LBEET Laboratory of Biochemical Engineering and Environmental Technology

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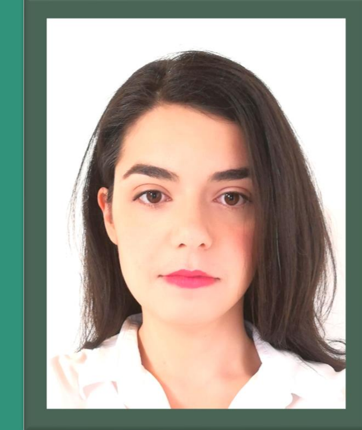
Chemical pretreatment and biotechnological exploitation of rice hull with microalgae towards the production of natural phenolic compounds



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Introduction

Rice hull is one of the most abundant lignocellulosic residues on earth, accounting for 20 % wt. of rice. It consists of approximately **36% cellulose**, **12 % hemicellulose** and contains high lignin (16%) and ash content (20 %), rich in amorphous SiO_2. Up to the present, only conventional methods, like combustion are utilized to combat the arising problem of its treatment. However, given its high carbohydrate content, more sophisticated biochemical processes could lead to a more thorough exploitation of this material. In order to exploit the carbohydrate content of rice hull, **chemical pretreatment** with dilute acid is essential to depolymerize the hemicellulose fraction, creating a liquid hydrolysate rich in soluble sugars, easily metabolized by microorganisms, like **microalgae**.

Microalgae are photosynthetic microorganisms, able to grow using different metabolic pathways (autotrophic, heterotrophic, mixotrophic), synthesizing thousands of **bioactive molecules**, including antioxidants, proteins, carbohydrates, pigments, fatty acids etc. Given the high cultivation costs for microalgae, **simple, economic nutrient sources** are necessary to render microalgae products cost efficient.

Our goal is to produce natural phenolic compounds from microalgae, utilizing rice hull hydrolysate as an economical substrate for their growth.

Phenolic compounds are important ingredients for several industrial sectors, including cosmetics, food, packaging etc., given their potent antioxidant activity. Lately, there is an emerging need in replacing the artificial phenols with naturally produced ones, based on concerns over the safety of the former. To this end, microalgae, could be an ideal source for natural phenols, given their advantages over terrestrial plants.

Materials & Methods

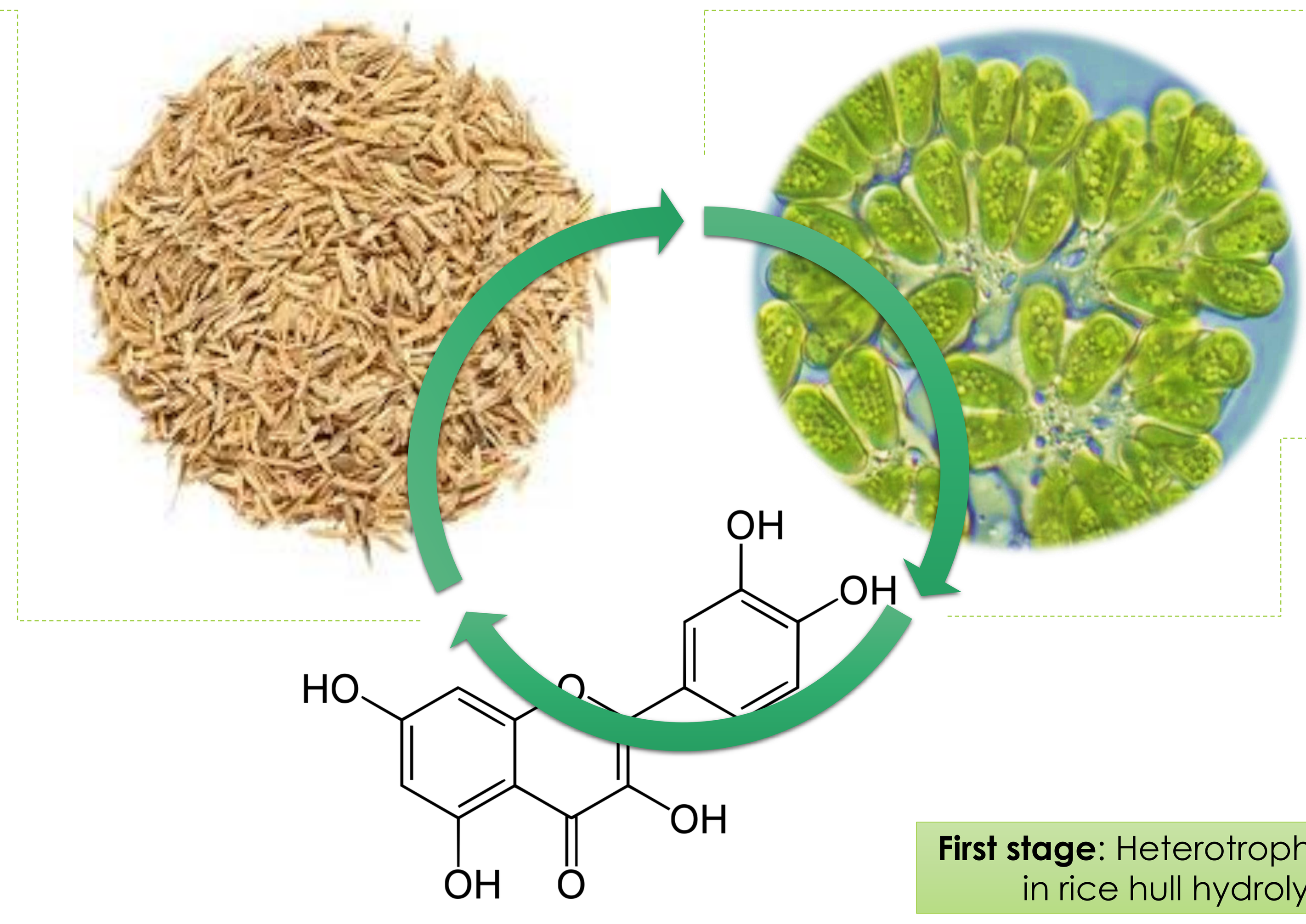
Chemical pretreatment of rice hull with dilute H_2SO_4: Parametric analysis

Find the **optimum** operational parameters for dilute acid hydrolysis that lead to a nutrient-rich liquid hydrolysate

- Temperature (100 -131 °C)
- H_2SO_4 Concentration (1 - 4%, v/v)
- Feedstock loading (4 -40%, w/v)
- Duration of treatment (15 -120 min)

Maximizing Monomeric sugars, NH_3-N, Phosphorus

Minimizing Inhibitors for microbial growth (furfural, HMF)



Microalgal growth in rice hull hydrolysate (heterotrophic conditions)

- Cultivation of microalgae ***Botryococcus braunii*** (SAG strain nr.: 30.81) in rice hull hydrolysate produced under optimum hydrolytic conditions.
- Find the optimum conditions for microalgal growth (Four concentrations of substrate tested, i.e. 10, 25, 50, 100 %, v/v)

Microalgal growth in rice hull hydrolysate for phenolics production (two-stage cultivation)

Since phenolics accumulation is triggered under autotrophic conditions, a novel **two-stage cultivation process** was utilized for the first time, combining utilization of rice hull hydrolysate as substrate towards the production of microalgal phenolic substances.

First stage: Heterotrophic growth in rice hull hydrolysate

Second stage: Autotrophic growth in synthetic medium (BG-11) for phenolics accumulation

Results & Discussion

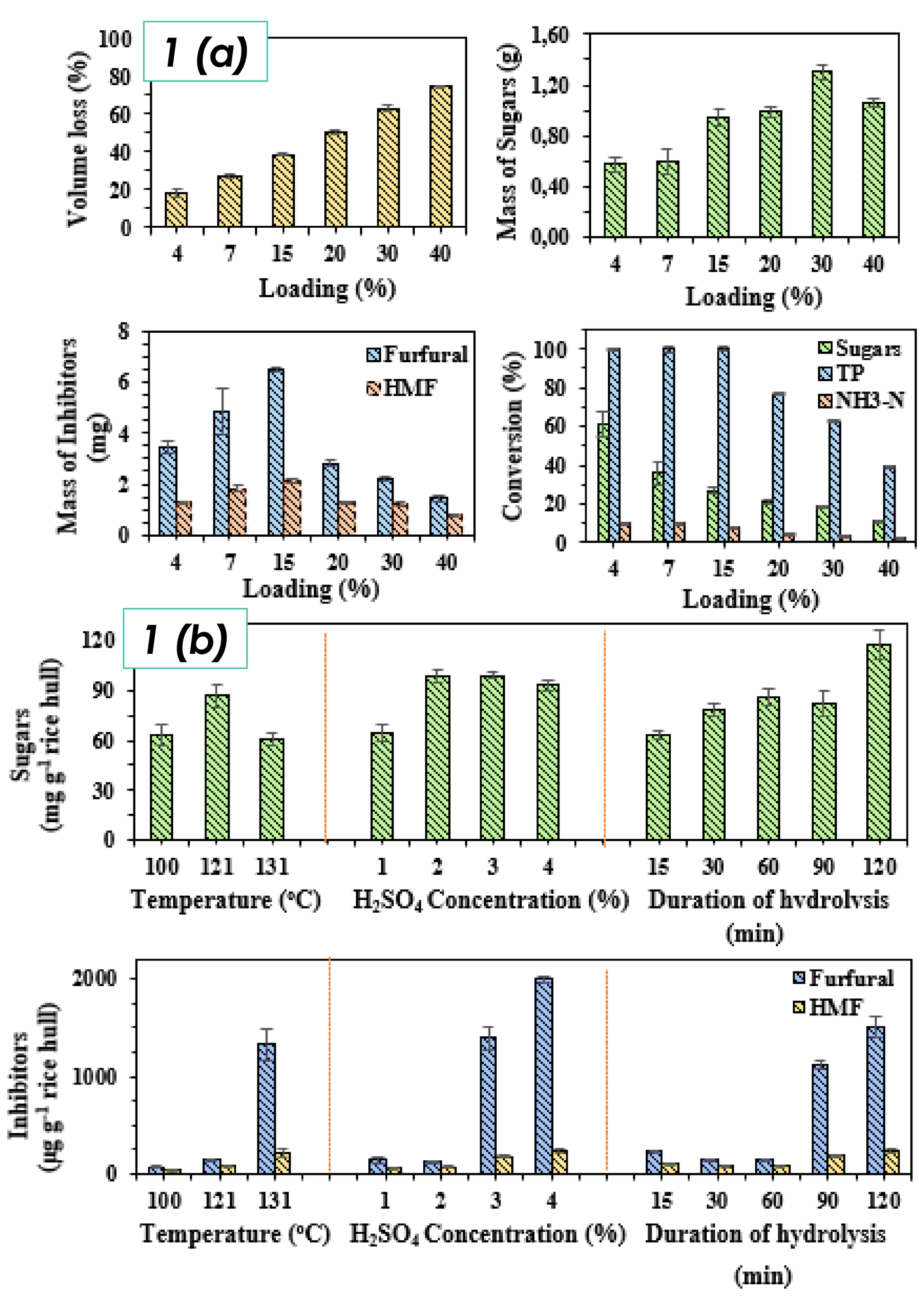


Fig. 1: Impact of operational parameters on composition of rice hull hydrolysate (liquid fraction); **(a)** Effect of loading on volume loss, mass of sugars, mass of inhibitors and conversion rates. **(b)** Effects of temperature, acid concentration and duration of pretreatment on yields of sugars and inhibitors.

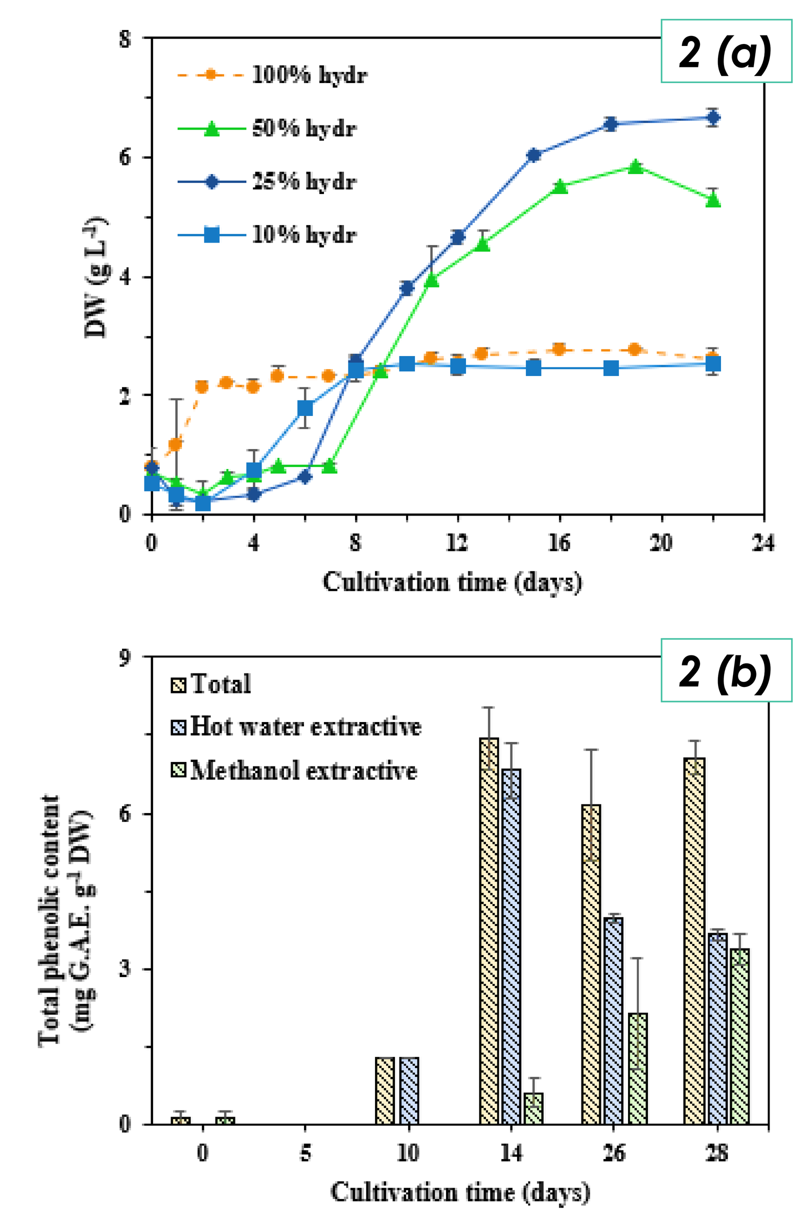


Fig. 2 (a): Microalgal growth of *B. braunii* in rice hull hydrolysate under heterotrophic conditions (four concentrations tested); **(b)** Accumulation of phenolic compounds from *B. braunii* during the second stage (autotrophic conditions) of its two-stage growth.

Chemical pretreatment of rice hull with dilute H_2SO_4: Parametric analysis

Among the four operational parameters tested, the impact of feedstock loading stood out, affecting positively the saccharification of structural carbohydrates, mainly hemicellulose, since sugars-mass was maximized at 30%, w/v loading, practically leading to more sugars-rich hydrolysates. Yet, sugars-yields were maximized at lower loadings (4%, w/v), indicating that higher feedstock loadings lead to more incomplete sugar depolymerization of the material. All the other tested parameters, had likewise a positive effect on the solubilization of hemicellulose to monomeric sugars, however under higher temperatures and stronger acidic conditions, inhibitors, such as furfural and HMF, were also produced suggesting degradation of the produced sugars to inhibitors.

Microalgal growth in rice hull hydrolysate (heterotrophic conditions)

The microalgae strain *Botryococcus braunii* was grown heterotrophically in rice hull hydrolysate produced under the optimum conditions, as previously described, testing four substrate concentrations (10 – 100 %, v/v). The two most productive substrate concentrations were 50% and 25% (v/v), where the maximum dry biomass concentration was reached (5.86 ± 0.03 g L^{-1} and 6.55 ± 0.10 g L^{-1} respectively), after 18 days of heterotrophic growth. Yet, the **optimum concentration** was achieved at **25%, v/v**, where nutrient-removal was more efficient.

Microalgal growth in rice hull hydrolysate for phenolics production (two-stage cultivation)

A two-stage cultivation process was followed aiming to maximize the microalgae phenolic content. According to obtained results, the heterotrophic cultivation of *B. braunii* in rice hull hydrolysate (first stage) followed by autotrophic cultivation in synthetic medium (second stage), **lead to accumulation of valuable natural phenolic compounds** in the produced biomass with the maximum value of **7.44 ± 0.60 mg G.A.E. g^{-1} DW** reached on the 14th day of the autotrophic stage.

Conclusions

The chemical pretreatment of rice hull with dilute H_2SO_4 proved to be efficient in depolymerizing the hemicellulosic fraction of the material.
The optimum pretreatment conditions were: **121 °C, H_2SO_4 2% (v/v), 60 min, 30% (w/v) feedstock loading**.

Microalgae *Botryococcus braunii* was able to sustain heterotrophic growth in rice hull hydrolysate for 24 days in all concentrations tested. Yet, it was unable to grow in 100% hydrolysate, possibly due to inhibition. The optimum substrate concentration was **25% (v/v)**, where **6.55 ± 0.10 g L^{-1}** of dry biomass were produced.

Microalgae *Botryococcus braunii* was able to maintain growth in a novel two-stage cultivation process, successfully **accumulating phenolic compounds** during the autotrophic stage of its cultivation. However, more research is necessary in order to optimize both parts of the process.

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