermentation of organic sludge is applied to raw sludge from the primary settling basins, excessive active sludge, or a mixture of both. Digested residuals are either disposed at sludge beds or mechanically dehydrated.

Biological methods of wastewater treatment, based on the use of immobilized microorganisms, are of great scientific and technical interest. They provide a solution for the problem of separation of microorganisms from wastewater treated with flow bioreactors, avoiding construction of additional units for segregation of sludge mixture and treated wastewater [Gvozdyak 1987; Rymovskaya and Ruchai 2008; Leikin *et al*. 2008; Zhukova and Morozov 2010; Degradation of phenol ... 2002, Doaa and Wafaa 2009]. Vertical anaerobic fluidized-bed bioreactors have recently become widespread. The surface of the carrier contains immobilized cells. These bioreactors work on the principle of complete displacement, in which each previous volume of fluid is displaced by the subsequent and does not mix with it. The principle of operation of the fluidized bed bioreactor is that the carrier

particles entrained by the upward flow of gas or liquid are suspended therein [Werther 2007]. With a properly selected high-speed mode, the carrier particles reach the upper expanding part of the bioreactor, stop rising and then return to the column, which allows them to be kept in the working area of the reactor, despite the continuous pumping of the medium [Serebrennikova et al. 2015].

Fluidized-bed mode of operation has several advantages, such as:

1. The 'clogging' of the working area of the reactor is prevented, which may occur in packed beds when using carriers with a developed surface area or active growth of microbial cells;
2. The liquid is distributed evenly within the load containing immobilized cells [Sokol and Korpal 2006].
3. The fluidized bed is replenished with microorganisms due to the regulated cell growth in the reactor, which allows maintaining the required amounts of active immobilized biomass [Shieh and Keenan 1986; Sokol and Halfani 1999].

Immobilized microbial cells have higher operation flexibility and are able to maintain viability and metabolic activity over long periods of time. Immobilization allows concentrating large amounts of active biomass, expands the residence time and prevents removal of biomass with increased volumes of wastewater [Hallas *et al.* 1992; Cassidy *et al.* 1996; Ilyina *et al.* 2004].

Biofilm ecosystem has higher resistance to the impact of unfavorable factors, than suspended bacteria. Biofilms are microbial cities, as they can comprise a great number of various microbial species, such as bacteria, protozoa, fungi and algae, each with specific metabolic functions [Watnick *et al.* 2000; Costerton *et al.* 1999; Tets 1998; Costerton *et al.* 2003; O'Toote *et al.* 2000, Davies 2003; Bester 2010]. Modern trends in biotechnology allow exploiting successfully the opportunities of the immobilized microflora to solve a wide range of tasks, including environmental ones.

A wide application of these methods is constrained by a small number of studies on the formation and populations of microorganisms of biofilms of an anaerobic fluidized-bed reactor.

METHOD

The studies were carried out on installations mounted in laboratory conditions. The installation scheme is shown in Figure 1. The reactor operated in a batch mode, the loading of effluents was processed through the upper part of the unit, and after the completion of the studies the sediment was pumped out. The anaerobic reactor was divided into two zones (fluidized-bed zones and separation zones) by a partition. As a loading, plastic cylinders and spheres were used.

The volume of each reactor was 25 L, the operating temperature was maintained at 30^0C, and the processing time of the cultivated medium was 2 days. Control reactor was operating simultaneously in an unloaded state to determine the effect of specified load on the substrate processing during biofilm formation. These facilities made it possible to carry out a complex of experimental studies of the processes of biological treatment with a fluidized bed both aerobically (with an open top of the unit and supplying oxygen) and anaerobically (with the hermetically sealed upper part of the unit).

As a test medium, liquid sediment was used from the primary and secondary settling tanks of a wastewater treatment plant.

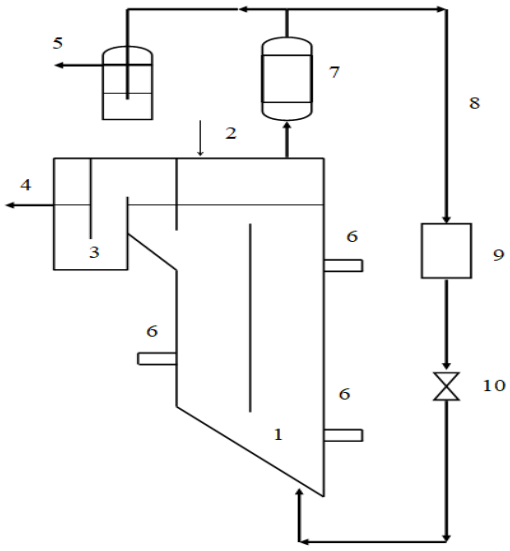

Fig. 1. Schematic diagram of an anaerobic reactor.
1. Biological reactor. 2. Supply of sewage for cleaning. 3. Hydraulic lock. 4. Output of

treated effluents. 5. Output of gas. 6. Sampling. 7. Cooling jacket. 8. Gas recirculation line. 9. Compressor. 10. Gas supply adjustment.

Sampling was carried out by taking the loading material from the upper, middle and lower parts of the installation. Samples were taken every 1 hour to study the growth rate of the biofilm. A sample of the loading with mature biofilm was fixated in vapours 25% glutaraldehyde for 30-40 minutes for further examination in a scanning electron microscope and in 5% formalin for topographic microscopy.

RESULTS AND DISCUSSION

The experimental studies of anaerobic fermentation processes were performed in the fluidized-bed reactor. The results of the tests allowed revealing the dynamics of immobilization processes and biofilm activity on the surface of the carriers.

During the study, observations were made of the behavior of the biofilm of an anaerobic fluidized-bed sewage treatment reactor. Biofilm on the surface of the carrier was formed and developed in several stages, similar to those described in the classical microbial growth: lag, exponential and stationary phases. So in the first stage (duration 4 hours), attachment of individual microbial cells contained to the loading surface was observed. In the second stage (duration 8-12 hours), fixation and formation of microcolonies which are firmly connected to each other and to the loading takes place. In the third stage (duration 16-20 hours), maturation of the biofilm takes place; the microcolonies are surrounded by a protective matrix, and special structures - pores, canals and cavities – appear in it. The fourth stage is characterized by growth – the maturation of the biofilm. At this stage, the biofilm is detached from the surface of the carrier, which is caused by a lack of nutrients in the lower layers of the biofilm; the processes of releasing the load from the microorganisms begin. However, the film is quickly restored after mechanical damage, and the mature biofilm re-emerges again within 24 hours.

It was also shown that aggregate-containing samples of the reactor medium coagulated in 15 minutes. It indicates possible agglomeration of small aggregates and formation of large bundles within the reactor.

Based on the observation data, a working hypothesis on the formation of aggregates of anaerobically mixed cultures, shown in Fig. 2, was adopted for further research, which helped to structure the research directions and explain the behavior of the biofilm population in the anaerobic reactor.

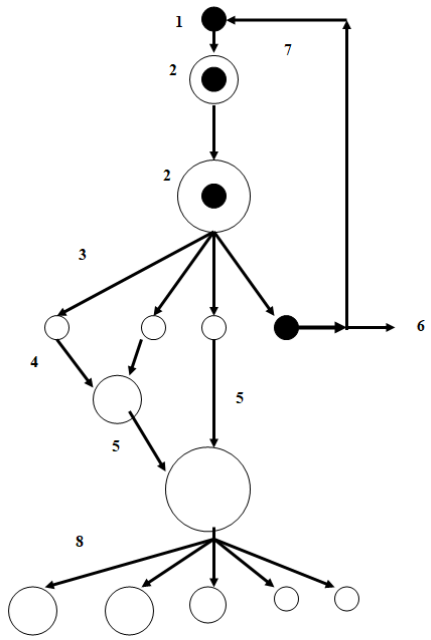

Fig. 2. Working hypothesis of the development of aggregates of anaerobically mixed culture. 1. Loading material. 2. Formation of biofilm on a carrier. 3. Separation of biofilm into smaller parts (fragments). 4. Adhesion of small parts (fragments); 5. Growth of aggregates. 6. Transfer of feed material from the reactor. 7. Return of the load to the biofilm formation process. 8. Fragmentation of large particles.

Optical microscopy revealed that the biofilm mostly consisted of heterogeneous microbial populations, dominated by two morphological families of bacteria – *Methanothrix* and *Methanosarcina. Methanothrix* was mostly present on the surface of the film, and *Methanosarcina* inhabited the lower layers of the film, attached to the load surface.

The presence of methane archaea of the genera Methanosarcina and Methanothrix is important in the anaerobic degradation of organic substances to methane. In the absence or lack of Methanosarcina and Methanothrix, anaerobic decomposition can result in acidic and acetogenous fermentation, which will lead

to accumulation of volatile fatty acids (oily, propionic and acetic), lowering the pH and stopping the process.

In the microbial composition of the film, heterogeneous bacterial populations were observed. *Bacilli*-, *cocci*-, *spirilla*- and *sarcina*-containing cysts of various size were present either separately or in the floccular fragments. *Sarcina*-containing cysts were found in large aggregates, being tightly attached to other bacteria. Biofilms also contained numerous bacterial spores and minute amounts of unicellular eukaryotes.

Different types of cells were combined into small, closely packed conglomerates or microcolonies. Some microcolonies contained only one morphological species, however, most microcolonies contained several different types of cells (Fig. 3). Long filamentous bacilli were the most frequent representatives of the surface microflora.

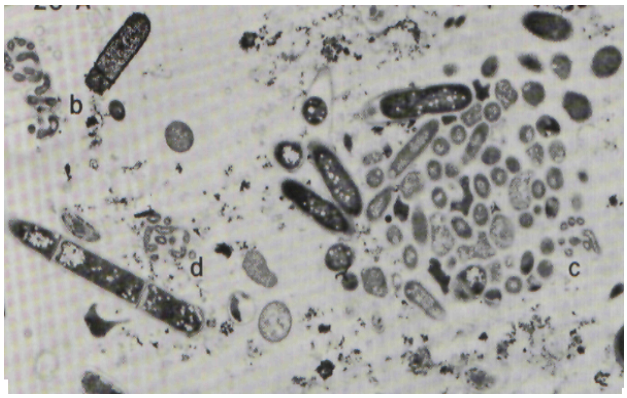

Fig.3. Microcolonies found in biofilms (magnified 9500 times).

Microbiological populations were embedded in the exopolysaccharide matrix with the highest density within the layers adjacent to the carrier. An extensive network of channels, going through the whole volume of the matrix and facilitating emission of gases, formed during substrate decomposition, and transfer of nutrients to the lowest layers of the film was registered. Cocci cells had less developed matrix than cells of any other type [Kadyseva, 2007].

Cell communities were combined into polymer matrices; these aggregates continuously increased in size due to bacterial growth and sought to coalesce among themselves. However, their growth was hampered by the presence of other large-sized aggregates. In addition, it was found that the position of individual bacteria in the aggregates is continuously changing – at the junction 'aggregates-liquid' and in the intervals between subaggregates it was possible to observe population differences. Often microcolonies formed, penetrating into the deeper layers.

It has been established that particles of a carrier with an excessive amount of adsorbed cells are most often concentrated in the upper part of the bioreactor and are eventually removed from it with a liquid stream.

The results of optical microscopy of biofilms showed:

- the density of the biofilm is high at the periphery and low in the center of the aggregates;
- biofilm thickness ranged from 1 to 3 mm in all reactors;
- the film was firmly attached to plastic load parts;
- the film on the inner surface of the reactor had no microbial disruptions, was uniform and solid.

Scanning electron microscopy revealed that the whole biofilm surface was irregular and rough (Fig.4), crater-like bulges with 100 to 500 mcm-wide dents were found mostly in thick parts of the film. Many crystals of various forms and up to 100 mcm large were embedded in the biofilm.

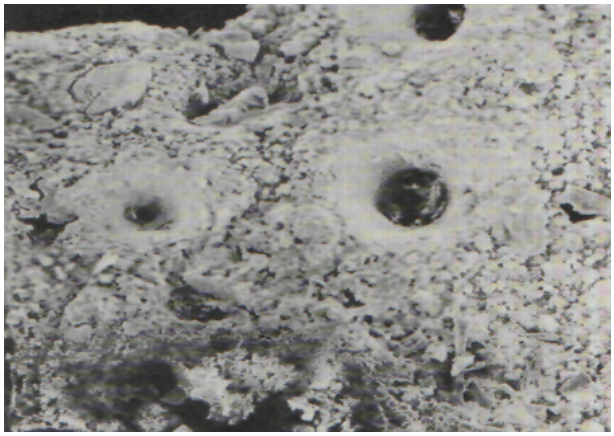

Fig.4. Scanning electron micrograph of biofilm attached to the load (magnified 800 times).

The results of current study are aimed at systematization of various methanogenic organisms in heterogeneous cultures, as the

existing studies of bacterial cultures have been focusing exclusively on testing pure and enrichment laboratory cultures. To further study the populations of biofilms of an anaerobic reactor, it is necessary to develop a biofilm formation model that corresponds to the stages of methane formation during anaerobic wastewater treatment, which will help develop control mechanisms for anaerobic treatment.

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