Investigation of probiotic agent influence on sewage quality and active sludge properties

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Abstract. One of the new and promising directions in the wastewater treatment technologies and treatment of precipitation is the use of the probiotic agents. A lot of brands of probiotics, which differ in their composition, concentration and cost are offered by the producers on the market. The research results of the “Pip Plus Water” probiotic influence on the concentration of organic contaminants in the model wastewater solution are presented in this article. An additional point is that the changes in the quality indices of the wastewater, which is taken from municipal sewage treatment facilities, because of the contact with a probiotic have been investigated. The characteristics, describing the active sludge compaction process before and after the addition of the probiotic agent, have been obtained. The preliminary study stage has showed that the probiotic agent being considered has a positive impact on the certain sewage quality indicators and the precipitation properties. The ability of “Pip Plus Water” to intensify primary precipitation, wastewater treatment by main indicators (suspended matters, COD) and to improve the sedimentation properties of the excess active sludge has been established.

1. Introduction
In order to preserve the natural environment, the normative documents [1] and sanitary-epidemiological authorities set rather stringent requirements to the wastewater treatment quality. Most CDP of Russia and CIS countries use the obsolete technologies and equipment that do not always allow achieving the required level of wastewater and precipitation treatment. Practically, there are no new methods that can provide an optimal correlation between the cost of resources and the quality of the result.

The issue of formation of large amounts of sludge, released in wastewater treatment processes is also very relevant. On the vast majority of CDP, the sludge is dried naturally on the sludge pits. This technology requires the alienation of the large ground plots and may be impractical in the environmental aspect.

In the current situation, it is relevant to consider new technologies that provide the required treatment efficiency without considerable increase of the cost of resources [1, 2]. One of these new technologies is the wastewater and its precipitation treatment method with the use of the probiotic agents.

2. Review
For the first time, the concept of probiotics was formed by the Nobel laureate biologist Ilya Mechnikov more than 100 years ago. He suggested that bacteria in yogurt can have a beneficial effect on the intestinal microbiome (1907) [3]. After this, D.M. Lilly and R.H. Stillwell (1965) [4], R.B.
Parker (1974), R. Fuller (1989), P. Lyons and R.J. Fallon (1992) and others continued to explore the idea and use the term "probiotic" - which means "for life."

According to the generally accepted definition, the probiotics are the preparations which contain living microorganisms and substances of microbial origin which, with the natural method of administration, have positive effects on the physiological, biochemical and immune responses of the host organism through the stabilization and optimization of its normal microflora functions [5-10].

The probiotic agents are widely used in medicine, veterinary medicine, food industry, cosmetics, etc. Recently, the preparations for the probiotic sewage treatment began to appear. The most common of these are: “Pip Plus Water” (Belgium), “SCD Bio Klean” (India), “Microbec” (Poland), “Oxidol” (USA), “Vodograi” (Ukraine), the probiotic of the company GK “Bio Vesta” (Novosibirsk, Russia).

The probiotic agents (probiotics) consist of probiotic bacteria and enzymes and do not contain chemical and mineral contaminants. The probiotic bacteria are non-pathogenic, non-toxic, they have high adhesive and antagonistic ability to pathogenic and opportunistic pathogenic bacteria. The technologies with the use of probiotics are referred to the environmental nature-friendly technologies. [8-13].

One of the modern wastewater treatment methods is based on the use of probiotic preparations with a large content of the probiotic microorganisms and enzymes designed for the rapid destruction of the organic substances in the wastewater, which allows to accelerate the process of their decomposition and to reduce the usual anaerobic processes, accompanied by the unpleasant odors and toxic gases’ release, significantly. This entails the ammonia, hydrogen sulfide and methane. In addition, the probiotic microorganisms, when ingested, absorb the substrate rapidly and do not leave any opportunities for the pathogenic micro-organisms’ development [11-13].

As a rule, probiotics include the anaerobic bacteria and sporogenous aerobic bacteria, which contribute to the processes of the water purification and the precipitation treatment at different stages. For example, the mechanic methods of water treatment can be influenced by Bifidobacterium and Lactobacillus anaerobic bacteria; the aerobic bacteria that make up the probiotic, such as Bacillus subtilis, Bacillus Licheniformis, Pumilus and Megaterium, and others [11-21], can influence biological purification.

As a result of the struggle for the nutrients and habitats, Bifidobacterium and Lactobacillus bacteria, produce a large number of organic acids and biologically active components, including antibiotics and hydrogen peroxide. Due to this, they expel the pathogenic bacteria and are able to inhibit the processes of the sediments’ anaerobic decay [11,12].

Lactobacillus anaerobic bacteria lead to the hydrogen peroxide formation in the water, which, after reaching the stage of the biological purification in the aerotank, decomposes under the influence of the activated sludge, forming the water and dissolved oxygen. In this case, according to the studies of a number of authors [11,12,16-20], there is an increase in the concentration of dissolved oxygen in the aerotank, which contributes to the effective biological decomposition of the organic contaminants.

The intensification of the biological wastewater treatment is also due to the vital activity of aerobic bacteria which are contained in the probiotic agents. Bacillus bacteria produce a large spectrum of the enzymes that help to break down the high molecular weight and hardly decomposable organic substances. As a result, the effect of the wastewater treatment on the indicators of biological and chemical oxygen demand (BOD and COD) should increase [12].

After the addition of the probiotic cultures to the waste water, the concentration of the beneficial microorganisms grows and the efficiency of the mechanical and biological wastewater treatment increases. According to published data, the probiotic microorganisms contribute to the oxygen production in the feed water and thereby create the possibility for the partial reduction of the air supply to the aeration tank and for the energy saving [11-17].

The following foreign and Russian scientists have been engaged in the probiotic agents’ application for the water treatment intensification: Miron A.R., Gvozdyak P.I., Serpokrylov N.S., Borisova V.Yu., Moldagulova N.B., Matrosova L.E., Markin V.V. [12].
The researches of the probiotic agents’ ability to improve the efficiency of the wastewater treatment have been carried out at the sewage treatment plants in Ukraine [14], Russia [15-17], Kazakhstan, Poland, Lithuania, Italy, France and the USA [2,8-10,18-21]. The results of the studies indicate that they are promising for the intensification of the wastewater and its precipitation treatment processes.

In the published works [11-20], three variants of introducing the probiotic agents in technological schemes of sewage treatment plants are considered: 1) before degritters; 2) before the preliminary setting tank; 3) before the aeration tanks. The most beneficial is the supply of probiotics before the preliminary setting tank. In this case, the beneficial effect of the probiotic is manifested at the stage of primary sedimentation, and at the stage of the biological purification simultaneously.

According to the data obtained by previous investigators [11-15,20], with the use of probiotics in the preliminary setting tanks, the putrefactive processes decrease, the release of foul gases diminishes, and partial disinfection of water and sediment occurs. The reduction of gas formation in the sediment ponds improves the precipitation regime of impurities and the qualities of raw sludge such as moisture-yielding ability and compactibility.

Existing data on the probiotics’ application in the process flowsheets of the wastewater treatment and sediment processing testify to the prospects for this direction development.

3. Problem

Nowadays, there are following major problems associated with the introduction of the probiotics in the technological schemes of the sewage treatment plants: 1) all produced agents have a different probiotic cultures’ composition and concentration; 2) there is no single methodology for determining the dose of the agent and the places of its introduction in the technological chain; in each individual case, it is determined experimentally, depending on the quality of the initial wastewater and the properties of the sediment; 3) in each specific case, a feasibility study of the new agent application is required; 4) also, the issues of the active sludge adaptation to the probiotics and its "habituation" to this biological agent remain unclear.

In any case, the topic of the probiotic agents’ introduction in water purification technology is interesting, it deserves serious attention and requires careful study.

4. Results

This article presents the results of the laboratory research carried out at the Department of Water Supply and Sanitation at Industrial University of Tyumen in 2018. The conducted experiments are included in the preliminary stage of the full-scale scientific work, which have been planned for several years.

At present, the influence of "Pip Plus Water" probiotic (Belgium) on the COD of a model wastewater solution has been studied. This preparation is a liquid which contains a large amount (6360000 per 1 ml) of Bacillus bacteria in a sporous state: Bacillus subtilis, Bacillus subtilis var. amyloliquefaciens, Bacillus licheniformis, Bacillus pumilus, Bacillus megaterium, and enzymes. The model wastewater solution (or synthetic waste water) was prepared by dissolving mineral salts and peptone in the faucet water. The initial concentration of COD in synthetic waste water is 180 mg / dm$^3$.

The experiment has been carried out at a known initial concentration of COD in a sample for 3 doses of "Pip Plus Water" probiotic (0.01; 0.05 and 0.1 cm$^3$ / dm$^3$) added to water samples.

The model solution samples have been mixed for 5 minutes with a probiotic, then the contact time has been maintained (60 minutes). Further, the concentration of COD in the sample has been measured by a photometric method on a Fluorat-02 liquid analyzer (END F 14.1: 2: 4.190-03). The obtained results are presented in Table 1 and in Fig.1.

As follows from the analysis of the obtained results, the following has been clarified: 1) the addition of a "Pip Plus Water" probiotic to the model wastewater solution (by COD) influences the change in water quality; 2) the overall effect of COD reduction in the model wastewater solution is
from 7.8 to 12.2%. It is noted that the effect of COD reduction on a model solution depends on the dose of the probiotic and the contact time: the smaller the probiotic dose and the shorter the contact time, the lower the COD concentration; 3) the best result (the effect of reducing the COD concentration 12.2%) has been achieved with a probiotic dose of 0.01 cm³/dm³ and a contact time of 60 minutes; 4) it is noted that as the treatment time and the probiotic dose increase, the COD concentration grows.

**Table 1.** COD concentration of the model (synthetic) wastewater with the "Pip Plus Water" probiotic agent addition, mg/dm³.

| Time interval | COD concentration in the solution (mg / dm³) with the "Pip Plus Water" probiotic dose |
|--------------|-----------------------------------------------------------------------------------|
|              | 0.01 cm³/dm³ | 0.05 cm³/dm³ | 0.1 cm³/dm³ |
| 60 min       | 158          | 163          | 170          |
| 120 min      | 160          | 165          | 166          |
| 180 min      | 165          | 170          | 178          |
| Maximum effect | 12.2%       | 9.4%         | 7.8%         |

Further, the effect of the "Pip Plus Water" probiotic on the municipal wastewater quality has been studied. The quality indicators of the wastewater, which is taken before the preliminary setting tanks at the MSTF of Tyumen (Russia) have been measured in laboratory conditions. The wastewater quality indicators (suspended substances, COD, total iron, pH indicator, individual cations and anions) have been determined in the initial water and after the contact with the probiotic.

The experiment has been carried out for 2 cases: 1) without any probiotic: after the preliminary settling of the sewage sample for 2 hours, the water qualitative indicators have been determined; 2) with the addition of the "Pip Plus Water" probiotic dose of 0.05 cm³/dm³: the wastewater sample is stirred for 5 minutes after the addition of the probiotic and is preliminarily settled for 2 hours, after this process the water qualitative indicators have been measured. All these experiments have been carried out in three parallels, based on the results of measurements, the mean value of the indicator has been calculated. The results of the experiment are given in Table 2.

The analysis of the obtained results has shown that the "Pip Plus Water" probiotic addition to a municipal wastewater sample, which is taken before the preliminary setting tank, affects the suspended solids’ concentration significantly. There is an improvement in the wastewater clarification degree with the addition of a probiotic, in comparison with simple precipitation, by a mean of 5.8%. The total clarification effect after 2-hours precipitation has achieved 84.2%.

![Figure 1. COD concentration change in the model wastewater solution, depending on the "Pip Plus Water" probiotic dose and the contact duration](image)
Table 2. Average values of wastewater quality indicators.

| Wastewater quality indicators | Initial water sample | After preliminary settling | After the probiotic addition and settling |
|-------------------------------|----------------------|---------------------------|-----------------------------------------|
| Suspended substances, mg / dm³ | 489                  | **105.4**                  | **77.0**                                 |
| COD mg / dm³                  | 341                  | 139.7                     | 139.0                                   |
| Total iron, mg / dm³          | 4.42                 | 1.79                      | 1.83                                    |
| Chlorides, mg / dm³           | 118.4                | 119.4                     | 120.7                                   |
| Sulphates, mg / dm³           | 68.17                | 67.24                     | 68.57                                   |
| Phosphates, mg / dm³          | 8.778                | 8.875                     | 8.558                                   |
| pH                            | 7.5                  | 7.5                       | 7.5                                     |

According to experiment, the influence of "Pip Plus Water" probiotic on the COD, pH, total iron, chloride, sulfate and phosphate indices is absent or not significant.

In order to clarify the "Pip Plus Water" probiotic action on the sedimentation properties of the sewage sludge, the compaction degree of the original excess activated sludge and the sludge with the addition of this probiotic, have been compared. The active sludge has been taken at functional municipal sewage treatment plants in Tyumen, from the secondary sedimentation tanks after aerotanks.

The experiment with the active sludge has been carried out for 2 cases: 1) the sludge compaction without any probiotic; 2) the sludge compaction with preliminary addition of the "Pip Plus Water" probiotic. The probiotic has been added in a volume of 0.5 ml per 1.5 l of sludge, after that the sludge has been stirred for 5 minutes, allowed to remain for 20 minutes and condensed in a prescribed manner: a sludge sample of 1.5 l is bottled into 3 standard 0.5 l cylinders and the height of the sludge interphase boundary as well as the volume of the sludge compact part have been recorded every 15 minutes during 3 hours.

Figure 2 shows the comparative characteristics of the active sludge strong compacting without any probiotic and with the use of a probiotic.

**Figure 2.** The initial activated sludge compaction degree and with the addition of the "Pip Plus Water" probiotic over the height of the interface between the "sludge" and "water" phases.

The initial interface between the phases "sludge" and "water" \( (h_0) \) has been formed in the first 15 minutes and has been: 12-13 cm - without probiotic and 10 and 10.5 cm – with probiotic.

The process of the phase boundary reduction \( (H) \), depending on the active sludge compaction duration \( (T) \), can be described by the equation of exponential dependence (1):
The coefficient of compaction for the initial activated sludge has been $k=0.007-0.009$ and it has increased to values $k=0.013-0.015$ for the sludge with probiotic.

As a result of studying the sedimentation properties of the activated sludge, it is confirmed that the addition of the "Pip Plus Water" probiotic to the active sludge, which is taken from the secondary settling tank, affects the sludge compaction degree. At the same time there is an improvement by 14-28% in the activated sludge compaction degree with the addition of a probiotic. The duration of achieving the same compaction degree, with the addition of a probiotic, is reduced by a factor of 1.5-3.2.

5. Discussion
Based on the results obtained with the model wastewater solution, it can be assumed that the probiotic agent, when added to the waste water during the primary sedimentation stage, has a minor effect on the organic substances’ content (by a maximum of 12.2-7.8%). With a probiotic dose and the contact duration increase, COD concentration grows; presumably, this is due to the probiotic bacteria proliferation and COD biomass expansion. It is also expected that the "Pip Plus Water" probiotic will be able to influence the content of the organic substances at the stage of biological purification.

The "Pip Plus Water" probiotic addition before the initial sedimentation will improve the effect of municipal wastewater clarification by more than 5%. Further, it is contemplated that there will be a purification effect increase on the organic substances as a whole at the biological treatment facilities. It has been confirmed that the "Pip Plus Water" probiotic improves the sedimentation properties of the sludge when added to the secondary sludge. Presumably, the activated sludge improves its water-yielding properties and will deliver the bound moisture at the mechanical dehydration facilities more effectively after the probiotic addition.

6. Conclusions
In general, the "Pip Plus Water" probiotic has a positive impact on the certain waste water quality indicators and the precipitation properties. The results obtained make it possible to draw the following conclusions:

1) It is noted that the COD reduction effect on a model solution depends on the probiotic dose and the time of the contact: the smaller the probiotic dose and the shorter the contact time, the better the result obtained. The best COD concentration reduction effect of 12.2% has been achieved with a probiotic dose of $0.01 \text{ cm}^3/\text{dm}^3$ and a contact time of 60 minutes. It is also observed that COD concentration grows with a treatment time and the probiotic dose increase. COD concentration grows with a probiotic dose and the contact duration increase. Presumably, this is due to the probiotic bacteria proliferation and the COD biomass expansion. Besides, it is assumed that the "Pip Plus Water" probiotic will have a greater effect on the organic matter content during the biological treatment stage.

2) The addition of the "Pip Plus Water" probiotic to the sample of the municipal wastewater, which is taken before the preliminary settling tank, affects the suspended substances’ concentration significantly. The total clarification effect, after a 2-hour precipitation, has achieved 84.2%. When the "Pip Plus Water" probiotic is added to the wastewater and then it is followed by the sedimentation, the wastewater clarification effect increases, which corresponds to the other authors’ studies.

3) The "Pip Plus Water" probiotic addition to the active sludge sample, which is taken from the secondary tank, improves the sedimentation properties of the precipitation. It is supposed that, the activated sludge improves its moisture-yielding properties, after the probiotic addition, and will deliver the bound moisture to the mechanical dehydration facilities more effectively.

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