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REVIEW ARTICLE

Technology and implementation of fermentative units for bioprotein production from natural gas

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Abstract

Objectives. *To conduct a comparative analysis of the features of a fermentation unit design for obtaining bioprotein from natural gas and determine the main technical and structural solutions used in the development of fermentation apparatus, which vary according to the method of organizing hydraulic and mass transfer processes.*

Results. *An analysis of publications devoted to the problem of developing technological equipment for conducting the process of obtaining a bioprotein from natural gas is presented. Using the comparative analysis, the key features of bioreactors and their internal elements are indicated according to the method of organizing the hydrodynamic regime. The main approaches to the technological development of fermentation units for obtaining bioprotein from natural gas are described and technical solutions used in the implementation of these structures are identified.*

Conclusions. *Fermenter designs for the cultivation of methane-oxidizing microorganisms vary according to the main approaches for implementing the hydraulic regime inside the apparatus. While one class of fermentation systems is based on the principle of volumetric mixing in*

the working space of the apparatus, with the possibility of including external circulation circuits, additional tanks, and auxiliary bioreactors in the system, the other main class relies on the principle of flow (displacement) in the tube space with subsequent release of the gas phase from the circulating culture liquid.

Keywords: *bioreactor, fermenter, fermentation, biomass, protein, flowsheet, methanotrophs, Methylococcus capsulatus*

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ОБЗОРНАЯ СТАТЬЯ

Технологическое и аппаратурное оформление ферментационного узла процесса получения биопротеина из природного газа

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Аннотация

Цели. *Провести сравнительный анализ особенностей аппаратурного оформления ферментационного узла процесса получения биопротеина из природного газа. Определить основные технические и конструкционные решения, применяемые при разработке ферментационных аппаратов, различающиеся по способу организации гидравлических и массообменных процессов.*

Результаты. *Проведен анализ литературы, посвященной проблеме разработки технологической аппаратуры для получения биопротеина из природного газа. С использованием метода сравнительного анализа были выявлены ключевые особенности конструкций биореакторов и их внутренних элементов, отличающихся способом организации гидродинамического режима в аппаратах. Описаны различные подходы к разработке оборудования для ферментационного узла процесса получения биопротеина, а также определены основные технические решения, используемые при создании данных конструкций.*

Выводы. *Установлено, что большинство конструкций ферментационных аппаратов, предназначенных для культивирования метанокисляющих микроорганизмов, базируется на реализации гидравлического режима внутри аппарата. Часть ферментационных систем построена на принципе объемного перемешивания в рабочем пространстве*

аппарата с возможным включением в систему внешних циркуляционных контуров, дополнительных емкостей и вспомогательных биореакторов, другая часть использует принцип движения потока (вытеснения) в трубном пространстве, с последующим выделением газовой фазы из рециркулирующей культуральной жидкости.

Ключевые слова: *биореактор, ферментер, ферментация, биомасса, белок, технологическая схема, метанокисляющие бактерии, Methylococcus capsulatus*

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INTRODUCTION

The rapid growth of the world's population poses the problem of providing humanity with the necessary food sources, in particular, protein, which contains essential amino acids. While the food market is currently dominated by proteins derived from plant and animal sources, a trend is developing towards the extraction of proteins from alternative sources. This group includes single cell proteins derived from unicellular bacteria or yeast organisms. For this purpose, bacterial cultures seem to be the most effective, since they grow faster and on a cheaper substrate [1]. As well as containing almost all essential amino acids, protein biomass derived from natural gas is richer in vitamins than that derived from vegetable sources (e.g., soy, oilcake, and meal) [2].

The use of meat and bone meal as animal-based sources of proteins involves certain restrictions linked to the source of its production. Periodic outbreaks of disease have led to bans on the use of animal meal in some countries around the world[1]. The use of fishmeal is complicated by the fact that the total volume of its production, currently at around 5 mln t/year, is significantly less than the demand, which is about 8–10 mln t/year. As a result, prices rise and the market is flooded with imitations and counterfeits [3].

Global trends in the use of feed ingredients are reflected in a significant increase in the demand for protein. According to 2019 data provided by *Global Market Insights*, annual global sales of protein supplements for livestock and aquaculture needs exceed USD 183 bn. A projected steady trend of sales growth implies that the figure will reach USD 220 bn by 2026. In Russia, the production of feed protein additives was projected to increase by 2.3 times, i.e., by 10.6 mln t for the period 2010–2019. The Russian market for protein is forecast to reach USD 4.7 bn by 2026[2].

The history of the development and evolution of microbial protein production processes in Russia demonstrates great successes in this field from the middle of the 20th century onwards. By 1980, there were 12 Soviet biochemical plants operating on the territory of the USSR, producing about 1 mln t of microbial protein. Some of the produce was supplied to the country's collective and state farms to meet the needs of the national economy, while the rest was exported[3].

The development of microbial protein production involved technologies for the production of paprin—feed yeast, whose production involves the use of paraffins as a raw material (substrate), and gaprin—a protein based on the cultivation of methane-oxidizing bacteria *Methylococcus capsulatus*.

[1] Food and Agriculture Organization of the United Nations. FAO/WHO Global Forum of Food Safety Regulators. BSE as a National and Trans-Boundary Food Safety Emergency. 28–30 January 2002. Marrakesh, Morocco. URL: https://www.fao.org/3/y2038r/y2038r.htm. Accessed October 11, 2022.

[2] Innopraktika. The animals will be fed with bacteria and bacteria will be fed with natural gas. Moscow, Russia. URL: https://innopraktika.ru/smi-o-nas/1583/ (in Russ.). Accessed September 22, 2022.

[3] Forum & Expo "ProteinTek". Food from Oil and Natural Gas. Moscow, Russia. URL: https://proteintek.org/novosti/1030/ (in Russ.). Accessed September 22, 2022

The main advantages of the gaprin production process are the non-pathogenicity of the main culture and the ability to grow cultures on methane-depleted gas, including associated gas. The use of the culture on an industrial scale using raw materials of a given quality was made possible by the selection of the initial strain found under natural conditions.

The question of the usefulness and applicability of microbial protein is currently under active consideration by specialized Russian research organizations, whose ultimate goal is to ensure the country's food security.

In accordance with the Priority 2030 program, an evaluation of the effect of gaprin on poultry productivity indicators is underway. The feasibility and economics of incorporating microbial proteins into industrial feed production technologies are also being evaluated. In terms of crude protein content, gaprin was shown to outperform fishmeal by 5% and equivalents grown from oil production wastes by 20–27.5% [4]. Compared to fishmeal, gaprin contains an order of magnitude more tryptophan (3.81 mg/kg vs. 0.6 mg/kg) and vitamin B_6 (35 mg/kg vs. 4.0 mg/kg), as well as containing vitamin B_{12} (up to 42 mg/kg).

The efficacy of gaprin as a feed additive has been reviewed in detail in [5]. The possibility of replacing fishmeal with gaprin in whitefish diets was investigated. Since gaprin does not reduce the growth rate of juveniles or cause deviations in physiological parameters, it can be used as an alternative to fishmeal.

When considering issues related to the use of bioproteins obtained from natural gas as feed additives, it is necessary to focus on the design of hardware and specific features associated with the technology used in their production process. The literature analysis presented below considers the main technical solutions used in the design of the reactor node involved in the process of obtaining bioprotein from natural gas along with the nodes of the technological chain connected to it.

MAIN TECHNICAL SOLUTIONS USED IN TECHNOLOGIES FOR OBTAINING MICROBIAL PROTEIN FROM NATURAL GAS

Technologies for obtaining microbial protein from natural gas can be generally represented by the group of technological blocks interconnected at various stages of production. Figure 1 shows the main stages of the process in the form of a flowchart.

The raw material preparation unit includes units for the preparation of working nutrient solutions, water treatment, and supply of oxygenated and methane-containing gases. The fermentation unit comprises a main reactor unit consisting of one or more fermenters designed for the cultivation of microbial proteins, which are supplemented by auxiliary capacitive and pumping equipment. The concentration unit may consist of several units for concentrating the biomass coming from the reactor unit, as well as the collection and transport of the spent culture fluid (SCF), comprising the light aqueous phase obtained following biomass concentration. The pre-condensed biomass is heat-treated in the inactivation unit. In addition to the drying and packaging unit of the finished products, an intermediate granulation stage may represent an additional block in the technological process.

In the context of the relationship of the reactor node with other nodes in the technological chain, the analysis of technologies for obtaining bioprotein from natural gas shows that one of the most often considered processes is the return of SCF from the separation unit directly into the bioreactor. This has a significant impact on the system of organizing the input of liquid flows into the fermenter, since the amount of fresh water injected into the bioreactor channel should be reduced by the amount of the incoming flow. In this case, it is important to determine the optimal entry points for the mineral nutrient components, which are supplied in the form of solutions that maintain the pH in the bioreactor and are returned directly to the reactor SCF.

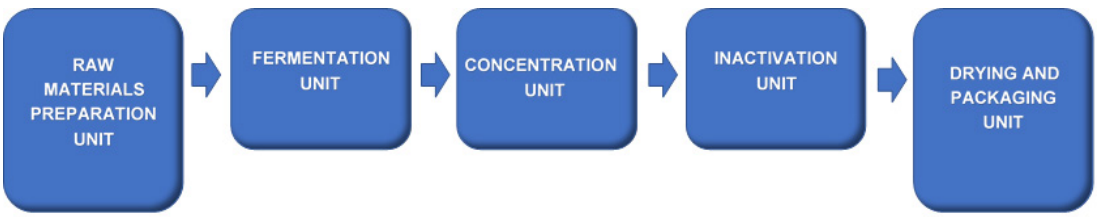

Fig. 1. Stages of the protein production process from natural gas.

Additional technological nodes may be introduced into the main production chain due to the specificity of the implementation of certain approaches to process optimization. The paper [6] describes the sequence of technological stages for obtaining microbial protein from natural gas. This comprises both the main technological units, such as fermentation, separation, inactivation, and drying, as well as auxiliary units—a system for extracting carbon dioxide from biomass entering the centrifuge (by reducing the pH), and an ultrafiltration system which can be used to obtain a more concentrated flow before its feeding to the drying unit. According to the data given in the source [7], fermenter biomass condensed in the centrifuges of the separation unit up to 80–90 g/L can be sequentially concentrated in an ultrafiltration unit up to a concentration of 220 g/L. Under ultrafiltration conditions, the amount of SCF returned to the bioreactor increases, having consequences for the system of organizing the input of flows into the fermenter.

The above-mentioned literature [6, 7] also mentions the need to ensure returns from the centrifugal separation system and, in particular, from the ultrafiltration system to the SCF digester. When considering the problem of reducing the water consumption of the fermentation system, the authors of [8] emphasize the possibility of returning the SCF after separation to the fermentation stage in a volume of up to 95% of the total amount of water supplied to the reactor.

The reciprocal relationship between the fermentation unit and the concentration unit involving the return of the SCF to the fermenter leads to the need for its purification, since the SCF sent to the apparatus comprises a certain quantity of organic compounds and accompanying microflora. The obvious solution to this problem is to introduce a culture liquid purification unit into the technological chain. One of the possible approaches discussed in [9] involves the following sequence: cooling the SCF obtained during separation to a predetermined temperature and feeding it to an additional aerobic fermentation, followed by returning the purified product to the main fermenter designed for the cultivation of biomass of methane-oxidizing bacteria.

The reactor unit, representing the key component in the technological chain of protein production from natural gas, involves hardware design and organization of the fermentation process. This determines the main parameters of the equipment for the further processing cycle of the synthesized bioprotein into a marketable product. In this context, specific features of the structural design of the bioreactors used in this technology will be further considered.

VARIATIONS IN THE STRUCTURAL DESIGN OF FERMENTATION EQUIPMENT FOR THE CULTIVATION OF METHANE-OXIDIZING BACTERIA

Despite the wide variety of bioreactor designs used in the microbiological industry, all the discussed fermentation devices are equipped with standard structural elements designed to provide optimal conditions for the biochemical process, as well as optimizing the underlying physical processes (hydrodynamic, thermal, and mass transfer) [10].

While the design of fermentation apparatus is based on standard structural solutions, it is necessary to take into account the specific characteristics of the cultivated organisms. When developing bioreactors for the cultivation of aerobic microorganisms, an important design criterion is the method of energy supply to the apparatus. Here, delivery options include gas-phase (barbotage and gas lifting devices), liquid-phase (ejection and jet devices), as well as combined energy delivery in both liquid- and gas phases (Fig. 2) [11].

The fermentation equipment used in the process of obtaining proteins from natural gas has a number of features that set it apart from other equipment used to grow aerobic microorganisms. The presence of an additional gas phase taking the form of natural gas injected into the digester leads to a significant increase in the total volume of gas distributed in the liquid phase. The input and distribution of natural gas by volume in the fermenter also affects the solubility of oxygen and carbon dioxide from the general gas phase of the apparatus.

In addition to the known standard design solutions, bioreactors are equipped with a large number of specific internal technical elements for the cultivation of methane-oxidizing bacteria. The structural elements of the fermenter perform local tasks related to the hydrodynamic component of the apparatus, such as eliminating water shocks during operation, reducing the amount of liquid carried away with the gas phase, as well as diverting and separating internal flows of the liquid phase or gas–liquid mixture.

Having identified the goal of developing a particular bioreactor design, it is important to consider the criteria that will be used to assess the effectiveness of its work. The most commonly used are the degree of oxygen conversion as a result of biochemical consumption and the volume coefficient of oxygen mass transfer. Methods for organizing the gas and liquid flows used in the various fermentation equipment designs have

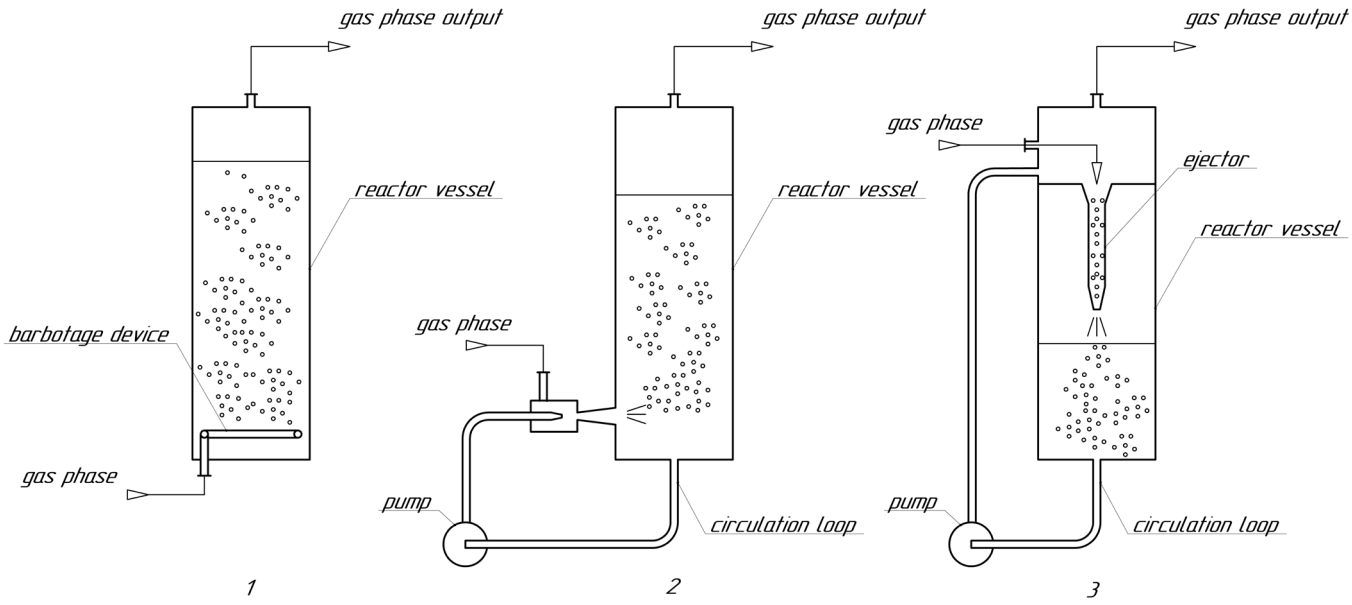

Fig. 2. Examples of bioreactors with different energy input methods:
(1) barbotage column apparatus (energy input with gas phase);
(2) ejection fermenter (energy input with liquid phase);
(3) jet fermenter (energy input with liquid phase).

a significant effect on the contact surface of the phases and, consequently, on the above parameters. The literature also describes effectiveness criteria based on the specific productivity of the process occurring in the apparatus, expressed as the amount of biomass produced per unit of reactor working volume, as well as the specific cost, expressed as the amount of energy expended per quantity of biomass produced.

The paper [12] presents the design of an apparatus based on the principle of separate supply of oxygen and natural gas to two separate fermenter sections connected by a circulating liquid phase. In the natural gas inlet section of the fermentation system, a gravitational ejector distributes the natural gas in the liquid phase towards the section where it is to be aerated. In the fermenter model presented, there are also basic technical solutions in the form of standard designs, such as barbotage and liquid phase circulation devices, which can be described in terms of a circulation pump. Additional sections in the apparatus are used to prevent gas saturation of the culture liquid by ensuring the removal of gaseous products of microorganisms from the reactor. While the authors failed to indicate criteria or parameters for evaluating the performance of this bioreactor, each described section comprises a separate local zone with different ways of organizing flows, implying separate mass exchange characteristics.

The main fermentation equipment presented in [13] is divided into sections to ensure a separate controlled process of dissolution of methane-containing and oxygen-containing gases. The combined technical solution uses mechanical mixing devices in auxiliary bioreactors, as well as a diffuser and a circulation pump, to create a predetermined distribution of flows in the volume of the main reaction zone of the column fermenter. For this design, which uses a mechanical mixing device for dissolving gas components, data on oxygen absorption reaching a value of 10 kg of $O_2/m^3 \cdot h$ with specific energy consumption of 0.3–0.4 kW·h/kg of O_2 are presented. Although the presented bioreactor design is claimed to provide a high mass transfer rate, it is important to note the technical complexity of this fermentation system, which has practical implications for its start-up and commissioning.

The principle of supplying of methane-containing and oxygen-containing gases to separate sections where they can be distributed in a liquid volume is presented in a bioreactor for growing methane-oxidizing microorganisms [14]. The sections in which the gases are dissolved are located on opposite sides of the body of the main part of the apparatus. Both sections, which are in the form of vessels that expand at the top, are equipped with turbine stirrers for dispersing a gas–liquid medium in each of them. After being fed into the central

circulation line of the bioreactor for mixing, a uniformly distributed gas–liquid mixture from each section is then discharged into the main volume of the apparatus. The circulation of the liquid phase in the apparatus and the relationship of the flows between the auxiliary sectors and the main volume of the fermenter are provided by pumping equipment installed on external circulation circuits. In terms of the method for organizing the process of dissolving methane-containing and oxygen-containing gases, the presented bioreactor model has similar features to the apparatus given in [13]. The developers of the fermenter state that the speed of the turbine agitators in the gas dissolving zones could reach 1400 rpm, which would certainly lead to technical difficulties in the operation of these zones, especially with increased bioreactor volumes as a result of scaling. While the authors indicate a productivity value of 4.4 kg of biomass/m^3·h with energy consumption for fermentation at 1.1 kW·h/kg of biomass, these data cannot be used to evaluate the mass transfer characteristics of the unit according to the above-mentioned criteria.

Considering the use of a gravitational ejector to ensure the mixing of gas and culture fluids in devices for cultivating methanotrophs, the fermentation devices presented in [15, 16] are worthy of note. The paper [15] presents a vertical apparatus equipped with an external circulation circuit, a gravitational ejector (jet aerator), a heat exchange device, and internal elements—a grate and a bump—for reorganizing the gas–liquid flow and separating the gas from the liquid. A special feature of the design is the presence of a liquid phase degasser installed on the inlet line to the flow inductor, which ensures the operation of the circulation circuit and the jet aerator. Rather than being removed from the system, the gas phase from the separator is sent to the overflow chamber of the aerator together with recirculated and fresh gases (oxygenated gas and methane). The operating principle of the vertical apparatus presented in [16] is based on the use of a jet aerator. The design features several circulation circuits operated by means of pumps and an internal jet aerator structure characterized by division into sections. The number of sections corresponds to the number of circulation circuits that provide sufficient energy to the gas–liquid jet leaving the jet aerator.

In bioreactors models involving various designs of jet aerators [10], average values of oxygen mass transfer coefficients in the range of 200–300 h^{-1} are achieved when ensuring the contact of the liquid and gas phases by means of jet aeration. However, the presence of a gas phase in the culture fluid entering the circulation circuits leads to additional pumping equipment requirements.

No additional capacitive sections are included in the fermentation apparatus model presented in [17]; instead, the main fermentation process takes place in a vertical volumetric apparatus. The gas phase is introduced into the fermenter in the lower part of the apparatus using ring bubblers. The liquid phase circulating through the pump is fed to a gas–liquid ejector. After entering the ejector from the upper part of the fermenter, the gas phase is mixed with the working liquid phase and introduced into the lower part of the apparatus. The use of a jet pressure liquid ejector on the external circulation circuit in the place of a gravity ejector entails significant adjustments to the requirements for the overall characteristics of the fermentation plant, in particular, the height of the bioreactor, which can be reduced in relation to its diameter. By introducing a gas–liquid mixture from the jet apparatus into the volume of culture liquid, the reaction volume of the bioreactor can be used as efficiently as in a design using a gravitational ejector. The authors of the publication indicate the concentration of absolute dry matter and reactor flow rate, from which it is possible to calculate the specific productivity of the process at 5 kg of biomass/m^3·h.

For further analysis of the group of fermenters based on jet pressure ejectors, we should also consider the designs given in [18, 19]. The described plants comprise fermentation systems consisting of three main units: a fermenter, a gas separator, and a storage tank. The units are equipped with liquid-phase recirculation circuits, which represent the working medium for the gas–liquid ejector, as well as remote heat exchangers. The gas separator device allows the circulation pump to run continuously without pressure loss due to the presence of bubble gas in the culture liquid produced in the fermenter. The basic differences between the systems described in [18] and [19] consist in the method for organizing the alignment of the flow rates of the gas–liquid mixture coming out of the main digester, as well as the degassed culture liquid entering the circulation pump from the gas separator and entering the ejector for mixing with the exhaust gas returned to the digester. In the prototype presented in [18], flow alignment is achieved by returning some of the culture fluid to the suction line of the recirculation pump. In the prototype presented in [19], the equilibrium in the fermenter–separator–pump–ejector–fermenter system is achieved by regulating the flow rates of the gas flows: fermenter–gas separator, gas separator–ejector, fermenter–ejector. The data presented in [18, 19] on the flow rate and concentration of absolute dry matter in the culture fluid can be

used to indicate the specific productivity of the process occurring in bioreactors of the proposed design in a range of 3.6–4.2 kg of biomass/m^3·h.

When considering structural solutions such as bioreactor zoning, pressure ejectors installed on remote recirculation circuits, and gravity ejectors built into the apparatus, a distinction should be made between fermentation equipment in which combined solutions are used. For example, in [20] the fermenter design comprises a vertical two-chamber apparatus in which communication between the chambers is achieved by several vertically oriented internal channels: a liquid phase flow line and a gas phase exchange system. The original technical solution of the design involves the use of two gravity ejectors together with pressure ejectors, each of which ensures the introduction of a gas–liquid mixture into its chamber. As mentioned above, jet irrigation cannot be used to achieve high mass transfer coefficients. However, the use of pressure ejectors makes a significant contribution to improving the mass transfer characteristics of the device. The authors claim that the presented bioreactor is capable of reaching 7 kg of biomass/m^3·h at an energy consumption of 1.3 kW·h/kg of biomass.

In addition to the widely used vertical fermenters for the cultivation of methane-oxidizing organisms, special designs are developed using original technical solutions. In [21], a horizontal apparatus is presented with a partition dividing it into two reaction zones: a zone for feeding oxygenated and methane-containing gases into the volume, followed by their dissolution, and a zone in which the liquid working phase circulates by means of specially shaped blades fixed to the rotor. By combining the types of blades installed on the structure, various technological tasks can be performed, for example, aeration of the circulating liquid and distribution of the gas phase in its volume. The proposed bioreactor differs significantly from other multi-section vertical devices designed for the cultivation of methane-oxidizing bacteria. The high specific energy consumption for fermentation here amounts to 2.1 kW·h/kg of biomass at a productivity of 4.2 kg of biomass/m^3·h.

A device for the cultivation of *Methylococcus capsulatus* methane-oxidizing microorganisms on the principle of energy supply with a gas phase is presented in [22]. The main body of the apparatus comprises a turbine agitator mounted on a shaft attached to a turbine driven by a jet of compressed air. The compressed air used to drive the turbine and ensure the operation of the mixer is then distributed throughout the volume of the fermenter to stimulate the cultivation process. The main advantage of this design is the use of compressed gas energy to drive the mechanical part of the bioreactor. However, due to the limited speed of the mixer, the oxygen uptake is below the typical range of 5–20 kg/m^3·h for mixers with a stirrer [10]. Accordingly, this design can be considered as an intermediate bioreactor for growing micro-organisms with an absolute dry matter concentration of no more than 4–5 g/L.

In some types of fermentation equipment for the cultivation of methane-oxidizing microorganisms, combined internal structural elements are used for ensuring multidirectional internal flows of a circulating gas–liquid mixture. An apparatus described in [23] comprises a vertical two-section fermenter with bubblers installed in each section for the introduction of natural and oxygenated gas, as well as nozzles located in the partitions at the entrance to each section. The nozzles are arranged in such a way that the gas–liquid mixture emerging from them in the upper part of the apparatus (from one section to another) enters internal tubular structural elements installed along the axis of the apparatus opposite each nozzle. By using special deflectors in each section of the fermenter, the gas–liquid mixture circulates in the space between the internal tubular structural elements and the wall of the apparatus to increase the useful working volume. In order to achieve uniform flow distribution in the working volume of this bioreactor design, it is necessary to ensure the exit velocity of the gas–liquid mixture from the nozzle in the range of 0.5–20 m/s. Here, a wide range of high-speed exit modes from the nozzle is assumed taking into account the cultivation process in different modes. The paper claims a specific productivity of 4.5 kg of biomass/m^3·h, but without providing information on energy consumption.

An additional group of devices for obtaining biomass from natural gas is represented by loop bioreactors or so-called U-shaped fermenters (Fig. 3).

A number of foreign publications have addressed the issue of optimizing mass transfer processes in this type of equipment to increase productivity. In particular, the paper [24] discusses development approaches related to the use of a multiphase model of a tubular bioreactor with forced mixing for assessing the degree of influence of mixing conditions and interphase mass transfer on the overall performance of closed-loop fermenters. Once a volumetric oxygen mass transfer coefficient of 360 h^{-1} is reached, the productivity of the process in a loop reactor is shown to be practically independent of the mass transfer characteristics of the apparatus. The paper [25] is devoted to an experimental study of the issues of energy consumption and increasing the efficiency of mixing

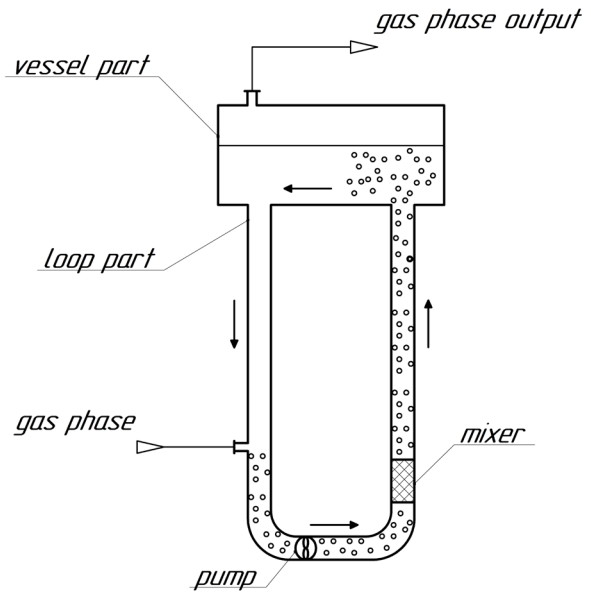

Fig. 3. Loop bioreactor.

in loop-type apparatuses. The increase in mixing efficiency is considered not only from the point of view of the static component of the bioreactor (mixer design), but also its dynamic component (pumping equipment). The cited literature sources illustrate the variety of approaches leading to the emergence of new and improved fermentation apparatus designs.

The design of the loop bioreactor in [26] is represented by two vertical piping sections for the ascending and descending flows of culture fluid, an axial circulation pump installed in one of the piping sections, and the upper cylindrical part of the apparatus where the gaseous phase is separated from the culture fluid. Gas flows into the unit are fed directly into the loop section of the system. Static mixers for ensuring uniform distribution of the gas in the culture liquid are located on the vertical sections of the loop part of the bioreactor.

The bioreactor design presented in [27] is considered according to the operating principle and design solutions used in loop fermenters. The apparatus is described in terms of an upper separation part, represented in the form of a horizontal cylindrical container, as well as two vertical pipelines for the circulation of a culture gas-saturated liquid and a horizontal pipeline section in the lower part of the bioreactor. The vertical sections of the pipeline are used to ensure the circulation of liquid and the supply of gases to the apparatus, as well as involving static mixers and additional means to control the pressure in the zones of the apparatus. By using special equipment, such as a pressure control valve, a nozzle or an axial pump placed in the loops of the bioreactor, pressure drops can be created in different parts of the apparatus to increase or decrease the solubility of gases circulating with the liquid.

Despite the variety of loop bioreactor technical designs involving the combined use of special internal structural elements located both in pipelines and in the gas separation zone, there are also descriptions of external geometric parameters [28]. In the presented design, this principle is articulated by the combined use of vertical and horizontal sections of the apparatus loop in which the gas–liquid mixture circulates, while the main part of the bioreactor loop occupies the horizontal plane.

The literature on loop design bioreactors includes a limited number of publications that address the issue of apparatus efficiency in terms of mass transfer. In [25], the presented values of the volumetric oxygen mass transfer coefficients of 400–3000 h^{-1} obtained in a loop reactor consider the optimal velocities of the gas phase and the correct choice of its entry points into the apparatus. In [26–28], the main problems associated with U-shaped (U-loop) fermenters include ensuring the specified pressure drops at various points of the bioreactor, as well as the timely supply of the transported culture liquid with a gas substrate and the removal of the gas phase containing carbon dioxide.

The loop bioreactor design presented in the patent [29] is characterized by a large number of static mixers located in the horizontal part of the loop and a vertical gas separator tank in which the hydraulic pressure of the liquid column is provided at the suction line of the circulation device. For this model, options for implementing pressure reduction zones at the inlet from the loop to the gas separator tank using equipment and special structural elements installed in the loop portion of the bioreactor are considered. Here, a given parametric control can be implemented for one or more consecutive steps.

One of the factors influencing the approach to the development of fermentation apparatus designs for the cultivation of methane-oxidizing bacteria is the problem of maintaining the concentration of dissolved carbon dioxide in the reaction volume of the fermenter. The fermenter design and technological installation described in [30] are aimed at maintaining the dissolved carbon dioxide content at a sufficient level to ensure high productivity. The bioreactor is made up of vertical sections for ensuring ascending and descending flows between two horizontal capacitive devices in which the culture fluid is to be degassed. Some of the technical solutions of the bioreactor are implemented using standard approaches to internal and external structural

elements: circulation in a closed circuit provided by a pump, gases entering the fermenter through bubblers, and uniform mixing of the culture liquid using static mixers. The most significant aspect of the design is the technical solution for transferring the gas from the degassing tanks to the carbon dioxide absorber. The purified waste gas is returned to the fermenter by means of a compression device for optimizing the cultivation process. As well as indicating the value of the achieved specific productivity of the process (5 kg of biomass/m^3·h), the authors give an example of a specific implementation of a laboratory fermentation system.

CONCLUSIONS

The presented literature analysis shows that all the considered designs of fermentation apparatuses intended for the cultivation of methane-oxidizing microorganisms are based on the basic approaches to the implementation of the hydraulic regime inside the apparatus. Here, one major class of fermentation systems is based on the principle of volumetric mixing in the working space of the apparatus, with the possibility of incorporating external circulation circuits, additional tanks, and auxiliary bioreactors into the system. The other class uses the principle of flow movement (displacement) in the tube space followed by release of the gas phase from the recirculating culture fluid. Internally, the most commonly used structural elements are static mixers placed in pipelines on the flow line of the culture liquid, bubblers that ensure the entry of oxygenated and methanated gases into the bioreactor, as well as nozzle devices and ejectors embedded in the main volume or placed in gravity and pressure circuits for ensuring the maximum degree of mixing of gas and liquid parts in the apparatus.

Authors' contributions

V.M. Kochetkov – concept of the research, writing and scientific editing the paper, drawing conclusions;

I.S. Gaganov – literature analysis on fermentation equipment designs, comparative analysis, and bibliography design;

V.V. Kochetkov – literature analysis on the technology for obtaining bioprotein from natural gas, technical editing the paper, and preparing illustrative materials;

P.A. Nyunkov – systematization of cited literature, critical analysis.

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