Closed biotechnological cycles for transport life support systems in deep space exploration

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Abstract. The integration of closed biotechnological cycles in life support systems (LSS) of autonomous transport systems (ATS) is a critical solution for deep space and planetary exploration. Prolonged autonomous existence of integral ecosystems depends on the degree of cyclic use of substances and the coefficient of closure of the ecosystem's mass-exchange processes. In missions lasting over two years, it becomes more beneficial to reproduce necessary substances inside the system instead of relying on external supplies. This research aims to explore the integration of closed biotechnological cycles, with a focus on the biotechnological cycles of methane and carbon dioxide, in the LSS ATS to achieve a high coefficient of closed circulation of substances and nature-like features. Two methods of ATS crew waste processing are described, and the potential of using methane for additional oxygen generation and food protein biosynthesis in the closed-loop cycle of substances transformation is investigated. Additionally, the possibility of applying methane biooxidation technology to existing biotechnological processes of feed and food protein production using methanotrophic bacteria in terrestrial conditions is discussed as a potential method for producing food animal protein in LSS ATS.

1 Introduction

The first human spacecraft used life support systems based solely on substance reserves [1]. Such systems based on stores of water, oxygen, and carbon dioxide absorbers are not economical in orbital spaceflight and cannot be used in long-distance space missions as basic systems because of mass and volume limitations. Therefore, complexes based on water and oxygen regeneration from human life products are used on space stations [2]. In the future, some biological and biotechnological links are expected to be added to LSS complexes. The cost of cargo traffic to orbit, though considerably reduced recently, is estimated today at several thousands of dollars per kg of payload. So, the minimum cost of delivery of 1 kg of cargo to the MSC today is about $3300/kg. [3].

The physicochemical regeneration complex LSS in principle cannot be completely closed, because there is no process of food regeneration in it, so it is required to have food

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reserves and a way of removing the unregenerated part of the waste of vital functions. Only a biotechnological regeneration facility can be enclosed. Analysis of the mass balance and the formation of the optimal structure of a closed regenerative biotechnological complex to ensure the maximum closure of the mass balance are fundamentally important.

First of all, we are talking about solving the problem of oxygen extraction from carbon dioxide, finding additional sources of oxygen and methane generation, and most importantly, the possibility of using the generated methane to obtain onboard ATS protein concentrate of animal origin for food reproduction, which will allow, along with higher plants, microalgae, and biological objects, to close regeneration complex LSS and on this basis to plan long-distance manned missions and planetary exploration.

2 The physical and chemical method of methane formation

The most common method of oxygen recovery from carbon dioxide is the Sabatier reaction with subsequent electrolysis of the resulting water [1, 4].

The Sabatier reaction is exothermic and is carried out at 260-300 °C using a catalyst:

\[
\text{CO}_2 + 4\text{H}_2 = \text{CH}_4 + 2\text{H}_2\text{O}
\]

\[
2\text{H}_2\text{O} = 2\text{H}_2 + \text{O}_2
\]

Due to the loss of some hydrogen with methane, the carbon dioxide cannot be fully utilised to produce water to extract oxygen from it. In the absence of methane pyrolysis, the Sabatier reaction allows only 61% of human excreted carbon dioxide to be used; the oxygen loss in this case will make 39% (0.27 kg/person/day) of the available carbon dioxide or 31.5% of all oxygen necessary for human breathing. Thus, when using the Sabatier process, an additional 0.18 + 0.30 = 0.48 kg/person/day of water electrolysis is needed [1].

As it can be seen from Sabatier formula, from one grammolecule (gm) of carbon dioxide one gm of methane and two gm of water are produced, which in terms of mass, makes: from 0.96 kg of CO2 emitted by one person per day to 0.35 kg of CH4 [4].

The missing 19% of oxygen is provided by the electrolysis of water at 800 - 10000°C with the removal of hydrogen:

\[
2\text{H}_2\text{O} = 2\text{H}_2 + \text{O}_2
\]

3 Ways for transformation of exometabolites of crew and waste from higher plants

3.1 Mass balance

The mass balance of major human waste products is given in Table 1, without taking into account atmospheric and water micronutrients [1].

<table>
<thead>
<tr>
<th>Intake, kg/day</th>
<th>Excretion, kg/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nutrition</td>
<td></td>
</tr>
<tr>
<td>Dry mass</td>
<td>0.60</td>
</tr>
<tr>
<td>Water</td>
<td>0.50</td>
</tr>
<tr>
<td>Respiration</td>
<td></td>
</tr>
<tr>
<td>Oxygen</td>
<td>0.86</td>
</tr>
<tr>
<td>Water</td>
<td>2.00</td>
</tr>
<tr>
<td>Cooking and drinking</td>
<td></td>
</tr>
<tr>
<td>Urina</td>
<td>1.20</td>
</tr>
<tr>
<td>Water</td>
<td>1.15</td>
</tr>
</tbody>
</table>

Table 1. The mass balance of major human waste products.
Similar data on crew waste were obtained at IBF SB RAS during studies at the BIOS-3 facility when growing fish and higher plants: Urine - 1500 ml man/day; Feces - 150 g man/day; Fish waste - 50 g man/day; Wheat straw - 375 g man/day.

Water balance is achieved by releasing an additional amount of water formed in the human body - 0.35 kg of so-called metabolic water.

The total amount of oxygen contained in exhaled human carbon dioxide is 0.70 kg/person/day, which is 81% of the oxygen necessary for human breathing. The remaining 19% of the oxygen (0.16 kg/person/day) would come from human excreted water (0.18 kg/person/day), which is consistent with the balance, since humans excrete 0.35 kg of metabolic ('extra') water per day. Theoretically, full utilisation of water excreted by the crew leaves a water reserve of 0.17 kg/person/day.

### 3.2 Pyrolysis method with the production of trace elements

Experimental prototypes of closed-loop LSS involving humans, such as, BIOS-3 complex (USSR - Russia), NEC, BIO-Plex, BIOSPHERE-2 (USA), CEEF (Japan), MELISSA (EU), China Cosmonaut Training Centre in Beijing (China), Yuegun-1 (China), SPACEnter CELSS of the Space Institute of Southern China in Shenzhen (China), showed that higher plants are the basis of future space LSS, and the role of physicochemical and biotechnological fertilizer preparation techniques is to accelerate the reduction processes of organic material so as to maximise yields, as they fulfil the most vital functions of reproducing oxygen, water and food for the crew [5].

Some protein food can be reproduced by fish, worms, and other heterotrophic organisms, and the use of bacterial cultures of microorganisms and soil-like substrates as biotechnological methods of utilising some plant waste is also being considered. The principle of liquid-phase oxidation of organic wastes by reactive oxygen species in an aqueous medium seems very promising for an artificial physical-chemical link of reducing agents in future LSS with a crew of several people.

Hydrogen peroxide (H2O2) is currently the most ecologically clean, effective, and easily reproducible type of oxidiser in the intrasystem cycle [6].

The consecutive technological subsystem of physical-chemical treatment of biological wastes for obtaining nutrient solutions for growing cultivated plants on hydroponics, developed in IBF SB RAS with respect to closed ecosystems of space purposes, consists of three main reactors: "wet incineration", urea decomposition and H2O2 synthesis. A simplified scheme of the technological process is shown in Figure 1 [3, 7, 8].

<table>
<thead>
<tr>
<th></th>
<th>Moisture through the lungs and skin</th>
<th>Condensation of atmospheric moisture</th>
<th>1.50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water consumption</td>
<td>2.5</td>
<td>Water removal (Metabolic 0.35)</td>
<td>2.85</td>
</tr>
<tr>
<td>Consumption</td>
<td>3.96</td>
<td>Removal</td>
<td>3.96</td>
</tr>
</tbody>
</table>
Fig. 1. Simplified scheme of the organic waste processing subsystem for high closed-loop LSS [3,7,8].

The method of biowaste fertilizer preparation for phototrophic link of closed-loop LSS uses nitric acid HNO₃ due to its synthesis in the existing chain of LSS circular processes and alkali - KOH due to its predominance in urea dialysis products [9].

The dried hardly soluble sludge is oxidized separately from basic solution in small volume of mixture HNO₃ + H₂O₂ 1:1 that gives higher concentration of reagents and decrease of pH value at the same amount of reagent moles, under the action of alternating current.

The method of preparation of fertilizers from biowaste for phototrophic link of closed-loop LSS uses nitric acid HNO₃ due to its synthesis in the existing chain of circular processes of LSS and alkali - KOH due to its prevalence in urea dialysis products [9].

The dried hardly soluble precipitate is oxidized separately from the basic solution in a small volume of HNO₃ + H₂O₂ 1:1, which gives a higher concentration of reagents and decrease of pH value at the same amount of reagent moles, under the influence of alternating current.

This method allowed to transfer more than 90% of nutrients to the dissolved ionic form and was much more effective than adding acid directly to the whole solution before the classical “wet digestion” [9, 10].

3.3 Methanogenic method with fertiliser and micronutrient production

Considering crew metabolites and waste from the phototrophic link of a closed LSS with a higher degree of integration of biotechnological cycles as raw materials, it is possible to produce methane in addition to fertiliser and from fertiliser a complex of microelements for the cultivation of higher plants and fermentation of microbial biomass for the balance of crew food.

This requires an anaerobic digestion process involving different groups of microorganisms. The main purpose of anaerobic digestion of biological waste is to produce biogas, consisting of methane and carbon dioxide in an approximate 50/50 ratio, and organic fertiliser, providing micronutrients for cultivation of higher plants and fermentation of biomass.

The anaerobic process of biowaste decomposition and biogas production can be divided into 4 stages:
In the first stage, the hydrolysis stage, aerobic bacteria rearrange cellulose, protein, carbohydrates, and fats into low molecular weight compounds such as sugar, amino acids, fatty acids, and water. Enzymes released by hydrolysis bacteria break down the organic constituents of the substrate into small water-soluble molecules and the polymers are converted into monomers. The process is significantly influenced by the pH level (4.5-6) and the time of residence in the tank.

In the second step, the acid-forming bacteria do further degradation. This phase is called the oxidation phase, where the pH is lowered. Individual molecules will pass into the bacteria cells where they continue to decompose. This process creates the anaerobic conditions necessary for methane bacteria. At pH 6-7.5, first of all unstable fatty acids, low-molecular-weight alcohols, carbon dioxide, carbon, hydrogen sulphide and ammonia are first produced.

The third step is very sensitive to temperature changes. Acid-forming bacteria create the precursors for the formation of methane from organic acids: acetic acid, carbon dioxide, and carbon.

In the fourth step, methane, carbon dioxide and water are produced in small quantities as a by-product of the methane bacteria. Ninety percent of all methane is produced in this step, and 70% of the methane comes from acetic acid. The formation of acetic acid in the third step is a factor in determining the rate of methane formation. Additionally, further amounts of CH₄ and H₂ are formed from CO₂ and H₂O.

All methanogenic bacteria are exclusively anaerobic. These reactions occur simultaneously, and methanogenic bacteria have considerably higher requirements for their conditions of existence than acid-forming bacteria; they require absolutely anaerobic medium and require more time for reproduction. Methane-forming bacteria are active within a temperature range of 5-70°C. At higher temperatures, the bacteria die, with the exception of a few strains that can survive up to an ambient temperature of 90°C. When the temperature drops below 5°C, the bacteria cease to be active. The higher the process temperature, the faster the decomposition occurs.

The average gas composition that can be obtained from exometabolites and phototrophic waste at an optimum fermentation temperature of 34°C corresponds to:

\[ \frac{CH_4}{CO_2} = 2 \]

50-87% methane, 13-50% CO₂, minor admixtures of H₂ and H₂S. After purification of biogas from CO₂, biomethane is produced. Biomethane is fully analogous to natural gas, the only difference is the origin. The gas yield can be up to 300 litres from 1 kg.

The main factors affecting the fermentation process are: Temperature; Moisture of the medium; pH level; C: N: P ratio; Surface area of the raw material particles; Substrate feeding frequency.

4 Ways to use methane

4.1 Using methane to regenerate oxygen

The decomposition of methane into hydrogen and carbon has now become one of the pathways for the development of hydrogen energy. Work on the production of hydrogen is carried out in several directions: electrolysis of water, steam conversion of methane, production of hydrogen from natural gas, the main component of which is methane. It turned out that by using appropriate catalysts, the temperature of methane pyrolysis to produce hydrogen and oxygen can be significantly reduced and the catalyst efficiency increased by
orders of magnitude. A great contribution to the study of this process has been made by employees of the G.K. Boreskov Institute of Catalysis SB RAS and Novosibirsk State Technical University, whose patents are presented in [11].

The process itself is expressed by the following equations:

\[
\begin{align*}
CO_2 + 4H_2 &= CH_4 + 2H_2O \\
2H_2O &= 2H_2 + O_2 \\
CH_4 &= C + 2H_2 \\
2CO_2 + 4H_2 &= CH_4 + 2H_2O + C + O_2
\end{align*}
\]

A technology was needed to produce hydrogen and nanofibrous carbon, providing an increased hydrogen-methane ratio in the decomposition products. For this purpose, a process of obtaining hydrogen and nanofibrous carbon material from methane by decomposition on a catalyst, at 700-750°C was proposed.

The proposed technology allows catalytic decomposition of methane with obtaining hydrogen and nanofibrous carbon material, maintaining the molecular correlation hydrogen: methane in gaseous decomposition products in the range (3:1) - (40:1).

A schematic of this technology is shown in Figure 2 [12].

![Fig. 2. Basic scheme of methane decomposition technology. 1 - outer glass, 2 - inner glass, 3 - catalyst bed, 4 - inlet nozzle, 5 - outlet nozzle, 6 - heater, 7 - vibration drive, 8 - rod.](image)

The technology was carried out in batch-type reactor with vibration bed of the catalyst. The reaction results in decomposition of the feed hydrocarbon into hydrogen, nanofibrous carbon, methane and other gaseous hydrocarbons. The carbon is deposited on the catalyst, forming granules of nanofibrous carbon material, and remains in the reactor. The gaseous reaction products including hydrogen and methane are removed from the reactor. The qualitative and quantitative composition of the gas mixture at the reactor outlet depends on the reaction temperature and the composition of the catalyst. When using catalysts described above, methane-hydrogen mixture with high hydrogen content and very low methane content can be obtained [13].
The best results were obtained in the following experiment [14]. A catalyst containing: 63 % Ni, 23 % Cu, 14 % SiO2 (mass percentages). With the help of vibration drive 7 the catalyst was set in fluidized state, then heater 6 was switched on and catalyst bed temperature was increased up to 500°C. After that methane was fed into reactor through inlet nozzle 4 and partially decomposed into hydrogen and carbon of nanofibrous structure passing through the catalyst bed. The vibration was carried out in the vertical direction with an amplitude of 1 mm and a frequency of 30 Hz. The products of the decomposition reaction being a mixture of unreacted methane and hydrogen were removed from the reactor through outlet nozzle 5. Carbon yield per 1 g of catalyst was 253 g during reactor operation. The hydrogen-methane molecular ratio in reaction products was 40:1 [12].

At present the domestic system of carbon dioxide concentration and processing by Sabatier reaction has been developed and is being tested. Similar types of systems are being developed and tested by ESA and NASA [4].

As indicated above, due to the loss of some hydrogen with methane in the Sabatier reaction, the carbon dioxide cannot be fully used to produce water to extract oxygen from it. In case of decomposition of methane into carbon and hydrogen, the latter returns to the reaction of hydrogenation of CO2 with complete extraction of oxygen from it.

4.2 Biosynthesis of food protein biomass

In addition to the physicochemical technology of methane use onboard ATS described above, there is an alternative possibility of using methane, which is its processing into a protein ingredient for crew foodstuffs. In addition to methane by the Sabatier reaction, biogenic methane can also be produced in the digester in the LSS ATS when recycling crew exometabolites and photophile waste.

On board, ATS additional water for methanotrophic biosynthesis can be produced by anaerobic digestion of exometabolites and waste to produce methane, fertilizers and trace elements. In addition, the methanotrophic fermentation of a protein product must have a closed water loop to prevent losses.

Production of food product by methane biosynthesis is possible as a duplicate system in terms of food provision in life support complex and during the flight to Mars with further operation of this system on the planet using Martian water, and probably with participation of Martian methane.

Methanotrophy is a field of biotechnology that uses obligate methanotrophic bacteria that oxidize methane of both geological and biogenic origin. This process has been going on in the Earth's nature for many millions of years, including the bottoms of seas and oceans, oil and natural gas deposits. Methanotrophy has created a living biological filter that traps the flow of methane into the atmosphere, keeping its nitrogen-oxygen composition suitable for life instead of the explosive methane composition. Besides, methanotrophic bacteria are the primary link in food chain in the world ocean [15].

Biomass of methanotrophic bacteria is characterized by high content of protein, essential amino acids, vitamins and microelements, contributing to complete balancing of fodder for animals of all kinds and ages. The process is adjusted to the input norms under the conditions of application in agriculture on earth, and after additional treatment, the biomass can be used in food products in the LSS ATS as well.

Depending on the producer strain, the microbial mass of methane-oxidizing microorganisms contains up to 79% protein, high amounts of vitamins (B12 - 42mg/g, biotin - 1.3 mg/g, riboflavin - 20 mg/g), a full composition of essential amino acids. The biomass is stable during storage for a year [20]. Methanotrophic bacteria are an important microbial source of many substances, including ectoin, polyhydroxyalkanoates and methanobactins, which are invaluable for the fields of biotechnology and biomedicine.
A schematic diagram of bioprotein production under terrestrial conditions is presented in Figure 3 [16]. The productivity of typical strains of bacterial microorganisms is estimated to range from 1 to 9 g per litre per hour. However, more productive strains with a productivity of 15 g/litre per hour have been bred. Thus, as a result of the conducted selection a strain of methane-oxidizing bacteria Methylococcus capsulatus GBS-15 was obtained, and when cultivated under industrial conditions on natural gas it can be used both as a part of association and as a monoculture. The strain has a high technological potential, in particular: it is resistant to short-term changes in temperature and pressure, is thermotolerant, non-pathogenic, and is not genetically modified [17].

![Schematic diagram of bioprotein production](image)

Fig. 3. Schematic diagram of bioprotein production [16]. 1-Methane, 2-Co2, 3-O2, 4-minerals, 5-U-fermentor, 6-tank, 7-centrifuge, 8-return water, 9-homogenizer, 10-drying chamber, 11-cyclone, 12-ready product, 13-sorption leaching unit.

The fermentation of biomass was successfully performed on other methanotrophic cultures. The author and his colleagues jointly performed methane-based fermentations on different nutrient media, which showed high productivity of a number of strains of methanotrophic microorganisms [18].

Figure 4 shows a laboratory setup for the fermentation of methanotrophic bacteria, the starting materials for the bioprocess and the possible types of biosynthesis target products are shown.

The most efficient process is cultivation carried out in continuous mode, as these microorganisms usually exhibit low specific substrate consumption rates. In order to reduce time and costs and to quickly switch the process to continuous mode, it is necessary to accelerate the growth of the applied organisms as much as possible during the scaling stage of the inoculum by controlling the substrate concentration in the medium.

The aim of our study was to investigate the effect of growth factors such as vitamins and different nitrogen sources (yeast extract, yeast nitrogen base with/without amino acids and tryptone) on the growth of methanotrophic bacteria such as: Methylomicrobium alcaliphilum BKM B-2133, Methylomicrobium methanica BKM B-2110 and Methylosinus trichosporium ob3b. All experiments were performed in 250 ml flasks on flasks and incubated at 30 °C under sterile conditions using pure cultures.

Figure 5 shows a graph of biomass concentration during a continuous process, the chemical elements required for fermentation are shown in the Table 2.
Fig. 4. Laboratory setup for the fermentation of methanotrophic bacteria.

Fig. 5. Graph of biomass concentration during a continuous process.

Table 2. Solution chemistry of media used to grow methanotrophs.

<table>
<thead>
<tr>
<th>Components g/L</th>
<th>LB</th>
<th>LB/2 MeOH</th>
<th>LB MeOH</th>
<th>NMS Trypt</th>
<th>NMS + Yeast</th>
<th>NMS Vit.</th>
<th>NMS Yeast</th>
<th>NMS YNB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Na₂HPO₄·5H₂O</td>
<td></td>
<td></td>
<td></td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>0.7</td>
<td></td>
<td></td>
<td>0.7</td>
<td>0.7</td>
<td>0.7</td>
<td>0.7</td>
<td>0.7</td>
</tr>
<tr>
<td>KNO₃</td>
<td>1</td>
<td></td>
<td></td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>MgSO₄·7H₂O</td>
<td>0.2</td>
<td></td>
<td></td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>0.02</td>
<td></td>
<td></td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>NaCl</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microelements</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamins</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tryptone</td>
<td>10</td>
<td>5</td>
<td>10</td>
<td>5</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yeast extract</td>
<td>10</td>
<td>5</td>
<td>10</td>
<td>5</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
LB – Lysogeny broth, NMS – nitrogen and mineral salts, YE – yeast extract, Vit. – vitamin mixture, YNB – yeast nitrogen base

Studies have shown that the use of enriched, concentrated media (LB, LB+MeOH) increases the microbial growth rate and biomass yield, compared to mineral media (NMS), by 60% and 20% on average, respectively. The addition of YE and tryptone to mineral medium, as well as the use of diluted LB, resulted in a slight increase in growth rate by 10 - 20% on average and increased the biomass yield by 20-60%. In turn, the addition of vitamins in NMS medium had the opposite effect and led to a 40-50% decrease in growth rate, while the biomass yield increased 2-3 times (from 0.13 to 0.46).

To produce 1 kg of bioprotein one of the technologies requires 2 m3 of methane gas, the fermentation process is continuous chemostat.

The results of current problems of protein production based on methane are discussed in detail on the website and in the author's monographs [19].

The carbon dioxide generated during protein fermentation by microalgae and higher phototrophic plants is transformed into oxygen for LSS ATS, or used in the Sabatier reaction as described above.

A high-speed flow centrifuge is used to extract the biomass from the culture liquid.

The use of biomass of methanotrophic bacteria directly in food has not been fundamentally investigated to date, as the aim was to provide livestock, poultry and fish farming with efficient animal protein for feed production. Methanotrophic protein was successfully investigated for safety of use at the Food Industry Research Institute, with the involvement of volunteers, and specifications and Norms for safe introduction into animal feed were developed for it.

Food-gaprin differs from feed-gaprin in the absence of nucleic acid components. This requires the resulting biomass to be homogenised and the nucleic acid components removed. The safety of food protein must be confirmed by additional testing. Assuming that the mass of 1.71 kg of methane is 0.71 kg, and that 1.0 kg of protein can be produced from 2.0 m3 of methane, 1.42 kg of methane is needed to produce 1.0 kg of haprin, but 2.5-4.0 times more oxygen is needed, depending on the strain. Thus, from 1.0 kg of methane, 0.70 kg of protein can be produced. In this volume of biomass, nucleic acids account for about 10%, resulting in 0.63 kg of safe food protein after their removal. In a small-scale food facility, the possible use of solid-phase cultivation can reduce the volume of the fermenter and the amount of centrifuge work. The possible use of protein in the form of a paste rather than in dry state would simplify drying - not drying the biomass to 10% moisture content [4].

According to the author's monograph [20], technology for intensification of biotechnology processes based on composite biostimulants (CBS) was developed, which allows to significantly increase efficiency of biotechnology production, in particular it was possible to increase growth rate of cell biomass of various yeast and bacterial cultures, as a result of its application biomass volume is increased by 15% on average. The method is adaptable to any biotechnological process and a small mass of biomass is required.

The rate of change of the biomass concentration in the fermenter during fermentation is determined by the sum of the biomass growth and cellular carry-over with the resulting culture liquid [16]:

\[ \frac{dx}{dt} = \mu X - DX = X(\mu - D) \]

Here \( \mu \) is the specific growth rate \( \mu = \mu_{\text{max}} S/(K_s + S) \), \( \mu_{\text{max}} \) is the maximum specific growth rate, \( S \) is the substrate concentration, \( K_s \) is the saturation constant numerically equal to the substrate concentration at which the specific growth rate corresponds to half the maximum growth rate, \( X \) is the biomass, \( D \) is the dilution rate, the ratio of the fed medium to the volume of the microbial suspension in the fermenter.
The concentration of the biomass in the fermenter increases if the growth rate of the cells exceeds the dilution rate and decreases in the opposite case. When $\mu$ and $D$ are equal, the culture is in an equilibrium state; the number of cells removed from the fermenter corresponds to the number of newly grown cells in the same time interval. The change in concentration of biomass and growth limiting substrate is described by the following equations:

$$\frac{dx}{dt} = X \left( \frac{\mu_{\text{max}} S}{K_s + S} - D \right)$$
$$\frac{ds}{dt} = D \left( S_0 - S \right) - \left( \frac{\mu_{\text{max}} X}{Y} \right) \left( K_s + S \right),$$

where $Y = \frac{dx}{ds}$ is the ratio of the amount of biomass produced to the amount of substrate used.

Continuous cultivation in a chemostat is based on the relationship between growth rate and substrate concentration, i.e., the chemostat is a self-regulating system. If the dilution rate is increased, cells start to be washed out of the fermenter and the biomass concentration drops. Decreasing the concentration entails less consumption of the substrate and the substrate concentration rises. Increasing the substrate concentration causes the growth rate to increase according to the level up to a value numerically equal to the new dilution rate. In this case, another equilibrium regime with new values of biomass concentration in the substrate arises. The capacity of a continuous cultivation process is equal to the biomass concentration multiplied by the volume of the microbial suspension flowing out of the fermenter:

$$P = D X$$

Here $P$ defines productivity of the continuous cultivation process in the chemostat; $D$ is dilution rate; $X$ defines the amount of biomass.

In order to ensure explosion protection, the author's patent [21] provides for separate dissolution of gases with a lowering of the temperature of the nutrient medium even before the fermenter. At stage 2 the gases are also separately fed into the fermenter through special hydrodynamic nozzles and dissolved in the culture liquid.

For closed modules of space stations and bases, the mass balance of biomass production is particularly important, which is difficult to determine and depends on the methanotrophic bacterial strain and a large number of characteristics of the production regime [4].

As an example, the following equation to describe methane oxidation by a culture [16] can be given:

$$6.25\text{CH}_4 + 7.92\text{O}_2 = C3.92\text{H}_6.50\text{O}_1.92 + 2.33\text{CO}_2 + 9.25\text{H}_2\text{O}$$

Here $C3.92\text{H}_6.50\text{O}_1.92$ show a simplified biomass formula, or in terms of methane molecule:

$$\text{CH}_4 + 1.27\text{O}_2 = C0.63\text{H}_1.04\text{O}_0.31 + 0.37\text{CO}_2 + 1.48\text{H}_2$$

Since methane and oxygen in space life support are derived from carbon dioxide, the above formulas for these substances must be added in this process:

$$\text{CO}_2 + 4\text{H}_2 = \text{CH}_4 + 2\text{H}_2\text{O}$$
$$2\text{H}_2\text{O} = 2\text{H}_2 + \text{O}_2$$
It follows that the mass of oxygen supplied for the reaction is 2.5 times the mass of methane, but after the electrolysis of the water formed by the reaction:

\[ 0.37\text{CO}_2 + 1.48\text{H}_2 = 0.37\text{CH}_4 + 0.74\text{H}_2\text{O} \]

we get that most of the oxygen can be returned to the biomass reaction:

\[ 0.74\text{O}_2 + 0.37\text{O}_2 = 1.11\text{O}_2 \text{gm or 0.87\%} \]

As a result, additional 182 g of oxygen, or 26% of the 700 g of oxygen contained in exhaled human carbon dioxide, would be needed per crew member in a space-based closed-loop life support system. This 182 g of oxygen can be obtained by electrolysis of additional water or from water resulting from biomass fermentation. The given reaction, as indicated, is an example from one of the bacterial strains. In it, 85% of the biomass is produced from methane, whereas according to current data, 70-75% is recommended.

As shown above, the Sabatier reaction produces 350 g of methane per day from 1 person. From this amount 245 g (70%) of protein is obtained and after purification from nucleic acids 220 g (90%) of dietary protein. However, the ratio of proteins, fats and carbohydrates in the human diet must be 1:1.2:4.6, with a total of 6.8 the amount of protein in the diet is 15%. Total dry weight of astronaut's ration is 600 g, i.e. in astronaut's daily ration protein amounts to 90 g, which is 40% of dietary protein per one crew member. In such a case, amount of oxygen required for protein production for a daily ration of one cosmonaut will make up 73 g. That is additional 10.5% of oxygen contained in carbon dioxide and 8% of daily amount of oxygen required for breathing of one cosmonaut.

With such an amount of extra oxygen, food protein production does not interfere with methane decomposition, and both systems can operate in parallel.

From the given balance calculations, it follows that with use of methane formed in Sabatier reaction, under condition of returning in further stages of methanotrophic reaction of formed carbon dioxide and water to produce food protein in the ration of cosmonauts, small amount of oxygen is required, which can enable to include such a system into life-support not only for planets bases, but also for interplanetary spacecrafts. If biogenic methane, produced in the digester during anaerobic digestion of metabolites and phototrophic waste, is also taken into account in the balance, all the described technologies can function and close the regeneration loop of LSS ATS.

Additional research is required to determine the composition of the LSS pneumohydro circuit, mass, power consumption, performance and reliability of the units comprising it in order to provide the ATS crew with food protein capable of operating in weightlessness and high radiation conditions.

Creation of an onboard LSS based on the use of the cycles described above will make it possible to fully solve one of the main tasks of regeneration - provision of the crew with full-rate food [4].

5 Conclusion

Integration of key vital biotechnological cycles creates the LSS of future ATS long-range, extended missions and the basis of bases for planetary exploration.

It has been shown that when carbon dioxide is hydrogenated using one of the most efficient technologies, the Sabatier reaction, the resulting methane can be used to form food protein.
Using in LSS of additional closed cycle with digester, it is possible to increase the volume of produced methane and to produce additional oxygen, water and food product of animal origin - biomass of methanotrophic bacteria.

It is shown that existing in terrestrial conditions biotechnological processes of fodder protein production from methane using methanotrophic bacteria for animal husbandry and fish breeding can be applied for food protein production in LSS ATS.

The safety of the food protein produced on board the ATS must be confirmed by additional tests.

Balance calculations on using methanotrophic process of food protein production for interplanetary flights, lunar and planetary bases were performed, amount of food protein produced from methane generated from daily carbon dioxide excretion by one cosmonaut was determined.

Possibility of simultaneous operation of two systems onboard a space object: methane decomposition and production of food protein from it was determined.

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