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EXTRACTION EXAMINATIONS OF MICROALGAE PROPAGATED FOR BIODIESEL ADDITIVES

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Because of the growing energy consumption of the world CO_2 emission is also growing. A part of this carbon emission can be captured by microalgae. These creatures like any other photosynthetizing organisms consumes it as carbon source. They set up their cells and makes lots of valuable compounds of it. Some of these compounds can be transformed into biodiesel or blending components.

We have studied the whole range of algae cultivation and processing is at the Department of Chemical Engineering at the University of Pannonia. The utilization of algacultures in experimental photobioreactors is examined, together with the optimization of the operational conditions both for artificial and natural light and different fertilizers. We made extraction experiments of dried algae.

Keywords: algae, biodiesel, lipides, lipid exraction

Introduction

Driven by the rising need for biofuels and by the necessity to capture carbon dioxide, autotroph organisms got into the spotlight of energetic research. With cultivation of these organisms we can feed back the carbon content of CO_2 into biological systems and we can get numbers of valuable organic compounds, among others biofuel, to reach ecological and economical benefits.

Algae production is the most promising solution amongst the alternatives because of its specific area necessity and high reproduction rate. Additional benefits are that there is no need to use growing fields and some wastewater may be used with nutrient supplementation.

The algae-based technology

Algae based fuel technology was mentioned in the beginning of the 1950's. Some pilot plant to cultivate algae as energy source was built in 1970's. Algae oil production for fuel technology was mentioned at first in the 1980's which lives its renessaince in 21st century [1, 2].

Algae species are applicable for energetic purposes wich produce lipids as more as it possible in their whole growing period. Some of these species' lypide content may be more than 40 percent of their own weight. These lypides mostly contents glycerine esthers of various C_{16}-C_{20} fatty acides. These compounds are applicable for biodiesel production [4].

Cultivation parameters of algae

Autotrophic organisms synthetizes complicated organic compounds which need them to build up their own cells. These organisms can be monocytae (microalgae) or other differentiated autotrophics (e.g. corn, soy beans).

Algae are a large goup of simple, typically autotrophic organisms. They are eucaryotic, autotrophic, unicellular or multicellular form.

Algae get nutrients and other compounds from aqueous solution (see *Fig. 1*)

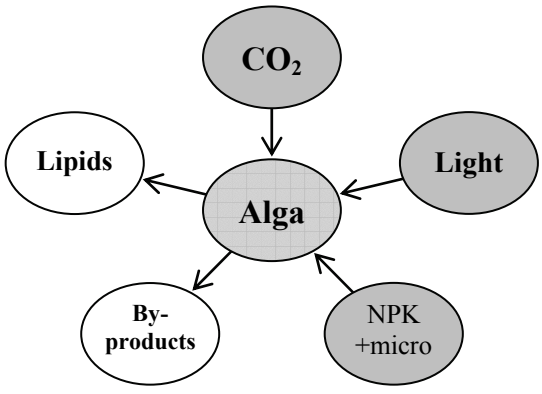

Figure 1: Algae cellfactory

CO_2 capture

On one hand they consume inorganic compounds and simple organic compounds, on the other hand they feed CO_2 in the form of hydrogencarbonate from dissolved gas mixture. We use CO_2 as a fetilizer to reach higher biomass productivity than the average [3]. Generally, we feed as a gas mixture about 5–30 vol% CO_2 and air is the rest. It is possible to grow algae in gas mixtures without air, but their oxygen content for the dark period is an essence. The applicable CO_2 concentration depends on the temperature and liquid fertilizer contents and concentration too.

Light source

Light has a special function since it supplies the energetic background of the biochemical reactions in the light period. Autotrophic organisms use only a part of total sunlight spectrum (400–700 nm) for photosynthesis. This range is 42.3% of the total spectrum. This is called photosynthetic active radiation (PAR). The average energy of the photones is 218 kJ/mol in this interval. The maximum theoretical photosynthetic energy efficience (PE) can be determined from these data given above. PE is 9% for the total spectrum of sunligh and it is 21.4% for the range of PAR.

For laboratory use we can applicate of several types of light sources. It is important to use that type of source wich spectra meets the needs of algaculture.

Inorganic nutrients

There are significant differences in nutrient requirement among species of the same alga genus. Accordingly, an optimal nutrient composition for a specified alga might not be applicable for another species in the same genus.

Commonly an optimized medium composition is only valid and applicable in the same circumstances as observed. It is an important to mention that in these systems single nutrient composition changes do not have effets of the same intensity than in combination with another nutrient(s). There are multilateral effects between nutient component concentrations, these connections might be a relevant information.

There are a lot of media recipes accessible. There are recommendations for the most algae species. Generally nitrogen (as nitrate), phosphate and potassium addditon to the media arean instance. There are other macro nutrients are also important: calcium, magnesium, suplphur.

There are micronutrients which must be added in small amount of concentration (above the effective and below the toxic amount). Generally applicated micronutrients are shown in *Fig. 2*.

These compounds are in the media is essence to reach high alga biomass concentration in a good condition.

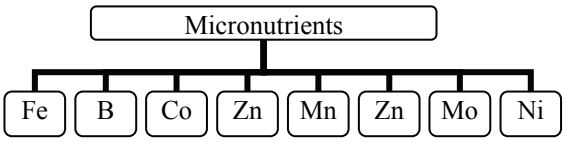

Figure 2: Generally applicated micronutrients for microalgae culturing

The first is getting acclimatized. It can lasts from a few hours to 1-2 days. It is probably affected by the change of environmental parameters. The second is the quick growth or exponentional phase when significant biomass multiplication is shown. In this phase batch harvesting is too early. After this, a maximum is reached in biomass concentration. The next phase is the decrease of biomass comcentration. In this phase algae should be harvested. At the end of this phase there are a few algae in population and the chances are that other harmful microorganisms have been proliferated.

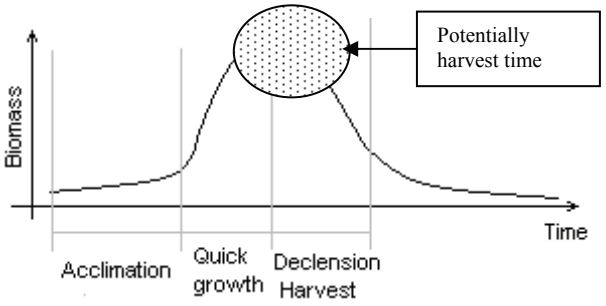

Figure 3: Population vs. age of population

We use quasi-continious systems. In this case part of the culture is harvested and restored with fresh medium. Its advantage is the ability of simple automation system but the disatvantage is the need of a proper harvesting schedule. It is important to avoid the proliferation of harmful microbes and important to monitor the accumulation of metabolites.

Photobioreactors

We use special photobioreactors (PBR) to keep specified cultivation parameters. Common expectations are specified below.

It is important that as much PAR type light as possible be accesible for the algae. Input and output streams must be safely and well built because the toxic CO_2 content of the gas mixture. These reactors must be designed to be resistant against environmental effects (wind, rain, sunlight, insects etc.). These algae suspensions must be well stirred, because degradation might be started in subsided algae conglomerate. Stirring is also important to keep algae cells at the side of absorbance wall for the optimal resistance time in the applicated light conditions.

These reactors must be designed for local microclimate and mostly mounted with cooling system. The planned cultivating volume affects the reactor geometry.

The largest volume can be reached in open pond systems. In this case we can keep those type of algae which are resistant against local microbes and environmetal effects. To avoid invasive species proliferation parameters must be well monitored. Generally, mechanical stirring is applied to maintain aeriation and stirring.

Another open type cultivation is the raceway system. In this case algae suspension flows in a canal. The thickness of the layer is between 100–500 mm.

Another type of cultivation can be in closed photobioreactors. These reactors have a well-defined area of light trasmitting wall. This is critical to the design. We should reckon with shadows of statically necessary elements on the light side. Inner or outer contaminations of walls must be regularly eliminated. Source of outer contaminations origin can be technical (eg. scratches) or other environmental (eg. dust). Important is the choice of optimal thickness of layer to reach sufficient mixing. A thoughtful reactor design and monitored inputs can assure a well balanced algae cultivating system with low risk of unwanted external effects.

Closed photobioreactors are built in two designs. The first is the pipe system, with the advantages of simple geometry and few shading element but it has the disadvantage of low area by volumetric unit. The second type is the panel with the advantage of high area of volumetric unit and the disadvantage of evolving idle spaces.

Algae culturing conditions

We used flat type PBRs (see *Fig. 3*). Some of them were in natural light and others were in artificial light (special fluorescent tubes). We mixed the 5–8 vol% CO_2 content gas mixture outside the reactor. Then this gas mixture were dispensed in the alga culture. This suspension contains the algae cells, and also the nutrients (modified BG-11 type medium).

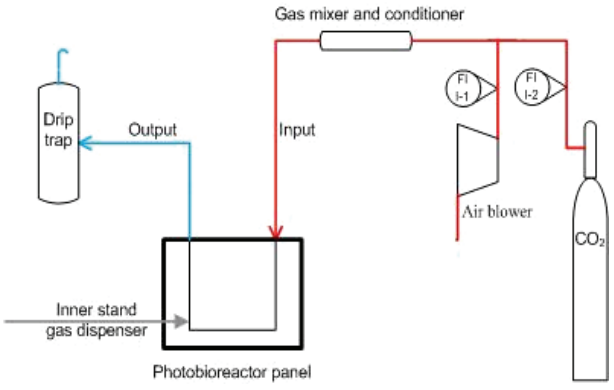

Figure 3: Algae PBR. Panel and its supplies

We let these cultures to grow up their biomass concentration to maximum 6 g/l (dried content). Generally we don't let them to reach the declension phase in PBRs because it is easier to concentrate the suspension and avoid the quality loss.

Pretreatment of harvested suspension

The harvested suspensions are concentrated and dried. The concentration method consist generally two steps. First is the flocculation and the second is filtration. We are test the applicability of membrane separation processes which may be promising solutions of this problem.

Next step is drying. We dry the algae cake 65 °C to avoid of the cells boiling. In occurrence of boiling, the cells are destrtoyed. This can causes easyer attack of microbes and other (mainly separational) problems in the following procedures.

Milling of dry alga can be also an important step if the algae cake were dried in blocks.

Extraction

Extraction can be carried out in two startegies. One of them is to extract oil from dry or moist material. The other is cell degradation come before extraction. The latter can be made by ultrasonic, microwave radiation, chilling shock, cell blast, enzymatic process. The aim of these methods that let intracellular compounds achievable for extracting material. But they can causes solvent-separation problems.

In *Table 1* we present some common used solvent systems. In complex solvent systems, the polarity order is kept.

Table 1: Common solvent systems for algae extraction

Extraction	*Solvent1*	*Solvent2*	*Solvent3*
Solid-liquid	Chloroform	Methanol	Water
	Hexane	I-Propanol	Water
	Hexane	Ethanol	Water
	Ethanol	1- buthanol	Water
	Acetone	hexane	
Supercritical fluid	CO_2, Water, methanol, buthane, penthane		
Novel techniques	Ultrasonic, Microwave, ASE, Cell-milking, Liquid dimethyl-ether		

Use of supercritical extraction is not so competitive but there are researches to get the optimal fluid-cosolvent pair.

There are more and more new algal oil extraction can be reached. Some of these keeps to solvent free technologies [19] others lead to new solvent base ones [20-22].

These solvent systems are not applcable right now beacause its high costs. By the way these solutions shouldn't be neglected. On *Table 2* there are the possibly application timeline of these procedures.

Experimental data

When we inspect measured data, we should count on with crosseffects. At first the growing method effects to both of algae culture and cell composition. When we have a good conditioned suspension, we try to concentrate it. Different flocculating and filtering methods effects differently to the amount of achiavable dry biomass and the latter technology parameters. When a concentrated, well dried, well milled alga is available we can start its extraction.

After the specific extraction method and time we should separate the residue from the new, organic suspension. This can be usually made by simple filtration.

On the other hand we must evaporate the solvent from extract.

If we make several processes on algae suspension from concentrating to drying, we should extract the widest range of compounds of biomass. Then we extracted chloroform methanol mixture which solved several other compounds than glycerol aldehydes of fatty acids such as colurs, eicosanol etc. These latter compounds may gives other benefits than energetic use of algae extract.

Table 2: Alga extraction with chloroform-methanol mixture

Algae grow ID	Extraction ID *E*	Operating conditions	Extraction time (h)	Lipide content (%)
T1	58	Natural	3	37.00
T2	59	Natural	3	21.10
T3	90	Natural	20	28.10
T4	88	Natural	20	21.10
T5	62	Natural	3	17.60
T6	63	Natural	3	18.80
T7	117	Natural	42	8.30
T8	65	Natural	3	11.40
TV602BM	75	Laboratory	3	10.20
TV605BM	78	Laboratory	3	10.40
TV6312BM	76	Laboratory	3	22.90

The rest of the samples had different lipide content considering their growing conditions..

Natural operating conditions means:

- natural light conditions
  - T1-T2: July-August, Chlorella V.
  - T5-T6: July-August: Scenedesmus A.
  - T3-T4: October-November Chlorella V.
  - T7-T8: October-November Scenedesmus A.
- Laboratory artificial light
  - E75, E78 Fluorescent tube, Scenedesmus A.
  - E76 Fluorescent tube, Chlorella V.

In this experiment the Chlorella V. had higher growing potential and also higher lipide content in each type of growing conditions. But we must be careful beacause the starter culture was stronger Chlorella than Scenedesmus. We found at later inspections that these suspensions was probably about to change.

Conclusion

Algae technology is lives its renessaince in the 21th century. We use flat panel PBRs to test the effects of parameters on algae growing, ang alga suspension processing at the whole technolgy line.

Several parameters have influence on the quality and quantity of the algae extract. At first we must keep our eyes on growing parameters to get strong and high concentration of biomass. We must make the necessary changes on suspension to get dry and easily processable biomass. We must extract a wide spectra of compounds from algae to get several valuable of it.

Extraction is a very important in algae technology but cross effects between growing and processing parameters are cannot neglible.

There are still a lots of challenge in algae technology. Some of them limited by well known parameters but others have some latent parameters which is before discover.

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