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Biotechnology

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SPECIAL PROPERTIES OF FREE-FLOATING AND IMMOBILILIZED ACTIVE SLUDGE BIOTIC COMMUNITY

The biotic community composition of free-floating and fiber-porous carrier immobililized active sludge from Ivatsevichi treatment plant aerotanks, functioning under nitri-denitrification conditions, has been studied. Differences in flock structure, species composition and proportion of main indicative groups of free-floating active sludge organisms for different technology lines have been revealed. About 50 species of organisms have been identified. The ability of the fiber-porous carrier to adsorb the considerable amount of sludge biomass has been shown.

Introduction. The systems using carriers for biomass immobilization are getting increasingly widespread in constituction and reconstruction of treatment facilities.

The elements made of fiber-porous nonwoven fabric proved to be the best carriers now being in use [1]. They are produced in the form of hollow cylindrical elements with an internal diameter of 45-55 mm and wall thickness of 5-10 mm. The carrier porosity is 65-80%, while the average pore diameter is 50-70 microns. Small fibers diameter (50-80 microns), high porosity of the carrier and the pore size comparable with that of the active sludge (AS) flocks, create favorable conditions for their retention. High concentration of bacterial mass, in turn, attracts organisms of higher trophic levels eating it. As the result, the specific biocenosis is formed on the carrier, differing from that in zones, not equipped with the carrier. This biocenosis is stable toward the change of its functioning conditions (drain composition, sewage pH, time of aeration, concentration of the dissolved oxygen in water, etc.). Application of the immobilized organisms improves purification from hardly oxidized organic substances, intensifies processes of nitrogen compounds removal, decreases the risk of active sludge swelling and increases oxidizing power of the aerotank [2].

Main part. The purpose of the work has been to determine the level of development and specific features for biocenosis of AS, free-floating and immobilized on the carrier.

The object of research was the sludge suspension selected from denitrification and nitrification zones of four technological lines at treatment facilities of Ivatsevichi. Inside each line, a separate sludge suspension circulation contour has been created, from secondary settling tanks to sludge regenerators and from nitrification zones to denitrification zones. As the result, despite identical composition of arriving sewage, these lines function in rather isolated mode. Nitrifiers of each technological line are equipped with fiber-porous carriers of biomass. For studying of the immobilized biocenosis, the carrier elements taken from nitrification zones of the 2nd and 4th lines have been used.

The study has been carried out during the period from November, 2012 to February, 2013.

The analysis of AS was performed using a microscope "Biological". For studying of freefloating sludge, the "calibrated drop" method was used [3]. Roiled sample was studied microscopically. Large hydrobionts were examined at $\times 100$ magnification. Smaller organisms localized within active sludge flocks and hardly distinguishable at smaller magnification, were examined at $\times 400$. The absolute quantity of organisms in the volume unit of sludge suspension and the total quantity of the organisms belonging to the main indicator groups were determined.

Identification of organisms was done by morphological features [4–5].

Flock characteristics were determined at $\times 40$, $\times 100$ and $\times 400$ magnifications.

The immobilized biocenosis was studied by microscopic examination of longitudinal and cross cuts of the carrier element at $\times 40$ and $\times 100$ magnifications.

The biomass retention ability of the carrier was determined by a mass difference between the carrier element from nitrification zones of 2nd and 4th lines dried up to the constant mass, before and after removal of AS biomass. For biomass removal from the carrier, it was treated with concentrated sulfuric acid at 85–90°C for 15–30 min, then washed with distilled water.

About 50 species of protozoa and metazoan have been found in the biocenosis of free-floating AS, and from 20 to 28 of these species occur together. In some cases it was difficult to identify the exact species, and identification was done to a genus. 7 species of testate amoebae and more than 3 species of naked amoebae, 9 species of attached, 9 – free-swimming, 2 – crawling ciliates and more than 5 species of suctorian, 3 species of flagellates, 5 types of rotifers are revealed. Nematodes, oligochaetes and tardigrades have been found as well. It has been established that in all four technological lines, the biocenosis composition shown only slight variations throughout the whole research period. However, the quantity of organisms of some species greatly varied in the sludge suspension samples selected from different lines. Total number of protozoa and metazoan within the studied period fluctuated from 7 to 16 thousand organisms/cm³ in 1st and 2nd line biocenoses and from 5 to 44 thousand organisms/cm³ in 3rd and 4th line biocenoses.

It is shown that biocenoses of all lines are subject to fluctuations of the number of organisms from the main indicator groups, that is characteristic for treatment facilities of small settlements (Fig. 1–2).

More favorable situation, both on species variety and on ratio of indicator groups was observed in the 1st and 2nd line biocenoses (Fig. 1). Peritrichous ciliates constituted the considerable share (30–70%) in them at all times, with most of them belonging to *Epistylis* and *Opercularia* genes. *Vorticella convallaria*, species has been found in considerable amounts, both the usual *V. convallaria* var. typica and the V. *convallaria* var. similis forms being noted. This fact provides the evidence of high concentration of the dissolved oxygen in nitrification zones of these lines.

Small testate amoebae of *Centropyxis* sp. which were present in the nitrification zones of the 1st and 2nd lines on AS flocks caused the increase their weight and better sedimentation. Naked amoebae in AS of these two lines were rather big. The share of free-swimming ciliates and hypotrichas was usually insignificant. The quantity of nematodes in these biocenoses was found to be insignificant that suggests good mixing and lack of stale zones. Rotifera and suctorian occured constantly, the fact demonstrating a high level of biocenosis development.

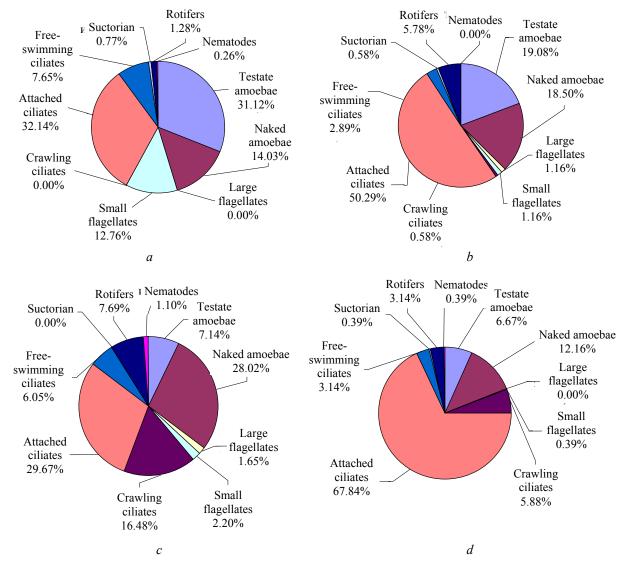


Fig. 1. Distribution of active sludge organisms by main indicator groups in the nitrification zone of the 2nd line: *a* – December, 2012; *b* – January, 2013; *c*, *d* – February, 2013

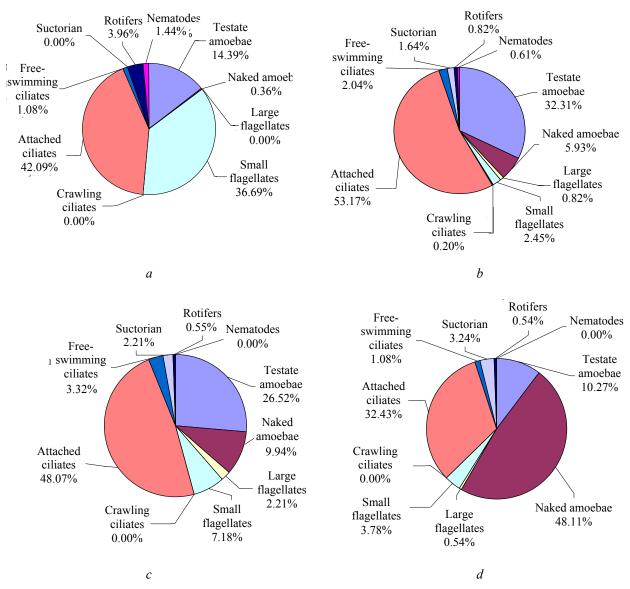


Fig. 2. Distribution of active sludge organisms by main indicator groups in the nitrification zone of the 4th line: *a* – December, 2012; *b* – January, 2013; *c*, *d* – February, 2013

In the 3rd and 4th line biocenoses, a great share of peritrichous ciliates (32-56%) has been observed as well (Fig. 2), however here, in addition to Epistylis and Opercularia genes and Vorticella convallaria species, common for treatment facilities, Vorticella submicrostoma species (tolerant to low oxygen concentration and higher loads by organic compounds) widely occurred. Another characteristic sign for the 3rd and 4th line biocenoses was periodic sudden population increases of small flagellates, small testate amoebae Pamphagus hyalinum and small flagellant amoebae. The total share of these indicator groups during the research period reached 50-60%. It indicates lower level of biocenosis development and can be a sign for sewage treatment process violations, in particular, caused by dissolved oxygen deficiency in the sludge suspension.

As a rule, alternation of aerobic and anaerobic zones in the same construction leads to formation of more dense, compact, well structured flock of active sludge. This has been observed for AS of the 1st and 2nd lines throughout the entire research period (Fig. 3 *a*, *b*). The average size of flocks here generally is 200–400 microns. At the same time AS flocks of the 3rd and 4th lines were of smaller size (50–170 microns), with loose edges (Fig. 3 *c*, *d*). Besides, there was a large number of small, poorly formed flakes in sludge suspension. The data obtained by microscoping correlate with sludge index indicator values that for lines 1 and 2 was within 80–100 cm³/g, and for lines 3 and 4 – 120–190 of cm³/g.

Sedimentation dynamics of AS (fig. 4) confirms this. Flocks of the 1st and 2nd lines almost completely precipitate within 15 min, while flocks of the 3rd and 4th lines undergo more uniform and slow sedimentation.

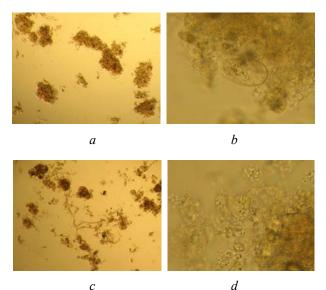
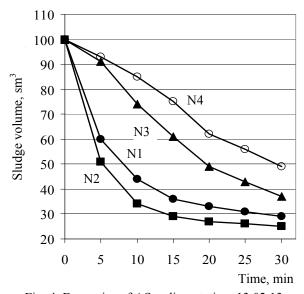
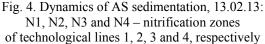


Fig. 3. Characteristic structure of active sludge flock of the 1st and 2nd lines $(a - \times 40, b - \times 400)$ and the 3rd and 4th lines $(c - \times 40, d - \times 400)$

Mass concentration of AS (a sludge dose) in sludge suspension of all lines was rather high, varying at $2.6-4.3 \text{ g/dm}^3$, the average being about $3.0-3.5 \text{ g/dm}^3$.

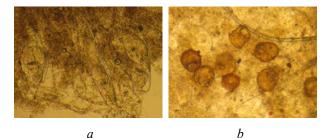




No AS filamentous bulking has been detected during the research on treatment facilities of Ivatsevichi, despite intake of sewage from the distillery with the high pollution level.

The biocenosis of immobilized AS (Fig. 5) is identical by specific composition to the biocenosis

of free-floating one, but contains much higher concentration of both the bacterial mass and higher trophic level organisms (rotifera, nematodes, predatory fungi, oligochaetes, etc.).



a bFig. 5. Biocenosis organisms immobilized on the carrier in the nitrification zones of the 4th line 30.11.12: a – rotifers; b – testate amoebae (×100)

It has been established that in pores of the fiber-porous carrier, a significant amount of AS flocks (Fig. 6) is collected. Retention ability of the carrier by biomass in the nitrification zone of the 2nd line in January-February was slightly higher, 0.109-0.112 g/g, while for the nitrification zone of of the 4th line it was within 0.093-0.100 g/g. This phenomenon can be explained by smaller packing density in the carrier pores for looser flocks of the 4th line. Retention ability of the carrier increased by 33–48% reaching 0.139–0.193 g/g, under mass development of large organisms, for example the oligochaetes, that was noted in the nitrification zone of the 4th line in November-December, 2012. Worms eat away sludge flocks from the pores of the carrier and promote renewing of biomass retained on it, improving mass exchange in this zone. However their mass development led to stripping of carrier and decreased its role in the nitrifier. Besides, violations of aeration procedure and decrease in dissolved oxygen concentration kill these organisms. Bodies of worms from the carrier get into sludge suspension, and their decomposition can lead to secondary pollution of water.

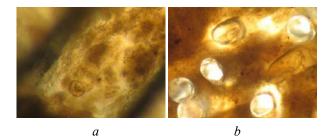


Fig. 6. The biocenosis immobilized on the carrier in the zone of nitrification of the 2nd line 16.01.13: a – active sludge on a surface of fibers (×100); b – active sludge in a time of an element of the carrier (cross cut of an element, ×40)

The total dose of the free-floating and immobilized active sludge in nitrification zones was up to $4.1-4.8 \text{ g/dm}^3$.

Conclusion. It has been shown during the research of free-floating active sludge of four technological lines at Ivatsevichi treatment facilities has shown that, despite the identical composition of initial sewage, autonomous recirculation of sludge on different lines leads to formation of biocenoses similar on their specific composition, but differing in quantitative ratio of organisms, both of various species and of the main indicator groups.

Free-floating active sludge of all lines is subject to considerable fluctuations of the number of organisms, that is caused by the change of sewage consumption and its pollution level by industrial dumpings. Upon the whole, biocenoses of the 1st and 2nd lines have higher level of development and better quality of flocks in their sludge suspension. Biocenoses of the 3rd and 4th lines, however, are subject to spontaneous rises of the number of organisms at the lowest trophic levels, their sludge suspension includes small loose flocks and has worse sedimentation characteristics. Thus, the higher destructive potential of the 1st and 2nd line biocenoses should be noted [2].

Introduction of the fiber-porous polymeric carrier into the nitrification zones promotes durable retention of the significant biomass amount in them, allowing to increase the sludge age. It is especially important for deep nitrification since the bacteria oxidizing ammonium nitrogen grow slowly.

Immobilized AS has virtually the same specific composition as free-floating, but with higher concentration of organisms. Retention ability of the carrier is lower in zones where the sludge suspension contains looser and poorly formed AS flocks, and it grows at increase of the number of the immobilized organisms from the highest trophic levels.

In the nitrification zones equipped with the fiber-porous polymeric carrier, the greater dose of sludge, as compared to the zones with free-floating sludge, is achieved. It causes more intensive pollution removal from sewage, provides deep nitrification, i.e. increases efficiency of treatment facilities.

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