# Comparison of different strategies for saccharification of algae R. *okamurae*.

**C. Agabo-García**[1], S. Ioannidou[2], D. Ladakis[2], Blandino, A[1].; Álvarez-Gallego[1], C.J.; Romero-García[1], L.I.; A. Koutinas[2]

*[1]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*
*[2]Department of Food Science and Human Nutrition, Agricultural University of Athens, Athens, Greece*

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Presenting author email: cristina.agabo@uca.es

The massive coastal arrival of seaweed *Rugulopteryx okamurae* to the Strait of Gibraltar and other Mediterranean Coastal areas has negative environmental and economic impacts. However, this environmental problem can be converted into an opportunity if it is applied to a circular economy approach.

It is possible to revalorize this seaweed, transforming this biomass into bio-based sugars as platforms for producing several compounds such as plastic, solvents and/or functional bio-products (Lips, 2022).
In this sense, enzyme hydrolysis is one of the most well-known processes used to obtain monomeric sugars able to be fermented in different bioprocesses integrated into a biorefinery. It is known that solid-state fermentation by application of *Aspergillus awamori* can be used as a biological pre-treatment to increase sugar release in the saccharification of *R. okamurae* (Agabo-García et al., 2023). However, this process could be also used for enzymatic production obtaining high-valuable products such as specific enzymes: laminarinase or fucoidanase. In this sense, Life Cycle assessment can be used to assess the environmental sustainability performance of scenarios.

The objective of this study is to assess the environmental performance of an *R.okamurae*-based biorefinery for the production of sugars in two different scenarios: extracting the enzymes and without extraction

## Methodology

Solid-state fermentation was performed by the fungus *Aspergillus awamori* for 5 days using a solid-liquid ratio of 1:3 at 30ºC and static conditions.

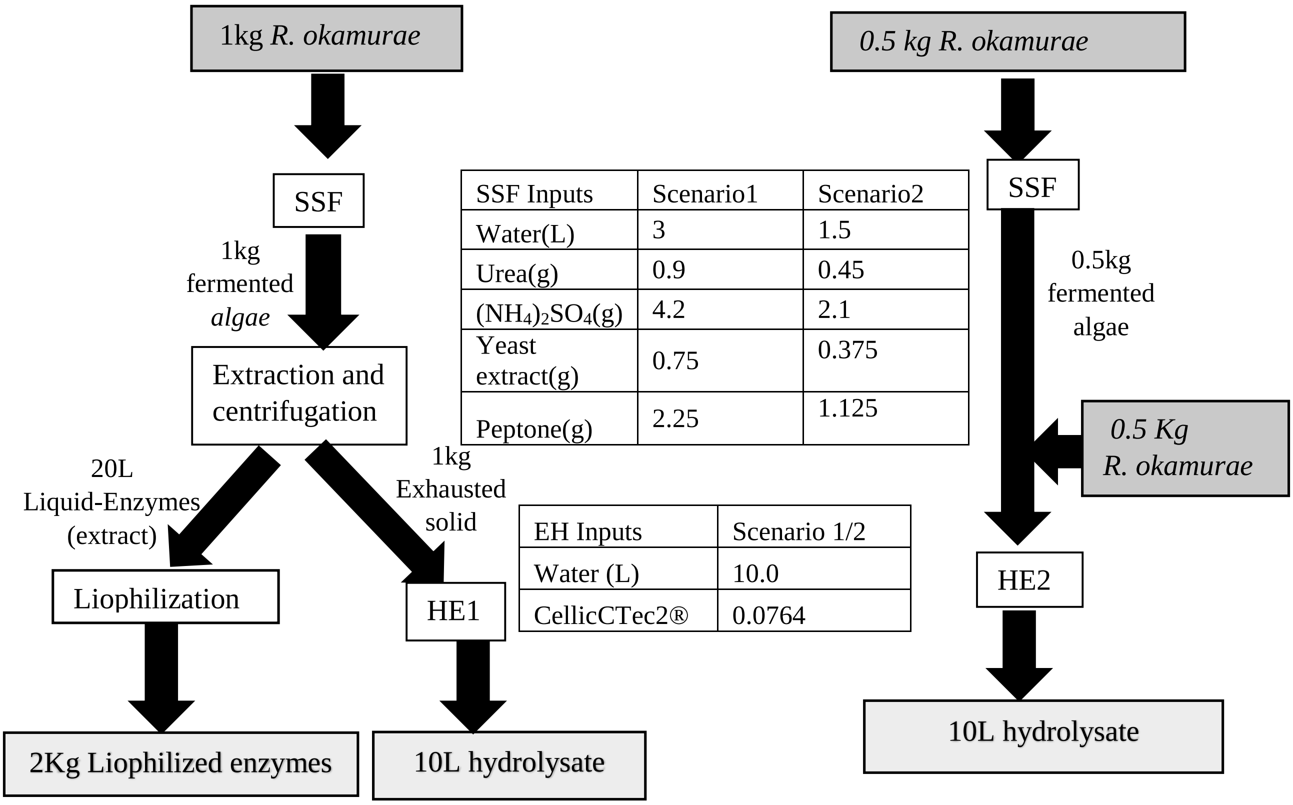
In scenario 1, after solid-state fermentation, it was developed the extraction of enzymes. This process was developed in a ratio of 1:20 weight of seaweed into volume of Tween 80 (0.1% v/v), for 30 min in a rotary shaker at 4 ºC and 150 rpm. Afterward, the resulting solid suspensions were centrifuged at 10,000 rpm and 4 ºC for 10 min. The supernatant liquor was lyophilized in a Virtis benchtop K lyophilizer at -60ºC and 100mt conditions for 4 days obtaining a solid lyophilized extract. On the other hand, the pellet obtained after centrifugation, named as "exhausted solid" was saccharified. For saccharification, 25 FPU/$g_{biomass}$ Cellic CTec2® (Novozyme®) and phosphate buffer (10% weight algae/volume buffer) were added to each Erlenmeyer flask and were incubated at 50 °C and 250 rpm in an orbital shaker during 72 hours. In scenario 2, half of the fermented and non-fermented algae was saccharified at the same operational conditions described above.

The Life Cycle Assessment (LCA) framework employed (ISO 14040 and 14044 standards) included the goal and scope phase, the inventory analysis, the impact assessment and the interpretation of results to estimate the final environmental impacts. The environmental impacts have been assessed with the LCA software GaBi using the CML2001 methodology (Ladakis et al., 2022). The life cycle inventory for the assessment is based on mass and energy balances that are estimated from the experimental data.

## Results and discussion

Using as a functional unit 1Kg of dried algae, in Scenario 1, it was obtained an enzymatic extract rich in alginate lyase (8.26 ± 0.01 IU/$g_{biomass}$) and 10L of hydrolysate rich in sugars with 5.22 ± 0.16 g/L (Figure 1). On the other hand, in scenario 2, it was obtained just 10L of hydrolysate with a higher concentration of sugars 10.02 ± 0.26 $g_{TRS}$/L (Figure 1). The Yield of EH2 was then, 113 ± 3 $g_{TRS}/Kg_{biomass}$ against 59.1 ± 1.9 $g_{TRS}/Kg_{biomass}$ of EH1.

Inventory results for both scenarios are presented in Figure 1. Based on these data, a "gate-to-gate" LCA was developed and different environmental categories, i.e. Global Warming Potential (GWP), Abiotic Depletion fossil Potential (ADP) Eutrophication Potential (EP) and Human Toxicity Potential (HTP), are reported.




| SSF Inputs | Scenario1 | Scenario2 |
|---|---|---|
| Water(L) | 3 | 1.5 |
| Urea(g) | 0.9 | 0.45 |
| $(NH_4)_2SO_4$(g) | 4.2 | 2.1 |
| Yeast extract(g) | 0.75 | 0.375 |
| Peptone(g) | 2.25 | 1.125 |

| EH Inputs | Scenario 1/2 |
|---|---|
| Water (L) | 10.0 |
| CellicCTec2® | 0.0764 |

**Figure 1.** LCI of both strategies for saccharification of algae *R. okamurae*.

## Conclusions

- ❖ Water inputs have a major environmental impact in both processes. Both processes reduce the Eutrophication parameter and can be integrated in sugar-based biorefinery process.
- ❖ The enzymatic product would increase the cost of the process but improve the sustainability of the saccharification.

## References

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