

# OPTIMIZATION OF PROTEIN EXTRACTION OF MICROALGAE FROM WWT: Effect of different variables on alkaline hydrolysis

Lorenzo Hernando, Ana<sup>1</sup>, Ruiz Vegas, Javier<sup>1</sup>, Bolado Rodríguez, Silvia<sup>1</sup>

<sup>1</sup> Department of Chemical Engineering and Environmental Technology, University of Valladolid, Valladolid, SPAIN ([ana.lorenzo@iq.uva.es](mailto:ana.lorenzo@iq.uva.es);

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## INTRODUCTION

Microalgae have potential as human and animal nutrition, emulsifier agents and a source of multiple bioactive compounds. They can be cultivated without competing for land or water with crops or even can be cultivated using wastewaters with high concentrations of N and P, and they generally have a favourable nutritional profile, having a protein fraction with an equilibrated amino acid profile, and a lipid fraction where essential PUFAs are present. Increasing interest in health qualities of algal proteins, as well as a strong financial need to valorise all fractions of the cultivated biomass has encouraged the development of alternative, safe and scalable technologies to access quality algal proteins. An appropriate cell disruption process should maximize the yield and the value of the compounds extracted, disintegrating most cells without contamination or degradation of the target compounds. For large scale production, it is also important that the disintegration process can be scaled up and that it is rapid. In addition, the integration of the cell disruption into the downstream processing must be easy and it should not have a negative impact on subsequent processing steps.

In this work, alkaline hydrolysis was tested as an appropriate method for extracting and solubilising proteins from microalgae biomass from pig manure wastewater treatment, evaluating the optimal conditions of NaOH concentration, temperature and time. Ultrasounds and microwave will be tested as additional treatments prior to alkaline hydrolysis to maximize yields and purity of extracts and minimising chemicals and energy requirements.

## MATERIAL AND METHODS

### Microalgae biomass

Microalgae biomass was kindly supplied by University of Almería. Microalgae biomass was cultivated in a thin-layer photobioreactor at a HRT of 3.3 days fed with pig manure wastewater diluted at 10%, harvested in October 2016 and freeze-dried and stored at 4°C prior to alkaline hydrolysis assays.

### Alkaline hydrolysis

Alkaline hydrolysis was performed at three different reaction times (0.5, 2 and 5 hours), temperatures (25, 40 and 55 °C) and NaOH concentrations (0.5, 1 and 2M). For these assays, 2.5g of freeze-dried biomass was placed in 100ml Erlenmeyer flask and NaOH solution – at the corresponding concentration - was added up to 50.0g, in order to obtain suspensions of 5% w/w. These suspensions were placed in an orbital shaker at 150rpm, at the corresponding temperature during the selected reaction time.

After alkaline hydrolysis, microalgae suspensions were centrifuged at 20000xg and 4°C for 15min. Supernatant was recovered and solid fraction (exhausted biomass) was stored at 4°C. Extracted protein was precipitated by adjusting the pH of the supernatant down to 2.5 (protein isoelectric point) by 2M HCl addition. This acidified suspension was centrifuged again under the same conditions, rich-protein pellet recovered and supernatant discarded. The pellet was finally dried at 35°C for 48h.

After complete drying, extracts were weighted and chemical composition of extracts was evaluated by analysing protein content, as well as carbohydrate, lipid and ash content. Total (g extract/100g biomass dw) and protein extraction yields ( $\text{g}_{\text{proteins}}$  in extract/100g $_{\text{proteins}}$  in raw biomass), as well as purity ( $\text{g}_{\text{proteins}}$  in extract/100g extract) were calculated.

### Analytical methods: Chemical composition of extracts

Protein content of raw biomass and protein extracts were quantified by Total Kjeldhal Nitrogen method (TKN). Carbohydrate content of extracts was determined using a modified NREL (National Renewable Energy Laboratory – USA) procedure. First, extracts were subjected to a concentrated acid hydrolysis for 1 h by adding 3 mL of H<sub>2</sub>SO<sub>4</sub> (72% w/w) at 30 °C to a 0.300g dry sample. Then, 84 mL of deionized water was added to dilute the acid concentration to 4% w/w prior to autoclaving at 121 °C for 1 h. Then, solid and liquid fractions were separated by filtration and liquid fractions analysed by HPLC-RI-UV. Lipid content was determined by Kochert method.

### RESULTS AND DISCUSSION

Figure 1 shows total extraction yield of alkaline hydrolysis of WWT microalgae biomass. As it could be expected, it was observed that the harsher the extraction conditions, the higher the obtained yields. Nevertheless, when two or more extreme conditions were applied (for example, 5h at 40 °C using 2M NaOH) a yield decrease was observed, indicating a possible degradation of extracts. Nevertheless, these total yields did not exceed 12% dw, showing that the application of a chemical pretreatment alone was not enough for obtaining rich-protein extracts.

(Purity and protein yields to be calculated soon)

### Figures

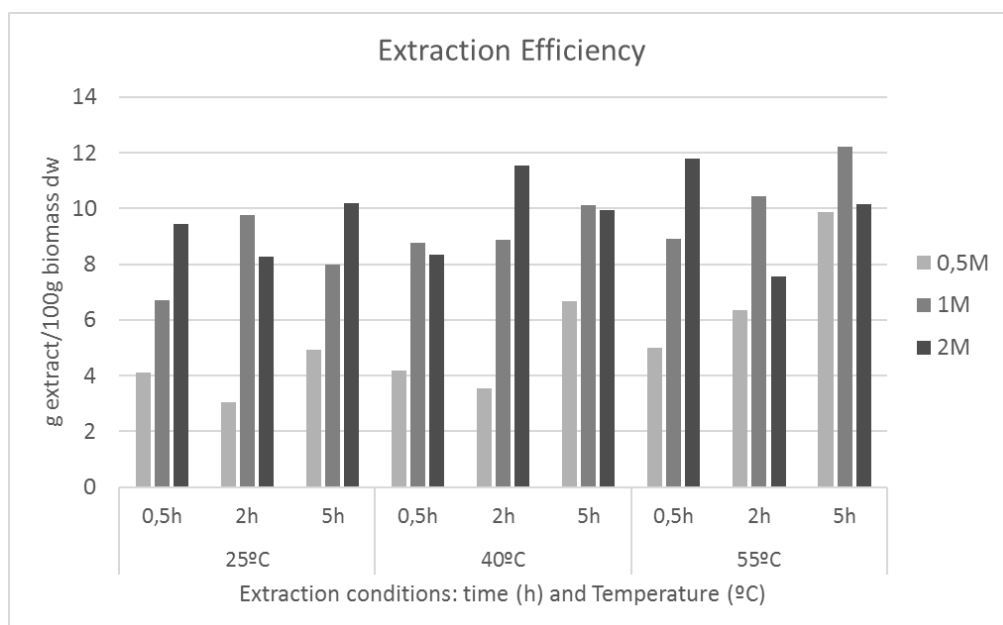


Fig. 1. Total extraction efficiency of alkaline hydrolysis of microalgae biomass, depending on NaOH concentration, temperature and hydrolysis time.

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### REFERENCES

- Günerken, E., D'Hondt, E., Eppink, M.H.M., Garcia-Gonzalez, L., Elst, K., Wijffels, R.H. (2015) Cell disruption for microalgae biorefineries. *Biotechnology Advances*, 33(2): 243-260.
- Becker, E. W. (2007) Microalgae as a source of proteins. *Biotechnology Advances*, 25: 207-210
- Gerde, J. A., Wang, T., Yao, L., Jung, S., Johnson, L. A., Lamsal, B. (2013) Optimizing protein extraction from defatted and non-defatted *Nannochloropsis* microalgae biomass. *Algal Research*, 2: 145-153



# OPTIMIZATION OF PROTEIN EXTRACTION FROM MICROALGAE GROWN IN WWs:

## Effect of operation conditions on alkaline hydrolysis

Ana Lorenzo Hernando<sup>1\*</sup>, Javier Ruiz Vegas<sup>1</sup>, Silvia Bolado Rodríguez<sup>1</sup>

Dpt. of Chemical Engineering and Environmental Technology, University of Valladolid,  
Dr. Mergelina s/n 47005. Valladolid, Spain

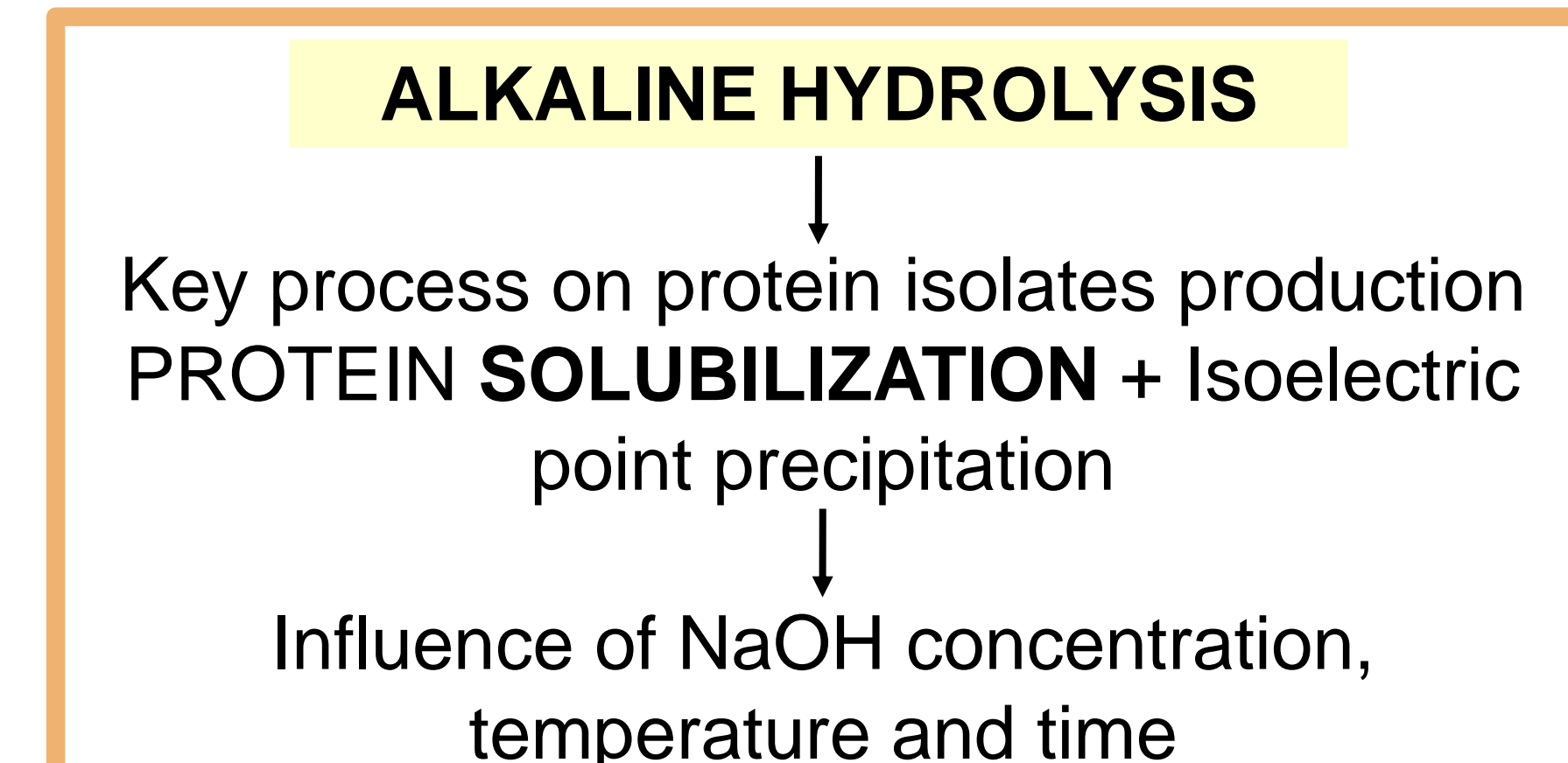
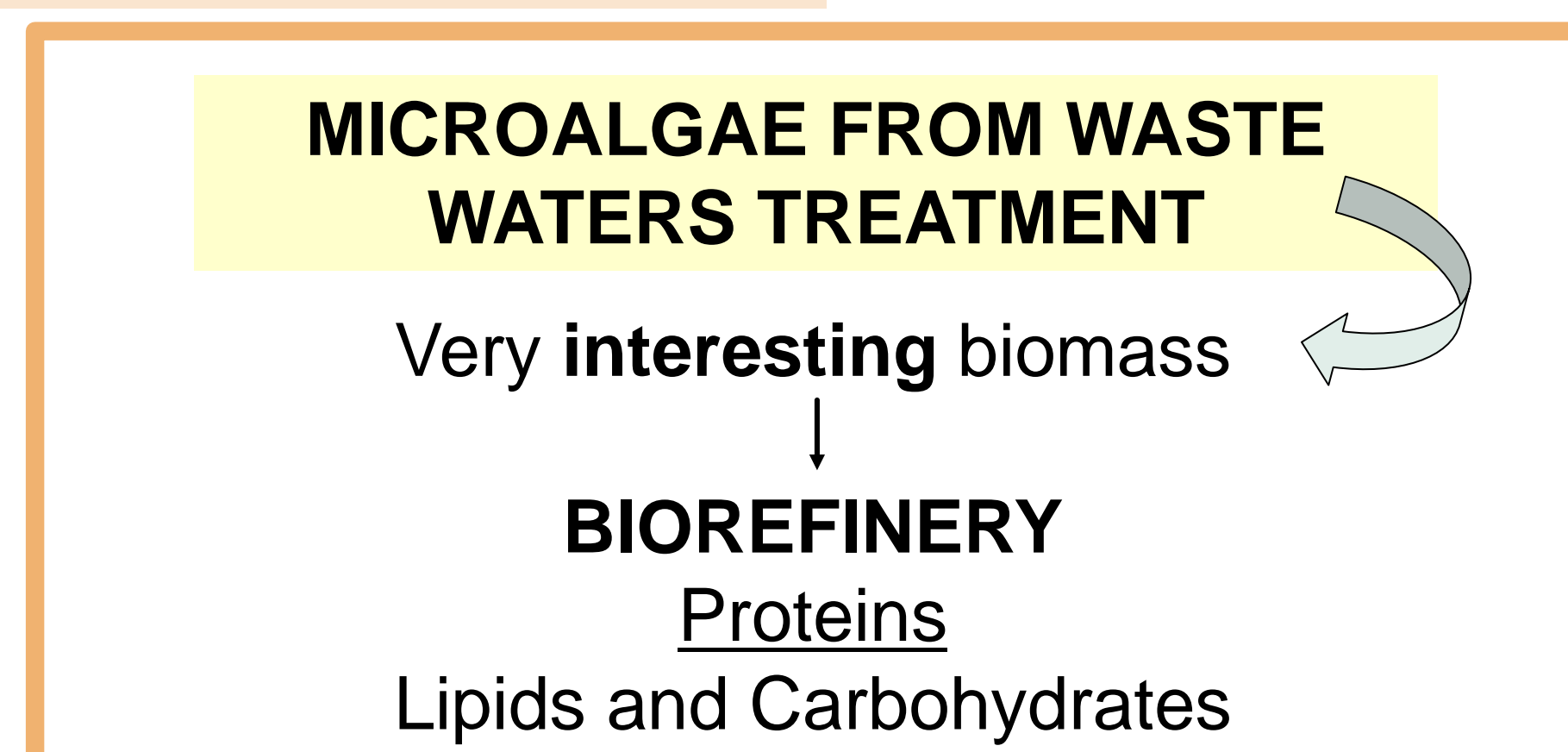
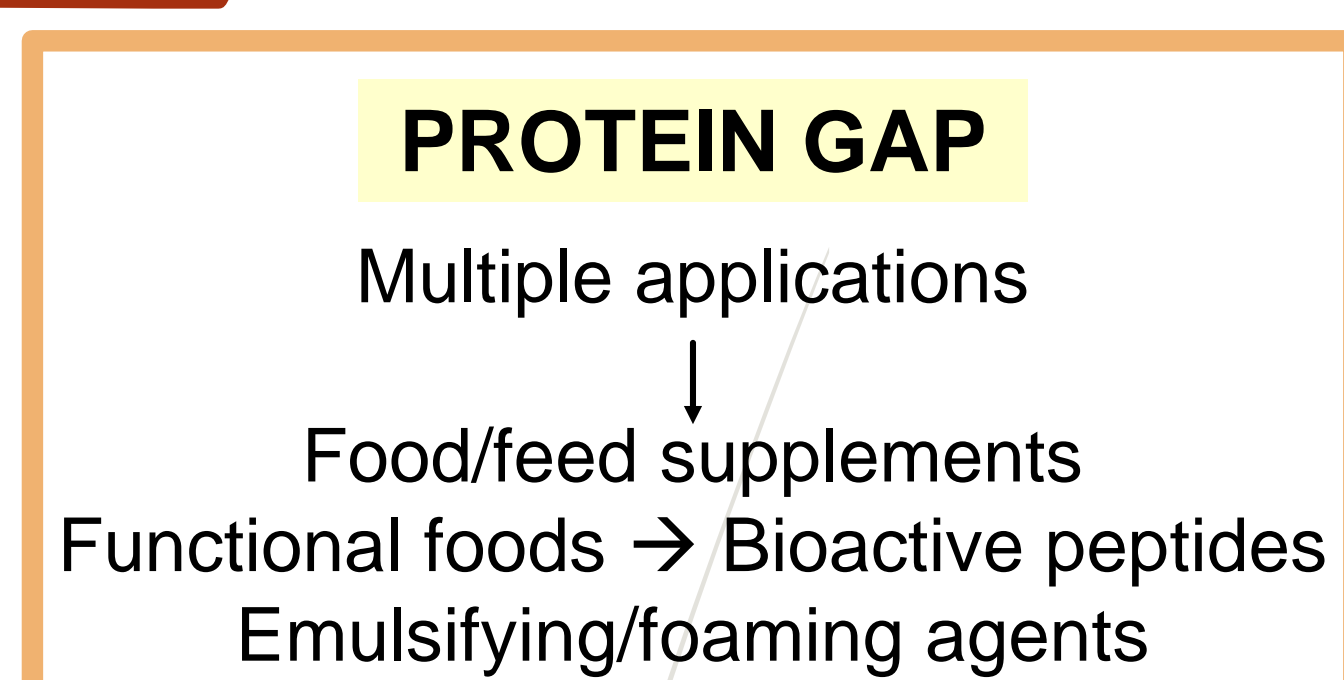
\* ana.lorenzo@iq.uva.es



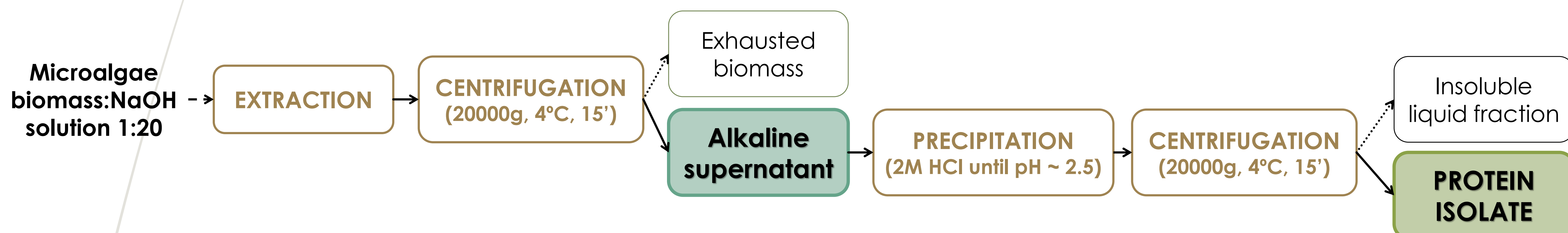
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### 1. INTRODUCTION



### 2. MATERIALS & METHODS



#### Alkaline hydrolysis:

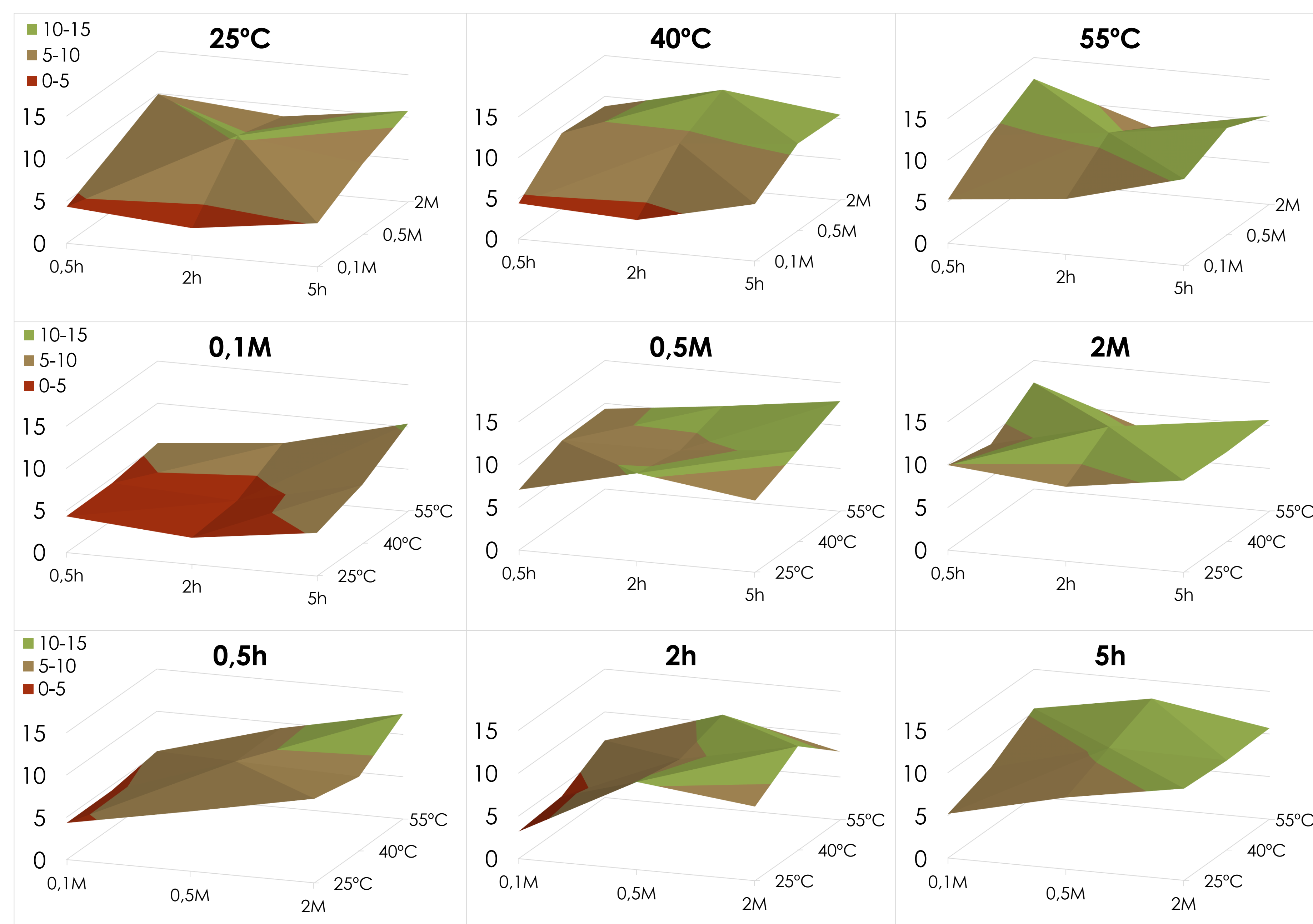
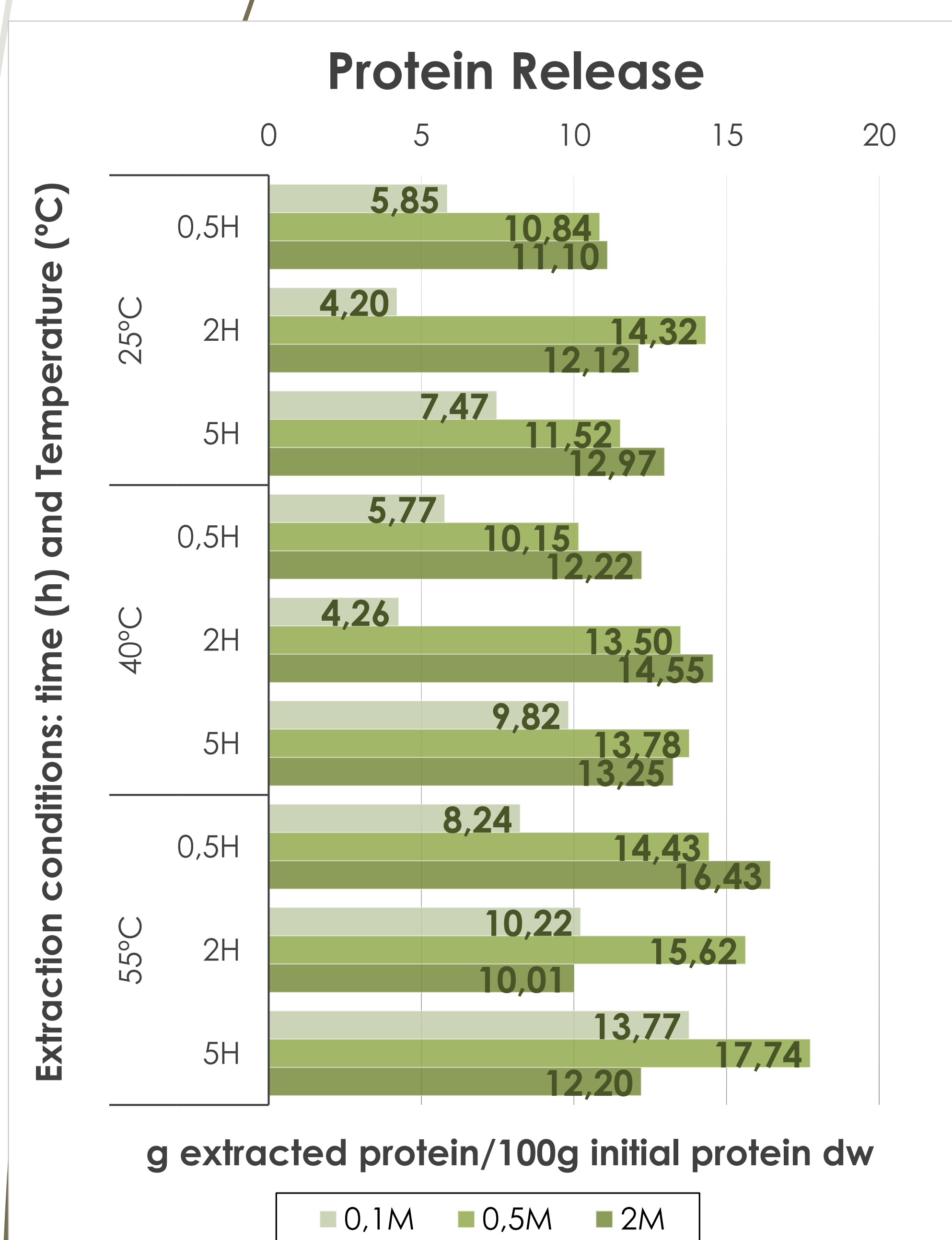
- 5% w/w of microalgae biomass in NaOH solution, 200rpm.
  - NaOH concentration: 0,1, 0,5 and 2M
  - Temperature: 25, 40 and 55° C
  - Time: 0,5, 2 and 5h

#### Protein isolates characterization

- Protein: NKT method
- Carbohydrate: NREL modified method
- Lipid: Kochert method
- Ash: 550°C, 24h



### 3. RESULTS



### 4. CONCLUSIONS

- Alkaline hydrolysis only caused slight microalgae protein solubilization, obtaining yields up to 13%.
- Maximum extraction yield (12.5%) was found to be for NaOH 0,5M, 55°C and 5h as operation conditions
- All protein isolates had a similar composition, independent of the applied extraction conditions (average protein content: 50%, av. lipid content: 10-20%, av. carbohydrates content: 5-10%, ash: 10-20%)
- The harsher the conditions, the lower the extraction yield and protein release

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#### References

- Günérken, E., D'Hondt, E., Eppink, M.H.M., Garcia-Gonzalez, L., Elst, K., Wijffels, R.H. (2015) Cell disruption for microalgae biorefineries. *Biotechnology Advances*, 33(2): 243-260.
- Becker, E. W. (2007) Microalgae as a source of proteins. *Biotechnology Advances*, 25: 207-210
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