RESEARCH ON THE IMPACT OF THE AIR-LIQUID JET MIXER UPON RING BIOREACTOR OPERATION
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ДОСЛІДЖЕННЯ ВПЛИВУ ПОВІТРЯНО-РІДИННОГО СТРУМІНЕВОГО ЗМІШУВАЧА НА РОБОТУ КІЛЬЦЕВОГО БІОРЕАКТОРА

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ABSTRACT
In the production of biological preparations for agriculture there are promising jet bioreactors with an external circulation ring, providing intense mixing of the medium along with a high degree of aeration. However, application of these bioreactors is limited by large shear loads on the microorganisms (microorganism cells) arising in the circulation pump. The article deals with one of the ways for solving this problem by using a low-capacity pump for the circulation of the fermentation medium according to a non-standard scheme of medium jet aeration in order to intensify the mass exchange processes. There are presented results of physical simulation of the mixing parameters of flows in an aerator-jet mixer and their impact upon the intensity of metabolic processes in the circulation ring of a bioreactor.

АНОТАЦІЯ
Застосування струменевих біореакторів із зовнішнім циркуляційним кільцем лімітується великими зрізовими навантаженнями на мікроорганізми (клітини мікроорганізмів), що виникають в циркуляційному насосі. У статті розглядається один із шляхів вирішення даної проблеми за рахунок застосування малопотужного насоса для циркуляції ферментаційного середовища з використанням нестандартної схеми струменевої аерації середовища для інтенсифікації масообмінних процесів. Наводяться результати фізичного моделювання параметрів зміщення потоків в аераторі – струменевому змішувачі та їх вплив на інтенсивність обмінних процесів в циркуляційному кільці біореактора.

INTRODUCTION

Mixing and aeration of the fermentation medium play one of the important roles in the deep cultivation of microorganisms. These processes directly affect the supply of nutrients and oxygen to the cells, as well as the removal of metabolic products. The technical and technological design both of mixing and aeration includes many engineering solutions. However, intensification of the metabolic processes between the liquid and the gaseous phases still remains an urgent task for most types of bioreactors. Among the devices providing intense mixing of the fermentation medium along with a high degree of aeration, it is worth highlighting jet bioreactors with an external circulation ring *(Stanbury P. et al., 2016)*. Mixing in devices of such a type is based on the pump circulation of the medium along an external ring (*Warmeling H. et al., 2016)*. Additional intensification of the mass exchange processes is achieved due to jet aeration of the fermentation medium *(Weber S. et al., 2018*). Such an organisation scheme of mixing and aeration also allows one to avoid using additional devices inside the device immersed into the fermentation medium (impellers, bubblers, etc.), which helps to improve the aseptic fermentation conditions.

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Despite the fact that general schemes for mixing and fermentation of the medium with the use of pump circulation have been known for quite a long time they have not found wide application in biotechnology since it is believed that cells in the impeller of the pump experience large shear loads. Therefore, jet reactors are now used mainly in chemical technology *(Warmeling H. et al., 2016; Pangarkar V., 2015).* Analysis of the studies made in the recent years on the inhibition of the biomass growth because it gets into the pump impeller during circulation *(Weber S. et al., 2018; Ughetti M., 2018)* showed that the shear loads have a much less pronounced negative effect on the cells than it was previously assumed. At the same time, it is noted that the intensity of the mass exchange processes in the jet bioreactors significantly exceeds a similar indicator for the most common devices equipped with mechanical impellers *(Dierendonck L., 1998; Weber S. et al., 2019*). It was also pointed out that the technological design of mixing and aeration of the fermentation medium in the jet bioreactors was more energy-efficient compared to the use of the bubble columns or fermenters with mixers *(Ughetti M., 2018; Weber S. et al., 2018; Bespalov I. & Hodorchuk V., 2017; Botton R. et al., 2009).*

The main technological obstacle limiting the application of jet reactors in biological processes remains the need to reduce the shear loads on the cells, which translates into a requirement to reduce the number of the pump impeller revolutions with a corresponding decrease in the mixing intensity of the medium. For the small-scale production of biological products this task is most acute since the application of the approach developed for industrial fermenters (with a volume of 40 m^3, or more), involving the use of multiphase pumps pumping the air-liquid mixtures along the circulation ring *(Stanbury P. et al., 2016)*, seems very costly and nonpurposeful. There are technical *solutions (Bespalov I. & Hodorchuk V., 2017; Krutyakova V. et al., 2019)* that look much more attractive, suggesting reduction in the shear loads by using low-capacity pumps and intensification of medium mixing by aeration in small bioreactors (100 l). Numerous studies were devoted to jet mixing of the fermentation medium and air in reactors with an external circulation ring *(Pangarkar V., 2015; Botton R. et al., 2009; Fadavi A. & Chisti Y., 2005*). One of the most common flow mixing schemes is implemented by means of a jet apparatus (JA) – an ejector *(Stanbury P. et al., 2016; Sharma D. et al., 2015; Fadavi A. & Chisti Y., 2005).* As a rule, a medium flow is supplied to the nozzle of such an ejector, but air is injected. The ready-made air-liquid mixture can be supplied under the level of the medium in the apparatus, or sprayed above the surface of this medium.

We have developed a new scheme of jet aeration of the fermentation medium in the bioreactor (Fig. 1), taking into account the advantages and disadvantages of the existing technical solutions.
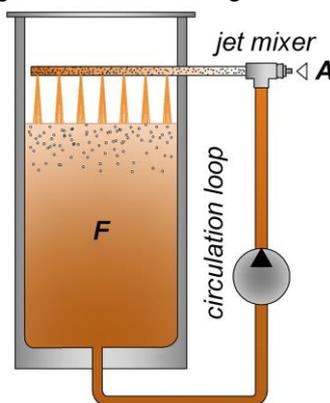

Fig. 1 - A scheme of mixing and aeration of the fermentation medium in a jet bioreactor
F – fermentation medium, A – air

The basis of this development is also the use of a JA – a mixer. The organisation of the working process in a jet mixer (JM) has two essential differences: 1) not the fermentation medium but air is supplied to the JM nozzle; 2) both flows (the air and the medium) are under pressure, i.e. the amount of the mixed air does not depend on the formation of negative pressures in the JA, and it allows one adjustment of the conditions of mixing. To improve mixing, the flows are supplied at right angles to each other, and the mixture flows into the bioreactor in thin jets through the air gap. The movement of the fermentation medium along the bioreactor ring is carried out using a low-capacity circulation pump. Analysis of the references to the JA *(Dutta N. & Raghavan K., 1987)* shows that the proposed scheme of organisation of the workflow is atypical not only for the jet bioreactors but also for the JA used in other areas. Therefore, the purpose of this study is qualitative assessment of the impact of the JM on the main characteristics of the bioreactor.

MATERIALS AND METHODS

Jet mixer (JM) is a simplified design of the mixer (JA) without additional compression of the mixed flow (Fig. 2), i.e. without a diffuser. The design is based on a standard equal-bore profiled T-joint *2* with a diameter of 15 mm, in one of the branches of which a steel nozzle *1* with a 1.8 mm flow section is installed. The nozzle is centred relative to the longitudinal axis of the T-joint and fixed with a gasket *4* inserted into the standard pipe reducer *3*.

a)   b)
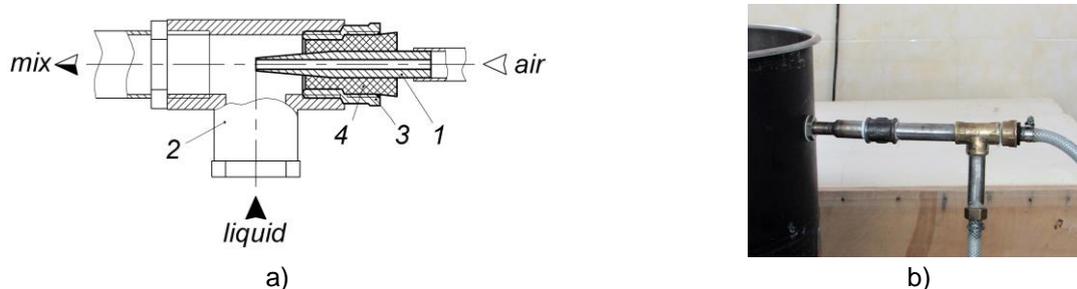
**Fig. 2 - A jet mixer scheme (a) and a photo of the JM installed before the inlet
of the bioreactor internal capacity (b)**
1 – nozzle; 2 – T-joint, 3 – pipe reducer; 4 – gasket

During the operation of the JM, the active flow (the compressed air) flows out of the nozzle *1* and admixes the passive flow (the liquid). The function of the mixing chamber in this case is performed by the flow part of the T-joint *2*. Final mixing of the flows takes place in the area between the JM and the sprayer installed inside the bioreactor. The experimental plant (Fig. 3) is designed to simulate the jet aeration process of the fermentation medium pumped by a pump along the external circulation ring of the bioreactor. The aeration conditions of the fermentation medium were simulated by using the JM 1 installed before the inlet of the bioreactor internal capacity *2* (ETI "Biotechnica", 100 l). The mixture obtained at the outlet of the JM was distributed over the surface of the liquid inside the container *2* using a sprayer *3*. In the experiments purified tap water was used as a working fluid.
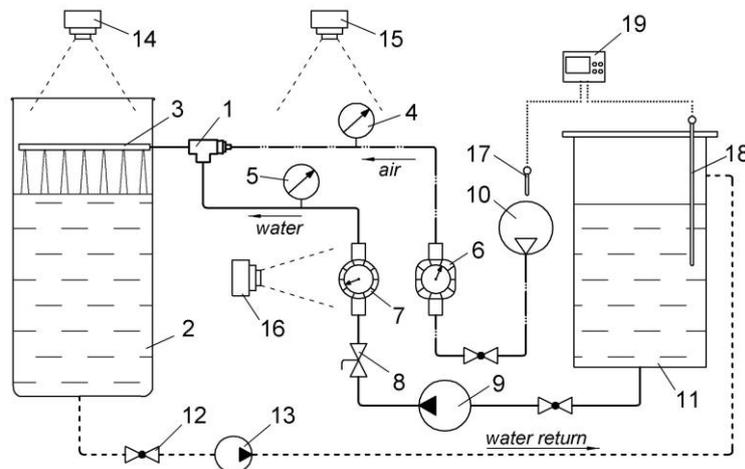
Fig. 3 - A diagram of the experimental plant
*1 – jet mixer; 2 – the internal capacity (100 l), 3 – sprayer; 4, 5 – pressure gauges; 6 – gas meter; 7 – water meter; 8 – manual control
valve; 9 – circulation pump; 10 – air compressor; 11 – auxiliary container with water (180 l); 12 – locking ball valve;
13 – pump for the returning water; 14, 15, 16 – digital cameras; 17, 18 – temperature sensors; 19 – temperature controller*

The operation of the fermentation medium circulation line was simulated using an auxiliary container 11 (ETI "Biotechnica", 180 l) from which water was supplied by the pump *9* (Wilo Star RS 15 / 5-3P) to the suction pipe JM *1*. The flow rate of the pump *9* was controlled by the throttling method using a manual control valve *8*. The return of water from the working container *2* was carried out using a pump *13* (Aquario AC 254-180). Besides, only the pump *9* was working during the experiments but the valve *12* on the water return line was closed. The aeration air is supplied to the nozzle JM *1* using a compressor *10* (ABAC FC2 / 50 CM2). The parameters of the water and the air flows were measured by means of pressure gauges *4* (MTI –1... + 3 bar) and *5* (MTI 0...0.6 bar), the gas meter *6* (Arsenal G 2.5), the water meter *7* (Novator LK-

15C). In order to record the readings of devices *4, 5* and *6, 7*, as well as the process of the mixture outflow into the container *2*, digital cameras *14-16* (Canon) were used. The air and the water temperatures were measured using the remote temperature sensors *17* and *18* (TERA RTD), the data of which were transmitted to the temperature controller *19* (RPC "ANT Electronics", TRC-2200-T).

Water from the container *11* (Fig. 3) was supplied to the JM *1* by means of the circulation pump *9*. The water flow was controlled by the tap *8*. Alongside with the water supply to the nozzle of JM *1*, compressed air was supplied using the compressor *10*. The flows were mixed in the JM and an air-water mixture entered the container *2* through the sprayer *3*. The outflow process of the mixture into the container *2*, as well as the readings of the pressure gauges *4, 5* and the flow meters *8, 9* were video captured using stationary cameras *14-16*. Before the beginning of experiments measurements were made of the air temperature at the inlet of the compressor *10* and of the water temperature in the container *11* by means of a temperature controller *19* with remote sensors *17* and *18*. The experiments were carried out for three modes of operation of the circulation pump, corresponding to three frequencies of rotation of the impeller (2350, 2630, 2720 rpm). In each operation mode of the pump, tests were conducted for four fixed values of the air pressure at the compressor outlet (1.0, 1.5, 2.0, 2.5 bar). Separate measurements were also performed for the parameters of the pump and compressor separate operations. At the beginning of each experiment the water level in the container *2* was 50 litres. The excess water, accumulated during the experiment, at the end of the tests was pumped into the auxiliary container *11* by means of the pump *13*.

The readings of the pressure gauges and flow meters were determined by processing the video recordings captured by cameras *15* and *16* (Fig. 3) in the software environment of the Sony Vegas Pro video editor. The video recordings of the instrument readings were previously synchronised according to characteristic peaks of the sound tracks, which made it possible to compare the parameters of the hydrodynamic processes taking place in the experiment simultaneously. In a frame-by-frame analysis of the video recordings instantaneous values of volumes and static pressures of water and air were determined. The time values for the corresponding frames of the videos were determined by the timeline scale of the Sony Vegas Pro. The flow rates of water and air were calculated on the basis of the measured values as a ratio of the volume passed through the flow meter during a period of time to the duration of this time period. Statistical processing of measurements was performed using the MS Excel spreadsheet according to a standard method *(Bulgakov V. et al., 2018)*. During the data processing the arithmetic mean values of the measured magnitudes, the mean root square deviation, were determined; the relative and absolute measurement errors were calculated for a confidence level of 95%. During the experiments the temperature of air and water, measured with the help of remote sensors *17, 18* (Fig. 3), remained unchanged, and it was 27^0C. Based on the values measured by the standard dependences, the average cross-sectional flow velocities, the dynamic and total pressures in the cross-sections were calculated before each of the flows entered the JM. The analysis of the flowing process of the mixture into the bioreactor container was carried out by processing the videos and photos obtained from the camera *14*.

RESULTS

To determine the performance parameters of the JA it is convenient to use dimensionless hydraulic characteristics, which are ratios of the corresponding flow parameters of the mixed media *(Lyamaev B., 1988)*. The basic characteristics of the JA of any type are the relative flow rate (the injection coefficient) and the relative pressure. The mathematical models of processes occurring in the classical JAs often consider the flow mixing as an auxiliary process accompanying the transfer of energy from the active flow to a passive one. In our case, mixing is the main result of the jet mixer operation, the processes of energy redistribution in the structural parts of the apparatus being secondary. Owing to such a change in the approach to the study of the jet processes, a need for strict attachment to classical dependencies in order to determine the relative flow rate and pressure of the JA becomes unnecessary.

The relative flow rate of the JM can be conveniently determined using the ratio of the volumetric flow rates:

$$q = \frac{Q_w}{Q_a},\qquad(1)$$

where q – the relative flow rate; Q_w, Q_a – the relative flow rates of water and air, [l/min^{-1}].

The use in dependence (1) of volumetric flow rates, rather than the mass ones, as in the classical JA, is determined by the fact that the passive flow is not sucked up due to the created vacuum but is pumped by

a separate pump. Therefore, the process is not based on inertia, the measure of which is mass, but on friction that occurs when the flows are mixed. Second, it is accepted in practice that the degree of aeration of the fermentation medium is estimated by the amount of air supplied per 1 litre of the medium. The relative flow rate, determined by formula (1), is a value inverse to the aeration degree. To determine the relative pressure, it is convenient to use the ratio of the total pressure of the water and air flows before the inlet of the plant:

$$h = \frac{H_a^t}{H_w^t},$$

(3)

where: h – the relative pressure; H_a^t, H_w^t – full pressures of the air and water flows at the jet mixer inlet, bar.

The characteristics of the jet mixer without adjusting the parameters of the water flow are shown in Fig. 4. Analysis of the graphs shows that an increase in air pressure at a constant rotational speed of the impeller leads to a decrease in the relative flow rate of the JM q, respectively, an increase in the proportion of air in the mixture. At the same time, an increase in the impeller speed at a constant air pressure, on the contrary, significantly increases q.
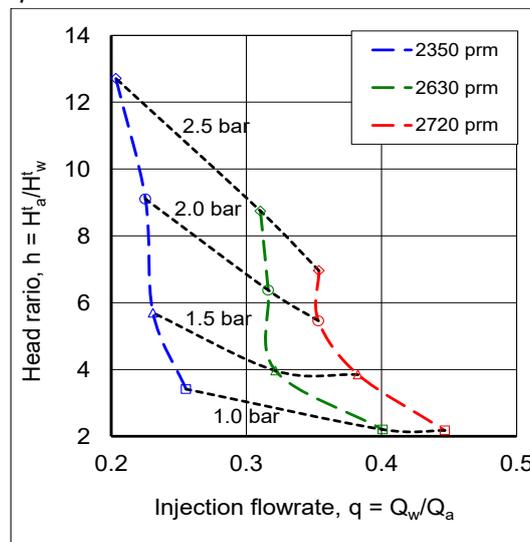

Fig. 4 - The dimensionless characteristics of the JM in the absence of the parameter adjustment of the water flow at the air pressures (1.0- 2.5 bar) and the revolution frequencies of the pump impeller (2350, 2630, 2720 rpm)

The parameter adjustment of the water flow allows one a slight expansion of the range of values of the relative flow rates ($q = 0.05...0.45$) and relative pressures ($h = 2...25$). Throttling of the passive flow leads to a pronounced decrease in the relative JM flow rate with a simultaneous increase in the relative pressure, which is observed in all the tested conditions. The aeration degree of the fermentation medium is inversely proportional to the relative flow rate of the JM q. Analysis of the experiments shows that even the use of the simplest JM design allows mixing the media in a wide range of volume ratios: 2.2...18.5 litres of air per 1 litre of water. Consequently, even in the case of a significant difference in the viscosity of the fermentation medium from the viscosity of water in the experiment and a corresponding reduction in the obtained range of the air-medium ratios, this jet aeration scheme can be used in the production of a sufficiently large range of biological products. Separately it is worth noting the advantage of the air supply through the JM nozzle in the proposed aeration diagram, which allows one the adjustment of the amount of the air supplied without changing the pump performance parameters. In the standard jet aeration schemes, air is injected with the fermentation medium. An increase in the viscosity of the medium during fermentation, caused by an increase in the concentration of microorganisms in the bioreactor capacity, leads to a decrease in the pump flow rate and a corresponding decrease in the amount of the injected air. Therefore, in order to maintain the required degree of aeration, it is necessary to increase the number of revolutions of the pump impeller, which means that the power consumption will increase. The use of the JM makes it possible to maintain the desired degree of aeration while increasing the viscosity of the medium by increasing the air pressure, i.e. without affecting the pump. Mixing of the fermentation medium is one of the key processes responsible for a mass exchange during the deep cultivation of microorganisms. In the jet bioreactors the most intense mixing of the fermentation medium takes place in the circulation ring *(Weber S. et al., 2019)*.

Therefore, it is necessary, first of all, to evaluate the impact of the JM upon the medium mixing intensity exactly in the circulation ring of the bioreactor. The mixing intensity is usually estimated applying the dimensionless criteria of hydrodynamic similarity, in particular, the Reynolds number *(Pangarkar V., 2015).* Intense mixing is achieved at the Re values corresponding to the turbulent flow regime, i.e. at Re > 3000. In order to perform the calculations, the pressure section of the water supply line of the experimental plant (Fig. 3), simulating the bioreactor circulation ring, was divided into two sections: before the JM (the first section) and after the JM (the second section), respectively. In the first section water moves through the pipeline, and in the second – the air-water mixture. Calculations of the Reynolds number for the first section (Table 1) indicated that in all the pump operation modes a mode of a turbulent flow took place. At the same time, it was pointed out that the change in the parameters of the air supplied to the JM nozzle did not have any significant impact upon the mode of the water flow in this section. Mixing of the fermentation medium in the circulation ring before the JM will occur sufficiently intensely with a minimal impact of the jet processes proceeding in the mixer itself.

Table 1

The Reynolds number for the first section of the water supply line (before the JM)

Air pressure, bar	The Reynolds number, Re		
2.5	4461	6702	7749
2.0	4830	6704	7681
1.5	4800	6703	7877
1.0	4800	6980	8267
	Velocity of the pump impeller, rpm		
	2350	*2630*	*2720*

A more complicated task is how to determine the flow conditions of the air-water mixture flow in the second section. The standard dependence for the calculation of the Reynolds number, in addition to the diameter of the JM outlet cross section and the flow velocity in it, determined on the basis of experimental data, includes density and the dynamic viscosity of the mixture. If the density of the air-water mixture can be calculated analytically as a ratio of the sum of the mass flow rates to the sum of the volumetric flow rates, it is not possible to calculate the exact value of the viscosity without conducting separate experiments. However, for a qualitative assessment of the mode of motion, it is possible to determine the boundaries of the interval into which the Re value of the mixture falls. For this it is sufficient to calculate the boundary values of the Reynolds numbers based on the boundary values of the dynamic viscosity of water and air. Based on the fact that the dynamic viscosity of the mixture should be less than the viscosity of water but more than the viscosity of compressed air, the desired value of the Reynolds number will fall into the range:

$$\mu_a < \mu_{mix} < \mu_w, \quad Re_a > Re_{mix} > Re_w \qquad (4)$$

where μ_a, μ_w, μ_{mix} – the dynamic viscosity of compressed air, water and mixture

Re_a, Re_w, Re_{mix} – the Reynolds number for compressed air, water and mixture

The results of calculation of the boundaries of the interval Re_a and Re_w for the second section of the circulation ring are given in Table 2.

Table 2

**The Reynolds number of the air-water mixture for the second section
water supply line (after the JM)**

Air pressure, bar	The Reynolds number of the air-water mixture, Re$_{mix}$		
2.5	$3.8 \cdot 10^4 \ldots 2.2 \cdot 10^5$	$5.8 \cdot 10^4 \ldots 3.2 \cdot 10^5$	$6.7 \cdot 10^4 \ldots 3.7 \cdot 10^5$
2.0	$4.2 \cdot 10^4 \ldots 2.3 \cdot 10^5$	$5.8 \cdot 10^4 \ldots 3.2 \cdot 10^5$	$6.6 \cdot 10^4 \ldots 3.7 \cdot 10^5$
1.5	$4.1 \cdot 10^4 \ldots 2.3 \cdot 10^5$	$5.8 \cdot 10^4 \ldots 3.2 \cdot 10^5$	$6.8 \cdot 10^4 \ldots 3.8 \cdot 10^5$
1.0	$4.1 \cdot 10^4 \ldots 2.3 \cdot 10^5$	$5.9 \cdot 10^4 \ldots 3.3 \cdot 10^5$	$7.1 \cdot 10^4 \ldots 3.9 \cdot 10^5$
	Velocity of the pump impeller, rpm		
	2350	*2630*	*2720*

It is evident from the data in Table 2 that the air-water mixture at the outlet of the JM moves in a developed turbulence mode in all tested modes. Comparison of the data in Table 1 and Table 2 shows that the JM plant allows increasing the Reynolds number by more than an order of magnitude, which indicates a significantly intensified medium mixing in the circulation ring of the bioreactor. During the JM operation there is an increase in the water pressure before the apparatus, observed by 10...34%, in contrast to the movement of water through the JM without mixing with air. Consequently, the flow of air flowing out of the nozzle will throttle the flow of the fermentation medium in the circulation ring. The throttling process is generally accompanied by a reduction in the flow rate. However, the decrease in the water flow rate was not observed in all the modes studied. So, at the frequencies of the pump impeller revolutions 2630 rpm and 2720 rpm the water flow rate decreased 7...13% but at a frequency of 2350 rpm, contrary to the increase in the water pressure, an increase was observed rather than a decrease in the water flow rate by 3...11%. The obtained increase in the water flow rate, when mixed with air, at minimum pump revolutions of the impeller exceeds the measurement error. Therefore, it is logical to assume that this effect is caused by a change in the nature of the jet processes occurring in the JM. In jet mixing the injection processes generally take place: a part of the energy of the active stream is spent on the injection of the passive stream. In the first two operating modes of the pump (at frequencies of 2630 rpm and 2720 rpm), the losses of the water pressure during mixing exceeds the injection pressure created by the air stream. As a result of this, the flow of water is throttled (propped) by the flow of air, and the water flow rate is accordingly reduced. In the third mode (at a frequency of 2350 rpm), the injection pressure exceeds the losses during mixing. Accordingly, a part of the energy of the nozzle stream is spent on the injection of an additional water flow. Thus, even in the case of mixing in the JM, the pressure flows induced by various compressors a possibility arises of useful application of the injection effects in a certain range of the flow parameter ratios. The impact of the jet mixer upon spraying of the medium was analysed by using the videos obtained during the experiments and the photos of the outflow of the air-water mixture into the bioreactor container, and by comparison of these data with the parameters of the air and water flows before the JM. This analysis shows that the intensification effect of the mass exchange processes during jet aeration, obtained on the basis of calculations, is confirmed by the results of visual observations of spraying the mixture in the bioreactor (Fig. 7). The pressure and the opening angle of the jets flowing from the sprayer increases with increasing the air pressure, and, at the same time, it little depends on the change in the number of pump impeller revolutions. Analysis of the photos also revealed that the process of the foam formation on medium surface in the bioreactor during the aeration of the medium is less intense than without aeration.

a)   b)
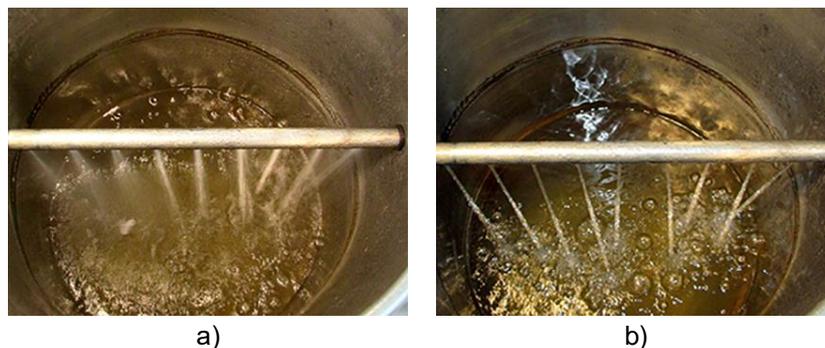
Fig. 5 - The outflow of the air-water mixture into the bioreactor at a frequency of the pump impeller revolutions of 2720 rpm with aeration at an air pressure of 1.5 bar and (a) and without aeration (b)

The study of video recordings confirmed that in case the jets pass through the intermediary air layer in the bioreactor container, excess air is removed from the mixture. In a maximum operating mode of the plant (2.5 bar – 2720 rpm) this process is accompanied by intense emission of the water dust that settles on the inner surfaces of the bioreactor container and the sprayer. Analysis of the photos indicates that the formation of the water dust is caused by a decreased size of the droplets suspended in the air flow and arising as a result of an increased air pressure. Consequently, at the maximum performance parameters of compressors it becomes possible to spray the liquid, circulating along the ring, onto the surfaces located above the sprayer. This effect can be used when sterilizing the internal capacity of the bioreactor by means of liquid disinfectants. At the stage of microorganism cultivation, the formation of dust is not desirable. To prevent its occurrence, the air pressure before the JM nozzle should not exceed 2 bar.

CONCLUSIONS

The plant JM on the circulation ring of a jet bioreactor allows intensification of the mass exchange processes, improvement of the aeration conditions of the medium, and reduced foaming at the fermentation stage. The obtained data correlate with the results of studies conducted by other authors on jet aerators in which a liquid, circulating along the reactor ring, is used as an active flow, the passive flow being air. Taking into account the low coefficient of efficiency of the JA, we proposed a concept of the organisation of the working process, focused on the improvement of the quality of mixing, and not on the injection of the secondary stream. That is why air was chosen as the active flow but the passive flow – the liquid – was made the pressure flow. This makes it possible to use a low-capacity circulation pump that not only lowers the cost of the electric power but also significantly reduces the shear loads on the cultural fluid, entering the impeller. The scheme of mixing the flows in the JM assumes useful application of the injection effects (an increased fluid flow rate) in a certain range of the flow parameters. Absence of a diffuser in the JM design contributes to great hydraulic losses during mixing, which limits the range of useful application of injection with a minimum number of revolutions of the pump impeller. Consequently, further research in this area will be aimed at developing a more advanced design of the JM.

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