Enhancing saccharification of Ru*gulopteryx okamurae* by specific enzymes

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Rugulopteryx okamurae (*R. okamurae*) is a brown seaweed of the *Dictyotacea* family, native to Asian countries and North America areas that has colonized European coasts since 2015 (Faria et al., 2022) causing ecological and socio-economical problems (Altamirano et al., 2019). Enzyme hydrolysis is one of the most well-known possibilities to revalorise this alga, converting this biomass into hydrolysates rich in fermentable sugars. Carbohydrates in *R. okamurae* constitute 60 % (on a dry basis) of its composition being alginate the main constituent. Therefore, in the saccharification of this macroalgae alginate degrading enzyme plays an important role. In this sense, it is known that some fungi can produce alginate lyases (Agabo-García et al., 2023) that can be used in saccharification processes instead of commercial ones reducing highly the cost of the process.

In this study. it has been tested the saccharification of *R.okamurae* by applying commercial cellulases and alginate lyase as well as a mixture of extracted lyophilized enzymes produced by *Aspergillus awamori* by solid-state fermentation in order to evaluate the condition with maximum release of sugars.

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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. 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. Afterwards, 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. For saccharification, 15 FPU/$g_{biomass}$ Cellic CTec2® (Novozyme®) and phosphate buffer (10% weight algae/volume buffer) were added to each Erlenmeyer flask and were incubated at 250rpm and optimal temperature conditions for alginate lyases (37ºC) during the first 3h and optimal condition for cellulases (50 °C) for the rest 69h. Samples were taken at different times and it was determined TRS by DNS method in order to obtain the kinetic of the process.

Results and discussion

In Figure 1, it can be seen the effect of supplementation with commercial and extracted alginate lyase on the subsequent release of total reducing sugars (TRS). The results showed that the lyophilized extract produced slightly higher values of TRS (1.37 ± 0.01 g/L) in comparison with only commercial AL (1.14 ± 0.05 g/L) due to the effect of other enzymes present in the extract such as laminarinase. So, this liophilized extract was used in combination with commercial CellicCTec2®, reaching a final TRS of 13.6 ± 0.18 g/L. Finally, the combination of three enzymatic agents (commercial CellicCtec2®, commercial AL and LE) produced 14.0 g ± 0.14 g/L; 1.5 times more sugars than using only commercial Cocktail CelliCTec2®.

This concentration of sugar is enough for subsequent fermentation processes. In this sense, maximal glucose production was obtained for CCT2+LE condition and the maximal uronic acid production was obtained at CCT2+LE. All the experimental data of TRS produced along the hydrolysis were normalized and fitted to simple first-order kinetics.

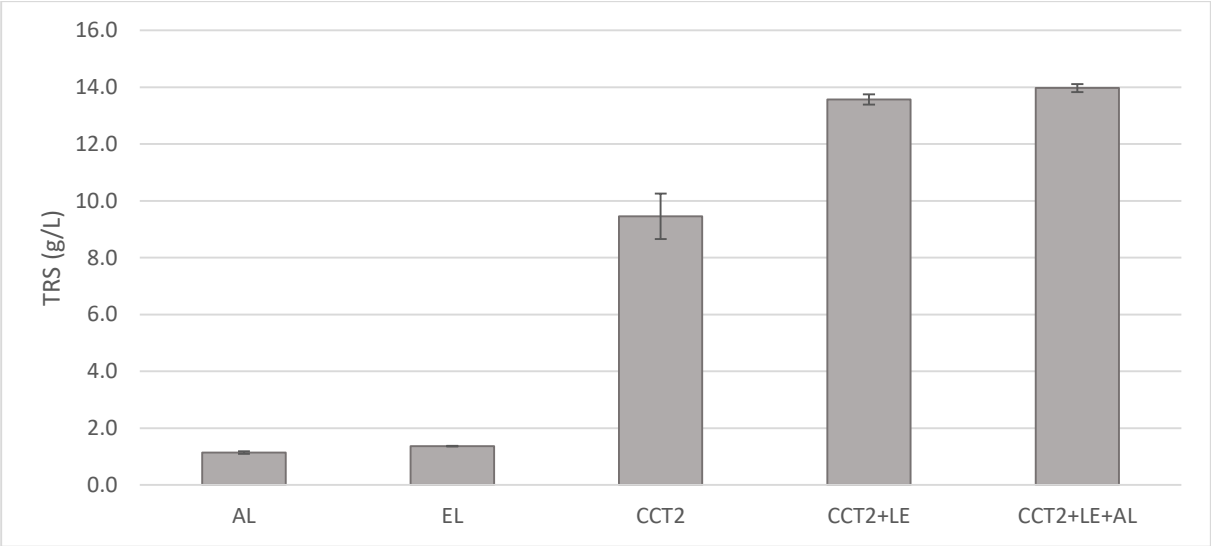

Figure 1. TRS (g/L) using different enzymatic agents in saccharification of *R. okamurae.*

Conclusions

The main conclusions are:

- ❖ The application of the alginate lyase enzyme increases the saccharification process of *R. okamurae*.
- ❖ *A. awamori* enzymes produced with *R. okamurae* as biomass can be used for increasing alginate degradation.
- ❖ The final hydrolysate is not only rich in uronic acid but also in glucose by the application of CellicCTec2® in combination with AL and LE being useful for subsequent fermentation bioprocess.
- ❖ The TRS release fit with first-order kinetics.

References

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