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GETTING OF BIOETHANOL FROM ALGAE EUGLENA VIRIDIS

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Досліджено методику отримання вуглеводів із водоростей Euglena viridis для виробництва біоетанолу. Для гідролізу полісахаридів був застосований антронний метод. Визначено, що вміст клітковини в культурі водоростей вищий, ніж вміст крохмалю

Ключові слова: вуглеводи, водорості, біоетанол

Исследована методика получения углеводов из водорослей Euglena viridis для производства биоэтанола. Для гидролиза полисахаридов был применен антронный метод. Определено, что содержание клетчатки в культуре водорослей выше, чем крахмала

Ключевые слова: углеводы, водоросли, биоэтанол

The methodic of carbohydrates getting from algae Euglena viridis for bioethanol production is researched. For hydrolysis of polysaccharides was applied Anthrone method. It is determined that the content of cellulose in the culture of algae is higher than the content of starch

Key words: carbohydrates, algae, bioethanol

Introduction

Today's world is facing two critical problems: high fuel prices and climatic changes. Experts suggest that current oil and gas reserves would suffice to last only a few more decades. It is well known that transport is almost totally dependent on fossil fuels, particularly petroleum-based fuels such as gasoline, diesel fuel, liquefied petroleum gas, and natural gas. Of special concern are the liquid fuels used in automobiles. Hence, there has been widespread recent interest in learning more about obtaining liquid fuels from non-fossil sources.

Petroleum is the mixture of a very large number of different hydrocarbons; the most commonly found molecules are alkanes, cycloalkanes, aromatic hydrocarbons, or more complicated chemicals like asphaltenes, each of them are ecologically harmful and even toxic. The Middle East produces 1/3 of the world's oil and has a major influence on worldwide crude oil prices that are near US$82 a barrel. To be independent from the petroleum there are developed new technologies using the renewable energy sources out of which biomass represents an important alternative. Biomass can be converted into the most preferred liquid form of the fuel including bioethanol, biodiesel, and biogasoline. Ethanol is the most widely used liquid biofuel. Domestic production and use of ethanol for fuel helps to reduce world economic crisis and global climate change because of carbon dioxide buildup.

Classification of Existing Methods of Researches

A large number of analytical techniques have been developed to measure the total concentration and type of carbohydrates present in algae. The carbohydrate content of a food can be determined by calculating the percent remaining after all the other components have been measured: %carbohydrates = 100 - %moisture - %protein - %lipid - %mineral. Nevertheless, this method can lead to erroneous results due to experimental errors in any of the other methods, and so it is usually better to directly measure the carbohydrate content for accurate measurements.

Chromatographic and Electrophoretic Methods

Chromatographic methods are the most powerful analytical techniques for the analysis of the type and concentration of monosaccharides and oligosaccharides in foods. Thin layer chromatography (TLC), Gas chromatography (GC) and High Performance Liquid chromatography (HPLC) are commonly used to separate and identify carbohydrates. Carbohydrates are separated on the basis of their differential adsorption characteristics by passing the solution to be analyzed through a column. Carbohydrates can be separated on the basis of their partition coefficients, polarities or sizes, depending on the type of column used. HPLC is currently the most important chromatographic method for analyzing carbohydrates because it is capable of rapid, specific, sensitive and precise measurements. In addition, GC requires that the samples be volatile, which usually requires that they be derivitized, whereas in HPLC samples can often be analyzed directly. HPLC and GC are commonly used in conjunction with NMR or mass spectrometry so that the chemical structure of the molecules that make up the peaks can also be identified.

Ca ydrates can also be separated by electrophoresis after they have been derivitized to make them electrically charged,

e.g., by reaction with borates. A solution of the derivitized carbohydrates is applied to a gel and then a voltage is applied across it. The carbohydrates are then separated on the basis of their size: the smaller the size of a carbohydrate molecule, the faster it moves in an electrical field.

Chemical Methods

A number of chemical methods used to determine monosaccharides and oligosaccharides are based on the fact that many of these substances are reducing agents that can react with other components to yield precipitates or colored complexes which can be quantified. The concentration of carbohydrate can be determined gravimetrically, spectrophotometrically or by titration. Non-reducing carbohydrates can be determined using the same methods if they are first hydrolyzed to make them reducing. It is possible to determine the concentration of both non-reducing and reducing sugars by carrying out an analysis for reducing sugars before and after hydrolyzation. Many different chemical methods are available for quantifying carbohydrates. Most of these can be divided into three catagories: titration, gravimetric and colorimetric.

The Anthrone method is an example of a colorimetric method of determining the concentration of the total sugars in a sample. Sugars react with the anthrone reagent under acidic conditions to yield a blue-green color. The sample is mixed with sulfuric acid and the anthrone reagent and then boiled until the reaction is completed. The solution is then allowed to cool and its absorbance is measured at 620 nm. There is a linear relationship between the absorbance and the amount of sugar that was present in the original sample. This method determines both reducing and non-reducing sugars because of the presence of the strongly oxidizing sulfuric acid. Like the other methods it is non-stoichemetric and therefore it is necessary to prepare a calibration curve using a series of standards of known carbohydrate concentration.

Enzymatic Methods

Analytical methods based on enzymes rely on their ability to catalyze specific reactions. These methods are rapid, highly specific and sensitive to low concentrations and are therefore ideal for determination of carbohydrates in foods. In addition, little sample preparation is usually required. Liquid foods can be tested directly, whereas solid foods have to be dissolved in water first. There are many enzyme assay kits which can be purchased commercially to carry out analysis for specific carbohydrates. Manufacturers of these kits provide detailed instructions on how to carry out the analysis. The two methods most commonly used to determine carbohydrate concentration are: (i) allowing the reaction to go to completion and measuring the concentration of the product, which is proportional to the concentration of the initial substrate; (ii). measuring the initial rate of the enzyme catalyzed reaction because the rate is proportional to the substrate concentration.

Physical Methods

Many different physical methods have been used to determine the carbohydrate concentration of foods. These methods rely on their being a change in some physicochemical characteristic of a food as its carbohydrate concentration varies. Commonly used methods include polarimetry, refractive index, IR, and density.

Molecules that contain an asymmetric carbon atom have the ability to rotate plane polarized light. A polarimeter is a device that measures the angle that plane polarized light is rotated on passing through a solution. A polarimeter consists of a source of monochromatic light, a polarizer, a sample cell of known length, and an analyzer to measure the angle of rotation. The extent of polarization is related to the concentration of the optically active molecules in solution by the equation a = [a]lc, where a is the measured angle of rotation, [a] is the optical activity (which is a constant for each type of molecule), l is the pathlength and c is the concentration. The overall angle of rotation depends on the temperature and wavelength of light used and so these parameters are usually standardized to 20°C and 589.3 nm (the D-line for sodium). A calibration curve of a versus concentration is prepared using a series of solutions with known concentration, or the value of [a] is taken from the literature if the type of carbohydrates present is known. The concentration of carbohydrate in an unknown sample is then determined by measuring its angle of rotation and comparing it with the calibration curve.

More sophisticated instrumental methods are capable of providing information about the molecular structure of carbohydrates as well as their concentration, e.g., NMR or mass spectrometry.

Immunoassays

Immuoassays are finding increasing use in the food industry for the qualitative and quantitative analysis of food products. Immunoassays specific for low molecular weight carbohydrates are developed by attaching the carbohydrate of interest to a protein, and then injecting it into an animal. With time the animal develops antibodies specific for the carbohydrate molecule. These antibodies can then be extracted from the animal and used as part of a test kit for determining the concentration of the specific carbohydrate in foods. Immuoassays are extremely sensitive, specific, easy to use and rapid [1].

Aim of the Work is getting of carbohydrates from starch and cellulose in the algal culture, which are extracted for further ethanol fermentation.

The Main Material

Algae have a tendency to have a much different makeup than does most feedstocks used in ethanol, such as corn and sugar cane. Ethanol from them is possible by converting the starch (the storage component) and cellulose (the cell wall component). Lipids in plant oil can be made into biodiesel, while the carbohydrates can be converted to ethanol. Algae are the optimal source for third-generation bioethanol due to

the fact that they are high in carbohydrates/polysaccharides and thin cellulose walls.

Fermentation process to produce ethanol includes the following stages:

(a) Growing starch-accumulating, cellulose-accumulating algae in an aqua culture environment;

(b) Harvesting the grown plants to form a biomass;

(c) Separation of carbohydrates from the biomass;

(d) Contacting the decaying biomass with yeast capable of fermenting it to form a fermentation solution;

(e) Separating the resulting ethanol from the fermentation solution.

Initiating decay means that the biomass is treated in such a way that the cellular structure of the biomass begins to decompose (e.g., cell wall rupture) and release the carbohydrates. It can be accomplished mechanically, non-mechanically. The yeasts used are typically brewers' yeasts (*Saccharomyces cerevisiae* and *Saccharomyces uvarum*) (fig.1) [2].

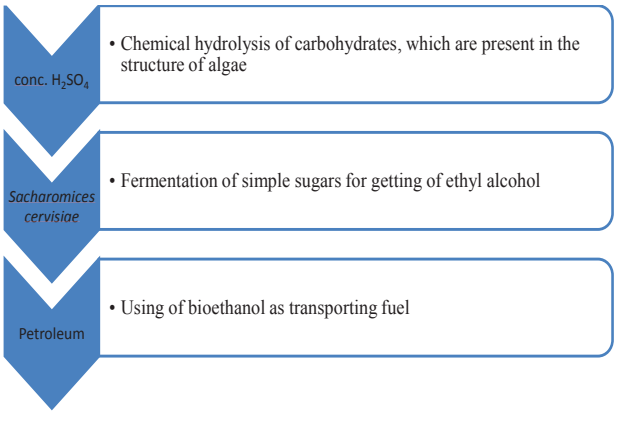

Fig.1 General scheme of getting bioethanol from algae

The producer of carbohydrates is taken algae *Euglena viridis* (fig. 2). The organisms are all capable of active movement, swimming by the lashing of the flagellum and the corkscrew-like turning of the whole body. They also perform a characteristic, rhythmical and contractile motion, termed euglenoid movement. The flagella show an axial thread with a protoplasmic sheath, and there are indications that the axial thread itself consists of a spiral strand of still finer fibres.

It refers to Kingdom - Protozoa, Phylum - Euglenozoa, Class - Euglenida, Order - Euglenales, Family - Euglenaceae, Specie - Euglena.

Fig. 2 Euglena viridis

Reproduction is effected by the longitudinal division of the body into two. This is called binary fission. In most instances the individual comes to rest, secretes an envelope of mucilage, and then proceeds to divide, beginning at the front end of the protoplast. In some species the cells so formed may round themselves off and divide again and again, so that a large number of spherical cells are formed all enclosed in the original membrane. In dividing while at rest *Euglena* differs from the majority of the other Flagellates, and shows an approach to the true Algae.

Cysts with thick walls are frequently found; they are generally spherical and the walls are striated. Such encystment may be only temporary, and the individual may retain its flagellum. If, however, the process is employed to tide the organism over a longer period of unfavourable conditions the enclosed cell may retract its flagellum.

The method used for hydrolysis of polysaccharides is Anthrone method. It is based on hydrolyzing the polysaccharides into simpler sugars by acid hydrolysis and estimating the resultant monosaccharides (fig. 3) [3].

Fig. 3 Steps of experiment

Materials

- 2.5 N HCl.
- Anthrone reagent: dissolve 200mg anthrone in 100ml of ice cold 95% H_2SO_4. Prepare fresh before use.
- Standard glucose: stock – dissolve 100mg in 100ml water. Working standard – 10ml of stock diluted to 100ml with distilled water. Store refrigerated after adding a few drops of toluene.

Procedure

1. Weight 100 mg of the sample in a boiling tube.
2. Hydrolyse by keeping it in boiling water bath for three hours with 5 ml of 2,5 N HCl and cool to room temperature.
3. Neutralize it with solid sodium carbonate until the effervescence ceases.
4. Make up the volume to 100 ml and centrifuge.
5. Collect the supernatant and take 0.5 and 1 ml aliquots for analysis.

6. Prepare the standards by taking 0, 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard. "0" serves as blank.
7. Then add 4 ml of anthrone reagent.
8. Heat for eight minutes in a boiling water bath.
9. Cool rapidly and read the green to dark green color at 630 nm.

There is such content of carbohydrates from different species of microalgae, which can be used for further production of bioethanol (table 1) [4].

Table 1

Carbohydrates content from various species of microalgae

№	Algae strain	Carbohydrate (% dwt)
1	Scenedesmus obliquus	10–17
2	Scenedesmus quadricauda	–
3	Scenedesmus dimorphus	21–52
4	Chlamydomonas rheinhardii	17
5	Chlorella vulgaris	12–17
6	Chlorella pyrenoidosa	26
7	Spirogyra sp.	33–64
8	Dunaliella bioculata	4
9	Dunaliella salina	32
10	Euglena gracilis	14–18
11	Prymnesium parvum	25–33
12	Tetraselmis maculat	15
13	Porphyridium cruentum	40–57
14	Spirulina platensis	8–14
15	Spirulina maxima	13–16
16	Synechoccus sp.	15
17	Anabaena cylindrical	25–30

Conclusion

The results of researches showed that the content of proteins in algae *Euglena viridis* may be useful in further production of bioethanol.

Algal resources are in general very widespread and abundant. Today they can exist in algae closed and open system. Such biomass materials can be supplied from a variety of resources at a low price and don't need much expense of fertile soils. Also the main advantage of algal biomass for bioethanol obtaining is non competition with food products.

The use of gasohol as an alternative motor fuel has been steadily increasing around the world and must be provided in Ukraine for solution of ecological and economical problems.

References

1. Edinger, R. Humankind's detour toward sustainability: Past, present, and future of renewable energies and electric power generation / Edinger R., Kaul S. – Washington.: Renew Sustain Energy, 2000. – 167 p.
2. Demirbas, A. Bioethanol: A realistic fuel alternative for diesel engines / Demibras A. – London.: Springer, 2008. – 128 p.
3. Cuff, D.J. US energy atlas / Cuff D.J., Young W.J. – New York.: McMillan, 1980. – 42 p.
4. Raven, P.H. Sustainability standards for bioenergy / Raven P.H., Evert R.F. and Eichhorn S.E. – New York.: Freeman and Company, 2006. – 103 p.