Cultivation of Microalgae for Biofuel

Antonia Bukovac[*], Tea Arvaj[*], Dario Orlić[*], and Marieke F. Buffing[* **]

[*] Summer School of Science 2015, S[3] program, Požega, Croatia
[**] ETH Zürich, Switzerland

ABSTRACT

Uses for microalgae vary from food to cosmetics, but we are interested mostly in biofuel. In this research we explored ways of growing algae and their growth conditions. We also made biofuel from triglycerides and extracted pigments from plants. Our main goal was to see which type of bioreactor is better and, from our results, we concluded that the continuous flow reactor was more efficient than the batch reactor.

KEYWORDS: microalgae, *C. sokokiniana*, bioreactor, biofuel, pigments

INTRODUCTION

Today's fuels pollute our environment and are a cause of global warming. Biofuels are more environment-friendly because they are made from biological material, and in the case of algae they make use of photosynthesis. In photosynthesis energy from the sun and carbon dioxide are used to make organic compounds, which means that the amount of CO_2 released via usage of biofuel is equal to CO_2 that is used to make it. Biofuel is mostly made from plants like palms, corn and soy [3], but microalgae (unicellular algae) could be a better alternative. Algae require much less water than plants and do not require fertile land [1]. Also, plants have other competitive mass uses as food and feed, which algae do not[3].

Unlike fossil fuels, such as oil and natural gas, that need to be extracted from non-renewable sources that will all be exploited in the near future, algae are grown in bioreactors. A bioreactor is a container which is used to regulate the conditions crucial for efficient cultivation, in our case algae growth. These conditions are light, temperature, carbon, nitrogen and phosphorous source, macronutrients, micronutrients and pH value. These conditions will be described in detail for the algae *Chlorella sorokiniana* in the chapter "Methods and materials". In a bioreactor algae are grown in a solution called medium which contains all necessary compounds for them. After enough algae are cultivated in a bioreactor they need to be separated from the medium. Filtration and centrifugation are mostly used for that propose [3]. Next, organic compounds used to make biofuel (lipids and oils) have to be extracted. Other compounds (e.g. β-carotene) can be extracted to make high value products. If the correct methods are used they can all be extracted from the same algae.

To make algae cultivation efficient and profitable, different bioreactors have to be explored. That was the purpose of our project: to see what type of bioreactors is better for microalgal cultivation.

METHODS AND MATERIALS:

Building bioreactors

We decided to make a continuous flow reactor (Fig. 1) and a batch reactor (Fig. 2). As the title of our project implies, we used only material we could buy in the local hardware shop and equipment we had in our laboratory.

To make the continuous flow reactor we used: a Buchner flask containing the culture-medium with algae, a tube with a filter at one end trough which we blew exhaled air so that the algae get more CO_2 (a filter was used so that bacteria from our mouth would not get in the reactor), a beaker to contain the outflow with algae, a separatory funnel and a bottle with new medium, tubes to connect all parts, a retort stand to keep the funnel and the bottle above the reactor, a cooling tank, a reflector light, aluminium foil to reflect light so all sides of reactor get equal amount of light and a thermometer. We used an overflow mechanism to get old medium out of the reactor.

Fig. 1 - Continuous flow bioreactor

For the batch reactor we used: an Erlenmeyer flask with yeast, an Erlenmeyer flask with algae and a tube that supplies the algae with CO_2 from the yeast container. We sealed both algae containers with cotton so that O_2 can get out of the container and no

Fig. 2 - Batch bioreactor

dust or insects get in the container.

Temperature in both bioreactors was regulated using cold water that was poured in the cooling tank and by changing the distance between the reflector and the bioreactors. We checked the temperature regularly and maintained it at 32 - 37 °C.

Medium preparation

For medium preparation, 1.80 g/L urea and 2.83 g/L HEPES were dissolved. After that per litre 10 mL stock I, 10 mL stock II, 10 mL stock III, and 1 mL stock IV were added (for stock solutions, see table 1). Subsequently 0.84 g/L $NaHCO_3$ was added as a carbon-source and the pH was adjusted to 6.7-6.8 with 4M NaOH.

Table 1 - Preparation of stock solutions. All stock solutions were filter sterilized and stored in fridge. The P-buffer was stored in dark. (*) The first salt was dissolved before second was added.

Name	**Component**	**100x stock (g/L)**
I. P-buffer	KH_2PO_4	74.0
	$Na_2HPO_4x2H_2O$	26.0
II. Ca-Mg-salts	$MgSO_4x7H_2O$	40.0
	$CaCl_2x2H_2O$	1.3
III. Fe (*)	EDTA ferric sodium salt	11.60
	$Na_2EDTAx2H_2O$	3.72
Name	**Component**	**1000x stock (g/L)**
IV. Micronutrients	H_3BO_3	0.062
	$MnCl_2x4H_2O$	12.980
	$ZnSO_4x7H_2O$	3.200
	$CuSO_4x5H_2O$	1.830

Inoculation (the introduction of algae to the medium)

The first time we inoculated the continuous bioreactor was with our maintenance culture, but the algae did not grow well so we inoculated it again with a more dense culture we had for situations like this. We inoculated the batch reactor with the same dense culture.

Monitoring growth: optical density and dry weight

We stirred the culture in the bioreactors using a magnetic stirrer before taking optical density and dry weight samples to make sure that the algae are homogeneously distributed throughout the reactor.

To measure the optical density of the algae we used a spectrophotometer. It measures the light absorbance of the algae over the light path of the cuvette. We used light that had a wavelength of 750 nm because light absorption of *Chlorella sorokiniana* is highest at that wavelength.

To measure the dry weight of the algae we took samples, made filters from filter paper, weighed filters, filtered samples to separate algae from medium using a vacuum pump, dried filters in an oven at 80°C for 16 hours, cooled the filters in a dessicator, weighed the filters with samples and calculated the dry weight of algae per volume.

Making biofuel

Algae products can be divided into two categories: bulk or low-value chemicals and high-value chemicals. Large amounts of bulk products are needed to make the production of bulk chemicals profitable. Biofuels are the most common bulk chemical, especially biodiesel. Biodiesel can be produced from plant and vegetable oils, including oil from algal biomass. Algal biomass consists of approximately 30% oil. Because of certain restrictions in time and equipment, we were compelled to use sunflower oil to make our biodiesel. The process for both sunflower and algal oil is identical because both oils consist of triglycerides; molecules that made from three fatty acids linked to a glycerol molecule by an ester bond. The triglycerides need to be converted to make biodiesel. The process we use is called a transesterification reaction (Fig. 3). The reaction converts one ester group to another by replacing the side groups of the ester bond. Alcohol is used as a catalyst, in our case the alcohol used was methanol. Glycerol was a waste product of the reaction.

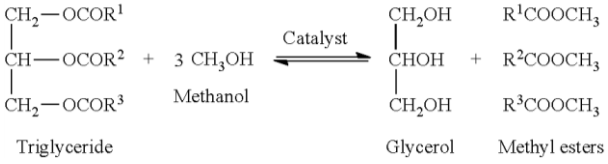

Fig. 3 - Transesterification of triglycerides.

Extraction of pigments from spinach and carrots

We did not have enough algae to extract pigments from, therefore we used carrots and spinach instead. The vegetables were shredded using a blender. For extraction, 100 g of acetone was added to 100 g of vegetable-paste in a jar, the jar was closed and left for two days at room temperature. We used 100 g of spinach and 100 g of carrots for pigment extraction. After two days pigments were extracted into the acetone. The extract was filtered with a tea strainer and then with filter paper.

Chromatography

To analyse the pigments qualitatively, we used paper chromatography. For this, chromatography paper was cut in stripes that could fit inside of a test tube, carrots and spinach pulp was put on the paper about 2 cm from bottom of the paper and left to dry. We put an organic solvent as a running liquid, 92% ethane propane and 8% acetone, and chromatography paper with vegetable paste in the test tubes and did it so that the paste was not submerged in the solvent.

Light absorption of pigments

Light created by a light bulb passed through a diaphragm and into our sample, after which it was refracted by a prism and projected on a white wall (Fig.4). This means that if a sample absorbed every colour except green we would see only green light on the wall instead of a full spectrum. With this experiment we wanted to see which wavelengths pigments in carrots and spinach absorb.

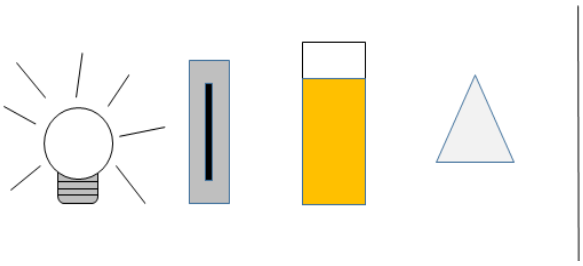

Fig. 4 - Setup for light absorption

Spectrophotometry of pigments

We put the pigment extracts in a quartz cuvette and used the spectrophotometer to measure light absorption of pigments at different wavelengths (390, 430, 470, 510, 550, 590, 630, 652, 665, 670, 710, 750 nm)

Results and discussion

The pH of the bioreactors

We measured the pH value at the beginning (6.7-6.8) and when we stopped our bioreactors. At the end pH was slightly alkaline (ca. 7.4), probably because $NaHCO_3$ was used as a carbon source, so when the algae used up CO_2, OH^- (a base) was formed.

$$NaHCO_3 \rightarrow Na^+ + HCO_3^-$$

$$HCO_3^- \rightarrow CO_2 + OH^-$$

Optical density

In the first part of graph (Fig.5) algae were growing, the second part - in the batch reactor growth slowed down, maybe because algae used up most nutrients and some of them did not get enough light, maybe some algae did not have access to enough CO_2. In the continuous reactor they were still growing, but the flow was too high so algae were "washed" out of the reactor faster than then they could grow. It was difficult to control the flow because the valve adjustment is not a very accurate method and we did not have a pump.

We had problems with optical density measurements because the algae were settling at the bottom of the bioreactors. We found out that the liquid in the bioreactors needs to be stirred before taking OD samples, but at first we did not know that. Two points that are not connected with the lines on the graph are inaccurate measurements caused by that problem.

Since optical density was our most accurate way of measuring the concentration of algae, we examined the bioreactors' efficiency using this method. At the end of measuring the batch reactor had a slightly higher concentration of algae, but if we take into account the outflow liquid from the continuous flow reactor and liquid from the reactor itself, we had about double the volume than in the batch reactor. Therefore the total yield, the amount of algae produced per time unit, is higher for the continuous reactor compared to the batch reactor. From this we conclude that the continuous flow reactor was more efficient.

Dry weight

Unfortunately this method supplied us with inaccurate measurements: the graph (Fig. 6) shows that the concentration of algae increased at the start, but then it plummeted below the starting point. That basically means that we ended up with less

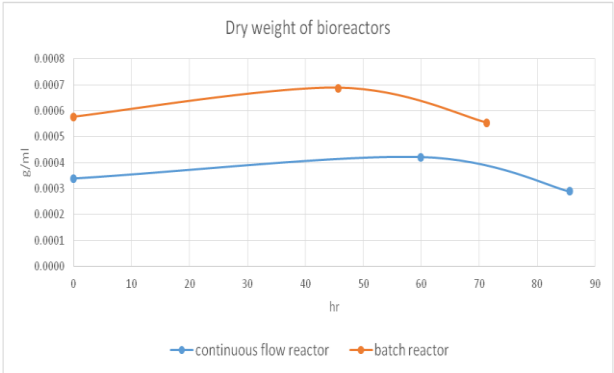

Fig. 6 – Dreigh weight (D.W.) of algae

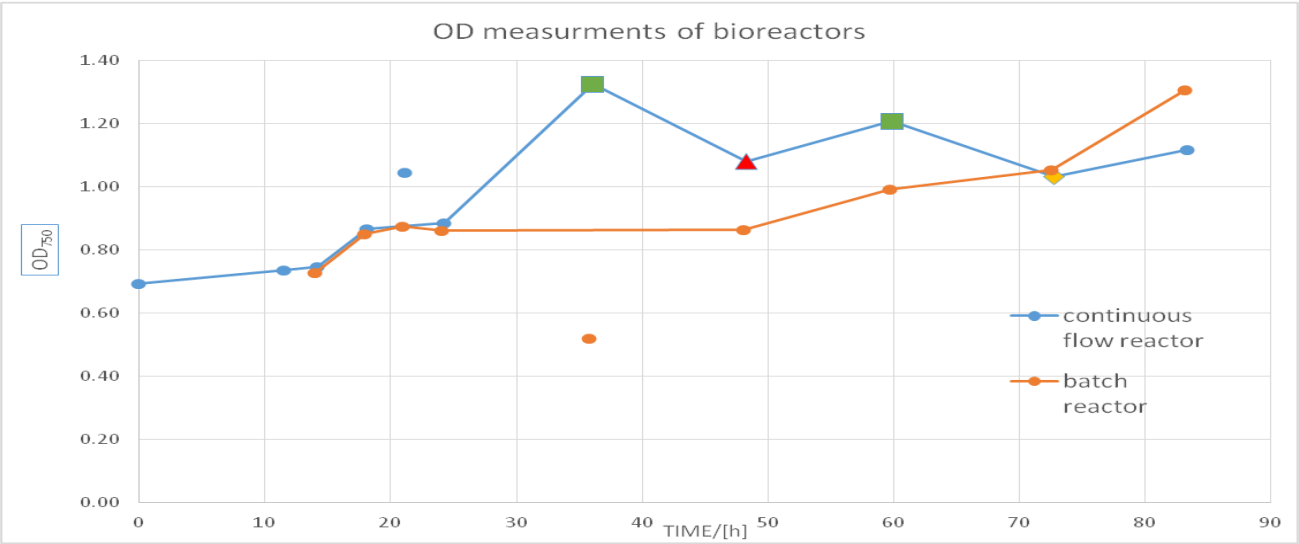

Fig. 5 - Orange line: batch reactor. Blue line: continuous flow reactor, with green points - flow was started, red point - flow was stopped, yellow point - flow was very slow because some cotton got stuck in the valve of the separatory funnel from which new medium was supplied to the reactor.

algae than what we started with, which is possible for the continuous flow reactor, but certainly not for the batch reactor. One explanation for this is that algae from the first two samples continued growing in the refrigerator - the final sample was taken immediately before the testing. Algal growth is unlikely because algae need light. However, if the algae indeed grew, it could be solved by keeping dry weight samples in the freezer instead of the fridge, or by measuring the dry weight immediately after taking the sample. Another reason might be that it was measurement error.

Spectrophotometry of pigments

For the spectrophotometry we used the same pigment extracts of carrots and spinach as we did for the experiment "light absorption of pigments". Here we have two graphs (Fig.7) both representing optical density: the upper one is ours containing the data that we collected; the lower graph we found on internet [2]. They have been positioned so that the X axis (wavelength in nm) coincides. We can see that the spinach pigment sample is mostly chlorophyll b and that the carrot is mostly carotenoids. We can see that the spinach measurement is almost the same as the chlorophyll b on the 2nd graph. The OD of carrot pigments does not match with the carotenoids and we do not know why.

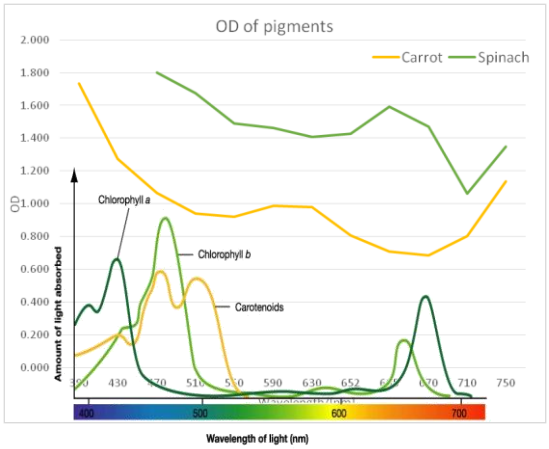

Fig. 7 - OD of pigments

Chromatography

With carrots, only carotenoids were visible at the top of paper (Fig. 8). With spinach, carotenoids and chlorophylls were visible. Carotenoids were at the top of paper, but chlorophylls did not move very far from the vegetable paste because they dissolve worse in the running liquid than the carotenoids.

Light absorption of pigments

This experiment it did not go well because the carrot sample was too diluted and the spinach sample was too concentrated. Because this was not our priority and we did not have enough time we decided not to continue and improve this experiment.

Fig. 8 - Results of cromatography. Left - carrots, 1st type of paper. Middle - carrots, 2nd type of paper. Right - spinach, 1st type of

Biofuel from sunflower oil

Using 100 mL of sunflower oil, we were able to produce 95.6g of biodiesel.

Biggest problems and mistakes

1. As already mentioned in the chapter "Optical density", after some time the algae started to settle at the bottom of the bioreactors. This caused two problems: we had inaccurate measurements of the algae concentration and in our continuous flow bioreactor the outflow tube was at the top of the flask which meant that the concentration of algae in the collection flask was lower than the concentration in the bioreactor. This problem could be solved by constant stirring with a magnetic stirrer, but that would require a different design because in the current setup, the cooling tank did not allow the use of a magnetic stirrer.

2. The second problem is that the flow in the continuous bioreactor was close to uncontrollable. We used a separatory funnel for that purpose, but, as you can see on the OD graph, we were not able to match the dilution rate (that depends on the flow) to the growth rate of the algae. The optimal situation would be that the concentration of algae is stationary meaning that the dilution rate and the growth rate are equal. That means that, over time, the algae would not dilute and they would not over-saturate the bioreactor causing inefficiency. This could be solved with a pump or a more precise valve or an electronic system that can control the flow.

3. The third point for further improvement is to take more accurate and frequent measurements of the conditions in the bioreactors.You may have noticed that when we had an inconsistency in our measurements we mostly inferred what went wrong. It would be much easier to figure out what is happening in the bioreactors if we knew all the conditions all the time.

CONCLUSION AND OUTLOOK

During our project, we have been able to reach a conclusion about our research question using the data we have gathered. We have analysed our bioreactors and have found that a continuous flow reactor will yield higher algae production than a batch reactor: the amount of algae produced per time unit is higher in the continuous reactor compared to the batch reactor. A continuous flow bioreactor can also be used to simplify production as it does not need to be dismantled to collect the algae. Unlike in a batch reactor, excess algae exit the bioreactor into the collection flask. We now know which reactor is more efficient, but overall efficiency could still be increased because of the many factors that influence algae production. We have just compared the basic construction of our bioreactors, but several more factors, such as carbon source or temperature, could have been altered to receive more precise results as to what the ideal bioreactor would be. We are still far from maximum yield and efficiency. Because of this, more research

into the topic is vital since algae seem to be one of the potential solutions to the search of alternative energy sources. There are many benefits to using algae in today's polluted and resource-lacking world which is desperately seeking clean energy alternatives. It is possible that algae and their various uses (such as a source of biodiesel) could be a part of the environmentally clean solution needed for long-term sustainability of our Earths ecosystems.

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