Added-value biorefinery products from invasive brown seaweed *Rugulopteryx okamuare.* A kinetic comparison.

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Keywords: dark fermentation, enzyme hydrolysis, rugulopteryx okamurae, kinetics, pretreatments.
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Since 2015, the coastal area of the Strait of Gibraltar has been colonised by the Asiatic seaweed *Rugulopteryx okamurae.* The massive coast arrival of this brown macroalgae has seriously affected the economy of the regional fishing and touristic sectors due to its fast spreading as invasive organism. This environmental problem is, in fact, an opportunity to apply the circular economy approach. On the other hand, between 19 and 23 million tonnes of plastic waste are dumped into the sea every year due to its low biodegradability (Bergmann et al., 2022). In this context, bioplastics production through biological processes may provide a solution to this problem.

Enzyme hydrolysis is one of the most well-known processes used to obtain monomeric sugars. Many of these, such as glucose D-galactose or D-mannitol are classified as fermentable sugars, which makes them a highly interesting substrate for subsequent biorefinery processes (Agabo-García et al., 2023). In fact, one of the possible ways of using sugars is as a substrate for bioplastics precursors synthesis processes, such as polyhydroxyalkanoates (PHA), involving both pure cultures and mixed cultures of microorganisms (Heng et al., 2017; Serafim et al., 2008). However, macroalgae cell structure is hard to fully degradate without the application of a previous pretreatment.

The objective of this study is to obtain total reducing sugars (TRS) by enzyme hydrolysis (EH) from the invasive seaweed *R. okamurae*. In addition, two different pretreatments will also be applied and compared: microwave (MW) and biological (BIO) pretreatments.

Materials and Methods

Pre-treatments

MW pretreatment was performed at temperatures from 160 °C to 220 °C for 20 minutes (Fernández-Medina et al., 2022). Otherwise, BIO pretreatment was carried out through as a solid-state fermentation (SSF) process by 10^8 cel/$g_{biomass}$ of the fungus *Aspergillus awamori* for 5 days at 30ºC using a mass-volume ratio of 1:3. Solid-state fermentation was developed in orbital shaker Type IKA-XX in agitation (250 rpm). The samples after pre-treatments were autoclaved at 121ºC 20 min in an autoclave type YY.

Enzymatic Hydrolysis

EH was carried out in non-pretreated and pre-treated samples using erlenmeyer flasks in Phosphate Buffer at pH 5. 344µl (25 FPU) of Cellic CTec2® (Novozyme®) were added to each Erlenmeyer flask and were incubated at 55 °C and 150 rpm in an orbital shaker during 50 hours. Finally, the first order kinetic model was applied using Origin Pro8® software according to the following equation:

$$P = \beta \cdot S_o\left(1 - e^{-kt}\right) = P_{max}\left(1 - e^{-kt}\right)$$

Where β is substrate-to-product yield coefficient, P is total TRS concentration (g/L) at any time, P_{max} corresponds to theoretical maximum value of TRS which can be obtained (g/L), k is EH rate constant (h^{-1}) and t represents time (hours). Results shown were normalized after eliminating the initial TRS.

Results and Disucssion

Pre-treatments solubilisation

Table 1. Characterization of liquid extracts after pre-treatments

Parameters	MW-160ºC	MW-220ºC	Bio-56 FPU	Bio-37 FPU
TOC (mg/L)	781	4775	1003	998
IC(mg/L)	42.8	64.6	12	74
TN(mg/L)	60	353	58	59
C/N	13.7	13.7	22.5	18.2
TRS(mg/L)	410	1730	338	338
$COD_{potential}$ (g/L)	0.438	1.85	0.361	0.361

In spite of biological pre-treatment obtained higher values of C/N, the highest solubilisation rates and reducing sugars concentrations were obtained by using microwave pretreatment at 220ºC as it can be seen in TOC, TRS and $COD_{potential}$ values shown in Table 1.

Enzymatic Hydrolysis yield and kinetics

The yields obtained for the reducing sugars production using both pretreatments are shown in Table 2. As it can be seen the optimal TRS yield was due to at 220ºC MW treatment. However, when the temperature decrease until 160ºC the results are similar than biological pre-treatment. So, we could find similar results using an ecological and economical process.

Table 2. TRS yields comparison between microwave and biological pretreatment

PT	Condition	RS Yield
MW-1day	160 °C	1.28
	220 °C	2.19
BIO-5 days	56FPU	1.33
	37 FPU	2.02

If the time evolution is considered, approximately the 90% of the final TRS were obtained were produced in the first 10 hours.

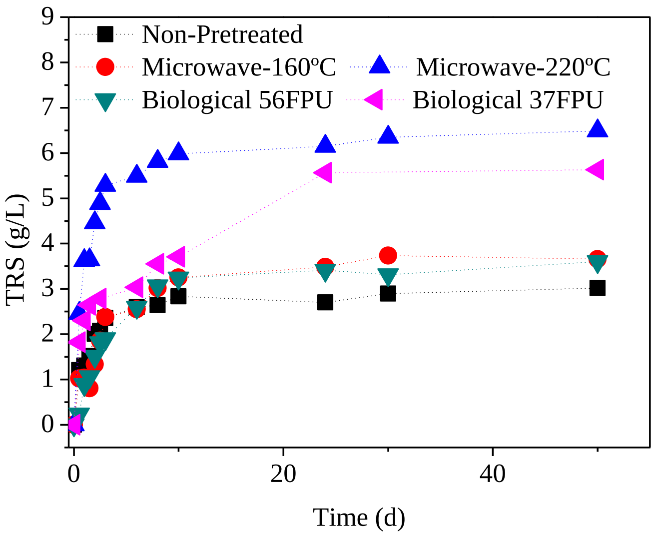

Figure 1. TRS release during EH assays

In this way, performing a kinetic adjustment to compare the main kinetic parameters associated with the effect of each pretreatment is highly interesting, especially in view of an industrial scale-up of the process.

Table 3. Enzyme hydrolysis kinetic parameters for microwave pretreatment and biological pretreatment

Kinetic parameter	NP	MW-160 °C	MW-220 °C	Bio-56FPU	Bio-37FPU
βS_0	2.60	3.34	5.69	3.47	4.3
k	0.56	0.23	0.68	0.28	0.0
P_0	0.20	0.25	0.35	-0.04	1.3
P_{max}	2.80	3.59	6.04	3.43	5.60
R^2	0.96	0.96	0.97	0.99	0.84

As it is shown in Table 3, the first order kinetic model finely fits the process behaviour ($R^2 > 0.95$ in all cases). It is noteworthy to highlight the kinetic constant (k) values, which is directly proportional to the reaction rate, and the maximum production (Pmax). Although the highest reaction rate and maximum yield is obtained by MW at 220 °C, it can be seen that BIO pretreatment achieves the same parameters values as MW pretreatment at 160 °C. The main conclusions are:

- ❖ MW pretreatment at 220°C gave the highest RS yields and reaction rate.
- ❖ In the first 10 hours, EH produce almost the 90% of the total RS.
- ❖ BIO pretreatment reached the TRS yields obtained by MW pretreatment at 160 °C in a more economical and sustainable way.

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Acknowledgements

This work is part of Project PID2019-104525RB-I00 funded by “Ministerio de Ciencia e Innovación”and the “Agencia Estatal de Investigación” MCIN/AEI/10.13039/501100011033) and by the European Union (ERDF A way of making Europe).

PhD. Cristina Agabo García was granted by a Posdoctoral Contract funded by Department of Economic Transformation, Industry, Knowledge and Universities from the Andalusian Government and Short stays in Foreign Research Centers in the framework of Universidad de Cádiz Research Plan

Mr. Pedro Fernández Medina was granted by a training contract of the “Operative Program for Junior employment 2014–2020″ of the “Regional Government of Andalusia” (Ref. GJ5Y6F-02).