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### CONSUMPTION OF PHOSPHATES FROM MEDIUM BY ACTIVATED SLUDGE BACTERIA UNDER VARIOUS CONDITIONS OF AERATION

The ability to consume phosphates from the medium has been assessed for bacteria isolated from activated sludge. There was selected an isolate, which cells has the highest level of phosphorus accumulation. It is shown that the most efficient consumption of phosphates from the medium per biomass unit is reached while incubating bacteria alternately under aerobic conditions and the conditions without aeration followed by the aerobiosis. The effect of the duration of the incubation stage without aeration on phosphates consumption has been studied.

**Introduction.** Due to the increase of compounds of nitrogen and phosphorus content in the city sewage and the aggravation of the problem of autrophication of reservoirs there was a change of priorities in the approach to sewage treatment. Now the main type of the pollution which is subject to removal, are considered to be biogenous elements – nitrogen and phosphorus. A large number of technological schemes is developed for the removal of nitrogen and the phosphorus base on alternation of zones with different level of aeration (aerobic, anoxic, anaerobic). The existence of various modifications of these schemes is caused by problematical character of providing conditions for combined effective nitrogen and phosphorus sewage treatment.

The creation of a strict anaerobiosis zone is a great problem because of the adverse relations of  $N_{av}/BOD$  and  $P/BOD$ , versions of schemes without preliminary primary settlement, with sediment acidification in primary settlers, with loading placement in the anaerobic zone for holding of the microorganisms carrying out fermentation are offered. The effective removal of phosphorus requires the increase in removal of excess sludge, while the age of sludge for providing nitrification should be not less than 5 days. The activated sludge contains not only aerobic and anaerobic bacteria, but also the representatives of aerobic microfauna for which the long period without oxygen is pernicious.

The optimal conditions for the development of the phosphate accumulating bacteria in activated sludge aren't determined. There are disembodied data on the influence of pH, some components of the environment on phosphate absorption by bacteria. So, it was established that volutin accumulation by the cells of activated sludge amplifies when alkalinizing acidic sewage water, at some stages of sewage treatment the increase of the quantity of cells with volutin under the microaerophilic conditions was observed [1]. For some species of bacteria, in particular *Brevibacterium casei*, for removal of phosphates from the medium the presence of ions of  $Mg^{2+}$ ,  $NH_4^+$  and  $\alpha$ -ketoglutarat [2] is required.

The aim of our work was to study phosphate consumption from medium under various condi-

tions of aeration by the bacteria allocated from activated sludge of treatment facilities. In the process of incubation of bacteria the order of alternation of aerobic conditions and conditions without aeration were changed, as well as the duration of the stage without aeration.

**Main part.** For allocation of pure cultures of the bacteria capable to accumulate phosphorus there was used sludge mixture from aerotanks of treatment facilities of the cities: Minsk, Mogilyov, Bobruisk, Malorita, Postavy, Klenovichi, Borovka, etc. To homogenize sludge mixture we performed the ultrasonic treatment with the frequency of 22 kHz during 30 s.

Primary determination of the phosphate accumulating ability of the bacteria allocated from activated sludge was carried out by staining the volutin granules in the cells with methylene blue of Leffler with the subsequent microscopic examination of the samples. Cytoplasm acquired blue colour, and volutin grains was stained violet-red.

In order to select isolates with the most expressed ability to accumulate phosphorus the overnight culture was incubated in nutrient broth with alternation of the aeration conditions: aerobiosis, anaerobiosis, aerobiosis. According to the difference of the phosphate content in the initial medium and after each stage of incubation, the amount of the absorbed phosphorus per dry biomass unit was counted. For the further research there were chosen isolates II and IV characterized by the highest level of phosphorus accumulation in cells.

Study of phosphorus consumption by the allocated bacteria was carried out according to the given scheme (fig. 1). Overnight culture of bacteria was brought into the nutritious broth and the incubation in ES-20 shaker incubator (200 RPM) was carried out. In the first series of experiments the aeration conditions of incubation were alternated (aerobic stage, stage without aeration, the aerobic stage, the incubation duration of each stage was 2 h), in the second series incubation was started with a stage without aeration and the duration of this stage was changed (1, 2 or 3 h). After the each stage of incubation bacterial mass

was separated by centrifugation at 8000 RPM within 20 min. Sediment was dried up at the temperature of 105°C and weighed. The content of phosphate-phosphorus in supernatant was determined by colorimetric method. 1 cm<sup>3</sup> of the mixed reactant was added to 1 cm<sup>3</sup> of investigated solution (10 cm<sup>3</sup> of 2,5% solution of ammonium molybdate, 10 cm<sup>3</sup> of 10% solution of ascorbic acid and 30 cm<sup>3</sup> of 2 N sulfuric acid). Samples were kept for 1 h in the thermostat at a temperature of 37°C, and then with the help of a photoelectric photometer KPK-3 the extinction at wavelength of 820 nm was determined. The concentration of phosphate-phosphorus was determined according to the calibration schedule.

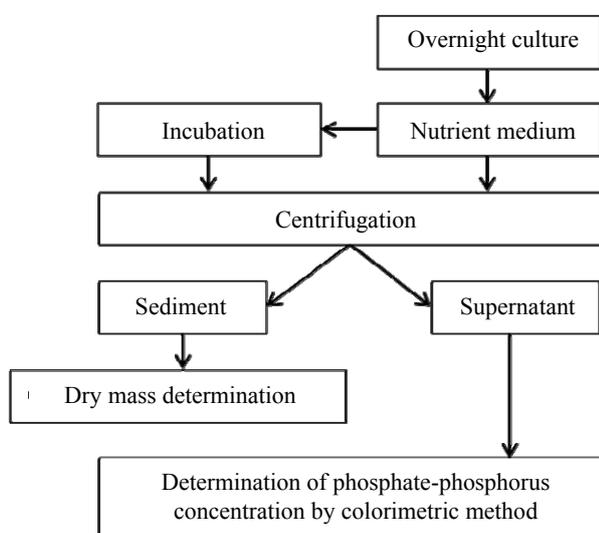


Fig. 1. Scheme of study the influence of aeration conditions on the ability of bacteria to absorb phosphate-phosphorus from the medium

According to the difference of phosphate-phosphorus concentration in a nutrient medium before and after each stage of incubation we determined the amount of the consumed phosphate-phosphorus. The value of the absorbed phosphorus per biomass unit was calculated.

At the first stage while incubating both isolates in the conditions of aeration there was observed the reduction of the phosphate-phosphorus content in the medium and the increase of concentration of phosphorus in biomass (Fig. 2, 3). At the subsequent stage of incubation in lack of aeration the same picture was fixed for isolate II, while in case of isolate IV there was an increase of the phosphate-phosphorus content in the medium due to the decrease of its content in biomass.

At the subsequent aerobic stage in both options the concentration of phosphate-phosphorus in the medium decreased again, and its content in biomass of bacteria increased either. As a whole, the quantity of the consumed phosphate-phosphorus

from the medium and accumulated phosphate-phosphorus in biomass of isolate II was approximately twice higher than that for isolate IV, therefore isolate II was used for further researches.

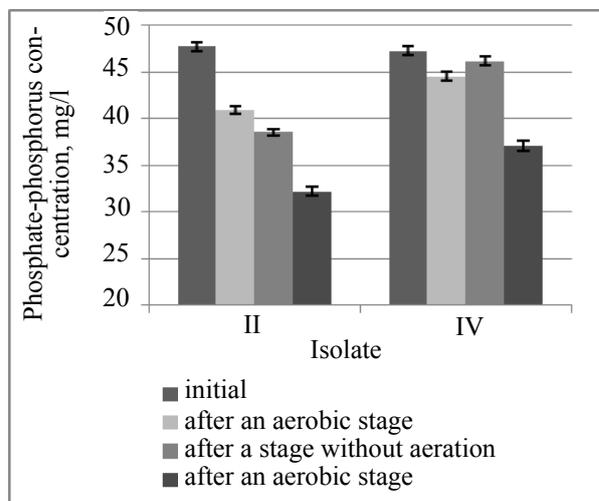


Fig. 2. Change of phosphate-phosphorus concentration in medium after incubation of bacteria under different conditions of aeration

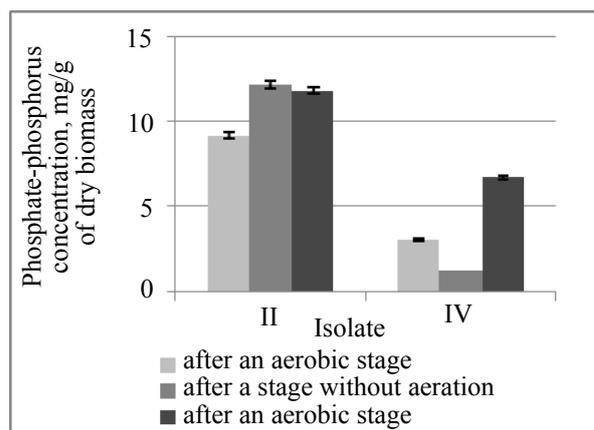


Fig. 3. Amount of the consumed phosphate-phosphorus per biomass of bacteria incubated under different conditions of aeration

In the second series of experiments the bacteria incubation was started with a stage without aeration, the duration of this stage was being changed. Such incubation led to insignificant decrease in the concentration of phosphate-phosphorus in the medium (Fig. 4). It should be noted that during the subsequent aerobic stage there also was no essential consumption of phosphate-phosphorus by the bacteria cells. There were determined no differences in the change of the phosphate-phosphorus content in the environment depending on incubation duration without aeration.

The amount of phosphate-phosphorus calculated per unit of bacteria biomass increased a little after the incubation stage under the aerobic condi-

tions and reached 3,0–3,3 mg/g of dry biomass (Fig. 5). It should be noted that consumption of phosphate-phosphorus by bacteria from the medium during the stage without aeration and the subsequent aerobic stage hasn't depended on the duration of the first stage. That fact showed that decrease in the consumption of phosphorus wasn't connected with the death of cells in aeration absence. Additionally the viability of cells was confirmed by the consumption of phosphate-phosphorus by cells and biomass increase at the subsequent aeration.

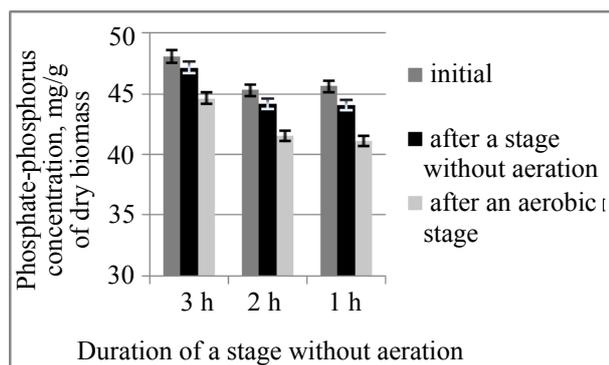


Fig. 4. Change of phosphate-phosphorus concentration in medium after different period of bacteria incubation without aeration

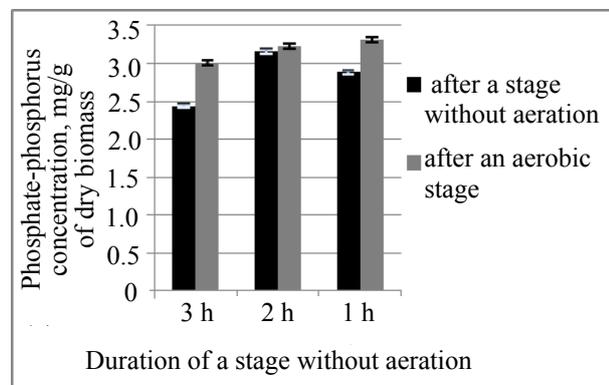


Fig. 5. Amount of the consumed phosphate-phosphorus per biomass unit after different period of bacteria incubation without aeration

At the same time, the consumption of phosphate-phosphorus from the medium and its accumulation in biomass was much more intensively when the incubation of bacteria was started with an aeration stage. The concentration of phosphorus in biomass of bacteria in this case reached 12–13 mg/g of dry biomass (Fig. 3).

**Conclusion.** After incubating the bacteria which were allocated from activated sludge and had shown the ability to accumulate phosphate-phosphorus in different conditions of aeration, we hadn't observed the regularities which the biological phosphorus removal is based on. According to biochemical model of this process under the conditions of aeration bacterium consume phosphate-phosphorus and allocate it into the medium under the anaerobic conditions while using easily oxidized material as a substratum (acetate) [3]. The authors of the review [4] underline that pure bacteria cultures which metabolism would correspond to this model aren't allocated from activated sludge, withdrawing of phosphorus is believed to be carried out by association of microorganisms, and the biological removal of phosphorus in the systems of activated sludge warrant further investigation.

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Received 28.02.2013