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BIOTECHNOLOGY MODULE FOR SPACE BIOLOGICAL LIFE SUPPORT SYSTEMS

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ABSTRACT: *For the cultivation of microorganisms light, feeding with carbon dioxide, minerals, stable temperature and pH of the environment are provided. Designs for closed circulating photobioreactors for algae Chlorella vulgaris are described for the purpose of their use in space. The main stages of different production for Chlorella vulgaris (strain ИФР № C-111) are presented. A microprocessor controlled module is proposed for terrestrial experiments.*

KEYWORD: *OPTIMAL CULTIVATION CONDITIONS FOR CHLORELLA, NUTRIENT MEDIUM, CARBON DIOXIDE SUPPLY, LIGHT SYSTEM, SPACE BIOTECHNOLOGY, BIOLOGICAL LIFE SUPPORT SYSTEMS, TEMPERATURE SYSTEMS, ALGAE,, FOTOBIOREACTOR,, BIOMASS, CONCENTRATION, STIMULATOR*

1. Lighting modules of space greenhouses

The American space biotechnology system (SBS) Veggie uses red, blue and green LEDs in the lighting module (LM). The dimensions of the vegetative cassette of Veggie are: 45 x 29 x 14.5 cm. The data in Table 1 are based on a 16-hour day/night cycle at high illumination power at a measurement distance of 32.5 cm. A calibrated quantum sensor (LI-190, Li-Cor Biosciences) is used [1, 2].

On January 22, 2018, the American SBS Advanced Plant Habitat (APH) was launched on board the ISS for the first test with WT Arabidopsis and Apogee semi-dwarf wheat. The maximum height of the plants is 45 cm with a growth area of 0.19 m^2. The plant tray size is: 45.4 cm x 40.8 cm x 5.1 cm, Figure 1. Three camcorders are used for video control: top; side and infrared. The LM off APH (Table 2) allows illumination of 0-1000 µmol $m^{-2}s^{-1}$ Photosynthetic Photon Flux (PPF) (measured at 15 cm distance), which can be changed in steps of 50 µmol m^{-2}s^{-1}. Light is measured with an internal quantum sensor (Li-Cor 190 CZ) and a red/far sensor (Skye 110, Skye Instruments, Powys, UK) [3].

Table 1 Parameters on the lighting module on Veggie [2].

Light level [μmol m^{-2} s $^{-1}$ ± %]	red 630 nm	blue 455 nm	Red 530 nm
Low	120 ± 10 %	30 ± 10 %	-
Middle	240 ± 10 %	60 ± 10 %	-
High	360 ± 10 %	90 ± 10 %	-
On/Off	-	-	30 ± 5 %
Max	550	150	100

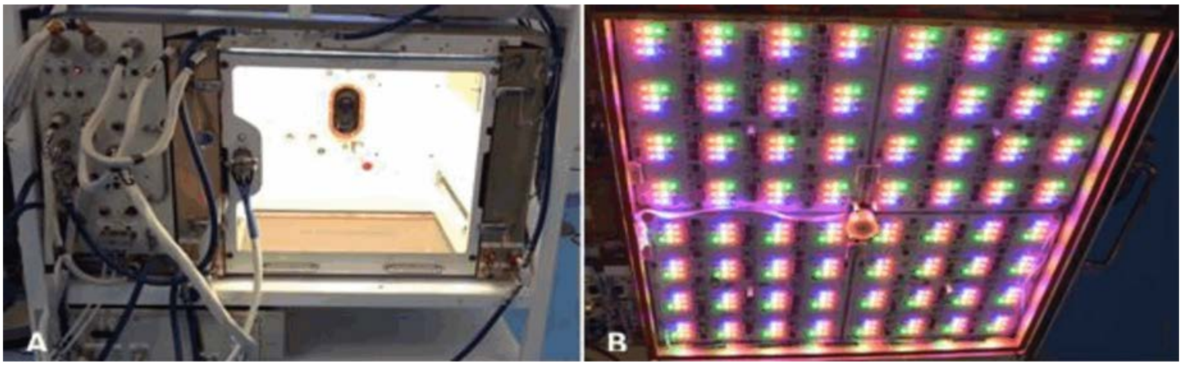

Fig. 1. ISS Advanced Plant Habitat and its lighting module [3].

Table 2 Lighting module on Advanced Plant Habitat [3].

Light	PPF [μmol m^{-2}s^{-1}]
Red (630 nm)	0-600 +/- 5 %
Blue (450 ± 10 nm)	0-400 +/- 5 %
Green (525 ± 10 nm)	0-100 +/- 5 %
White (4100 K)	0-600 +/- 5 %
Far red (730 nm)	0-50 +/- 5 %

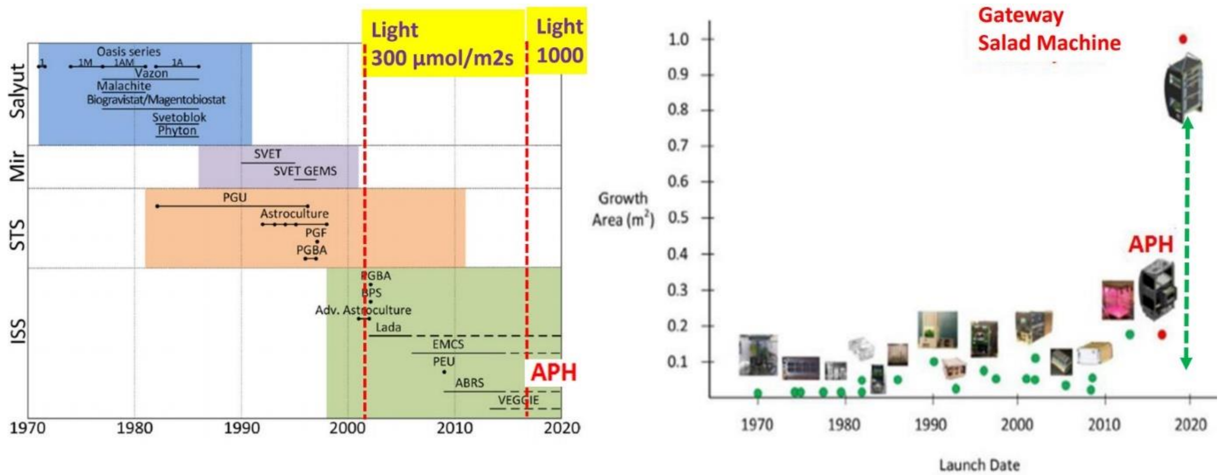

Fig. 2. Comparison of parameters of SBS and light modules [4].

In 2017, the European SBS "EDEN ISS" under the "Horizon 2020" project was tested in the Antarctic for year-round addition of fresh produce for the crew of the German Neumayer III station. The LEDs are water-cooled at 20 °C. The LM is managed via an IP protocol over an Ethernet cable. Each wavelength is individually controlled. The following spectra were used: 15 % blue (400-500 nm); 10 % green (500-600 nm); 75 % first red (600-700 nm); 2 % second red (700-750 nm). Each light source has a temperature sensor and temperature protection. The light distribution is designed to produce the best uniformity at 15 cm. There is a UV-LED based disinfection system for the water. Different SBS and LM flying in space and their parameters are shown on Figure 2. SBS with purple colour (SVET-2 flight lighting module) is described in detail in [5].

The Bulgarian on the ground project SVET has a LM equipped with: Cree® XLamp® 7090 XR; 30 LED spots with RGB-LEDs and 6 LED spots with Red-LEDs, [6]. Photosynthetic Photon Flux Density (PPFD) is in the range 0–400 $\mu mol\ m^{-2}\ s^{-1}$. Lettuce and radicchio plants are grown at 70 % red, 20 % green and 10 % blue light composition [6]. A new concept for an advanced SVET-3 for the ISS, based on the Bulgarian experience is described in [7]. There a conceptual block-diagram of the SVET-3 is presented.

2. Lighting modules of photobioreactors

The aim of the work is to study main stages of different parameters of microalgae Chlorella vulgaris (CV) in artificial conditions.

Four types of photosynthetic reaction centers are known: P700 (higher plants in photosystem I); P680 (higher plants in photosystem II); P870 (purple bacteria) and P840 (green sulphur bacteria) [8, 9]. Figure 3 shows absorption spectra of pigments and photosynthesis [9].

Figure 4 shows for one selected LM: the absorption spectrum on CV (green); LEDs (blue, yellow and red) and resulting spectrum (black). LEDs have up to 80 % power efficiency compared to fluorescent lamps up to 50 %. The service life of new LEDs is 50-100 thousand hours when the luminous flux is reduced to 30 %. The physical properties of led systems allow them to be placed inside the suspension of microalgae, and so it is better to utilize the energy of light [9].

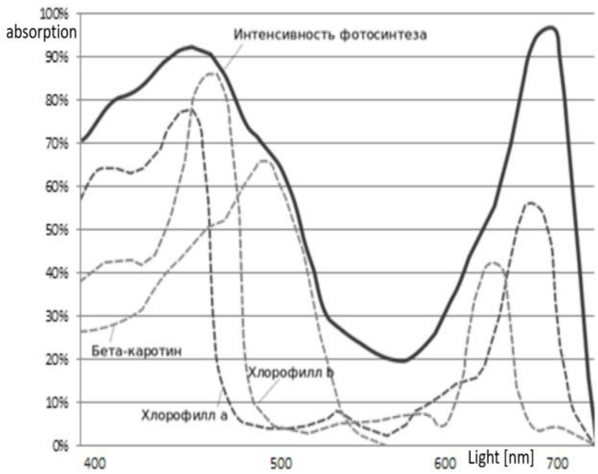

Fig. 3. Absorption spectra of pigments and spectrum of photosynthesis [8].

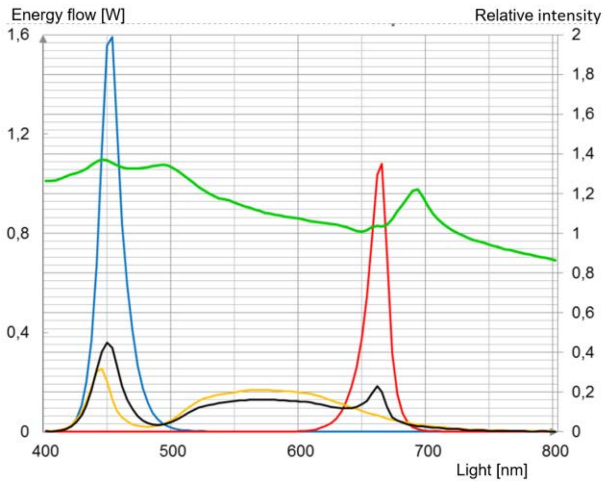

Fig. 4. Light parameters of Chlorella vulgaris and his LEDs modul. [9].

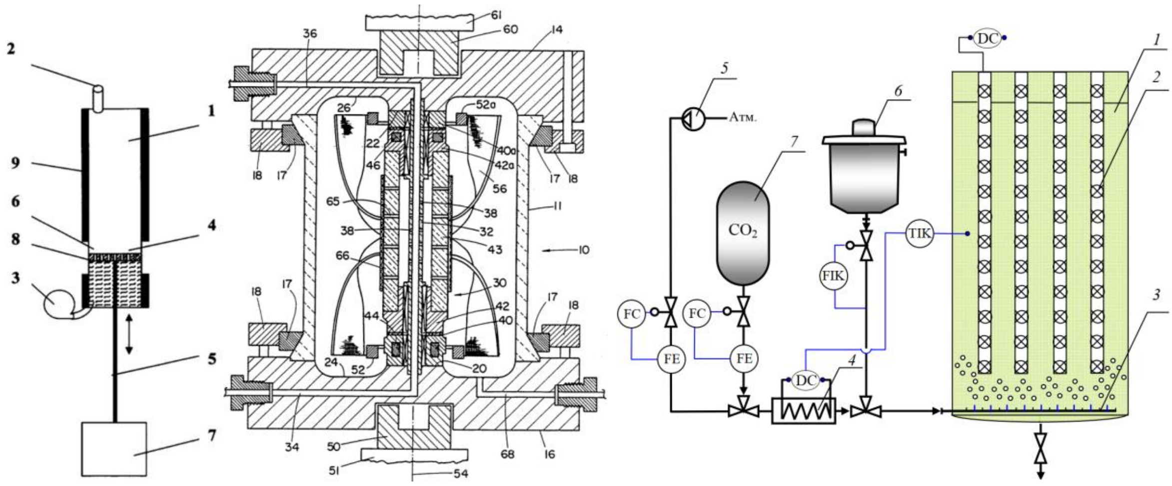

Fig. 5,6,7. Patents for bioreactors on microorganisms [10, 11, 15].

Figures 6 and 7 show two patented microorganism reactors, working in microgravity, and Figure 8 is a patented on the ground reactor, [10, 11,].

Suitable microorganisms for potential space application are described in [13, 14].

3. Specifics of Chlorella vulgaris growth and collection

The most popular forms for bioreactors are: cylinder with external LM; rectangular parallelepiped with external LM and cylinder with internal LM, [14]. For each of the types of tanks different positions of LEDs can be chosen, and lighting parameters in the whole volume, with a given type, quantity, and location various kinds of lamps, can be calculated with the Dialux software environment. Dialux can calculate: lighting color; reflection loss and the texture of the surface; full General 3D view of the illumination of the tank and a graphical representation of the light distribution over a given surface, [14]. As a result of modelling (after 12 hours) light flux decreased by half due to the growth of microalgae CV;

increased concentration is reducing the transmission capacity of the medium.

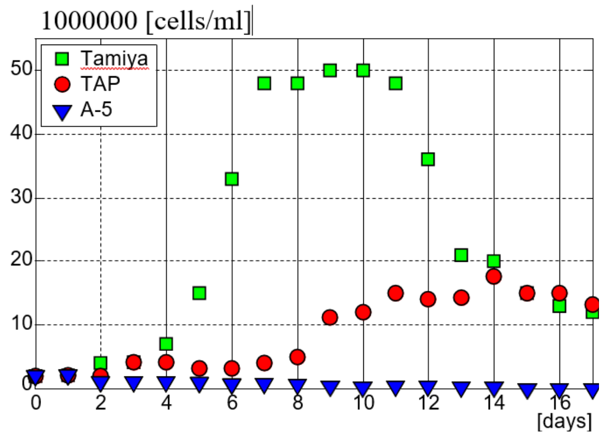

Fig. 8. Dynamics of Chlorella vulgaris biomass growth on nutrient media [16].

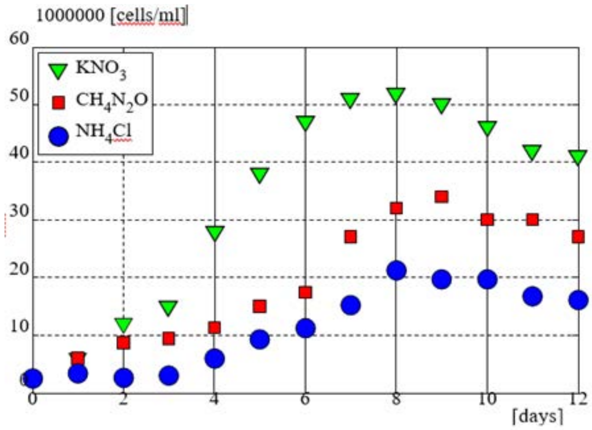

Fig. 9. Biomass growth on nutrient Tamiya with 3 nitrogen sources [16].

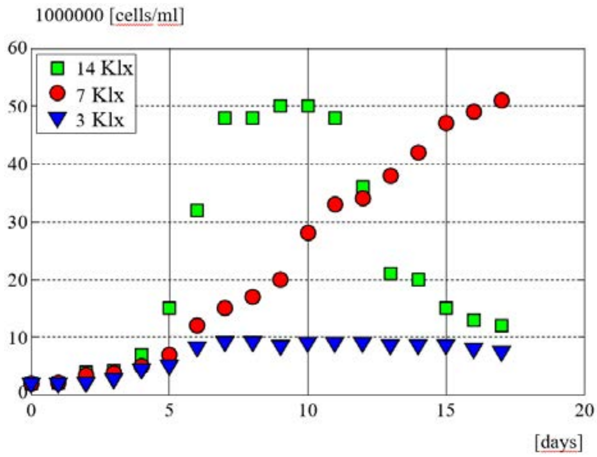

Fig.10. Dynamics of biomass growth at different light levels [16].

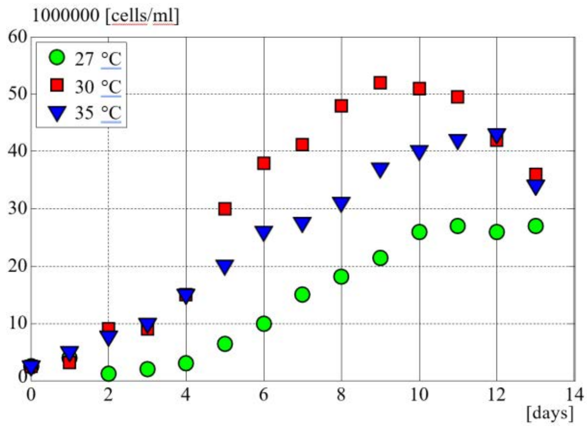

Fig. 11. Dynamics of biomass growth depending on temperature [16].

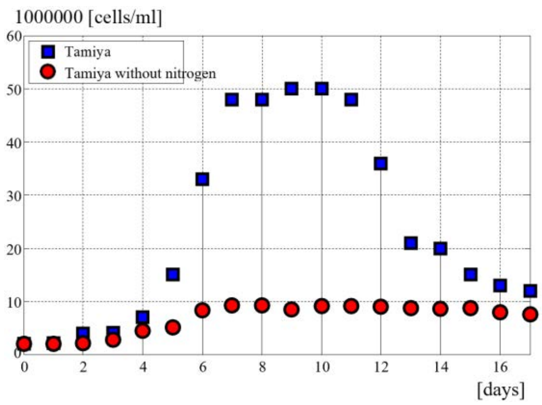

Fig. 12. Biomass growth in standard and nitrogen-depleted Tamiya media [16].

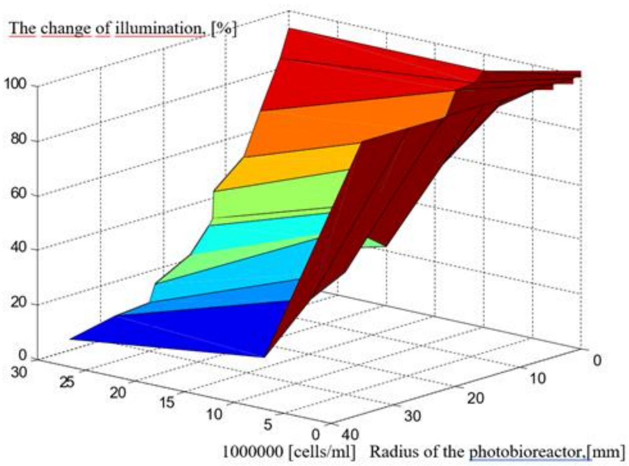

Fig. 13. Dependence of illumination level on photobioreactor radius and cell concentration in suspension [16].

With changing the nutrient composition, we obtain a CV product with the desired composition with a different ratio of proteins and fats. On a medium rich in nitrogen, CV can accumulate from 40 to 88 % of crude protein and 5 % fat. In the absence of nitrogen and excess carbon in the nutrient medium, on the contrary, - 88 % fat and 5 % protein. Biofuel production technology of the CV have two periods: the accumulation of microalgae biomass to a concentration of 55 ... 60 million cells/ml and above and the creation of stressful conditions for cells that inhibit the reproduction of CV and leads to the accumulation of lipids in cells. Figures 8 shows the CV biomass growth in different nutrient media and Figures 9 show the influence of three different nitrogen sources [16]. Additional methods to achieve a high concentration of CV cells are shown on Figures 10,11,12. The level of illumination of the CV suspension at a depth of 30 mm is only 7 % of the light intensity at the surface of the photobioreactor- Figure 13. Light uniformity can be improved with strong bubbling. Methods of stimulation of the CV are described in [17, 18, 19].

4. Results

For the measurement and management of the SBS, a microprocessor system (41 analog and 11 digital inputs, 11 relay and 8 digital outputs, measurements and control over digital protocol) with two LM, Figure 14, and software (for visualization and adjustment) Figure 15 and LM are developed [20]:

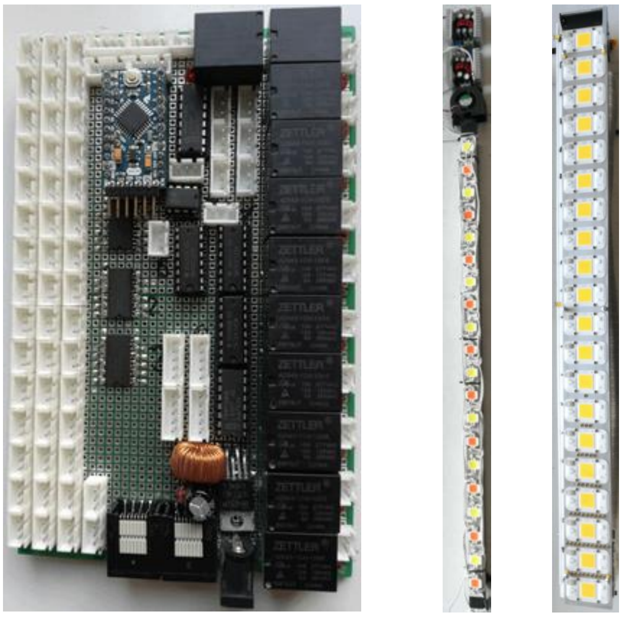

Fig. 14. Microprocessor system and LM.

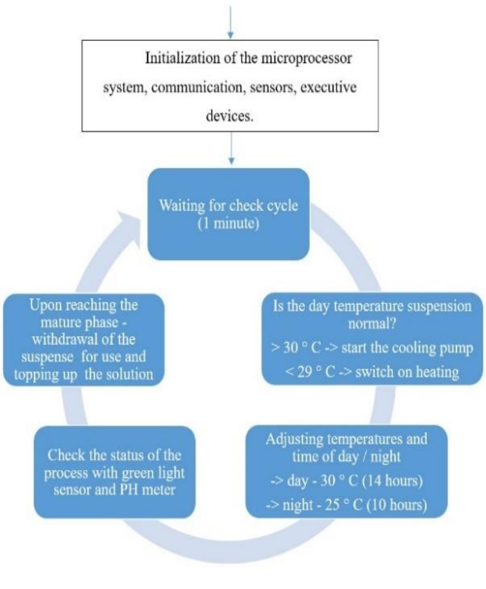

Fig. 15. Software algorithm.

A mathematical model capable of describing the biomass processes of CV cells, subsidy and lipid accumulation. The input variables are *S* - concentration of nitrogen-containing salts and *I* - light. Here are the internal parameters: K_S=1.06 g/l semi – saturation constant; K_i=8.4 g/l – inhibition constant, K_I – constant semi-saturation by light, μ_{max} – maximum unit growth rate. The process of inhibiting biomass growth by increasing the concentrations of the S substrate, when I_{const}=5.6 Klx is described (1) by the Andrews equation, [19]:

$$\mu(S) = \mu_{max} \frac{S}{K_S + S + S.S/K_{ing}} \tag{1}$$

The effect of тhe I on the nutrient growth of microalgae (2) is described by the Michaelis-Menten equation, when S_{const}=5600 and K_I=15 Klx:

$$\mu(S) = \mu_{max} \frac{I}{K_I + I} \tag{2}$$

The graph described by the Andrews equation has a clear extreme. By it, can determine (3) at what amount of limiting substrate the specific growth rate will be maximum. To do this, we will find a derivative of S and equate it to zero:

$$S + 2\frac{S.S}{K_i} - K_S - S - \frac{S.S}{K_i} = 0 \tag{3}$$

$$S_{opt} = \sqrt{K_S.K_I} = \sqrt{1.06 \cdot 8.4} = 3.2\ g/l \tag{4}$$

5. Conclusion

The biological parameters of CV allowing cheap, efficient and flexible control with microprocessor system are presented. Methods for control a reactor LM for photosynthetic microalgae experiments are proposed. With real time measurement of light parameters, the automatic control system will keep the tank efficiency of photobioreactor installation. Concepts of Algae bioreactor are discussed. The dynamics of optical density of suspension during the growth of culture will be measured, visualised and saved. Methods for the maximum accurate measurement of the biological parameters of Chlorella vulgaris will be used in parallel testing of 2 identical bioreactors. The first is a control, with a standard growing medium, and the second is experimental with a change of only 1 (subsequently with 2 and 3 cultivation parameters), intended to optimizing certain output properties of a CV, used mainly on the Earth. This two SBS will carry out research into the application of a CV in systems for securing the life of astronauts microgravity in orbit, on the surface of the moon, and on Mars.

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