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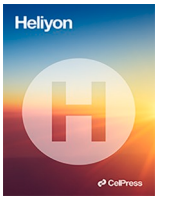

Research article

Investigation of the production and dietary features of oyster mushrooms for a planned lunar farm

N.S. Manukovsky [a,b], V.S. Kovalev [a,*], S.V. Trifonov [a,b,c], O.V. Hranovskaya [a,c]

[a] Institute of Biophysics SB RAS, 50/50, Akademgorodok, Krasnoyarsk, 660036, Russian Federation
[b] Reshetnev Siberian State University of Science and Technologies, Krasnoyarsky Rabochy Avenue, 31, Krasnoyarsk, 660037, Russian Federation
[c] Siberian Federal University, 79, Svobodny Avenue, 660041 Krasnoyarsk, Russian Federation

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ABSTRACT

In our previous work, we organized a project mainly to design a lunar mushroom farm. In this work, we proceeded to study the features of the production and consumption of oyster mushrooms in that project. Oyster mushrooms were grown in cultivation vessels containing a sterilized substrate. The fruit yield and mass of the spent substrate in the cultivation vessels were measured. A three-factor experiment was carried out with the subsequent application of the steep ascent method and correlation analysis in the R program. These factors included the density of the substrate in the cultivation vessel, its volume, and the number of harvesting flushes. The data obtained was used to calculate the process parameters: productivity, speed and degree of substrate decomposition, and biological efficiency. The consumption and dietary features of oyster mushrooms were modeled in Excel using the Solver Add-in. In the three-factor experiment, the highest productivity amounting to 272 g of fresh fruiting bodies/(m3*day) was obtained with a substrate density of 500 g/L, a cultivation vessel volume of 3 L, and two harvest flushes. The application of the method of steep ascent showed that it is possible to increase the productivity by increasing the substrate density and reducing the volume of the cultivation vessel. In production, there is a need to tally the substrate decomposition speed with the substrate decomposition degree and the biological efficiency of growing oyster mushrooms, since these process parameters have a negative correlation. Most of the nitrogen and phosphorus passed from the substrate into the fruiting bodies. These biogenic elements could limit the yield of oyster mushrooms. It is safe to set the daily intake of oyster mushrooms at 100–200 g while maintaining the antioxidant capacity of the food set.

1. Introduction

With mushrooms having been added to the list of space foods [1], the current task is to develop an efficient farm project. In fulfilling this task, a lunar farm for growing oyster mushrooms was designed [2]. The farm consists of two modules connected to a greenhouse. On average, one process cycle lasts 66 days, requires 86 kg of plant waste, and yields 28 kg of fresh mushrooms at a substrate density in the cultivation vessels of 300 g/L. Additional modules can be used to increase oyster mushroom production.

The prospects of using oyster mushrooms in a bioregenerative life support system (BLSS) depend on the estimated quantity of raw

* Corresponding author.
E-mail address: kovalev49@mail.ru (V.S. Kovalev).

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materials for its cultivation. The traditional raw material for growing oyster mushrooms is wheat and rice straw [3]. At that, these species are included in the list of the staple crops recommended for cultivation in the BLSS greenhouse [4]. In the BIOS-3 and Lunar Palace-1 BLSS, wheat occupied 83% and 58% of the total sown area, respectively [5,6]. In addition to wheat and rice, soybeans, potatoes, sweet potatoes, peanuts, and beans are the staple species. The inedible biomass of these species could presumably be mixed with straw. For example, it has been found that a substrate containing a mixture of wheat straw and potato tops in a mass ratio of 3:1 gives a higher yield of fruiting bodies than a substrate containing straw without additives [7]. Thus, BLSS is expected to have a good raw material base for growing oyster mushrooms.

The production capacity of the designed farm was calculated using our unpublished data obtained in a one-factor experiment. We studied the effect of the substrate density in the cultivation vessels on the yield of fruiting bodies and substrate decomposition. Other factors including the volume of the cultivation vessel and the number of harvested flushes were constant: 3 L and two flushes, respectively. It is impossible to fully understand the estimated performance of the lunar farm until other values of these two factors have been tested. A combination of these factors could be studied in a factorial experiment, followed by an analysis of the results obtained in relation to the yield of fruiting bodies and substrate decomposition. In this case, the unpublished data mentioned above could help in planning and interpreting the factorial experiment.

Spawn running is known to take place in sterile or non-sterile conditions [8]. The substrate used in non-sterile conditions should favor the spawn running and prevent the reproduction of harmful microflora. The biological selectivity of such a substrate is ensured by its pretreatment and the presence of protective microflora [9]. However, biological selectivity does not guarantee protection of the substrate from infection. It may lead to the spread of harmful microorganisms across the entire volume of the mushroom farm. There is no reliable information on the incidence of substrate infections in the long-term non-sterile production of oyster mushrooms. This information can only be obtained from one's own experience.

After complete colonization of the sterilized substrate, the oyster mushroom spawn is transferred to non-sterile conditions to obtain fruiting bodies. At this stage of oyster mushroom cultivation, the colonized substrate is not susceptible to infection, as it is difficult for harmful fungi and bacteria to find unoccupied sites of the substrate. As such, in our practice, it is rare for the colonized substrate to be infected, despite the fact that the sterilized substrate lacks biological selectivity. The infection frequency may increase after several fruit flushes when the oyster mushroom spawn begins to die due to ageing. The efficacy of spawn running under sterile conditions is confirmed by its use in the industrial production of oyster mushrooms [10]. Therefore, in our experiments, we decided to grow the spawn under sterile conditions.

Spawn running and fruiting occurs due to the degradation of plant waste, which is the substrate for growing oyster mushrooms. This process is very important in BLSS, as it ensures the return of biogenic elements in the form of spent mushroom substrate [11] or vermicompost [12] to the plants. Thus, the mushroom farm could be considered as part of the BLSS food production unit and, concurrently, as part of the waste processing subsystem. Therefore, exploring the substrate decomposition speed is an urgent task. It is also important to identify the chemical changes of the substrate during decomposition. This should be done to identify factors limiting the growth of oyster mushrooms, adjust the composition of the untreated substrate, and assess the suitability of the spent substrate for further use in BLSS.

Apart from productivity and substrate decomposition speed, two more parameters are used to measure oyster mushroom production. These are the biological efficiency [13] and the substrate decomposition degree. The second parameter can be used as a measure of the maturity of the substrate, that is, its readiness for further use. Biological efficiency reflects the success of using the substrate to obtain fruiting bodies. The relationship between these parameters is the object of this research.

The volume of mushroom production depends on its consumption by the lunar base crew. In turn, the consumption of mushrooms and other foods is regulated according to dietary standards. The designed lunar farm was assumed to have a production capacity capable of satisfying the needs of 14 crew members in one process cycle. This number was derived from the diet of Waters et al., 2002 [14]. A 10-day menu cycle for 6 crew members has 7 mushroom dishes. Each crew member could consume 30 g of fresh fruiting bodies per day. The upper limit of oyster mushroom consumption was not determined. Dietary restrictions that prevent increased consumption of mushrooms are also unknown. The problem of possible mushroom overproduction can be solved by conservation of fruiting bodies. In this case excess spent substrate can be used in the same manner as its bulk.

The radiation emitted by protons and particles with a high atomic mass and energy is of particular concern when it comes to the health of astronauts during their stay in space. Cosmic radiation is known to induce oxidative stress in astronauts during long stays on the International Space Station [15]. Therefore, it is of interest to predict how the inclusion of oyster mushrooms in the space diet will affect the antioxidant capacity of food.

The main objective of this work was to refine the oyster mushroom production and consumption factors to be able to apply the results on a future lunar mushroom farm.

The following tasks were set in the work.

- to study the influence of the substrate density in the cultivation vessel, the volume of the cultivation vessel, and the number of harvested flushes on the productivity and biological efficiency of oyster mushroom cultivation, as well as on the speed and degree of substrate decomposition;
- to determine the factors limiting the growth of oyster mushrooms and outline ways to use the spent substrate in BLSS;
- to model the effect of the oyster mushroom consumption on the fulfillment of space nutrition requirements and the antioxidant activity of the food set.

2. Methods

2.1. Object of the study and calculation of production parameters

The object of the study was the oyster mushroom *Pleurotus ostreatus (Fr.) Kumm* obtained from an agro-industrial enterprise located in the Zarechye settlement of the Moscow Region.

The influence of the substrate density in the cultivation vessels, the volume of the cultivation vessel, and the number of harvest flushes collected on the production parameters was studied.

The productivity was calculated using the formula:

$$P = nY/t \quad (1)$$

where n is the number of cultivation vessels placed in 1 m^3 of the growing chamber.

Y – weight of fresh fruiting bodies harvested from the cultivation vessel;
t – production time, i.e., time from sowing to fruiting bodies harvesting.

Growing fruiting bodies leads to an increase in the space occupied by the cultivation vessel. In view of this, the dimensions of the space occupied by a 1 L cultivation vessel were taken to be equal to 11 × 15 × 22 cm, 2 L – 13 × 18 × 27 cm, and 3 L – 16 × 21 × 31 cm. The placement of the cultivation vessels in the growing chamber was modeled in PowerPoint. It was established that 192, 105, and 72 fruit bearing cultivation vessels with volumes of 1 L, 2 L, and 3 L, respectively, can be placed in 1 m^3 of the growing chamber.

The following formula was used to calculate the substrate decomposition speed:

$$R = n(S_0 - S_r) / t \quad (2)$$

where S_0 is the initial dry weight of the substrate in the cultivation vessel;

S_r– residual dry weight of the substrate in the cultivation vessel;

The substrate decomposition degree was calculated using the formula:

$$D = 100(S_0 - S_r) / S_0 \quad (3)$$

The biological efficiency was defined similar to Ref. [8]:

$$BE = 100Y/S_0 \quad (4)$$

2.2. Oyster mushroom growing method

Wheat straw was used as the substrate for growing oyster mushrooms. The moisture content of the straw was brought up to 67–70% by immersing it in a measured amount of water. The moistened straw was distributed into cultivation vessels, which were 1 L, 2 L, and 3 L glass jars.

To fill the cultivation vessels, we used two fractions of straw with a particle size of 3–6 cm and a size of <2 mm. For densities of 50, 100, 200, and 300 g/L, 3–6 cm fraction particles were used. The density of 600 g/L was obtained from finely ground straw. To create a density of 400 g/L and 500 g/L of coarse-shredded straw, time and effort would be required to compact the straw in the cultivation vessel. Therefore, in these cases, a mixture of coarse and finely-grounded straw was used in a mass ratio of 3:1 and 3:2, respectively.

The moistened straw was sterilized in an autoclave at a pressure of 1 atm for 1 h. After the straw had cooled, the oyster mushroom was sown. Mycelium grown on the surface of a solid nutrient medium in Petri dishes was used as an inoculum.

Spawn running took place in a thermostat at 25 °C without air exchange and internal lighting. Cultivation vessels containing the spawned substrate were transferred from the thermostat to the climatic chamber for fruiting.

The air temperature and relative humidity in the climatic chamber were adjusted to 20–22 °C and 80–90%, respectively. Cultivation of oyster mushrooms in a climatic chamber was carried out in daylight. 1–3 flushes of fruiting bodies were harvested.

The homemade climatic chamber had a working volume of 0.2 m^3. The frame and cover of the chamber was made of stainless steel and its side panels were made of plexiglass. A water sprayer was installed in the center of the chamber cover. The bottom of the chamber had a hole for condensate drainage and air flow. The temperature and humidity of the air required for fruiting and the low concentration of carbon dioxide in the climatic chamber were maintained by switching on and the duration of operation of the water sprayer, which was adjusted manually.

The masses of fresh fruiting bodies and spent substrate in the cultivation vessels were determined. Also, the chemical analysis of the straw, spent substrate and fruiting bodies was performed.

2.3. Designs of one-and three-factor experiments

The only factor that was varied in the one-factor experiment was the initial density of the wet substrate in the cultivation vessel.

This was set at seven levels: 50, 100, 200, 300, 400, 500, and 600 g/L. Other factors, including the volume of the cultivation vessel and number of collected fruit flushes, were not changed. Oyster mushroom was grown in 3 L glass jars. Two flushes of fruiting bodies were harvested. The data obtained was used to reduce the number of procedures that were performed in the subsequent three-factor experiment.

The factors that were used in the three-factor experiment were the substrate density, volume of the cultivation vessel, and number of harvested flushes (Table 1).

The design of the three-factor experiment with three levels of each factor in coded form was borrowed from the paper of Yao et al. [16]. The response functions were the oyster mushroom growing parameters: productivity, substrate decomposition speed, substrate decomposition degree, and biological efficiency.

2.4. Sampling, analytical procedures and subsequent calculations

The sample received for analysis was milled and thoroughly mixed. Two analytical samples were taken from it by the quartering method [17]. Analytical samples were added to preliminarily dried and weighed beakers. Beakers and analytical samples in beakers were weighed and placed in a drying cabinet. Analytical samples were dried to constant weight at a temperature 105 ± 2 °C. The mass ratio of moisture in analytical sample W in percent was calculated by the formula:

$$W = 100 \times (m_1 - m_0) / (m_1 - m) \quad (5)$$

where m_1 is the mass of moist substrate with a beaker;

m_0 - mass of dried substrate with a beaker;
m - mass of the empty beaker.

The result of the analysis was taken as the arithmetic mean of the results of two parallel determinations. The mass ratio of dry weight in analytical sample DW in percent was calculated by the formula:

$$DW = 100 - W \quad (6)$$

To determine the ash content of analytical samples, the crucibles were placed in a muffle furnace and calcined at a temperature of 575 ± 25 °C [18]. After calcinations, the crucibles were cooled to room temperature and weighed. Then the dried analytical sample was placed in the calcined crucible and their total weight was determined. The crucible with the analytical sample was transferred to a muffle furnace, where it was kept at a temperature of 575 ± 25 °C for at least 3 h or until visible traces of carbon compounds disappeared. The ash content of the sample was calculated by the formula:

$$\text{Ash content} = 100 \times (m_{ash} - m_{cont}) / (m_{od} - m_{cont}) \quad (7)$$

where Ash content is the mass of ash, referred to the mass of the sample dried at 105 °C, expressed in percentages.

m_{ash} - mass of the crucible with ash, g;
m_{cont} - mass of the empty crucible, g.
m_{od} - the initial mass of the crucible with a sample dried at 105 °C, g.

Trends in changes in the chemical composition of the substrate during the cultivation of oyster mushrooms were identified by examining three samples of the substrate from the experimental group 9 (Table 1). The chemical composition of three samples of the untreated substrate (wheat straw) and fruiting bodies was also studied.

The content of cellulose, hemicellulose, and lignin in the untreated and spent substrates was determined by the detergent method [19]. The carbon content in the samples was measured on a Flash EA 1112 series; Thermo Finnigan, Milan, Italy analyzer. Crude fat

Table 1
Design of a three-factor experiment in coded form.

No of experimental group	Density	Volume	Number of flushes
1	−1	−1	1
2	−1	0	0
3	−1	1	−1
4	0	−1	0
5	0	0	−1
6	0	1	1
7	1	−1	−1
8	1	0	1
9	1	1	0

Factor levels in decoded form. Density: 1 - 300 g/L, 0–400 g/L, 1–500 g/L. Volume: 1 – 1 L, 0–2 L, 1–3 L. Number of flushes: −1 – one flush, 0 – two flushes, 1 – three flushes.

content in the samples was determined by the Rushkovsky method [20]. The content of the available carbohydrate in the dried analytical samples of fruiting bodies was determined by the following equation:

$$Carbohydrate\ (g\ /\ 100\ g\ sample) = 100 - [(fat + protein + ash + crude\ fiber)\ g\ /\ 100\ g] \quad (8)$$

where fat, protein, ash and crude fiber is the content of these substances in the tested material [21].

The total nitrogen content was determined by the Kjeldahl method [22]. The protein content of the wheat straw and spent substrate samples was determined by multiplying the total nitrogen content by a factor of 6.25 [23], while a factor of 4.38 was used to calculate the protein content in the fruiting bodies [24].

The total phosphorus content in the samples was determined by the molybdate method [25]. Potassium was determined by flame photometry [26]. Calcium and magnesium were determined by the complexometric method with Trilon B by back titration [20].

Based on the results of the analysis, the carbon balance equation was compiled for the cultivation vessel:

$$C_{init} = C_{spent} + C_{CO2} + C_{fb} \quad (9)$$

where C_{init} is the initial mass of carbon in the untreated substrate;

C_{spent} - mass of carbon in the spent substrate after the completion of oyster mushroom cultivation;
C_{CO2} - mass of carbon in the composition of carbon dioxide released due to the decomposition of the spent substrate;
C_{fb} - mass of carbon in the fruiting bodies.

Multiplying each term in equation (9) by $100/C_{init}$ gave this equation expressed as a percentage.

The mass of carbon C_{CO2} was determined from equation (9):

$$C_{CO2} = C_{init} - C_{spent} - C_{fb} \quad (10)$$

The distribution of biogenic elements between the spent substrate and fruiting bodies, expressed as a percentage, was determined according to the equations:

$$m_{iss} = 100 m_{is} \,/\, \left(m_{is} + m_{if}\right) \quad (11)$$

$$m_{ifb} = 100 m_{if} \,/\, \left(m_{is} + m_{if}\right) \quad (12)$$

where m_{iss} is the proportion of the i-th element in the substrate, %;

m_{ifb} - proportion of the i-th element in the fruiting bodies, %;
m_{is} - mass of the i-th element in the spent substrate in the cultivation vessel, g;
m_{if}- mass of the i-th element in the fruiting bodies grown from the cultivation vessel, g.

The content of the unidentified substances in the substrate samples was calculated as the difference between 100% and the sum of the identified substances as a percentage.

2.5. Statistical analysis

Descriptive statistics and Fisher's LSD post-hoc test were used to determine the standard deviations of means and to find the significant differences between the experimental variants. ("BioStat", AnalystSoft Inc). Each experimental variant was set up in three replicates. Thus, 21 and 27 cultivation vessels were used in one-and three-factor experiments, respectively.

The correlation between the oyster mushroom growing parameters was determined in the R program by means of the function "cor". The steepest ascent method was applied to determine the direction of increasing productivity [27].

2.6. Modeling the fulfilment of space nutritional requirements with an increase in oyster mushroom consumption

The mathematical model included a list of plant and animal species recommended for the bioregenerative life support system (Appendix). This list was compiled based on previously published proposals [28–34].

The edible biomasses of the species were considered as the food ingredients. Canola oil, peanut oil, chilies, quail eggs, sugar, water, and table salt were added to these ingredients. The combination of these ingredients was assumed to form a food set. The nutritional contents of the ingredients were taken from open databases [35–37].

The daily nutrient intakes were prescribed according to NASA nutritional requirements [38]. Fluoride, iodine, chromium, trans fatty acids, biotin, and cholesterol were excluded due to the absence of complete and reliable information on these nutrients in the databases. The remaining nutrients were divided into two groups [39].

The first group included 15 nutrients, which were magnesium, potassium, manganese, zinc, vitamin B5, vitamin B6, vitamin B12, vitamin C, vitamin D, vitamin E, vitamin K, thiamin, riboflavin, folate, and niacin. The standard daily intakes of the first-group nutrients are expressed in exact values. In the mathematical model, they are given the index k (formulas 13, 14). The objective function

(13) was designed to minimize the discrepancy among standard and the calculated daily intakes for group k-nutrients.

The second-group nutrients were assigned the index i (formula 15). This group included 16 nutrients, which were fluid, protein, carbohydrate, fat, fibre, iron, calcium, phosphorus, sodium, copper, selenium, vitamin A, saturated fat, n-3 fatty acids, n-6 fatty acids, and animal protein. The daily intakes of the second-group nutrients have a lower and upper limit, or one of them. Given these assumptions, the mathematical model can be expressed as follows:

Minimize:

$$f = \left[q^{-1} \sum_{k=1}^{q} \left((m_k / b_k) - 1 \right)^2 \right]^{0.5} \tag{13}$$

Subject to:

$$m_k = \sum_{k=1}^{q} a_{kj} P_j \tag{14}$$

$$m_i = \sum_{i=1}^{v} a_{ij} P_j \tag{15}$$

$$m_{ilower} \leq m_i \leq m_{iupper} \tag{16}$$

$$P_{jlower} \leq P_j \leq P_{jupper} \tag{17}$$

where b_k is the standard daily intake of the k_{th} nutrient of the first group for men;

m_k - calculated daily intake of the kth nutrient for men, dependent variable;
q - number of nutrients in the group k;
m_i - calculated daily intake of the ith nutrient for men, dependent variable;
a_{kj} - content of the kth nutrient in 1 g of the jth ingredient;
a_{ij} - content of the ith nutrient in 1 g of the jth ingredient;
v - number of nutrients in the group i;
P_j - calculated daily intake of the jth ingredient, independent variable;
m_{ilower} - lower bound for the calculated daily intake of the nutrient m_i;
m_{iupper} - upper bound for the calculated daily intake of the nutrient m_i;
P_{jlower} - lower bound for the calculated daily intake P_j;
P_{jupper} - upper bound for the calculated daily intake P_j.

The calorie content of the daily diet was calculated using the Atwater formula:

$$Cal = 4m_{protein} + 9m_{fat} + 4m_{carbohydrate} \tag{18}$$

where $m_{protein}$ is the daily protein intake;

m_{fat}- daily fat intake
$m_{carbohydrate}$ - daily carbohydrate intake.

Cal was set equal to 2800 kcal. Compliance with daily nutritional requirements was verified when using 0, 200, 400, 600, 800, and 1000 g of oyster mushrooms in the daily food set. In modeling, the oyster mushroom dosage was set through alignment of the lower and upper bounds (Formula 17). For example, $P_{lower} = P_{upper} = 400$. The value of the objective function (13) and the number of nutritional imbalances were determined for each oyster mushroom dose. Nutritional imbalance in terms of some nutrient was recognized in the case of $m_i < m_{ilower}$ or $m_i > m_{iupper}$. The lower bound for the daily intakes P_{jlower} of all ingredients except oyster mushrooms was set to zero, while the upper bound was not set.

The antioxidant capacity of the daily set of ingredients was calculated using the formula:

$$AD = m^{-1} \sum_{j=1}^{r} a_j P_j \tag{19}$$

where m is the mass of the daily set of ingredients, g.

a_j - antioxidant capacity of the j-th ingredient;
P_j - mass of the j-th ingredient in a daily set of ingredients;
r - number of ingredients in the set.

The antioxidant capacity is expressed in μ mol of Trolox equivalent/100 g of the daily food set. The values of a_j were taken from the Superfoodly database [40]. The diet containing oyster mushrooms was modeled in Excel using Solver Add-in.

3. Results and discussion

3.1. Influence of substrate density on oyster mushroom production in the one-factor experiment

A significant increase in the productivity from 200 to 283 g/(m3*day) was observed with an increase in substrate density from 300 to 500 g/L. At a substrate density of 600 g/L, a decrease in the productivity was observed, although the yield of fresh fruiting bodies for two waves was the highest at 371 g (Fig. 1).

The reason for the decrease in the productivity was the long seed-to-harvest time of 151 days. A dense layer of mycelium formed between the cultivation vessel wall and the substrate. It is clear that air had been blocked from entering the lower part of the cultivation vessel. Spawn running in this zone was significantly slow. This problem could be solved by introducing an inoculation hole in the substrate [41]. To prevent the destruction of the inoculation hole, it is advisable to use a perforated tube around which to place the substrate in the cultivation vessel. In the subsequent three-factor experiment, substrate density was used as a factor in the range of 300–500 g, since the best productivity was obtained therein.

3.2. Production features of oyster mushrooms obtained in the three-factor experiment

The highest productivity of the fresh fruiting bodies 266–272 g/(m3*day) was obtained in 7–9 groups of the three-factor experiment. However, the differences between these values are not significant (Table 2). The spent mushroom substrate with the highest speed of 156 g/(m3*day) was produced in group 7.

Biological efficiency in the three-factor experiment varied within 33–73% (Table 2). The highest biological efficiency of 71–73% was noted in groups 1, 6, 8.

It is of interest to compare the obtained results with the biological efficiency indicators when using other substrate preparation methods for spawn running in non-sterile conditions.

Kerketta et al., 2019 [42] evaluated chemical (carbendazim), hot water, lime (2%), and plane water (control) methods of substrate pasteurisation to study their impact on the biological efficiency of different *Pleurotus* species. The most effective was the chemical method, which allowed obtaining a biological efficiency of 123%.

A lower biological efficiency of 5.6–65.5% was obtained when *Pleurotus ostreatus* was grown on a substrate consisting of a mixture of wheat straw and sunflower husks after aerobic fermentation of the substrate [43].

Treatment of Digitaria decumbens grass in alkaline water for 24 h followed by cultivation of oyster mushrooms on the treated substrate allowed obtaining a biological efficiency of 95.1% [44].

Therefore, the biological efficiency indicators obtained in our experiment (Table 2) are inferior to those obtained after treating the substrates with carbendazim [42] and alkaline water [44]. However, this conclusion does not negate the method of spawn running in

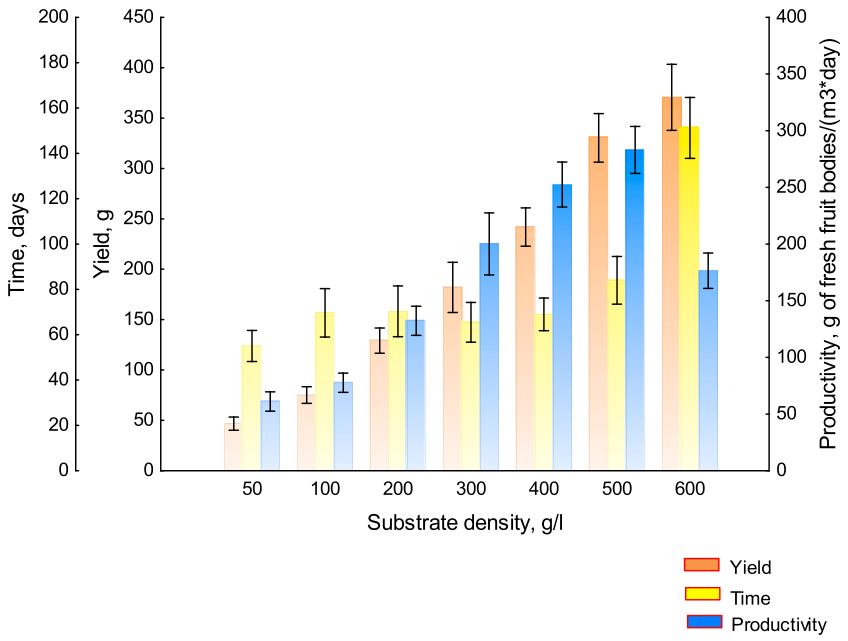

Fig. 1. Influence of substrate density in the cultivation vessel on the yield, seed-to-harvest time, and productivity.

Table 2
Data obtained in the three factor experiment.

No of group	Substrate density, g/l	Volume of vessel, l	Number of flushes	Productivity	Substrate decomposition speed	Substrate decomposition degree, %	BE, % *
1	300	1	3	201 ± 6^{a}	99 ± 8^{a}	36 ± 6^{a}	73 ± 7^{a}
2	300	2	2	209 ± 9^{a}	104 ± 4^{a}	29 ± 3^{b}	58 ± 5^{b}
3	300	3	1	176 ± 5^{b}	107 ± 6^{ab}	23 ± 2^{c}	38 ± 4^{c}
4	400	1	2	257 ± 6^{c}	125 ± 4^{c}	28 ± 4^{b}	57 ± 4^{b}
5	400	2	1	225 ± 6^{d}	132 ± 5^{c}	21 ± 2^{c}	36 ± 5^{c}
6	400	3	3	231 ± 9^{d}	116 ± 4^{bc}	35 ± 4^{a}	71 ± 5^{a}
7	500	1	1	266 ± 8^{e}	156 ± 6^{d}	20 ± 3^{c}	33 ± 4^{c}
8	500	2	3	271 ± 4^{e}	135 ± 4^{c}	35 ± 2^{a}	71 ± 5^{a}
9	500	3	2	272 ± 6^{e}	137 ± 6^{c}	29 ± 4^{b}	58 ± 6^{b}

The values of the parameters are means ± standard deviations of triplicate determination.
Means with same letter in each column are not significantly different ($p < 0.05$) as calculated by the Fisher LSD Test.
*BE – biological efficiency.
Units.
Productivity: g of fresh fruiting bodies/(m^3 *day).
Substrate decomposition speed: g of dry weight of substrate/(m^3 *day).

sterile conditions used in this study.

First, all factors when comparing different substrate treatment methods should be identical. In the above comparison, this condition is not met: different strains of oyster mushroom were cultivated on different substrates. Secondly, biological efficiency is not the only indicator. We give preference to the frequency of infections in the oyster mushrooms growing as applied to BLSS. Unfortunately, it is difficult to find objective information on the frequency of infection in published sources. Therefore, we have to rely on our own experience: the lowest frequency of infection is observed when using the spawn running in sterile conditions.

The results of the three-factor experiment (Table 2) were used as a starting point for finding a way to increase the productivity using the steepest ascent method. Table 3 shows that the increase in productivity could be achieved by increasing the substrate density and reducing the volume of the cultivation vessel.

A high density of 600 g/L and a small volume of 1.5 L will probably not lead to growth retardation, as was the case when using a 3 L vessel (Section 3.1). The trend towards a decrease in the volume of the cultivation vessel is explained by the fact that this reduces the time t from sowing to harvesting (formula 1). At that, the number of cultivation vessels n in the growing chamber working volume increases from 72 to 192. Due to the terms t and n, small-volume cultivation vessels can compete with larger-volume vessels, despite the relatively small yield from one small-volume vessel.

The steep ascent method offers to compensate for the small volume of cultivation vessels by increasing the density of the substrate therein. The steep-ascent method also shows the transition from the second to the third flush of the harvest. In practice, the number of flushes harvested can only take integer values. Therefore, an experiment is required to choose between using two or three flushes of fruiting.

A review of the correlation relationships allows distinguishing between two groups of parameters: 1) productivity and substrate decomposition speed; 2) substrate decomposition degree and biological efficiency. There is a strong positive correlation within groups. On the contrary, there is a negative correlation between the substrate decomposition speed and the second group of parameters (Table 4). The substrate decomposition degree can reflect its suitability as a fertilizer [45], and a feed for vermiculture [46]. It is therefore necessary to find a compromise between the productivity and the substrate decomposition speed, on the one hand, and the substrate decomposition degree and biological efficiency, on the other.

Table 3
Path of steepest ascent from ridge analysis.

Distance from center	Substrate density, g/l	Volume of cultivation vessel, l	Number of flushes	Productivity, g/(m^3*day)
0	400	2.0	2.0	234
0.5	448	1.9	2.1	253
1.0	497	1.8	2.2	272
1.5	545	1.7	2.2	291
2.0	594	1.6	2.3	311
2.5	642	1.5	2.4	330

Table 4
Correlations between oyster mushroom production parameters.

Parameters	Productivity	Speed of substrate decomposition	Degree of substrate decomposition	Biological efficiency
Productivity	1.00			
Speed of substrate decomposition	0.82	1.00		
Degree of substrate decomposition	0.08	−0.43	1.00	
Biological efficiency	0.11	−0.43	0.99	1.00

3.3. Substrate conversion when growing oyster mushrooms

Oyster mushroom growing led to changes in the chemical composition of the substrate. The trends of the changes are illustrated using the example of an oyster mushroom growing in the 9th experimental group with the highest productivity of 272 g of fresh fruiting bodies/(m3 *day). The cultivation factors of oyster mushrooms in this experimental group were as follows: density of the moistened substrate – 500 g/L, volume of the cultivation vessel – 3 L, and number of harvest flushes – 2 (Table 2).

The largest share in the untreated substrate (wheat straw) was occupied by cellulose, hemicellulose, and lignin, accounting for 36.2%, 30.1%, and 19.6%, respectively (Table 5). In the spent substrate, the content of cellulose and hemicellulose slightly increased, while the content of lignin decreased. On the contrary, in the work of Pandey et al., 2014 [47], a decrease in the content of cellulose and hemicellulose and especially lignin from 24.3% to 12.12% was obtained.

The straw conversion product is oyster mushroom fruiting bodies. The protein content in fruiting bodies is 22.6% (Table 6), while in straw it is 3.0% (Table 6). The main share in the composition of the fruiting bodies is occupied by carbohydrates - 42.4%. A similar chemical composition of fruiting bodies was obtained in the work of Economou et al., 2020 [48].

After growing oyster mushrooms, the moisture content of the substrate increased from the initial value of 67%–76%, possibly due to the formation of metabolic water, which should be included in the mass balance equation for oyster mushroom growth. However, it is not possible to accurately determine the mass of metabolic water. Therefore, carbon balance (formula 9) may give a better idea of substrate conversion and fruiting bodies formation.

When growing oyster mushrooms, the carbon of the untreated substrate passes into the spent substrate, carbon dioxide, and fruiting bodies. After two flushes of fruiting, the largest proportion of carbon – 72% remained in the substrate, 22% was lost in the form of carbon dioxide, and only 6% passed into the fruiting bodies (Fig. 2).

In fact, the yield of fungal biomass exceeded 6%, since the biomass of the substrate mycelium should be added to the biomass of the fruiting bodies.

Table 5
Chemical composition of straw and spent substrate.

	Cellulose	Hemicellulose	Lignin	Protein	Fat	Ash	OS*
Wheat straw	36.2 ± 5.2	30.1 ± 5.1	19.6 ± 4.5	3.0 ± 0.4	1.2 ± 0.2	6.6 ± 1.3	3.3
Spent substrate	38.5 ± 6.3	32.4 ± 5.1	18.6 ± 5.6	1.2 ± 0.4	1.1 ± 0.2	8.2 ± 1.5	2.2

All data expressed as % of dry weight. *OS - other substances.

Table 6
Chemical composition of fruiting bodies.

	Protein	Fat	Fiber	Ash	Carbohydrate
Fruiting bodies	22.6 ± 4.7	4.4 ± 0.53	21.9 ± 5.5	8.8 ± 0.7	42.4

All data expressed as % of dry weight.

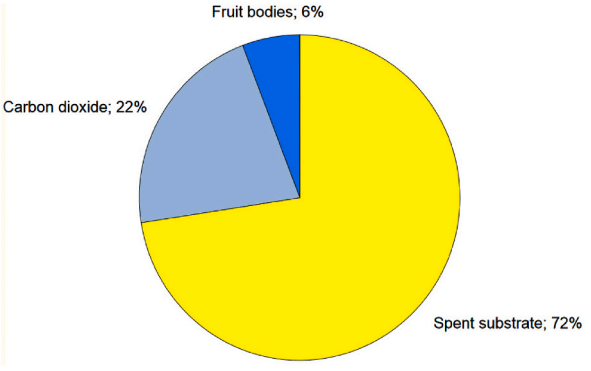

Fig. 2. Distribution of carbon among the products of the substrate conversion after two harvest flushes of oyster mushrooms.

Table 7
Content of biogenic elements and C/N ratio in the wheat straw, spent substrate and fruiting bodies.

	C	N	P	K	Ca	Mg	C/N ratio
Wheat straw	42.9 ± 6.5	0.5 ± 0.1	0.1 ± 0.02	0.6 ± 0.1	0.6 ± 0.1	0.1 ± 0.02	85.8
Spent substrate	41.7 ± 6.9	0.2 ± 0.03	0.04 ± 0.01	0.4 ± 0.1	0.8 ± 0.1	0.1 ± 0.02	208.5
Fruiting bodies	40.0 ± 6.4	5.2 ± 0.8	1.1 ± 0.3	3.8 ± 0.5	0.03 ± 0.005	0.2 ± 0.02	7.7

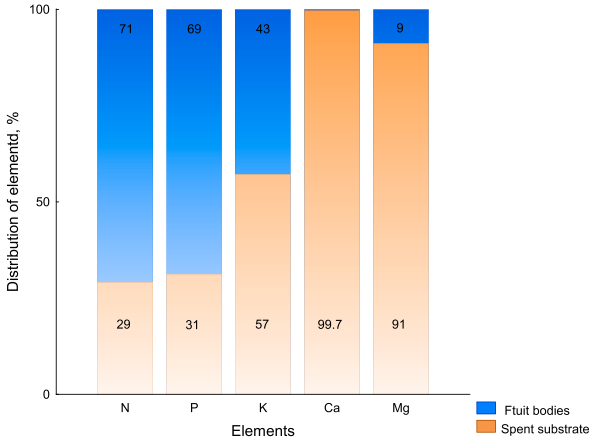

Fig. 3. Distribution of biogenic elements between the spent substrate and oyster mushroom fruiting bodies.

The C/N ratio in the untreated substrate was 85.8, increasing to 208.5 in the spent substrate (Table 7). This result contradicts published data, according to which the C/H mass ratio in the spent substrate decreases [23,48].

Such a large increase in the C/H ratio of the spent substrate is explained by the fact that the main part of the total nitrogen passes into the fruiting bodies. A C/N ratio of 208.5 prevents the spent substrate from being used as plant fertilizer, since the recommended C/N ratio is 24 [49]. To further treat the spent substrate in the fertilizer, it can be applied to the vermiculture with the addition of a nitrogen source. It is also possible to mineralize the spent substrate by the physicochemical method [50,51] and/or re-use by mixing it with some fresh substrate [52].

The data presented in Table 7 served as the basis for calculating the distribution of biogenic elements between straw and its conversion products.

The C/N ratio in the straw was found to be 85.8, while in the fruiting bodies – 7.7 (Table 7). Similar fruiting body C/N ratios of 7.51–13.69 were obtained when growing *Pleurotus ostreatus* mushroom on plant residues from the Amazon [53]. Therefore, the fruiting bodies can be considered as the nitrogen accumulators.

More than half of nitrogen and phosphorus passed into the fruiting bodies from the substrate – 71% and 69%, respectively (Fig. 3). It is these elements that can limit oyster mushroom fruiting.

Adding these elements to the straw could induce an increase in the oyster mushroom yield. The source of the nitrogen supplement could be soybean [54], and the source of nitrogen and phosphorus – vermicompost [55].

A large mass of spent substrate (Fig. 2) indicates insufficient cultivation efficiency. It has been shown that the spent substrate has the potential for re-cultivation of oyster mushrooms after mixing with a portion of fresh substrate and sterilization [56]. This increases the efficiency of using the substrate.

Another reserve in improving oyster mushroom production parameters can be the particle size of the substrate. A substrate composed of large particles can cause poor compactness in the cultivation vessel and, consequently, lead to a poor harvest.

Reducing the particle size can lead to an increase in the compactness of the substrate. However, this may interfere with the air supply to the mycelium. The solution to this problem could be finding the optimal particle size or determining the optimal ratio of different-sized particles in the substrate.

Dzulkefli, Zainol, 2018 [57] studied the contribution of five factors to the spawn extension rate. Substrate type and particle size contributed 1.15–2.63%, with particle size contributing the least. Against the background of these four factors, the fifth factor – substrate pretreatment, stands out with a contribution of 59.30%. However, only two particle sizes of 0.5 and 2.5 cm were used in the experiment. Therefore, the conclusion about the small contribution of the particle size can be limited by the scope of a specific study.

3.4. Influence of consumed oyster mushrooms on the dietary and antioxidant properties of a food set

In the absence of oyster mushrooms, 5 nutritional imbalances in a food set are present: excess of iron, saturated fatty acids, omega-6 fatty acids, and a lack of pantothenic acid and vitamin D. These imbalances were noted earlier when studying NASA food standards in relation to the bioregenerative life support system [39,58].

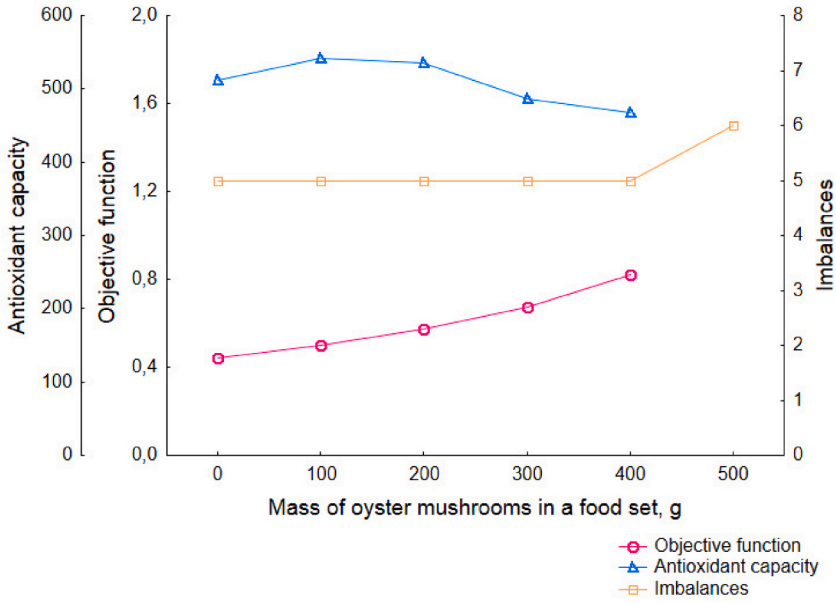

Fig. 4. Effect of oyster mushrooms consumed on the objective function of the modeling, food antioxidant capacity, and number of nutritional imbalances in a food set. Antioxidant capacity is expressed in μg Trolox equivalent/100 g of the food set.The objective function values were calculated using formula (13).

The space standard stipulates that the contribution of saturated fat to the caloric content of food should not exceed 7% [38]. Compliance with this standard is possible by reducing the amount of animal proteins and violating their consumption standard – 2/3 of the total amount of vegetable and animal proteins in food. Therefore, animal protein sources with a lower content of saturated fat should be included in the food set.

As the amount of oyster mushrooms in the food set increases, the objective function (Formula 13) continuously increases, which indicates an increase in the discrepancy between the calculated and standard intakes (Fig. 4). The extent to which this increase is critical could be determined by the maximum allowable tolerances for the intake of some nutrients [59]. However, the applicability of these tolerances to the space diet requires substantiation.

When consuming 500 g of oyster mushrooms, the animal protein consumption standard is not fulfilled due to the deficiency of the "methionine + cysteine" amino acid pair. It is known that the pair is limiting in the oyster mushrooms [60]. Thus, the sixth nutritional imbalance appears in the food set (Fig. 4).

The antioxidant capacity of the food set increases to 540 units of Trolox equivalent when 100 g of oyster mushrooms are included in it. A further increase in the oyster mushroom consumption leads to a decrease in the antioxidant capacity. No officially recognized space standard for the antioxidant capacity of food set exists yet. Therefore, it is impossible to determine how critical the decrease in the antioxidant capacity of food set to 480 units of Trolox equivalent with an increase in the daily consumption of oyster mushrooms to 500 g is.

In the current study, we model the space diet at the ingredient level. This is specified in formulas (13-17) of Section 2.6. Earlier, Olabi, 2001 [61] and Waters et al., 2002 [14] modeled the space diet at the dish level. These authors included 7 mushroom dishes on the menu. Such an approach would seem closer to practice. However, the mushroom content in the dishes was constant. The variables were the only masses of the dishes. This considerably reduces the ability to adapt the diet to the space nutrition requirements. Moreover, the impact of oyster mushroom content in the diet on the fulfilment of the space nutrition requirements remains unknown.

This study aimed to help solve these problems. We can now determine the boundaries of the variable oyster mushrooms content in the dishes and the total amount of mushrooms in the diet. Modeling a space diet with a variable content of ingredients in dishes could be done using our previous study [58].

4. Conclusions

The influence of the substrate density, volume of the cultivation vessel, and number of harvested flushes of fruiting on the productivity, substrate decomposition speed, substrate decomposition degree, and biological efficiency was studied.

It was established that the best oyster mushroom productivity of 272 g/(m3*day) is obtained at a substrate density of 500 g/L. Forecasting the productivity by the steep-ascent method showed that it is advisable to increase the substrate density to 600 g/L and above and reduce the volume of the cultivation vessel to 1.5 L.

The lunar mushroom farm is likely to be part of a bioregenerative life support system. It will therefore be important to ensure the recycling of the spent mushroom substrate. The substrate decomposition degree may be an indicator of its maturity, i.e., its readiness

for inclusion in recycling. In practice, it is necessary to study the dependence of the maturity of the spent mushroom substrate on the number of harvest flushes. At that, a negative correlation of −0.43 was found between the speed and degree of the substrate decomposition. Therefore, the speed and degree of the substrate decomposition should be consistent. Also, it is of interest to study the effect of the spent substrate maturity on the effectiveness of its use as a fertilizer for plants and animal feed. This primarily applies to wheat and worms, since these species have already been tested in BLSS and experimental BLSS models.

The oyster mushroom cultivation parameters could be improved if the deficiency of nitrogen and phosphorus in the substrate is removed. The spent mushroom substrate can be converted into fertilizer by vermicomposting or physical-chemical treatment. To improve the sanitary and hygienic condition of the mushroom farm, it is advisable to use a sporeless strain of oyster mushroom in the future.

The relationship between the mass of oyster mushrooms consumed and the fulfilment of NASA nutritional requirements was studied. It was shown that consumption of 500 g of oyster mushroom per day violates the animal protein consumption standard. The acceptable daily intake of oyster mushrooms is 100–200 g. Here, additional nutritional imbalances do not appear, and the antioxidant capacity of the food set is not significantly reduced.

It should be borne in mind that the physical conditions on the Moon and Earth differ significantly: on the Moon, the gravity force is about 6 times less and there is no dipole magnetic field. It is necessary to test oyster mushroom cultivation using a small-sized climate chamber on the Moon. The test results would help clarify the oyster mushroom growing parameters. The data obtained in the study could be a reference point in the development of a technology for growing oyster mushrooms on a lunar farm.

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Author contribution statement

Nikolay Sergeevich Manukovsky, PhD; Vladimir Stepanovich Kovalev, PhD; Sergei Victorovich Trifonov, PhD; Olga Viacheslavovna Hranovskaya: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Data availability statement

I have attached the Figures in stg format as supplemental materials, which are in the manuscript in the jpg format. If necessary, stg files can be edited in the “Statistica” program and then inserted into the manuscript.

Additional information

Supplementary content related to this article has been published online at [URL].

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e15524.

Appendix

List of plant and animal species recommended for a bioregenerative life support system.

Oyster mushroom (*Pleurotus ostreatus*), wheat (*Triticum aestivum* L.), rice (*Oryza sativa* L.), oats (*Avena sativa* L.), quinoa (*Chenopodium quinoa* L.), millets (*Panicum miliaceum* L*),* sorghum (*Sorghum bicolor* L.), sweet potato (*Ipomoea batatas* L.), potato white (*Solanum tuberosum* L.), beet (*Beta vulgaris* L. *var conditiva Alef.*), broccoli (*Brassica oleracea* var. *Italica*), Kale (*Brassica oleracea* L. *convar. acephala*), chard (*Bēta vulgaris subsp. Vulgaris var. vulgaris*), peas (*Pisum sativum* L.), cabbage (*Brassica oleracea* L. convar. Capitata (L.) Alef. Var alba DC), lettuce (Lactuca sativa L. *var. longifolia*), carrot (*Daucus sativus*), squash (*Curcubita pepo* L.), pumpkin (*Cucurbita pepo* L.), soybean (*Glycine* max L. *Merr.*), Peanut (*Arachis hypogaea* L.), Pinto beans (*Phaseolus vulgaris Pinto Group*), chickpeas (*Cicer arietinum*), lentil (*Lens culinaris*), cowpeas (*Vigna unguiculata*), strawberries (*Fragraria x ananassa Duchesne*), tomato (*Lycopersicon esculentum* L.), melon (*Cucumis melo* L. *var. Cantaloupensis Naud*), onion (*Allium cepa* L.), garlic (*Allium sativum* L.), quail (*Coturnix*), pig (*Porcula salvania*), tilapia (*Oreochromis niloticus*).

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