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SOLID-LIQUID EXTRACTION OF CHLOROPHYLL FROM MICROALGAE FROM PHOTOAUTOTROPH OPEN-AIR CULTIVATION

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Among industrial pollutants, strict quotas limit the emission of carbon dioxide in the European Union. The capturing and deposition of carbon dioxide requires significant expenditures. One of the newer solutions for the reduction of the carbon dioxide emissions is provided by algae technology. In this technology, the absorption of carbon dioxide is achieved by photosynthesis. Besides the reduction of pollutants, algae technology has another advantage by supplying valuable products, such as natural pigments, proteins, vitamins and oils from algae biomass. Since it has a high reproduction rate, algae cultivation can be a feasible substitute for plants traditionally used in the production of chlorophyll. The viability of the technology is dependent on whether or not the processing can be done economically. An investigation was carried out in order to compare and contrast two extraction methods (Soxhlet extraction and leaching) and four solvents (acetone, diethyl ether, ethanol and methanol) to determine the most effective method for the extraction of chlorophylls from dried microalgae (*Chlorella vulgaris sp.*). It was concluded that methanol is the most effective solvent for the extraction of both chlorophylls *a* and *b* using both Soxhlet extraction and leaching.

Keywords: microalgae, extraction solvent, extraction, chlorophyll, spectrophotometry

Introduction

Energy demand has been rapidly increasing throughout the world, which is mainly met by the combustion of fossil fuels that results in an increase in the concentration of greenhouse gases in the atmosphere. However as a possible remediation strategy, carbon dioxide levels can be reduced with the use of algae technology [1]. Algae technology is of significant interest in research and development since it is a 'green' technology that is capable of both decreasing the emission of pollutants and serving as a renewable energy source. The algae absorb carbon dioxide for photosynthesis while producing a number of valuable components [2]. Microalgae are a collection of various microscopic species capable of photosynthesis and are typically found in water with an exceptionally high reproduction rate. For propagation, they mainly need light, water and CO_2. Some species can even survive in waste water. Algae are good alternatives for plants that are traditionally used for the production of chlorophyll. Appropriate conditions are required for the successful cultivation of algae (solar energy, temperature, pH, and mixing) [3, 4]. However, the critical point of algae technology is neither cultivation nor processing. The biggest complication concerns the concentration of the microalgae suspension and the subsequent extraction of the valuable components due to the high investment costs and long operational times [5]. There is, however, an increasing demand for microalgae. The reason for this is that their oil content can be as much as 50% of their body mass, making them important in the production of biodiesel. The natural pigments, proteins and vitamins extracted from microalgae are primarily used by the pharmaceutical, cosmetic and food industries [3, 6].

Chlorophyll has been used for centuries as a traditional remedy for unpleasant body odours, for the neutralisation of the odours of stool and urine, and detoxification or sterilisation of wounds. Nowadays, its use is even more widespread. It is used as a food additive, food colouring, and nutritional supplement, especially because of its detoxifying and excellent basifying effects. It is also a strong antioxidant, which inhibits the harmful oxidative processes in the body and enhances the protection of cells and tissues [7, 8]. However, the processing of algae still needs to be improved with respect to determining the optimal methods, pieces of equipment, solvents, and parameters for the efficient and economic extraction of chlorophyll. Extraction is a process that can be carried out in a number of ways. There are also a number of solvents and preparation methods available. Also, variables such as pressure, temperature, the efficiency of contact and time all play an important role [9, 10]. Chlorophyll is sensitive to extreme light exposure, pH values and temperatures [7-9]. When choosing the solvent, one has to consider the sensitivity of the extracted component. A number of considerations are important, too: density, viscosity, heat of evaporation, price, effect on the environment and health. It is also essential that the

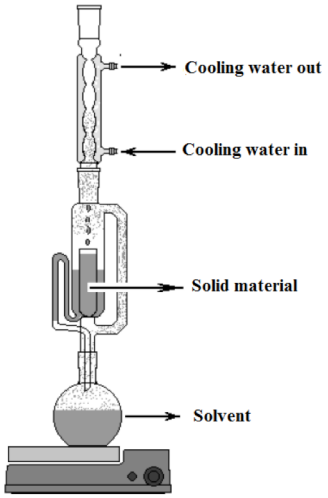

Figure 1: Soxhlet extractor [12]

solvent does not react with or cause damage to the extracted component and is not corrosive. Different methods can be used for different microalgae species [9-10].

Experimental

In advance of the extraction experiments the algae suspension (*Chlorella vulgaris sp.*) was concentrated, dried at 60 ºC until it reached a constant mass, then ground in a ball mill. Chlorophylls *a* and *b* products were obtained by the methods detailed in the following sections from this ground algae powder, which contains approximately 4 wt% water.

The Soxhlet Extraction Method

One of the most well known pieces of equipment for laboratory scale solid-liquid extraction is the Soxhlet extractor. In a Soxhlet extractor multiple fractional distillations are carried out, the extraction is always done with the pure condensate [10].

The first step of the extraction was to fill a cellulose casing with 1-3 g of algae powder. The filled casing was placed in the middle part of the piece of equipment (*Fig.1*). The lower round-bottom flask was filled with 400 cm^3 of solvent. The Soxhlet extraction was carried out with methanol, ethanol, and acetone. Pumice and a magnetic stirring rod were placed into the lower flask to ensure proper boiling. The cooling water was set to a continuous flow and then the heating was turned on. After the extracting solvents had reached their boiling points and started to evaporate, they condensed in the reflux condenser and dripped back down onto the algae powder at which point the extraction of the chlorophylls started. The liquid level continuously rose in the middle section until it reached the overflow pipe letting the chlorophyll extract pour back into the lower flask. A sample of 4–6 cm^3 was taken at the end of every cycle. The extraction continued as long as the absorption spectra measured by the spectrophotometer did not show a significant change.

The Method of Leaching

Leaching was carried out with so-called 'cold solvents' at room temperature. Samples of 0.1 g; 0.5 g; and 0.75 g of algae powder were measured into a test tube, then 5–5 cm^3 of solvent was added. The list of solvents was the same as for the Soxhlet extraction method with the inclusion of diethyl ether. The weight of the solvent was also measured and recorded in order to have reference data in the later phases of the experiment. The samples were mixed with Vortex and centrifuged for 2 minutes at 4000 rpm. A sample was taken from the top layer after centrifuge and its absorbance measured with a spectrophotometer [11, 12].

Calculations

The chlorophyll contents of the given samples were calculated with empirical formulae according to *Eqs.(1)-(8)*.

$$c_{\text{chlorophyll-a}}(90\%\ \text{methanol}) = 15.65 \cdot A_{666} - 7.34 \cdot A_{653} \quad (1)$$

$$c_{\text{chlorophyll-b}}(90\%\ \text{methanol}) = 27.05 \cdot A_{653} - 11.21 \cdot A_{665} \quad (2)$$

$$c_{\text{chlorophyll-a}}(96\%\ \text{ethanol}) = 13.95 \cdot A_{665} - 6.88 \cdot A_{649} \quad (3)$$

$$c_{\text{chlorophyll-b}}(96\%\ \text{ethanol}) = 24.96 \cdot A_{649} - 7.32 \cdot A_{665} \quad (4)$$

$$c_{\text{chlorophyll-a}}(100\%\ \text{acetone}) = 11.75 \cdot A_{662} - 2.35 \cdot A_{645} \quad (5)$$

$$c_{\text{chlorophyll-b}}(100\%\ \text{acetone}) = 18.61 \cdot A_{645} - 3.96 \cdot A_{662} \quad (6)$$

$$c_{\text{chlorophyll-a}}(95\%\ \text{diethyl ether}) = 10.05 \cdot A_{662} - 0.76 \cdot A_{644} \quad (7)$$

$$c_{\text{chlorophyll-b}}(95\%\ \text{diethyl ether}) = 16.37 \cdot A_{644} - 3.14 \cdot A_{662} \quad (8)$$

where A_λ is the absorbance at λ (in nm) wavelength, and $c_{\text{chlorophyll-a}}$ and $c_{\text{chlorophyll-b}}$ denote concentrations of chlorophyll *a* and chlorophyll *b* in μg cm^{-3} [12] as a function of solvents.

In Soxhlet extraction, the mass of the chlorophyll in the extract was calculated by the multiplication of the measured chlorophyll concentration values by the volume of the liquid in the round bottom flask. This also enabled the calculation of efficiency in mg of chlorophyll per g of dry algae units (relative to 100% dry algae powder).

In the leaching experiments, the chlorophyll concentration results calculated with the empirical formulae were multiplied by the volume of the solvent that resulted in the relative chlorophyll mass values in the given samples. Knowing the mass and water content of the algae, the efficiency of chlorophyll extraction relative to the mass of the dry algae can be calculated. These results were normalised to match the measurements and average values were calculated.

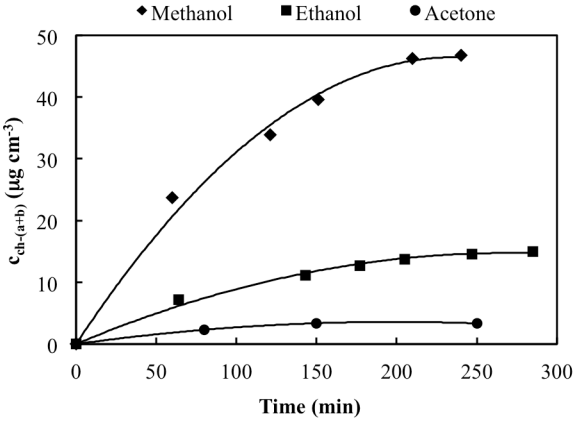

Figure 2: Changes in chlorophyll concentration during the Soxhlet measurements

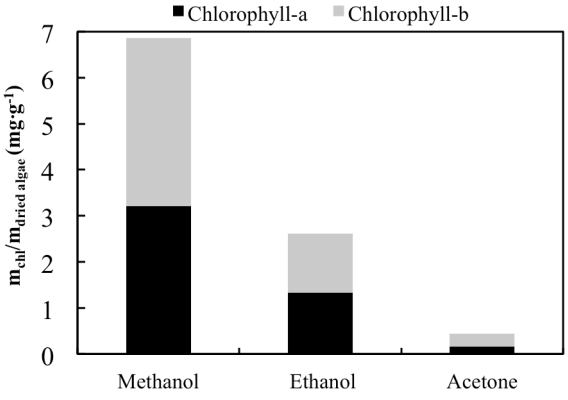

Figure 3: The efficiency of chlorophyll extraction of the various solvents during Soxhlet extraction

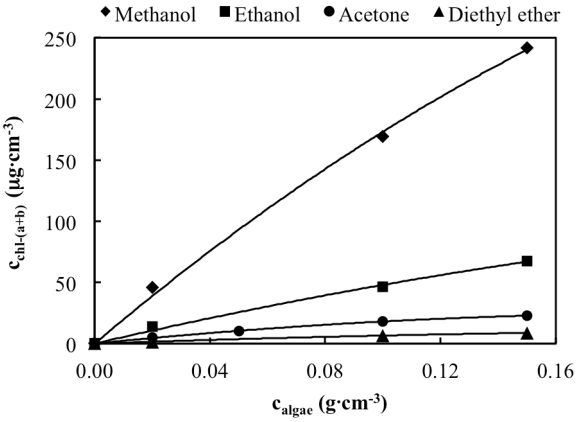

Figure 4: Change in chlorophyll concentration as a function of mass during leaching

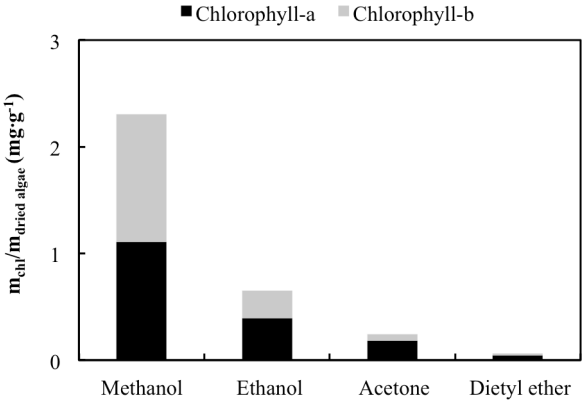

Figure 5: Efficiency of leaching using the various solvents

Results and Discussion

Soxhlet Extraction

The chlorophyll concentration measured changed according to a saturation curve as shown in *Fig.2*. It can be concluded that under the given circumstances the solvents reached their maximum efficiency and there was no need for longer extraction times. The ability of the solvents to extract chlorophyll relative to dried algae mass during the Soxhlet extraction is given in *Fig.3*.

The experiments were carried out using acetone, ethanol and methanol. It can be concluded that under the conditions of Soxhlet extraction, methanol is the most potent extracting solvent of chlorophyll *a* and *b* from the algae powder, followed by ethanol and acetone respectively. It can also be pointed out that acetone and methanol are more effective for the extraction of chlorophyll *b* while ethanol is more effective for the extraction of chlorophyll *a*. The latter experiment was not carried out with diethyl ether due to health and safety considerations.

Extraction by Leaching

Figs.4 and *5* illustrate the results of leaching. *Fig.4* depicts the concentration of chlorophyll for different masses of algae powder introduced into the system. The curves approach a saturation point in concentration, but do not reach the maximum. When we added progressively more and more algae powder to the same volume of solvent we observed increasing saturation behaviour. *Fig.5* presents the chlorophyll extracting ability of the various solvents when mixing 0.1 g of algae powder and 5 cm^3 of solvent. As expected, the best result was obtained by utilising at least 0.1 g of algae powder of. The efficiency ranking was similar to that of the Soxhlet extraction. Methanol proved to be the most efficient solvent, followed by ethanol, acetone and diethyl ether respectively.

The results are summarised in *Table 1,* which contains mg of chlorophyll per g of dry algae yields. This comparison enables the different solvents to be ranked and extraction methods, which is as follows according to their efficiency: methanol, ethanol, acetone, and diethyl ether. Additionally, significantly more chlorophyll can be extracted from ground algae with Soxhlet extraction than with leaching. In the

Table 1: Comparison of the chlorophyll (Chl.) extraction results

	Soxhlet extraction			Leaching		
	Chl. *a*	Chl. *b*	Chl. *a* & *b*	Chl. *a*	Chl. *b*	Chl. *a* & *b*
acetone	0.164	0.270	0.435	0.183	0.059	0.242
ethanol	1.329	1.290	2.619	0.395	0.259	0.654
methanol	3.206	3.657	6.862	1.109	1.199	2.307
diethyl ether	n/a	n/a	n/a	0.044	0.017	0.062

experiments conducted with acetone, it was observed that the extraction efficiency of chlorophyll *a* or chlorophyll *b* is greatly dependent on the conditions. Methanol, the solvent that proved to be the most efficient, could be removed later from the chlorophyll by low temperature evaporation.

Conclusions

From a systematic investigation of solid-phase extraction of chlorophyll *a* and chlorophyll *b* using Soxhlet extraction and leaching as a function of the employed solvent, it was found that the most efficient solvent is methanol. In both extraction techniques, the extracted amount of chlorophyll using methanol as a solvent is approximately an order of magnitude higher than in acetone and close to three to four times greater than the results of ethanol.

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