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RESEARCH ARTICLE

Increasing the efficiency of bioreactor operation for cultivation of methane-oxidizing bacteria under conditions of decreasing carbon dioxide concentration in the cultural liquid

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Abstract

Objectives. The work set out to develop a bioreactor that incorporates a carbon dioxide removal unit within the apparatus gas phase, which is capable of operating without the need for supplementary compression apparatus. As part of testing the developed equipment in order to ascertain its capacity for enhanced biomass production, the principal fermentation system parameters that facilitate the optimal bioreactor productivity in conditions of carbon dioxide removal from the apparatus gas phase were identified.

Methods. A series of tests were conducted on the fermentation unit with the objective of controlling the oxygen and carbon dioxide content in the gas phase of the bioreactor. This was achieved using an in-line gas analyzer fitted with electrochemical sensors. The oxygen and carbon dioxide content in the gas phase was determined by means of gas chromatography. The oxygen and natural gas flow rates were determined using a thermal electronic flow controller equipped with thermoresistive elements. The oxygen content of the cultural liquid was determined by means of an optical oxygen sensor with integrated transducer. The pH level in the bioreactor was monitored and maintained using an electrochemical pH sensor.

Results. The efficacy of the newly devised jet-type bioreactor design, which permits the incorporation of a carbon dioxide removal unit into the fermentation system without requiring supplementary compression apparatus, was evaluated through experimentation. The system was tested with the carbon dioxide removal unit included in the design, resulting in a 64% increase in bioreactor productivity and a 18% reduction in oxygen consumption as a component of the gas supply.

Conclusions. The operational parameters of a technological bioreactor that facilitate a stable continuous process of bacterial cultivation were identified.

Keywords

bioreactor, fermenter, fermentation, biomass, protein, carbon dioxide, ejector, methane-oxidizing bacteria, *Methylococcus capsulatus*

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НАУЧНАЯ СТАТЬЯ

Повышение эффективности работы биореактора для культивирования метанокисляющих бактерий за счет снижения концентрации углекислого газа в культуральной жидкости

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Аннотация

Цели. Разработать конструкцию биореактора, позволяющую включить в свой состав узел удаления углекислого газа из газовой фазы аппарата, функционирующий без использования дополнительного компрессионного оборудования; провести испытания разработанного оборудования с целью увеличения его производительности по биомассе; определить основные параметры работы ферментационной системы, при которых достигается максимальная продуктивность биореактора в условиях извлечения углекислого газа из газовой фазы аппарата.

Методы. Проведена серия испытаний ферментационной установки с осуществлением контроля содержания кислорода и углекислого газа в газовой фазе биореактора поточным газонализатором с электрохимическими сенсорами. Контрольное определение содержания в газовой фазе кислорода и углекислоты проводилось методом газовой хроматографии. Расход газовых компонентов (кислорода и природного газа) измерялся с помощью теплового электронного регулятора расхода с терморезистивными элементами. Содержание растворенного в культуральной жидкости кислорода определялось оптическим датчиком кислорода со встроенным преобразователем. Уровень pH в биореакторе контролировался и поддерживался с помощью электрохимического pH-датчика.

Результаты. Разработан и испытан биореактор струйного типа. За счет использования внутренних рециркуляционных потоков в ферментационную систему интегрирован узел удаления углекислого газа без применения дополнительного компрессионного оборудования. В процессе испытаний системы с включенным в конструкцию узлом извлечения углекислого газа достигнута увеличенная на 64% продуктивность биореактора и снижен на 18% расход кислорода, как компонента газового питания.

Выводы. Определены технологические параметры работы биореактора, при которых проходит стабильный процесс непрерывного культивирования бактерий.

Ключевые слова

биореактор, ферментер, ферментация, биомасса, белок, углекислый газ, эжектор, метанокисляющие бактерии, *Methylococcus capsulatus*

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INTRODUCTION

The process of obtaining bacterial biomass from natural gas is currently under active consideration by both research organizations that study the properties of methane-oxidizing bacteria and industry technology companies [2–3]. In the context of current trends of establishing Russian protein and vitamin concentrate production facilities, it is necessary to pay particular attention to the hardware design of the process, since this will ultimately determine the scalability boundaries of the process along with desired volumes of feed protein synthesis.

The technological line for the production of protein from natural gas employs a combination of standard and specialized equipment, including centrifugal and dosing pumps, capacitive equipment, and specialized food equipment. The line utilizes centrifugal separators, spray dryers, and high-temperature product processing systems that act as a pasteurizer. However, the most important equipment in the technological chain of bioprotein production is the bioreactor.

The process of cultivating methane-oxidizing bacteria is conducted on a gas substrate, which has considerable influence on the bioreactor design. The primary characteristics of the fermentation equipment designs, along with an overview of the majority of the documented bioreactor models for protein production from natural gas, are presented in [4]. Furthermore, it is essential to address the matter of carbon dioxide present in the gas phase of the fermenter, which is released by methane-oxidizing microorganisms.

The effect of carbon dioxide on bacterial growth has attracted the attention of researchers since the beginning of the 20th century [5]. With the accumulation of material subjected to analysis, two main areas were identified in which this issue became of paramount importance: food preservation and management of microbiological processes [6]. From a practical standpoint, the inhibitory effect of carbon dioxide on bacterial growth is most relevant to the question of food preservation [7]. The scope of challenges confronting researchers was considerably extended with the advancement and intensification of biotechnological procedures. In [8], the issues related to the presence of carbon dioxide in bioreactors are considered from two distinct perspectives: firstly, in terms of its effect on microorganisms, and secondly, in terms of solubility and diffusion in an aqueous medium. In this context, the advancement of fermentation apparatus for technological procedures wherein carbon dioxide is dispersed into the operational volume, which is generated as a consequence of microbial metabolic processes involved in the cultivation of methane-oxidizing bacteria, is a significant undertaking. This is primarily due to the necessity of guaranteeing the solubility of both oxygen-containing and natural gas in the presence of carbon dioxide in the cultural liquid (CL), which is continuously released by bacteria.

Observing that the data on the impact of carbon dioxide on the growth of methanotrophs are inconclusive, V.V. Lalov[1] noted the need for further investigation into the question of whether this component of the gas phase inhibits bacterial culture growth. The most interesting results concerning the effect of limiting and inhibitory concentrations of components of the gas phase and mineral medium on the growth of *Methylococcus capsulatus* are presented in the publication of R.R. Gayazov[2]. The growth of a *Methylococcus capsulatus* methane-oxidizing bacteria culture (strain VSB-874) obtained at the *VNIISINTEZBELOK* Institute (Russia) was shown to be inhibited with an increase in the partial pressure of carbon dioxide in the gas phase of the bioreactor. The publication [9] presents a method for cultivating bacteria of the genus *Methylococcus*, which is of practical importance in studying the effect of carbon dioxide on the growth of microorganisms. The authors propose the use of compression equipment for the removal of a portion of the carbon dioxide from the gas phase of the bioreactor and its partial return to the liquid phase of the apparatus.

The *GIPROBIOSINTEZ* scientific and technical center considered the results of previous studies on the impact of carbon dioxide on methane-oxidizing bacteria. During the commissioning of pilot plants for the production of protein from natural gas, modifications were made to the bioreactor in order to facilitate the return of the carbon dioxide-free components of the gas supply to the process without the use of compression equipment. Furthermore, tests were conducted on the modified apparatus to obtain empirical data regarding the impact of the purification system for the gas phase of the bioreactor on its overall productivity.

EXPERIMENTAL

Schematic diagram of the fermentation unit

In order to demonstrate the technical feasibility of removing carbon dioxide from the gas phase of the bioreactor, a fermentation system is proposed. The details of this system are outlined in the patent [10]. The schematic diagram of the fermentation unit is shown in Figure.

The unit comprises a fermenter (*1*) with a working volume of 15 L, a centrifugal pump (*2*), an ejector (*4*), a tank for CL recovery (*7*) and a carbon dioxide removal unit comprising two adsorbers (*5a*, *5b*). A centrifugal pump installed on the circulation circuit of the bioreactor is used to pump the CL into the ejector. The gas phase emerging from the upper portion of the bioreactor, which is carried along by the process medium (CL), also enters the ejector. The gas–liquid mixture is introduced into the lower section of the bioreactor working volume via the ejector. The primary technical solution of the proposed fermentation system is the installation of a purification system, specifically a carbon dioxide removal unit, on the gas phase outlet line from the bioreactor to the ejector. This system consists of two alternately operating

[1] Lalov V.V. *Analysis and synthesis of energy-technological systems for the production of fodder protein from natural gas.* Diss. Dr. Sci. (Eng.). Moscow, 1991. 416 p. (in Russ.).

[2] Gayazov R.R. *Limitation and inhibition of METHYLOCOCCUS CAPSULATUS growth by components of mineral medium and gas phase.* Diss. Cand. Sci. (Biol.). Russian Academy of Sciences. Institute of Biochemistry and Physiology of Microorganisms. Pushchino, 1992. 127 p. (in Russ.).

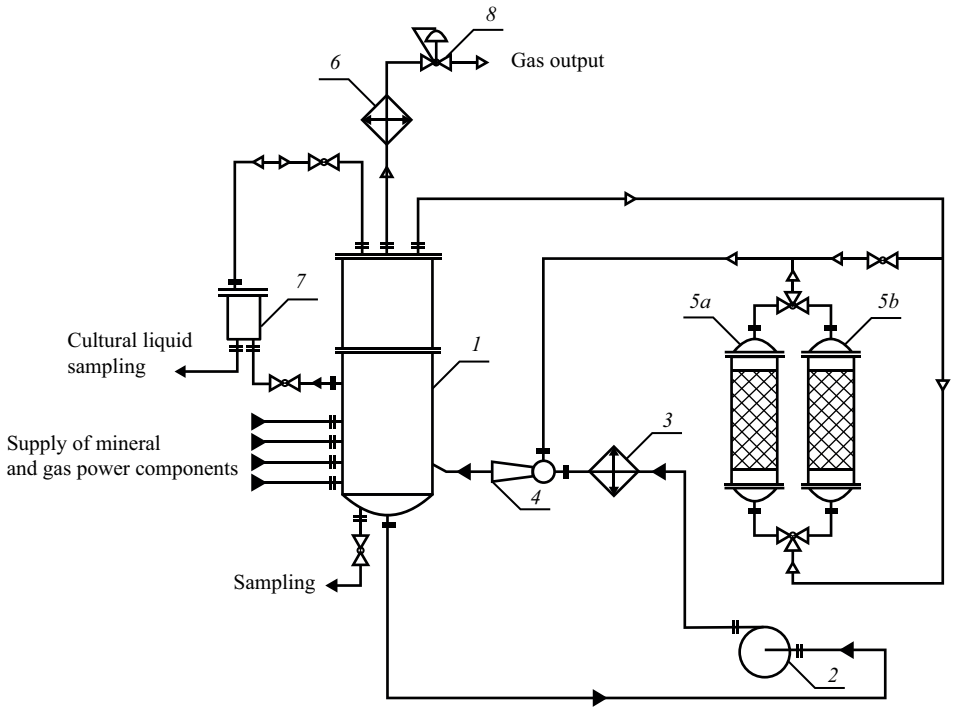

Fig. Modified fermentation unit for methane production from natural gas: (*1*) bioreactor; (*2*) centrifugal pump; (*3*) heat exchanger; (*4*) ejector; (*5a*, *5b*) adsorbers; (*6*) condenser; (*7*) cultural liquid collector; (*8*) upstream pressure regulator [10]

adsorbers. The principal benefit of this configuration is the capacity to link the purification apparatus to the gas recirculation circuit in advance of the pressure jet ejector. This enables the return of the gas phase purified from carbon dioxide to the fermenter without the need for supplementary compression apparatus.

Carbon dioxide removal unit

The gas recirculation line incorporates a bypass line with control valves for ascertaining the ratio of gas directed to the adsorbers for purification to the quantity of gas entering the ejector directly from the fermenter for mixing with the CL. In the carbon dioxide removal unit, which is situated within the fermentation unit and used to separate gases present in the gas phase, a variety of purification techniques may be employed. These include the use of amine solutions for absorption, aqueous potassium carbonate solutions, and membranes for the selective gas separation. In order to exhibit the parametric values of the fermentation process obtained for the modified bioreactor design, a more cost-effective adsorption process is proposed in the purification unit. A group of adsorbents with selective affinity for carbon dioxide was selected based on their absorption capacity at a gas phase pressure in the bioreactor (p_{react}) of 2 barg. Values of carbon dioxide capacity at given pressures and absorption temperature t_{ads} are given in Table 1.

In light of the aforementioned parameters, the adsorbent AMSORB® PLUS was identified as the optimal choice. The composition of the adsorbent is presented in Table 2.

When selecting an adsorbent, it is essential to consider not only the capacity and selectivity of the material in question, but also its chemical composition. The proposed adsorbent comprises components and solutions that are employed as calcium sources and introduced into the bioreactor to facilitate the bacterial cultivation process.

Bioreactor operating modes

In order to evaluate the efficiency of the fermentation system, its operation was tested in two modes: firstly, without a purification system, and secondly, with the use of a carbon dioxide removal unit, which was represented by two adsorbers.

In order to achieve the first mode of operation (Mode No. 1), the gas phase from the bioreactor was fed into the ejector via a bypass line by which means it was mixed with the CL. This bypass line circumvented the adsorbers. The apparatus was supplied with gas supply components: natural gas, oxygen and air in the ratio of 1 : 0.9 : 1. The process gas flow rate was regulated by means of electronic mass flow rate controllers (F-201CV) produced by *Bronkhorst High-Tech* (Netherlands). These controllers comprise a thermal electronic flow rate

Table 1. Absorption capacity of adsorbents

Adsorbent	Manufacturer	t_{ads}, °C	p_{react}, barg	Carbon dioxide (CO_2) capacity, mmol/g (mgCO_2/g)	Reference
Molecular sieve X13	*Zeochem* (Switzerland)	30	2	4.6 (202.4)	[11]
Zeolite NaY	*UOP* (USA)	25	2	5.0 (220.0)	[12]
Activated carbon	*Samchunri* (Korea)	45	2	3.0 (132.0)	[13]
Calcium hydroxide $Ca(OH)_2$	*Aldrich Chemical Company* (USA)	650	–	10.7 (470.8)	[14]
AMSORB® ($Ca(OH)_2$-based)	*Armstrong* (United Kingdom)	36.1	–	4.0 (176.9)	[15]
AMSORB® PLUS	*Armstrong* (United Kingdom)	35–40	–	7.8 (345)	[16], [17]

Table 2. Composition of AMSORB® PLUS adsorbent

Component	Content, wt %
Calcium hydroxide $Ca(OH)_2$	77.0–88.0
Calcium sulfate pentahydrate $CaSO_4 \cdot 5H_2O$	0.6–1.5
Calcium chloride $CaCl_2$	2.0–3.5
Water H_2O	10.0–18.0

controller with thermoresistive elements. The process was conducted at a temperature of 42°C. The bioreactor was provided with a solution of essential mineral nutrients required for optimal culture growth, as well as a continuous flow of ammonia water to maintain a pH range of 5.6–5.8 for CL. The pH in the bioreactor was measured using an EasyFerm Plus ARC 225 electrochemical sensor developed by *Hamilton* (USA). The content of dissolved oxygen in CL was determined using a VisiFerm DO Arc 225 H2 optical oxygen sensor (*Hamilton*, USA). The requisite flow velocity within the apparatus (in excess of 0.25 h^{-1}) was achieved by introducing process water into the apparatus. The reactor was pressurized at 2 barg to ensure that the carbon dioxide content in the circulating gas phase—and, consequently, in dissolved form in the CL—would not lead to disturbance of the cultivation regime by inhibiting the growth of methane-oxidizing bacteria (Footnote 2).

Upon identifying the second operational mode (Mode No. 2) of the fermentation system, the gas phase from the bioreactor was directed to the ejector via the purification unit. The apparatus was provided with the gas supply components comprising natural gas, oxygen and air. The flow rate ratio of the fed components was modified to values of 1.0 : 0.7 : 1.6 throughout the course of the experiment. The reactor is pressurized to 4 barg while maintaining the other process conditions. The carbon dioxide removal unit was constructed in such a way as to regulate the ratio of circulating gas entering directly into the ejector and being subsequently directed to the adsorbers. The control mechanism enables the delivery of a target load (gas flow with CO_2 content) to each operational adsorber, while ensuring that the carbon dioxide content in the gas phase does not exceed the required value for a pre-determined period of adsorber operation. The composition of the gas phase in the bioreactor was monitored using a MAG-6 gas analyzer (Russia, TU 26.51.53-016-70203816-2021[3]) with built-in sensors to determine the volume fraction of oxygen and carbon dioxide. The content of the methane, oxygen and carbon dioxide components was continuously recorded. In accordance with the data given in Gayazov's work (Footnote 2), the volume content of carbon dioxide in the gas phase was selected to be 3% to exclude its inhibiting effect on plant growth at a set apparatus pressure of 4 barg. Chromatography was used to determine the content of the gas phase components. Twice a day, a sample was taken from the gas phase of the bioreactor and analyzed on a chromatograph of the Chromatec-Crystal 5000.2 brand (*Chromatec*, Russia) using a column Mss. 316 2 m × 3 mm NaX 60/80 (*Chromatec*, Russia). Helium was used as a carrier gas; the detector temperature was 180°C; the column temperature was 60/180°C. Each sample was analyzed at least twice.

[3] TU 26.51.53-016-70203816-2021. Description of the measuring instrument type. MAG-6 multicomponent gas analysers. Moscow: Federal Agency for Technical Regulation and Metrology. Order No. 1984 of 10 August 2022.

The carbon dioxide purification unit was commissioned in a working fermentation system at a volume content of carbon dioxide in the gas phase of the bioreactor of 3 vol %. A portion amount of AMSORB® PLUS adsorbent (United Kingdom) was added to two pre-washed and drained adsorbers, one of which had been commissioned. The operating time of the adsorbent placed in the volume of the apparatus was determined by the level of carbon dioxide in the gas phase of the bioreactor. When the carbon dioxide content in the gas phase of the bioreactor rose above 3% by volume, the gas flow was switched to a parallel adsorber filled with a fresh portion of adsorbent. The spent adsorbent was replaced in the adsorber. The adsorber was started up with a new batch of adsorbent according to the work cycle.

RESULTS AND DISCUSSION

The above-described technical solution has been tested and confirmed to work in fermenters that incorporate jet devices as part of their design. The location of the purification unit on the internal gas recirculation line allows the ratio of the amount of gas entering the purifier and via the bypass line directly into the bioreactor to be adjusted using control valves. By means of this control mechanism the purification system can be operated in several bioreactor operating modes characterized by variable values such as the pressure in the apparatus, the composition of the oxygenated gas, and the circulation rate of CL.

According to a comparative analysis of the bioreactor operation modes, the maintenance of consistent performance within the cultivation system is achieved by integrating a gas purification unit into the operational process even with an increase in pressure from 2 to 4 barg.

The parameters of the fermentation system in two comparative modes of operation are presented in Table 3.

As evidenced by the data, the implementation of Mode No. 2 resulted in a notable increase in biomass concentration within the bioreactor, reaching 15.5 g/L at an elevated flow rate from 0.27 to 0.3 h^{-1}. The operation of the fermentation plant in carbon dioxide removal mode allowed the fermentation system's productivity to be increased to a value of 4.6 g/(L·h) (including an increase in system pressure). Accordingly, the enhancement in productivity in comparison to Mode No. 1 reached a level exceeding 64%.

The implementation of Mode No. 2 of operation reduced the oxygen supply to the process by 18% while simultaneously increasing air consumption by 60%. In Mode No. 1, when air flow was provided to the fermenter

Table 3. Fermentation system operation parameters for two modes

Parameter	Mode No. 1 System parameters without using a carbon dioxide removal system (adsorber)	Mode No. 2 System parameters using a carbon dioxide removal system
Input parameters		
Fermenter pressure, barg	2.0	4.0
Air flow rate, nL/h	250	400
Natural gas flow rate (96 vol % methane), nL/h	250	250
Oxygen flow rate (93 vol %), nL/h	220	180
Dilution rate, h^{-1}	0.27	0.3
Output parameters		
The content of oxygen and carbon dioxide in the gas phase, vol % Oxygen Carbon dioxide	 22.7 3.9	 23.2 3.2
Productivity, g/(L·h)	2.8	4.6
Biomass concentration, g/L	10.4	15.5
Dissolved oxygen content, mg/L	1.2	6.6

at a flow rate exceeding 400 nL/h, productivity did not exceed 2.8 g/(L·h).

CONCLUSIONS

The proposed fermentation plant design, which incorporates a carbon dioxide purification unit in the internal circulating gas line, allows the cultivation of methane-oxidizing bacteria with increasing pressure in the bioreactor without the need for additional energy consumption to return purified gas to the system. From the perspective of enhancing the solubility of the gas components of nutrition (methane and oxygen), it is recommended to investigate the impact of elevated pressure within the fermenter on the process productivity. This approach will also optimize the consumption indicators for the gas components of the power supply to ensure a stable process of protein production from natural gas.

The observed increase in the crucial productivity parameter of the bioreactor by over 64% underscores the need to select and implement technological processes that for effectively purifying gas phase from carbon dioxide. The utilization of membranes for selective gas separation in conjunction with the absorption process involving amine solutions represents a promising avenue for investigation in the context of the purification unit.

Authors' contributions

V.M. Kochetkov—research concept, developing the design, scientific editing, formulating the conclusions.

I.S. Gaganov—structural designs, writing the paper, designing the bibliography.

D.V. Tolkin—planning and conducting the experimental work.

V.V. Kochetkov—conducting the experimental work, technical editing, preparing the illustrative materials.

P.A. Nyunkov—systematization of publications, critical analysis.

The authors declare no conflicts of interest.

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