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THE USE OF *CHLORELLA VULGARIS* BEIJER. IN BIOREMEDIATION ACTIVITIES

L. M. CHEBAN

Yuriy Fedkovych Chernivtsi National University,
Ukraine, 58012, Chernivtsi, Kotsiubynsky 2 Str.
e-mail: l.cheban@chnu.edu.ua

The article focuses on the possibility of applying the green alga Chlorella vulgaris Beijer. culture to bioremediation activities. Two types of wastewater were simulated, agricultural (ACW) and domestic (DW). The experiment was conducted under laboratory conditions in 500-ml Erlenmeyer flasks. The ratio of the amount of the algal culture and the wastewater volume was 1:10. The content of NO_3^-, NO_2^- and NH_4^+ in the composition of the wastewater was tested before and after cultivating the alga; during cultivation, the pH of the culture medium and the optical density of the Ch. vulgaris culture were monitored. The indicators of the amount of various forms of nitrogen and the pH level show that simulated domestic and agricultural wastewater can serve as an alternative nutrient medium for growing green algae. The use of Ch. vulgaris for the treatment of domestic and agricultural effluents allows avoiding almost completely their nitrate and ammonia pollution. The amount of biomass obtained within 25 days of cultivating Ch. vulgaris on agricultural sewage was two times higher than in the control Tamiya medium. Resulting Ch. vulgaris algal mass with the proteins content of 55% and lipids reaching 30% can match various needs being used a source of protein or lipids.

Keywords: algae, Chlorella vulgaris Beijer., bioremediation, wastewater

Introduction. An emerging and promising trend of science and technology, environmental biotechnology deals with the problems of treating industrial and communal wastewater, processing industrial, agricultural and household waste and the like. To this end, environmental biotechnology exploits the ability of microorganisms to use inorganic compounds as an energy source for converting various organic substances into compounds available to other organisms. Of particular interest are pollutants that can be used as raw materials to obtain the target products of microbiological synthesis (Delgadillo-Mirquez et all, 2016; Arumugam et all, 2011).

All production systems, in addition to a large amount of dirt and suspended insoluble components, produce wastewater abundant in nutrients, for example, nitrogen and phosphorus compounds. The excessive release into the environment of these chemicals which are considered the main water pollutants associated with the activities of spinning mills, agricultural and fish farms (Norvill et all, 2016; Cheunbarn and Cheunbarn, 2015; Mook et all, 2012), can lead to eutrophication of natural bodies of water. However, nitrogen and phosphorus compounds are the main nutrient components in the composition of classical algae growing media (Edmundson and Wilkie, 2013; Zolotaryova, 2009). Microalgae utilize nutrients, including nitrogen and phosphorus, from wastewater, thus ensuring bioremediation and the production of by-products and biofuels on an ongoing basis (Fenton, 2012; Hemaiswarya et all, 2011).

There are compelling examples of the utilization of algae in bioremediation water treatment activities. Thus, schemes have been developed for cyanobacterium spirulina (Habib and Kohinoor, 2018) and green algae of the genus *Desmodesmus* cultivating on wastewaters of fisheries and recirculating aquaculture systems (Cheban et all, 2015). It is the nitrates, phosphates and urea of these discharges that are the main nutritional factors for growing microalgae and obtaining valuable biomass. The effectiveness of the use of *Chlorella zofingiensis* to remove pollutants from wastewater of livestock farms has also been confirmed: 10-day period of cultivation yielded in the removal of about 80% of nitrogen and 90% of the total phosphorus content (Prodip and Omprakash, 2017; Rajesh et all, 2015).

Among algae, there is a group of organisms capable of metabolizing carbon-containing substrates under conditions of mixotrophic cultivation, and this ability has been successfully demonstrated during treatment of sugar plants discharges (Matamoros et all, 2015; Xu et all, 2006).

To implement bioremediation activities, algal cultures are selected that actively grow without forming toxic exometabolites. Among such cultures, stable and fast-growing species of *Chlorella*, *Scenesdesmus*, and *Nannochloropsis* prevail (Norvill et all, 2016). The most promising in this regard is the culture of unicellular green alga *Chlorella vulgaris* Beijer.

Ch. vulgaris is an optional autotrophic microorganism that is capable of both autotrophic and mixotrophic growth (Heredia-Arroyo et all, 2011).

This is a species of microscopic unicellular green alga in the form of microscopic, 2 to 10 microns in diameter, non-motile globules. The cells are coated with a solid bilamellar cellulosic membrane, its cellulase cell wall can easily be converted to a technically affordable fuel (Guiry and Guiry, 2020).

All algal wastewater treatment technologies involve purification of discharges combined with obtaining valuable algal biomass.

The aim of the work is to assess the possibility of bioremediation activities using *Chlorella vulgaris* Beijer for the treatment of simulated wastewater.

Materials and Methods. The study was carried out using unicellular alga *Ch. vulgaris*. The algal culture we used for work was previously obtained from the collection of M.G. Kholodny Institute of Botany of the National Academy of Sciences of Ukraine and now is kept in the collection of the Institute of Biology, Chemistry and Bioresources of the ChNU.

Ch. vulgaris is non-colonial, non-flagellar unicellular alga, 2-10 microns in size. The cell is surrounded by a strong cellulose sheath that provides resistance to chemicals. *Chlorella* does not form aggregates. The control *Chlorella* culture was grown on Tamiya medium (Zolotaryova et all, 2009).

For bioremediation activities, two types of wastewater were modelled:

DW – domestic wastewater (10 g of food waste and kitchen detergents dissolved in 1000 ml of water);

ACW – agricultural wastewater (10 g of poultry manure dissolved in 1000 ml of water.)

Prepared models were kept for 24-hour infusion and then filtered.

The alga was grown in 1000 ml conical flasks. 500 ml of the selected medium and 50 ml of the initial algal culture were added to each flask.

Cultivation was carried out in a climatic room at room temperature and 6 hr photoperiod and lasted 25 days.

By the beginning of cultivation and upon its completion, the content of various forms of nitrogen was measured in the wastewater. For this, test systems of the company “Ptero” were used. The pH of the wastewater was controlled with a portable pH meter (WaterproofpH-Temp).

During the cultivation, the optical density of the culture was measured every 5 days. At the end of the cultivation, the algal biomass was harvested and the content of proteins and lipids in it was analyzed.

The optical density of the culture was analyzed spectrophotometrically on KFK-2MP at a wavelength of 750 nm (Hevorhyz and Shchepachyov, 2008).

At the end of the cultivation, the biomass was separated from the centrate by settling with sedimentation period of 24 hours. All calculations were carried out in terms of dry weight. The amount of protein was determined by the Lowry method (Lowry et all, 1951). The lipid content was determined according to the standard method using a phosphovanillin reagent (Knight, 1972).

All studies were repeated three times. The figures present the results in the form of average values and deviations from the average values. Statistical calculations were performed in the MX program.

Results and discussion. The implementation of bioremediation measures involves the treatment of wastewater of different origins using biological objects of various levels of organization. When choosing a culture of a biological agent for introducing it into purification systems, it is necessary, first of all, to evaluate the possibility of growing such an organism when there is a certain set of chemical components in the effluent. In order to do this, it is necessary to carry out a chemical analysis of the effluents, which will make it possible to predict the possibility of growing certain crops in each specific case.

Ch. vulgaris, like most green algae, is able to use various sources of nitrogen to actively increase biomass. To grow this alga, two types of wastewater, domestic (DW) and agricultural (ACW), were simulated. The control medium was the classical Tamiya medium for growing green algae.

It was observed that the simulated wastewaters, like the classical Tamiya medium, contain a sufficient amount of NO_3^+ and NH_4^+ (Fig. 1). NO_2^- was detected in trace amounts with both types of wastewater, 0.075 mg / L nitrite ions were observed only in domestic waters (DW). Given the amount of nitrogen compounds, these wastewaters can be regarded as alternative nutrient media.

During *Ch. vulgaris* cultivation, pH was monitored every 5 days (Table 1). The pH of the cultivation medium is one of the limiting factors for the successful cultivation of algae. The optimal pH values for the cultivation of freshwater algae are in the range of 6.5-8.5. Changes in pH will inevitably lead to a slowdown in the growth of algae. Lowering the pH to neutral values or even acidic can lead to the death of the culture. The pH values of the two simulated effluents were slightly different: for ACW, a pH value of 6.8 was recorded, which is close to the lower critical boundary of the pH range for growing algae. Clearly, the differences in pH between the two effluents are due to differences in their chemical composition. However, even in the course of growing, equalization of the acidity of both applied alternative media was registered. Throughout the cultivation of *Ch. vulgaris*, the pH of the medium remained within the recommended limits.

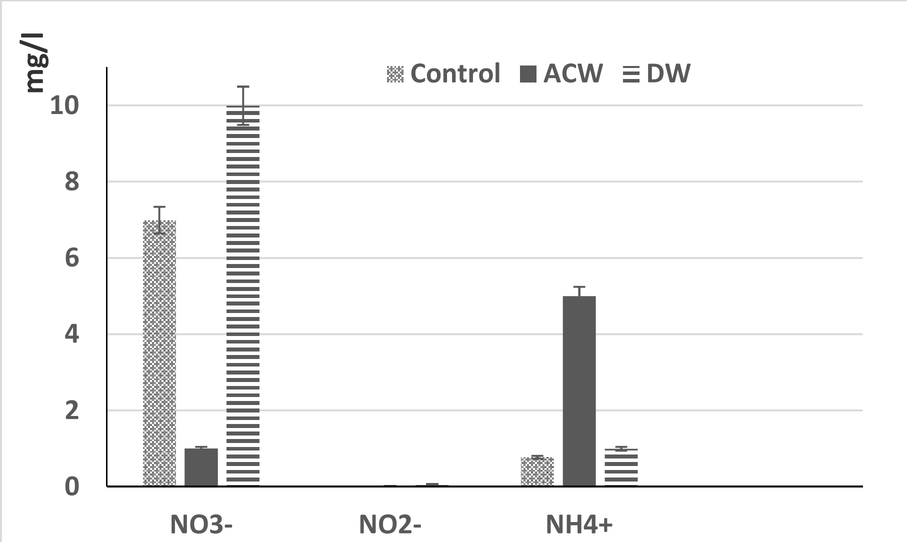

Fig. 1. The content of nitrogen compounds in the wastewater

Table 1.

Changes in pH during the cultivation of Chorella vulgaris on alternative media

Days	Type of wastewater		
	Control	ACW	DW
1	7,0	6,8	7,6
5	7,0	6,9	7,6
10	7,1	7,0	7,5
15	7,2	7,1	7,3
20	7,2	7,3	7,3
25	7,4	7,5	7,4

Thus, in terms of both the content of basic nutrients and the buffer properties, the wastewaters tested for cultivation are a fairly good growth medium. Whether it is suitable for growing *Ch. vulgaris* can only be said by estimating algae growth under these conditions. The peculiarities of the growth of unicellular algae are best monitored by spectral research methods. Changes in the number of algal cells in a culture lead to changes in the density of this culture. It is known that in photosynthetic cells the amount of chlorophyll correlates with the amount of biomass (Hevorhyz, 2008), which can be recorded by photocolorimetric measurements at 750 nm. This is how we could observe the growth rate of *Ch. vulgaris* in effluents (Fig. 2.). We see that *Chlorella* exhibits fairly intense growth in all the media used. Growing on DW gives the same picture as growing on a control medium. On the 20th day of cultivation, the culture reaches its maximum density, and therefore the maximum number of cells. In this case, we obtain values similar to the control ones (0.5). But when applying ACW, we observe a completely different picture: up to 15 days inclusive, the alga grows more slowly, with a lower culture density; then the density of the culture starts to increases rapidly. On the 25th day of cultivation, in contrast to the other two cases, the active growth continues. The density of culture *Ch. vulgaris* at this stage is twice as high as in the control Tamiya medium and domestic wastewater.

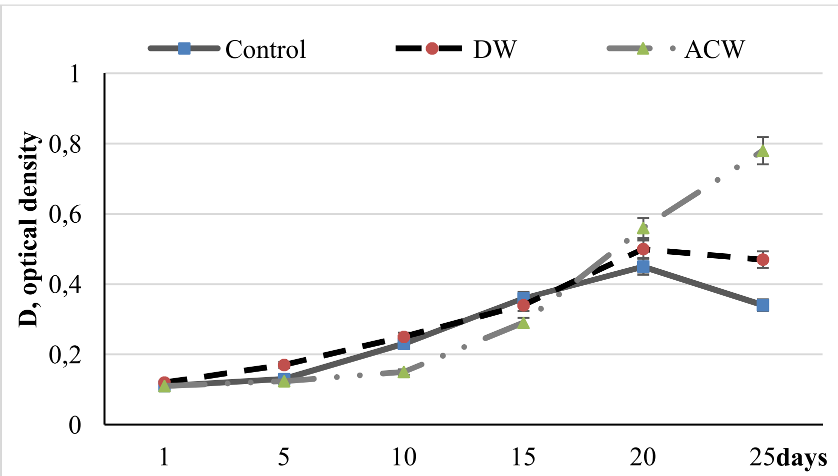

Fig. 2. Chorella vulgaris culture density when grown in wastewater

In our opinion, the explanation for this fact is the composition of agricultural wastewater: as already mentioned, the NH_4^+ content in them is quite high. It is known that algae can use the ammonium form as a source of nitrogen, but only after the exhaustion of NO_3^- reserves (Mook et all, 2012). Probably, this explains the active growth of *Ch. vulgaris* culture on ACW at the final stages of cultivation.

At the end of the cultivation, *Ch. vulgaris* biomass was removed from the nutrient medium by filtration through a plankton net. The obtained filtrate was transparent, with no cell remainders, had a yellowish colouring, there were no insoluble components in it. Later this filtrate was analyzed for residual amounts of nitrogen (Table 2).

Table 2.

The content of nitrogen compounds in wastewater after algae cultivation

Nitrogen compounds	Control	ACW	DW
NO_3^-	1 mg/l	-	2 mg/l
NO_2^-	-	-	-
NH_4^+	-	0,5 mg/l	-

After algae cultivation, no trace amounts of nitrite nitrogen were found in any of the media used. In ACW after bioremediation measures, a low content of NH_4^+ (0.5 mg/l) was recorded. At the end of algae cultivation on DW, the NO_3^- level decreased from 10 mg/l to 2 mg/l.

Thus, the use of algal cultures for the treatment of domestic and agricultural wastewaters can almost completely eliminate nitrate and ammonia pollution of such waters. Effluents treated in this way can be re-introduced into agricultural production cycle or used as process water.

A sufficient amount of nutrient factors in the water allows algae to quickly build up biomass, which is a potential source of valuable compounds. For example, the algal mass proteins, after bioremediation, may become a source of proteins, which makes it suitable as a feedstock in aquaculture or in the meat and poultry industry. If concentration of lipids is high enough, algal mass may become a raw material for producing biodiesel.

Table 3 presents the results of the analysis of the content of total protein and lipids in *Ch. vulgaris* biomass after the wastewater treatment.

Table 3.

Total protein and total lipid content of Chorella vulgaris biomass in wastewater cultivation

Version of medium	**Total protein content,** mg/g	**Total lipid content,** mg/g
Control	535± 34,2	273±13,6
ACW	480±31,8	342±18,9
DW	574±28,5	267±21,3

It has been established that the largest amount of protein in the biomass of *Chlorella* is found when grown on domestic wastewater. Under these conditions, the amount of *Chlorella* is about 57% of the biomass of the algae – even more than in the control classical medium Tamiya. The protein content in *Ch. vulgaris* biomass grown on ACW is slightly lower (48%), however, this biomass is rich in lipids (34% of the dry weight). In the biomass grown in DW the lipid content was 27%, which is comparable to the control culture.

Thus, we show the possibility of using the green alga *Ch. vulgaris* for bioremediation activities. The wastewater to be treated must contain nitrogen and phosphorus compounds in quantities sufficient for the algae. After carrying out treatment procedures, the water becomes sufficiently purified and can be reused in the production cycle, and the resulting *Ch. vulgaris* biomass can serve as a source of protein or lipids, in accordance with needs.

Conclusions. The possibility of using green algae *Ch. vulgaris* for wastewater treatment within bioremediation procedures is shown. The method has been validated for the treatment of simulated agricultural and domestic wastewater. It was found that growing algae in wastewater of various compositions makes it possible to significantly reduce the amount of nitrogen compounds in the effluents. In the 25-day process of cultivation on wastewater, the density of *Ch. vulgaris* culture increases by 2.5 times, and the resulting algal mass is rich in proteins and lipids.

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ЗАЛУЧЕННЯ *CHLORELLA VULGARIS* BEIJER. ДО БІОРЕМЕДІАЦІЙНИХ ЗАХОДІВ

Л. М. Чебан

У роботі досліджували можливість застосування культури зеленої водорості Chlorella vulgaris Beijer. для реалізації біоремедіаційних заходів. Було змодельовано два види стічних вод – сільськогосподарські (СГС) Та побутові (ПС). Спосіб реалізовувався в лабораторних умовах в колбах Ейленмеєра об'ємом 500 мл. Співвідношення між кількістю культури водорості та об'ємом стічних вод становило 1:10. У складі стічних вод до та після культивування водорості перевіряли вміст NO_3^-, NO_2^- та NH_4^+. В процесі культивування контролювали рівень pH живильного середовища та оптичну щільність культури Ch. vulgaris. За показниками кількості різних форм азоту та рівнем pH змодельовані побутові та сільськогосподарські стоки можуть слугувати альтернативним живильним середовищем для вирощування зелених водоростей. Застосування Ch. vulgaris для проведення процедури очищення побутових та сільськогосподарських стоків дозволяє практично повністю уникнути нітратного та амонійного забруднення таких вод. Протягом 25 діб вирощування Ch. vulgaris на сільськогосподарських стоках вдалося отримати кількість біомаси, що у 2 рази перевищує показники і на контрольному середовищі Тамія. Отримана альгомаса характеризуються вмістом білків на рівні 55 % та ліпідів – близько 30 %. Отриману біомасу водорості Ch. vulgaris у подальшому можна застосовувати як джерело білка чи ліпідів відповідно до потреб.

Ключові слова: водорість, Chlorella vulgaris Beijer., біоремедіація, стічні води

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