The CRG-PVA hydrogels study of properties with various nanoparticles and their application for cultivation of phototrophic microorganisms

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Abstract. In this work we are demonstrate results of researches of the hydrogels based on carrageenan and polyvinyl alcohols properties with various nanoparticles Al₂O₃ and ZrO₂ and their application for phototrophic microorganisms Chlorella vulgaris cultivation. X-ray diffraction of samples was carried out. Research on the cultivation of microalgae using the developed hydrogels has also been conducted, and the increase in productivity with the use of gels on average by 20% compared to the control sample has been shown. The highest productivity is observed with concentration 0.5% of ZrO₂ nanoparticles. We conclude that using of hydrogels in the developed photobioreactors possess a perspective.

1. Introduction

At the present time in the world actively developing technologies based on using phototrophic microorganisms (PM) – microalgae and cyanobacteria. The reason of their utilization is that PM need only light, carbon dioxide and small amount of mineral additives in water for grow [1, 2]. At the same time, a wide range of products can be obtained from various PM, including biofuels, vitamins, substances for cosmetics or pharmaceuticals, feed and food additives and fertilizers [3, 4]. Also PM is the promising organisms for carbon dioxide fixation [3].

The reduction of biomass cost is one of the main challenges of microalgae based refinery development [5]. This cost strongly depends on the capital expenses for the photobioreactor itself, in which the cultivation are carried out and lighting expenses [6]. For elaboration a photobioreactor construction, the developers try for ensure the achieving highest as possible concentration of biomass. Although, in the later stages, the growth of biomass is strongly inhibited by the lightless in the deep of culture medium and the growth of biomass is limited only by surface layers [7, 8]. Thus, it is necessary to use either pipes of small diameter or shallow ponds, which leads to increase photobioreactor volume and rise cost of the equipment themselves [2, 7]. For another thing, these factors impose limitations on overall dimensions and construction solutions of photobioreactors, which limits their use in a number of areas, such as, for example, air purification with carbon dioxide fixation in large parking lots and buildings with a many people inside (shopping malls, train stations and etc.)
[9, 10]. For such applications, photobioreactors must fit into existing structural and technical solutions, so that on the one hand they avoid reconstruction, on the other hand, take into account social factors of people's behavior in these buildings [11]. Overcome of all these technical limitations is possible by use approaches that allow providing illumination in the depth of the culture medium [12]. One approach is to create conditions for the reflection of light within the cultivated environment, by placing various materials with a high albedo there [13]. The aim of this work is to analyze the possibility of application such materials with zero buoyancy based on polymer hydrogels consisting of carrageenan (CRG) and polyvinyl alcohol (PVA). The selection of such a system was due to its biocompatibility, high stability and the ability to immobilize various nanoparticles within it. For example, earlier it was shown the possibility of creating photocatalytic hydrogels based on CRG PVA with titanium dioxide nanoparticles immobilized therein [14]. The hydrogels remained stable, despite the photocatalytic activity of titanium dioxide. In our work we use hydrogels with various concentrations of nanoparticles. As nanoparticles Al$_2$O$_3$ and ZrO$_2$ were chosen due to its inertness and biocompatibility to microalgae strains. Also, those nanoparticles can gives strong white color to hydrogels. Our experiments in this stage will be provided by cultivation of PM in flasks with and without hydrogels presence, to demonstrate proof-of-principal. Future perspective is developing of new type of photobioreactors that can be applied in the existing buildings to make modern cities closer to CO$_2$ neutral status.

2. Materials and methods

2.1. Preparation of hydrogels

For preparing each of the gels, 10 ml CRG solution with a concentration of 2.5 g/l and 10 ml PVA solution with a concentration of 2.5 g/l too, were initially prepared at a temperature of 70 °C. The solutions were then mixed together and nanoparticles were added at the same time. Al$_2$O$_3$ and ZrO$_2$ from BDT company (Russian Federation) were used as nanoparticles. The dimensions of the nanoparticles were: 68±10 nm of aluminum dioxide; 74±10 nm of zirconium dioxide. Samples with concentrations of 0.25%, 0.50%, and 0.75% were prepared for each type of nanoparticles. The resulting mixture was stirred for an hour at 500-550 rpm at a temperature of 70°C. After this, the gels were cooled to room temperature and subjected to four cycles of freezing/thawing. The freezing temperature was -12 °C and the thawing temperature + 25 °C. The number of cycles and temperatures were chosen in accordance with the results of the studies presented in [14, 15].

2.2. Microalgae cultivation

The object of the study was the Chlorella vulgaris GKV1 culture from the collection of the National Research Center "Kurchatov Institute". Chlorella vulgaris GKV1 microalgae were grown on a Basal medium [16] with the following composition (g/l): KNO$_3$, 1.25; KH$_2$PO$_4$, 1.25; MgSO$_4$·7H$_2$O, 1; CaCl$_2$, 0.0835; H$_3$BO$_3$, 0.1142; FeSO$_4$·7H$_2$O, 0.0498; ZnSO$_4$·7H$_2$O, 0.0882; MnCl$_2$·4H$_2$O, 0.0144; MoO$_3$, 0.0071; CuSO$_4$·5H$_2$O, 0.0157; Co(NO$_3$)$_2$·6H$_2$O, 0.0049; EDTA·2Na, 0.5, the nutrient medium was prepared in filtered tap water, the pH of the nutrient medium was adjusted to 7. Cultivation was carried out in 250 ml in Erlenmeyer flasks. Gels (900 mm$^3$ in size) with aluminum and zirconium nanoparticles of different concentrations were placed in the flasks. The flask without gel was a control. Stirring was carried out by installing the flasks on a shaker with a frequency of 110 rpm. The culture temperature was maintained at 24 ± 2 °C by air conditioning in the room. The culture grew under conditions of constant illumination during the day and night at an intensity of 1100 lux. Growth control was performed by measuring the optical density of the culture at the same time each day on a Thermo Scientific Genesys 10S UV-Vis spectrophotometer at a wavelength of 680 nm.

2.3. Swelling study

The swelling study was carried out in according to [14]. We used the dried samples to determine the swelling capacity of the hydrogels and its dependency with time. The swelling capacity (SC) was
determined by immersing a weighted amount of dried hydrogel nanocomposite into 10 mL of nutrient medium and allowed to swell at room temperature for 5 min. Then, they were removed from aqueous solutions and blotted with filter paper to remove surface water and then weighed again. Then the swelling/weighting steps were repeated with the constant time interval of 5 min until the mass change in three consecutive measurements would remain constant with the accuracy of 0.003 g. The Ohaus PA64C analytical balance was used in those studies.

The degree of swelling \( \alpha(t) \) at every given moment of time \( t \) was calculated by the formula

\[
\alpha(t) = \frac{m(t) - m_0}{m_0} \times 100\%
\]

Where \( m_0 \) – is the mass of the initial (dry) sample in g, \( m(t) \) – is the mass of sample in swelling study in g.

The swelling of all samples of composite hydrogels with different concentrations (0.25%, 0.5%, 0.75%, and 1%) of nanoparticles (\( \text{Al}_2\text{O}_3 \), \( \text{ZrO}_2 \)) in their composition was determined in a similar manner.

2.4. X-ray diffraction

The X-ray diffraction research was carried out on a Bruker Advance diffractometer using the characteristic line Cu Kα1 at a wavelength of 1.54 Å. Samples of hydrogels were preliminarily placed in distilled water for 2 hours to ensure their swelling. The samples were then placed in a drying chamber and dried at 60°C for 24 hours. In the experiment, the correlation of the intensity of scattered X-ray radiation on the scattering angle in the \( \theta/2\theta \) scheme is measured (the angle of incidence is equal to the angle at which the scattered radiation is recorded). Diffraction maxima are observed at angles satisfying the Wolf-Bragg condition:

\[
2d \sin \theta = n\lambda
\]

where \( d \) – interplanar distance, \( \theta \) – half the angle between the incident and recorded X-rays, \( n \) – diffraction degree, \( \lambda \) – X-ray length.

2.5. Measurement of mechanical characteristics of hydrogels

Measurements of mechanical characteristics are carried out at room temperature (23 ± 2°C) in a universal testing machine of Instron company (model 5965) in the regime of constant speed of clamping. For fixing samples in uniaxial tension tests, standard pneumatic clamps from a set of universal testing machine are used. Samples before the tests were conditioned at a temperature of 23 ± 2°C for at least 24 hours.

The procedure for testing samples under uniaxial tension is governed by GOST 11262-80. Dimensions of the working part of the sample – 10 x 3.5 x 0.5 mm. Speed of expansion of clamps - 1 mm/min.

Calculation of elastic moduli under uniaxial tension is carried out in accordance with GOST 9550-81. Calculation of the strength of deformation at break is carried out in accordance with GOST 11262-80.

The values of the mechanical parameters are obtained by averaging the measurement results in each series of at least 5 samples. The measurement error is defined as the average deviation from the mean values of the mechanical parameters.

3. Results and discussions

3.1. Research properties and characteristics of hydrogels

The presence of crystalline nanoparticles in the samples was determined by X-ray diffraction analysis. For both samples, the presence of crystal structures corresponding to those nanoparticles that were used in the synthesis process was recorded, which confirms their fixation in the gels (figure 1).
Observation of swelling of the obtained hydrogels showed the following results: all hydrogels containing aluminum dioxide for half an hour absorbed 1500 - 1700% water from their weight, which is a high index of swelling. At the same time, gels containing zirconium dioxide swelled much worse, gaining 250-400% water. This difference in swelling indicates a greater hydrophilicity of hydrogels with aluminum dioxide nanoparticles.

The results of observation the mechanical properties of gels in a dry form are presented in table 1. From the presented results it can be seen that the gels (with aluminum dioxide) lose their mechanical strength as the content of nanoparticles increases therein. The same results was observed for gels with titanium dioxide nanoparticles [14]. Gel with 0.75% zirconium dioxide has more mechanical strength than gel with concentration 0.25% and 0.5%. A24, A34, A44 and are gels with a content of 0.25%, 0.5% and 0.75% of aluminum dioxide, respectively; Z24, Z34 and Z44 are gels with a content of 0.25%, 0.5% and 0.75% of zirconium dioxide, respectively; CRG + PVS is a gel without nanoparticles and K is a control flask in which the biomass was cultured without gels.

Table 1. Mechanical properties of hydrogels. Where: $\sigma_{\text{max}}$ - maximum tensile stress, $\varepsilon_b$ - tensile strain, $E$ - Young's modulus.

| Sample code | $\sigma_{\text{max}}$ [MPa] | $\varepsilon_b$ [%] | $E$ [MPa] |
|-------------|----------------------------|------------------|-----------|
| Z24         | 17.3±4.0                   | 0.6±0.1          | 3273±415  |
| Z34         | 15.9±5.3                   | 0.7±0.07         | 2867±300  |
| Z44         | 38.7±6.9                   | 1.3±0.03         | 3311±510  |
| A24         | 46.8±2.34                  | 1.9±0.09         | 3306±300  |
| A34         | 27.5±2.1                   | 0.9±0.2          | 3677±449  |
| A44         | 24.9±1.24                  | 0.9±0.045        | 3418±350  |
| CRG+PVS     | 49.2±0.6                   | 15.0±0.1         | 2055±60   |

Experimental data on the cultivation of microalgae with gel are presented in tables 2 and 3 for the first and second cultivation, respectively. It should be noted that the optical density measurements were carried out in a sample of the culture medium and this method did not take into account the fouling of gels by microalgae biomass.

Consider the results of the first cultivation. From table 2 it can be seen that all variants of gels allowed to provide more effective cultivation in comparison with a control sample without gel. In this
case, the gels containing zirconia are largely fouling by biomass, so we can expect that a gel with 0.5% zirconia provides the greatest amount of biomass during cultivation under the conditions chosen in the experiment.

**Table 2. Results of the first cultivation (gel designations are deciphered in the text).**

| Fermentation time, h | A24 | A34 | A44 | Z24 | Z34 | Z44 | CRG+ | K |
|---------------------|-----|-----|-----|-----|-----|-----|------|---|
| 0                   | 0.142 | 0.142 | 0.142 | 0.142 | 0.142 | 0.142 | 0.142 | - |
| 24                  | 0.12  | 0.162 | 0.105 | 0.121 | 0.12  | 0.11  | 0.13  | 0.143 |
| 48                  | 0.181 | 0.213 | 0.168 | 0.188 | 0.2   | 0.189 | 0.334 | 0.173 |
| 96                  | 0.237 | 0.268 | 0.228 | 0.234 | 0.249 | 0.267 | 0.375 | 0.2 |
| 168                 | 0.369 | 0.417 | 0.337 | 0.377 | 0.375 | 0.383 | 0.422 | 0.271 |
| 192                 | 0.422 | 0.453 | 0.367 | 0.435 | 0.423 | 0.398 | 0.473 | 0.319 |

Area with settled cells from the total area of the gel, %

5 10 1 85 90 90 5 -

Subsequently, using the same gels, repeated cultivation was carried out under the same conditions (table 3). At the same time, gels with zirconium dioxide were biomassed to a lesser extent, and at the same time the greatest amount of biomass was observed in the flasks with them. Gels with aluminum dioxide, on the contrary, were more prone to fouling during re-cultivation. This effect can be explained by the greater hydrophilicity of the gels and their ability to contain a larger volume of water and biomass that could remain after the first cultivation.

**Table 3. Results of the second cultivation (gel designations are deciphered in the text).**

| Fermentation time, h | A24 | A34 | A44 | Z24 | Z34 | Z44 | CRG+ | K |
|---------------------|-----|-----|-----|-----|-----|-----|------|---|
| 0                   | 0.05 | 0.05 | 0.048 | 0.053 | 0.05  | 0.049 | 0.048 | 0.053 |
| 24                  | 0.052 | 0.066 | 0.065 | 0.064 | 0.057 | 0.056 | 0.072 | 0.066 |
| 48                  | 0.035 | 0.034 | 0.069 | 0.053 | 0.057 | 0.062 | 0.109 | 0.079 |
| 72                  | 0.06  | 0.026 | 0.116 | 0.087 | 0.109 | 0.096 | 0.174 | 0.101 |
| 144                 | 0.049 | 0.028 | 0.242 | 0.230 | 0.214 | 0.202 | 0.296 | 0.136 |
| 192                 | 0.059 | 0.019 | 0.283 | 0.28  | 0.288 | 0.279 | 0.375 | 0.072 |
| 312                 | 0.525 | 0.2  | 0.51  | 0.368 | 0.581 | 0.403 | 0.506 | 0.123 |

Area with settled cells from the total area of the gel, %

60 100 30 20 20 20 10 -
4. Conclusions
The synthesis of novel CRG-PVA hydrogels with Al$_2$O$_3$ and ZrO$_2$ nanoparticles was achieved by proposed method. The presence of nanoparticles in the gels was determined by X-ray diffraction analysis. The tensile strength gel with aluminum dioxide lose as the content of nanoparticles increases therein, whereas gel with 0.75% zirconium dioxide has more mechanical strength than concentration 0.25% and 0.5%. The gels containing aluminum dioxide has swelling about for 4-6 fold higher than gels with zirconium dioxide. Research on the cultivation of microalgae using the developed hydrogels has also been conducted, and the increase in productivity with the use of gels on average by 20% compared to the control sample has been shown. The highest productivity of microalgae is observed with concentration 0.5% of ZrO$_2$ nanoparticles. The possible application of this work will include possess hydrogels with nanoparticles in the developing photobioreactors for increase productivity of microalgae.

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