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Study of the scaling possibility of the process of obtaining feed protein from natural gas

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The carbon substrate is an important aspect in the industrial biotechnology. Thereupon the target product obtaining using relatively cheap non-food raw materials is important. Based on this, microorganisms that are capable of using C1-compounds are of interest, starting from the extremely reduced compound – methane, up to the extremely oxidized compound – carbon dioxide.

The implementation of the technology of obtaining microbial biomass from natural gas as a raw material is a promising, innovative task of biotechnology. The effectiveness of its implementation is determined by the raw material resource, which provides the possibility of creating high-capacity enterprises, as well as the significance of the resulting product – microbial biomass that is characterized by a high protein content (more than 70 %) and high biological value. This will reduce the protein deficiency in animal feed, will ensure an increase in the productivity of fattening farm animals, poultry and fish. The solution to the problem will reduce the import dependence of the Russian Federation on high-protein feed additives (soy, fish, meat and bone meal, etc.).

The efficiency of the technology of obtaining methane-oxidizing bacteria biomass largely depends on the technological potential of the used microorganism culture.

Previously [1, 2] the ability of an associative culture of methane-oxidizing bacteria to develop on natural gas was justified, in the process of cometabolism participate bacteria capable of using as carbon and energy sources the co-oxidation products of methane homologues present in natural gas: ethane, propane and butane, which at a certain concentration inhibit growth of an obligate methane-oxidizing culture.

In the developing of obtaining microbial biomass technology of based on natural gas, in 2016 a new strain of *Methylococcus capsulatus* GBS-15 was selected, which has a complex of industrially valuable properties: the ability to develop at a flow rate up to 0.36 h^{-1} on a mineral medium without addition of the growth factors in a wide temperature range of 38–45 ℃, pH 5.6–5.9 and in an atmosphere of methane:air (from 1:2.5 to 1:3).

Stable continuous fermentation on natural gas is possible only for an associative methane-oxidizing bacteria culture (with the dominance of the producer of 85–98 %) and heterotrophic satellite bacteria using products of methane homologues incomplete oxidation, and also potentially products of cell autolysis. In fermentation the associative culture the activity of its growth and resistance to stress effects of certain physicochemical parameters noticeably increase.

The isolation of associated bacteria was carried out from the fermentation process carried out in a fermenter V = 200 l, using natural gas with a methane concentration of 96 %, concentration the main homologues of ethane of 2.2 %, propane of 0.8 %, butane of

0.13 %. The fermentation was carried out with a gradually increasing flow rate from 0.1 h^{-1} to 0.27 h^{-1}. The flow rate was increased after culture growth had stabilized. The studing of the microbial profile using autoselection processes of the established associative culture while the selection of a pure culture of *Methylococcus capsulatus* GBS-15, made it possible to identify 3 heterotrophic satellites. It was found that *Cupriavidus gilardii* predominate among the associates (up to 15 %), and the rest in total no more than 0.5 %. Isolated cultures of methane-oxidizing bacteria and heterotrophic satellites were identified by 16S rRNA and deposited at State Research Institute of Genetics and Selection of Industrial Microorganisms of the National Research Center "Kurchatov Institute". The studies have been carried out in State Research Center for Applied Microbiology and Biotechnology and the report on of non-toxigenicity, non-pathogenicity and harmlessness of the main producer, heterotrophic associates and the feed product has been obtained. LLC "GIPROBIOSINTEZ" has a technology for obtaining protein feed product, including a full production cycle: starting from sowing an industrial apparatus to obtaining a dry feed product. The cultivation of methane-oxidizing bacteria can be carried out in fermenters of different volumes, with a scaling factor of 10. The fermentation is carried out in reactors with a volume of 5 l, 10 l, 15 l, 200 l and 3 m^3. Cultivation is carried out in fermenters with a volume of 5 l, 10 l, 15 l, 200 l and 3 m^3. All fermenters (except 5 and 10 l) operate at positive pressure. The fermentation of an associated culture in a chemostat mode in fermenters with a stirrer with a volume of 5 l and 10 l makes it possible to obtain a productivity of 2.3-2.5 g/l per hour or more. When cultivating in ejection-type fermenters with a volume of 15 l and 200 l, operating at an overpressure of 1.8–2.0 bar, the productivity is about 3 g/l per hour. The increasing the overpressure to 4.0 bar in fermenter with a volume 3 m^3 makes it possible to achieve a productivity of about 4 g/l per hour.

Fermenter volume	Overpressure in fermenter, bar	Mixing device type	Dry weight, g/l	Flow rate D, h^{-1}	Productivity, g/l per hour
5 l	–	stirrer	10.0	0.23	2.3
10 l	–	stirrer	8.9	0.28	2.5
15 l	1.8	ejector	11.0	0.26	3.0
200 l	2.0	ejector	10.5–11.2	0.25	2.6–2.8
3 m^3	2.0–4.0	ejector	14.5–18.0	0.2–0.25	2.8–4.0

The ongoing work in improving the design of the fermenter gives grounds for obtaining higher productivity.

References

1. Galchenko V.F. Methanotrophic Bacteria. Moscow: GEOS, 2001.
2. Patent WO 2010/069313 A2/ U-shape and/or nozzle U-loop fermenter and method of fermentation (DK).