Enhancing saccharification of *Rugulopteryx okamurae* by specific enzymes

C. Agabo-García, C.J. Álvarez-Gallego, A. Blandino, L.I. Romero-García

Department of Chemical Engineering and Food Technology, Wine and Agri-Food Research Institute (IVAGRO) and International Campus of Excellence (ceiA3), University of Cádiz, Puerto Real, Andalucía, 11510, Spain (cristina.agabo@uca.es;pedro.ernandez@uca.es;carlosjose.alvarez@uca.es; ana.blandino@uca.es;luisisidoro.romero@uca.es)

Introduction

Rugulopteryx okamurae is an invasive brown seaweed that has affected European coasts since 2015 causing ecological and socio-economic problems (Faria et al., 2022). Enzyme hydrolysis (EH) or saccharification is one of the most well-known possibilities for revalorizing this alga, converting the biomass into hydrolysates rich in fermentable sugars. In the EH of this macroalga, alginate-degrading enzymes play an important role. It is known that some fungi can produce alginate lyases (AL) (Agabo-García et al., 2023) that can be used in EH processes instead of commercial ones, significantly reducing the cost of the process.

References

Agabo-García, C., Romero-García, L. I., Álvarez-Gallego, C. J., & Blandino, A. (2023). Valorization of the invasive alga Rugulopteryx okamurae through the production of monomeric sugars. Applied Microbiology and Biotechnology, 1–12.

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Faria, J., Prestes, A. C. L., Moreu, I., Martins, G. M., Neto, A. I., & Cacabelos, E. (2022). Arrival and proliferation of the invasive seaweed Rugulopteryx okamurae in NE Atlantic islands., 65(1), 45–50.

Objective

The objective of this study was to test the EH of *R. okamurae* by applying commercial cellulases and alginate lyase (AL), as well as a mixture of extracted lyophilized enzymes (LE) produced by *Aspergillus awamori* through solid-state fermentation, in order to evaluate the condition that maximizes sugar release.

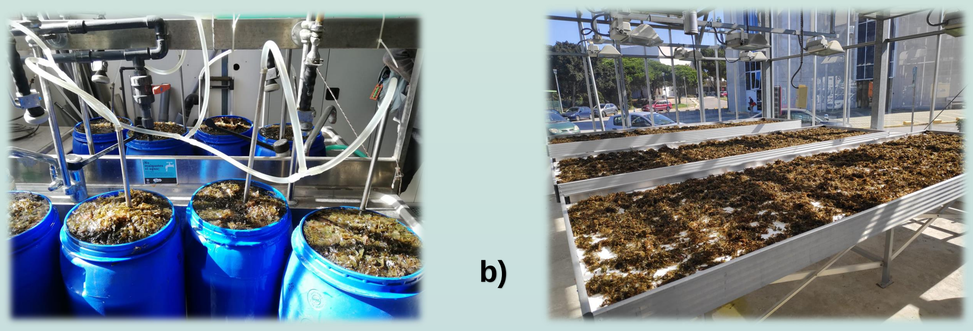

Figure 1. Algae samples conditioning (a) Washing; (b) Drying

Material and methods

Solid-state fermentation (SSF) was performed by the fungus *Aspergillus awamori* for 5 days using a solid-liquid ratio of 1:3 at 30ºC under static conditions. Enzyme extraction was carried out using a ratio of 1:20 weight of seaweed to volume of Tween 80 (0.1% v/v), for 30 min in a rotary shaker at 4 ºC and 150 rpm. Afterwards, the resulting solid suspensions were centrifuged at 10,000 rpm and 4 ºC for 10 min. The supernatant liquor was lyophilized at - 60 ºC and 100 mT for 4 days, obtaining a solid lyophilized extract. For EH, 15 FPU/g-biomass of Cellic CTec2® (Novozyme®) (CCT2) and phosphate buffer (10 % weight algae/volume buffer) were added to each Erlenmeyer flask, and then incubated at 250 rpm under optimal temperature conditions for alginate lyases (37ºC) during the first 3 h, and for cellulases (50 °C) for the remaining 69 h. Samples were taken at different times and total reducing sugars (TRS) were determined by the DNS method to study the kinetics of the process

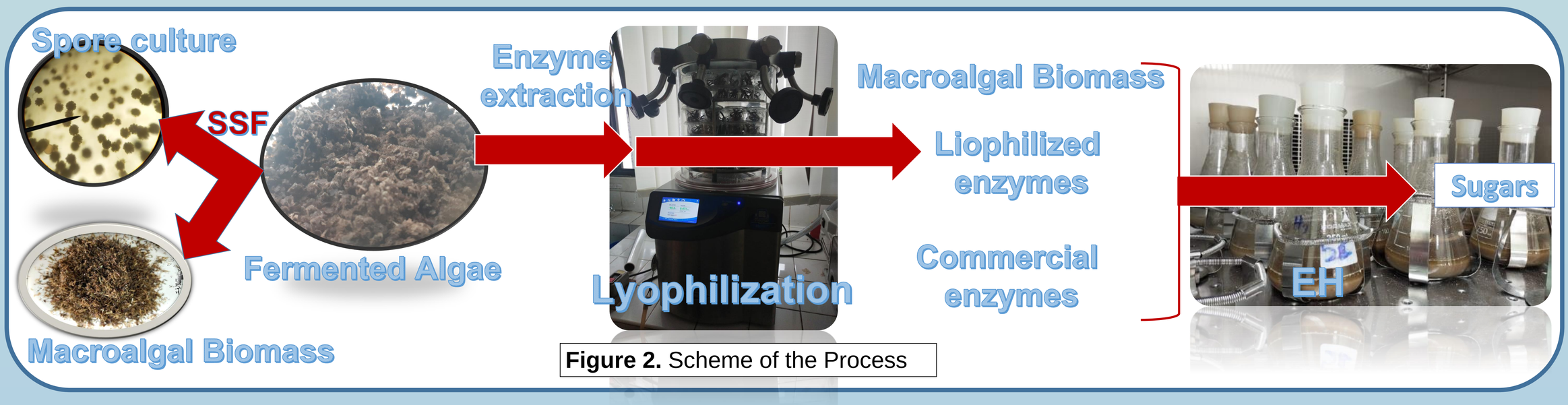

Figure 2. Scheme of the Process

Results and Discussion

The results showed that the LE produced slightly higher values of TRS (1.37 ± 0.01 g/L) compared to using only commercial AL (1.14 ± 0.05 g/L) due to the effect of other enzymes present in the extract, such as laminarinase. Therefore, this LE was used in combination with commercial Cellic CTec2® (CCT2), reaching a final TRS of 13.6 ± 0.18 g/L. Finally, the combination of three enzymatic agents (CCT2, AL, and LE) produced 14.0 g ± 0.14 g/L; which is 1.5 times more sugars than using only the commercial CCT2.

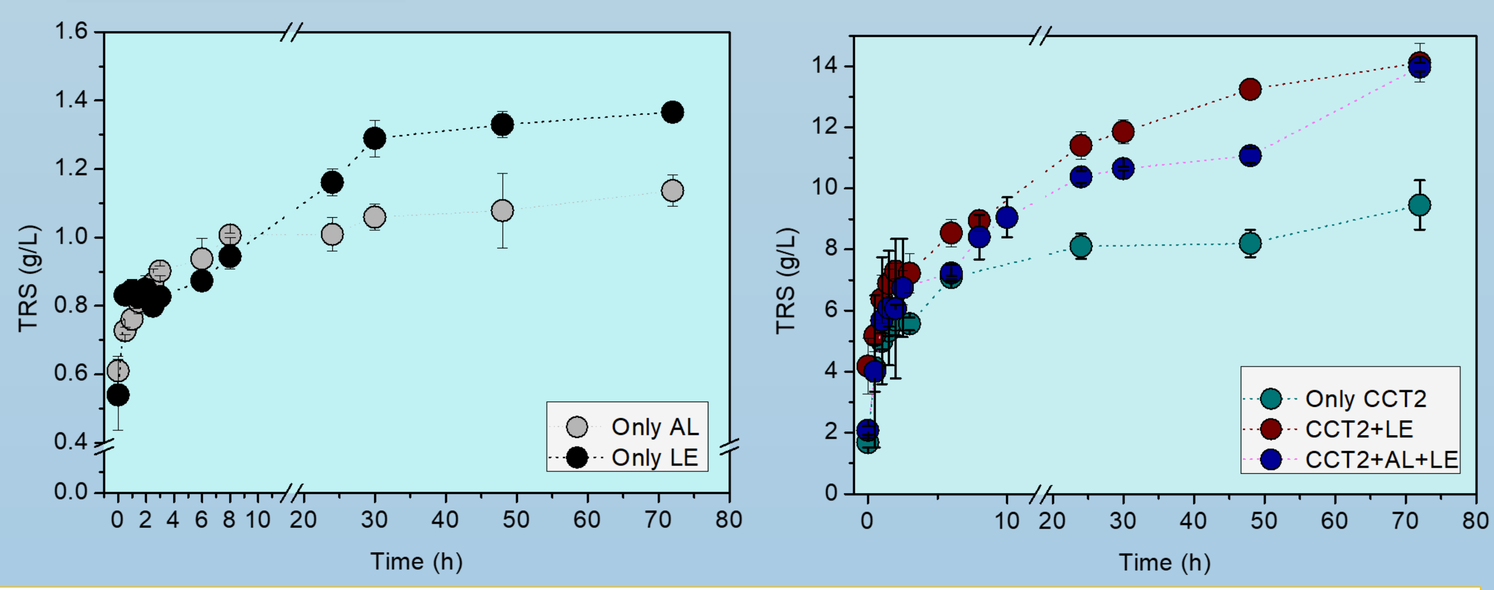

Figure 3. Time evolution of TRS during the hydrolysis of pretreated seaweed using Cellic CTec2®

Conclusions

- ✓ **The use of the alginate lyase enzyme enhances the saccharification process of *R. okamurae*.**
- ✓ **Enzymes produced by *A. awamori* growth using *R. okamurae* biomass as a solid substrate can enhance alginate degradation contained in the seaweed.**
- ✓ **The release of TRS follows a first-order kinetics**
- ✓ **A combination of three enzymatic agents (CCT2, AL and LE) generates sufficient sugars for subsequent fermentation processes.**

Proyecto *PID2019-104525RB-I00* financiado por MCIN/ AEI /10.13039/501100011033

Consejería de Transformación Económica, Industria, Conocimiento y Universidades de la Junta de Andalucía

Proyecto *TED2021-130891R-I00* financiado por MCIN/AEI /10.13039/501100011033 y por la Unión Europea Next GenerationEU/ PRTR