Techno-economical evaluation of protein extraction for microalgal biorefinery

Y W Sari¹,², J P M Sanders³, and M E Bruins³
¹Biobased Chemistry and Technology, Wageningen University, P.O. Box 17 6700 AA Wageningen, the Netherlands
²Biophysics Division, Department of Physics, Bogor Agricultural University, Jl. Meranti, Gedung Wing S, Kampus IPB Darmaga, Bogor 16680, Jawa Barat, Indonesia
³Food and Biobased Research, P.O. Box 17, 6700 AA Wageningen, the Netherlands

E-mail : yessie.sari@apps.ipb.ac.id

Abstract. Due to scarcity of fossil feedstocks, there is an increasing demand for biobased fuels. Microalgae are considered as promising biobased feedstocks. However, microalgae based fuels are not yet produced at large scale at present. Applying biorefinery, not only for oil, but also for other components, such as carbohydrates and protein, may lead to the sustainable and economical microalgae-based fuels. This paper discusses two relatively mild conditions for microalgal protein extraction, based on alkali and enzymes. Green microalgae (Chlorella fusca) with and without prior lipid removal were used as feedstocks. Under mild conditions, more protein could be extracted using proteases, with the highest yields for microalgae meal (without lipids). The data on protein extraction yields were used to calculate the costs for producing 1 ton of microalgae protein. The processing cost for the alkaline method was € 2448 /ton protein. Enzymatic method performed better from an economic point of view with € 1367 /ton protein on processing costs. However, this is still far from industrially feasible. For both extraction methods, biomass cost per ton of produced product were high. A higher protein extraction yield can partially solve this problem, lowering processing cost to € 620 and 1180 /ton protein product, using alkali and enzyme, respectively. Although alkaline method has lower processing cost, optimization appears to be better achievable using enzymes. If the enzymatic method can be optimized by lowering the amount of alkali added, leading to processing cost of  € 633/ton protein product. Higher revenue can be generated when the residue after protein extraction can be sold as fuel, or better as a highly digestible feed for cattle.

1. Introduction
Owing to the scarcity of fossil feedstock, there is an increasing demand for biobased fuels. Microalgae are considered one of the most promising biobased feedstocks, as their productivity in converting carbon dioxide into lipids exceeds that of oilseed crops [1-3]. However, currently, microalgae based fuels have not yet been produced at large scale. In depth works are being conducted to enable microalgae-based fuel production in a sustainable and economical way. Applying a biorefinery concept may aid here. Microalgae biorefinery uses the overall composition of the algae, and next to lipids, including carbohydrates and proteins [3].
Methods on microalgal protein extraction have been developed [4]. However, the yield is still considered low, limiting commercial protein production from microalgae. This low protein yield is due to the complexity of microalgal cell wall and microalgal protein properties. Extreme extraction conditions can be used to obtain substantial cell wall degradation, but this may reduce functional protein properties.

This paper discusses two relatively mild conditions for microalgae protein extraction. Green microalgae (*Chlorella fusca*) with and without lipid are used as the feedstock. Two protein extraction techniques were used; alkaline and enzyme assisted extraction. Data on the yield of extracted protein were used to calculate cost for producing 1 ton of microalgae protein.

2. Materials and Methods

2.1 Materials
Microalgae meal (*Chlorella fusca*) and non–deoiled microalgae (microalgae) (*Chlorella fusca*) were obtained from Ingrepro B.V., the Netherlands. Protex 40XL (activity: 52 MPU/ml) was obtained from Genencor (Danisco) International Oy, Denmark.

2.2 Protein content analysis
Protein content in starting material and hydrolysate was determined using DUMAS analysis (FlashEA 1112 series, Thermo Scientific, Interscience). Methionine was used as a standard for the calibration and a constant of 6.25 was used as a nitrogen-protein conversion factor to calculate the protein content in the samples.

2.3 Protein extraction
Alkaline protein extraction was conducted as described in [5] while enzymatic assisted protein extraction was conducted as described in [6] with some minor modifications.

2.3.1 Alkaline method
Protein extraction was conducted, according to [5], at three ascending temperatures: starting with a one day incubation at 25°C, followed by one hour at 60°C, and ending with a one hour incubation at 120 °C. Total protein yield obtained after three- step protein extraction was defined as mass of extracted protein relative to mass of protein in the starting material.

2.3.2 Protease assisted method
Protein extraction was conducted using Protex 40XL, a mixture of proteases, according to [6]. The enzyme dosage in this study was 1 and 5% (volume enzyme per weight of protein). Total protein yield obtained was defined as mass of extracted protein relative to mass of protein in the starting material.

2.4 Cost calculation
Cost analysis in this study focused on the main processing cost: biomass, NaOH, and heating. In the case of the enzymatic method, enzyme costs were added. Biomass costs were calculated based on the prices of microalgal meal and microalgae, which were € 230 [7] and € 1650 [3] per ton dry mass. NaOH cost were determined based the amount of NaOH required to extract protein per ton dry biomass. The required NaOH mass was determined by lab scale experiments. Mass biomass used on lab scale was 10 g. NaOH cost was determined as:
Cost_{NaOH} = m_{NaOH} \times p_{NaOH}

and

m_{NaOH} = \frac{m_{NaOH \text{ lab scale}}}{m_{biomass \text{ lab scale}}} \times m_{biomass}

with

Cost_{NaOH} = \text{cost NaOH} (\text{€})

m_{NaOH} = \text{mass NaOH required} \text{ (ton)}

p_{NaOH} = \text{price NaOH} (\text{€/ton}) = \text{€ 288/ton} [8]

m_{NaOH \text{ lab scale}} = \text{mass NaOH required during lab scale experiment} \text{ (g)}

m_{biomass \text{ lab scale}} = \text{mass dry biomass during lab scale experiment} \text{ (g)}

m_{biomass} = \text{mass dry biomass} \text{ (ton)}

The energy costs were calculated in relation to the thermal energy required to heat up the mixture of biomass and solvent. Specific heat capacity for water is 4.2 \text{ J.g}^{-1}.\text{K}^{-1} \text{ and for biomass is assumed to be 1.3 \text{ J.g}^{-1}.\text{K}^{-1}(at 25 \text{ °C})}[9]. The ratio of biomass to water used in the calculations is 1:9.

The thermal energy required was calculated as:

Q = \left( (m_{water} \times c_{water}) + (m_{biomass} \times c_{biomass}) \right) \times \Delta T

The thermal energy in Joules is then converted to kWh values (1 \text{ J} = 2.7 \times 10^{-7} \text{ kWh}). The price per kWh used in the calculations, \text{€ 0.041 kWh}, is the price of natural gas for a medium-sized industrial consumer [10].

Assuming that 50\% of heat involved in the extraction can be recovered then the final energy cost is defined as 30\% x calculated energy cost.

The enzyme costs used in this calculation were based on the amount of enzyme that was used

Cost_{enzyme} = m_{enzyme} \times p_{enzyme}

and

m_{enzyme} = \frac{m_{enzyme \text{ lab scale}}}{m_{biomass \text{ lab scale}}} \times m_{biomass}

with

Cost_{enzyme} = \text{cost enzyme} (\text{€})

m_{enzyme} = \text{mass enzyme required} \text{ (ton)}

p_{enzyme} = \text{price enzyme} (\text{€/ton}) = \text{€ 1000/ton enzyme solution.}\footnote{Personal communication with Sanders, J.P.M (2014). Commercial price of enzyme is €100/kg active enzyme. Assuming that Protea 40XL has the concentration of active enzyme for 10 g/L solution, then the solution costs are €1/L or €1000/ton.}

m_{enzyme \text{ lab scale}} = \text{mass enzyme required during lab scale experiment} \text{ (g)}

m_{biomass \text{ lab scale}} = \text{mass dry biomass during lab scale experiment} \text{ (g)}

m_{biomass} = \text{mass dry biomass} \text{ (ton)}
3. Results

3.1 Protein extraction
Microalgal protein has been extracted using alkali [5] and enzymes [6]. For both methods, microalgal meal (with prior lipid removal) protein was more easily extracted as compared to protein from microalgae (Fig 1.). This finding confirms the benefit of oil extraction prior to protein extraction [5].

The three-step alkaline extraction of microalgal meal resulted in 32.7% (w/w) protein extraction yield. A higher protein yield was obtained when enzymes were used to aid in the extraction. As much as 73.2% (w/w) of microalgal protein can be extracted from microalgal meal using 5% proteases (Fig. 1). Less microalgal protein was extracted when less enzyme was used. The protein extraction yield decreased to 62.7% w/w at 1% enzyme. Despite of this decrease, the 1% enzyme aided extraction still showed higher yield than the alkaline method, indicating the technical superiority of the enzyme assisted method in extracting microalgal protein.

![Figure 1. Protein extraction yield from microalgal meal and microalgae using alkali (left) and proteases at 1 and 5% (enzyme volume/protein weight).](image)

3.2 Cost calculation
To gain insight in the cost effectiveness of alkaline and enzyme assisted protein extraction, economic calculations were performed on part of the processing cost including cost of biomass, chemicals (NaOH for alkaline protein extraction, NaOH and enzyme for enzymatic protein extraction), and energy for heating. Biomass cost are the main contributor to the processing cost (Table 1). Figure 2 indicates that lipid removal has substantial effect on costs for production. It is therefore advisable to de-oil microalgae prior to protein extraction.
Table 1. Detailed processing cost calculation for producing 1 ton microalgal protein

| Cost required (€/ton protein) | Biomass | Chemicals | Heating | Enzyme | Total |
|-------------------------------|---------|-----------|---------|--------|-------|
| Alkaline Microalgae meal      | 1948    | 10        | 490     | 0      | 2448  |
| Alkaline Microalgae           | 33422   | 77        | 1173    | 0      | 34672 |
| Enzymatic Microalgae meal (1%)| 984     | 333       | 35      | 16     | 1367  |
| Enzymatic Microalgae (1%)     | 11970   | 426       | 45      | 27     | 12468 |
| Enzymatic Microalgae meal (5%)| 858     | 308       | 30      | 70     | 1266  |
| Enzymatic Microalgae (5%)     | 10141   | 338       | 38      | 115    | 10631 |

Due to the unavailability of a reliable industrial microalgae market price, the processing cost for microalgae protein depicted in Fig. 2 will probably not reflect the actual possible industrial protein production cost. The deviation on the predictive market price for microalgae is very high. In the calculation we cite € 1650/ton microalgae [3], which is already very high, while some also mentioned values of microalgae as high as € 25000/ton microalgae [7, 11].

4. Discussion

With the current low 17.6% protein yield from the alkaline extraction from microalgae, industrial microalgae protein production is far from being feasible. De-oiling increases protein yield to 32% protein from microalgae meal (with alkaline method at elevated temperatures). Looking at the still low yield, it seems that further optimization is required to extract microalgae protein. Protease-assisted-protein extraction has been developed for extracting more protein from microalgae and microalgae meal. With protease addition, the protein yield for microalgae and microalgae meals increased from 17.6 to 49.1% and from 31.7 to 73.2%, respectively (using 5% enzyme dosage).

The enzymatic assisted method in general required lower cost as compared to alkaline method. Without protease, protein production from microalgae requires 26 k€/ton protein (using alkali, see Fig. 2). Addition of 1% and 5% protease reduces the cost to 13 k€ and 11 k€/ton protein, respectively. Yet, this value is still too high for industrial production. The benefit of de-oiling microalgae brings protein
production closer to industry. Here the addition of 1% and 5% protease to microalgae meal solution reduces the cost to 1367 € and 1266 €/ton protein, respectively.

This makes the enzymatic method on microalgae meal economically preferred for microalgal protein production. However, 1266 €/ton protein is still much too high. In comparison, only 350 €/ton protein is required to produce 1 ton soybean protein concentrate [12]. The details on the microalgal protein cost calculation (Table 1) points out that biomass cost contribute most to the processing cost per ton of product A further optimized extraction method may contribute to higher extraction yield. If protein extraction yield can be increased to 90%, biomass cost can be reduced to € 555/ton protein (see Table 2). Table 2 indicates total processing cost for producing 1 ton microalgae meal protein with optimized method (90% yield).

Table 2. Processing cost for producing microalgae meal protein with optimized method (€/ton protein)

| Method      | Biomass | Chemicals | Heating | Enzyme | Total |
|-------------|---------|-----------|---------|--------|-------|
| Alkaline    | 555     | 15        | 50      | 0      | 620   |
| Enzymatic   | 555     | 562       | 18      | 45     | 1180  |

For a theoretical 90% yield, the alkaline method is less costly than the enzymatic one (see Table 2). However, in practice, it is rather difficult to increase the yield up to 90%. In this study, alkaline protein extraction was conducted overnight with increased temperature up to 120 ̊C. Still, most protein remained in the biomass.

Optimization of the enzymatic method appears more feasible. Cost minimization of the enzymatic method should first focus on the chemical cost. The enzymatic method has higher chemical cost compare to that of alkaline method due to use of a pH-stat during the extraction process. If the extraction can be performed in the absence of pH-stat and optimized for a fixed, but lower amount of alkali, then less chemicals will be required. Assuming as low a cost as € 15/ton protein (similar to the alkaline method without enzyme, see Table 2) the production cost may be reduced to € 633/ton protein, using 5% enzyme on microalgae meal.

In addition to an improved extraction yield or lower chemicals usage, biorefinery of other components from the microalgae is required to meet economic feasibility. In this case, the residual microalgal fraction after protein and oil extraction can be used for fuel or for feed with increased digestibility, due to the alkaline treatment. By refining microalgae into more than one or two products a feasible process may be achieved.

5. Conclusion

We examined the technical and economic feasibility of protein extraction from microalgae meal and microalgae, using alkali or proteases. For both methods, de-oiling microalgae prior to protein extraction increased the protein extraction yield. Enzyme assisted extraction resulted in higher protein yield as compared to alkaline method. Therefore, enzymatic extraction is preferred from an economical point of view. A reduction in chemicals cost due to excessive buffering is however needed to further reduce production cost. When combined with additional revenue from the residue after protein extraction, biorefinery of microalgae may become a feasible process.

References

[1] Konur O, 2011 Applied Energy 88(10) 3532-3540.
[2] Wijffels R H and M J Barbosa, 2010 Science 329(5993) 796-799
[3] Wijffels R H, M J Barbosa, and M H M Eppink, 2010 Biofuels, Bioproducts and Biorefining 4(3) 287-295

1 Biomass, chemicals, and power cost only.
[4] Sari Y W, W J Mulders, J P M Sanders, and M E Bruins, 2015 Biotechnology Journal 10(8): p. 1138-1157
[5] Sari Y W, U Syafitri, J P M Sanders, and M E Bruins, 2015 Industrial Crops and Products 70 125-133
[6] Sari Y W, M E Bruins, and J P M Sanders, 2013 Industrial Crops and Products 43 78-83
[7] FAO, 2010, Rome, Italy.
[8] Alibaba, NaOH price. [cited 2014 July 12] Available from: www.alibaba.com
[9] Dupont C. R Chiriac, G Gauthier, and F Toche, 2014 Fuel 115 644-651
[10] Eurostat 2014 Energy price statistics [cited 2014 October 27] Available from: http://epp.eurostat.ec.europa.eu/statistics_explained/index.php/Energy_price_statistics
[11] Pulz O and W Gross, 2004 Applied Microbiology and Biotechnology 65(6) 635-648
[12] Mustakas G C and V E Sohns, Soy process, equipment, capital, and processing cost. [cited 2014 August 27]; Available from: http://naldc.nal.usda.gov/