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

P. Papavasileiou[1], S. Koutras[1], T. Tarsanis[1], E. Koutra[1], M. Kornaros[1]

[1]Department of Chemical Engineering, University of Patras, Patras, Achaia, 26504, Greece
Keywords: dilute acid chemical pretreatment, rice hull, microalgae, natural phenolic compounds
Presenting author email: papavasileiou@chemeng.upatras.gr

The term lignocellulosic biomass describes an enormous group of agro-industrial residues, consisting of mainly cellulose and hemicellulose, natural sugar-based polymers, as well as lignin, a complex aromatic polymer. Lignocellulosic residues, nutrient-rich and abundant, hold an immense potential for their further exploitation, as economic raw materials either for energy production (directly or indirectly) or for synthesis of several bio-based platform chemicals, including various value-added products, through the application of several advanced biological, chemical or thermophysical treatments. However, given the vastness of origin of these by-products, as well as the several technological bottlenecks that still need to be overcome during their processing, more research is needed towards the establishment of more efficient exploitation technologies, aiming to achieve a full transition towards a circular economy (Bilal et al., 2020).

Given the complex nature of their polymeric components and especially when biological processes are selected for the exploitation of lignocellulosic materials, specific pretreatments, like enzymatic or chemical, are usually applied to them to improve the processes' efficiency. Amongst these methods, chemical pretreatment with dilute acid is one the most reliable, fast, applicable, and effective methods, affecting primarily the structure of hemicellulose, and of cellulose at a smaller rate, yet presenting the disadvantage of potentially allowing the formation of inhibiting compounds, like furfural or HMF, undesirable for biological growth (Avci et al., 2013).

Microalgae are unicellular, photosynthetic microorganisms, eucaryotic and procaryotic (cyanobacteria), found in every aquatic environment on earth, extremely versatile and resilient. From a biotechnological aspect, they are considered as microscopic biological factories, able to synthesize an extremely large number of bioactive substances, including lipids, carbohydrates, proteins, pigments, antioxidants etc., with remarkable bioactivities, useful in several industrial sectors, like pharmaceuticals, cosmetics, aqua-cultures, food, etc (Koutra et al., 2018). For that purpose, research regarding microalgae cultivation has been in the forefront over the last decades, aiming to produce several marketable products from their biomass in an economic and sustainable way; yet solutions are still needed to encounter several issues during both upstream and downstream processing of the produced biomass. Among the several bioactive substances produced from microalgae, phenols, important natural antioxidants, used as additives in cosmetics, food products etc., are among the least studied and thereby exploited ones, hence their study presents a great potential (Safafar et al., 2015).

The present study is an effort to compromise the utilization of a lignocellulosic material, rice hull, as an economical substrate for the cultivation of microalgae towards the exploitation of its phenolic content. The focus of this investigation is directed towards three distinct topics: first, a parametric analysis of the chemical pretreatment of rice hull with dilute sulfuric acid is conducted, in order to discover the optimum hydrolytic conditions for an advanced sugars-solubilization; second, the exploitation of the liquid fraction of sugars-rich rice hull hydrolysate as a culture substrate for microalgae strain *Botryococcus braunii* is tested under heterotrophic conditions, aiming to discover the specific culture conditions for advanced biomass productivity; third, a composite two-stage culture of *B. braunii* utilizing both autotrophic and heterotrophic conditions is employed towards the acquirement of natural phenolic compounds from its biomass.

Regarding the study of chemical pretreatment of rice hull, the impact of four parameters at specific ranges, namely temperature (100 – 131ºC), duration of pretreatment (15 – 120 min), acid concentration (1 – 4%, v/v) and feedstock loading (4 – 40 %, w/v) was evaluated aiming to maximize the sugar-content and minimize the inhibitor-content of the produced hydrolysate. Based on our results, all tested parameters affected positively the saccharification of the hemicellulose fraction from rice hull, increasing the sugar-content of the hydrolysates, however inhibitors, mainly furfural, were formed as well when higher temperatures and stronger acidic conditions were applied to the material, suggesting degradation of the produced sugars to inhibitors. To sum up, the optimum pretreatment conditions were achieved at 121ºC, H_2SO_4 2% (v/v), 60 min, 30%, (w/v) feedstock loading.

Next, 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 – 25 %, v/v) to reach the most adequate culture conditions for our species. The two most productive substrate concentrations were 50% and 25%, (v/v) with maximum biomass concentrations 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.

Given the fact that under heterotrophic culture conditions, as described above, no phenolic compounds were produced from microalgae biomass, a two-stage culture process was utilized to derive the desirable product from our species biomass. According to our study, heterotrophic culture of *B. braunii* in rice hull hydrolysate (first stage) followed by autotrophic culture in synthetic medium (second stage) lead to the accumulation of valuable natural phenolic compounds in the produced biomass which reached its maximum value of 7.44 ± 0.60 mg G.A.E. g^{-1} DW at 14^{th} day of autotrophic culture. However, further optimization of this novel process is essential towards an advanced and more efficient cultivation of this species for phenolics production.

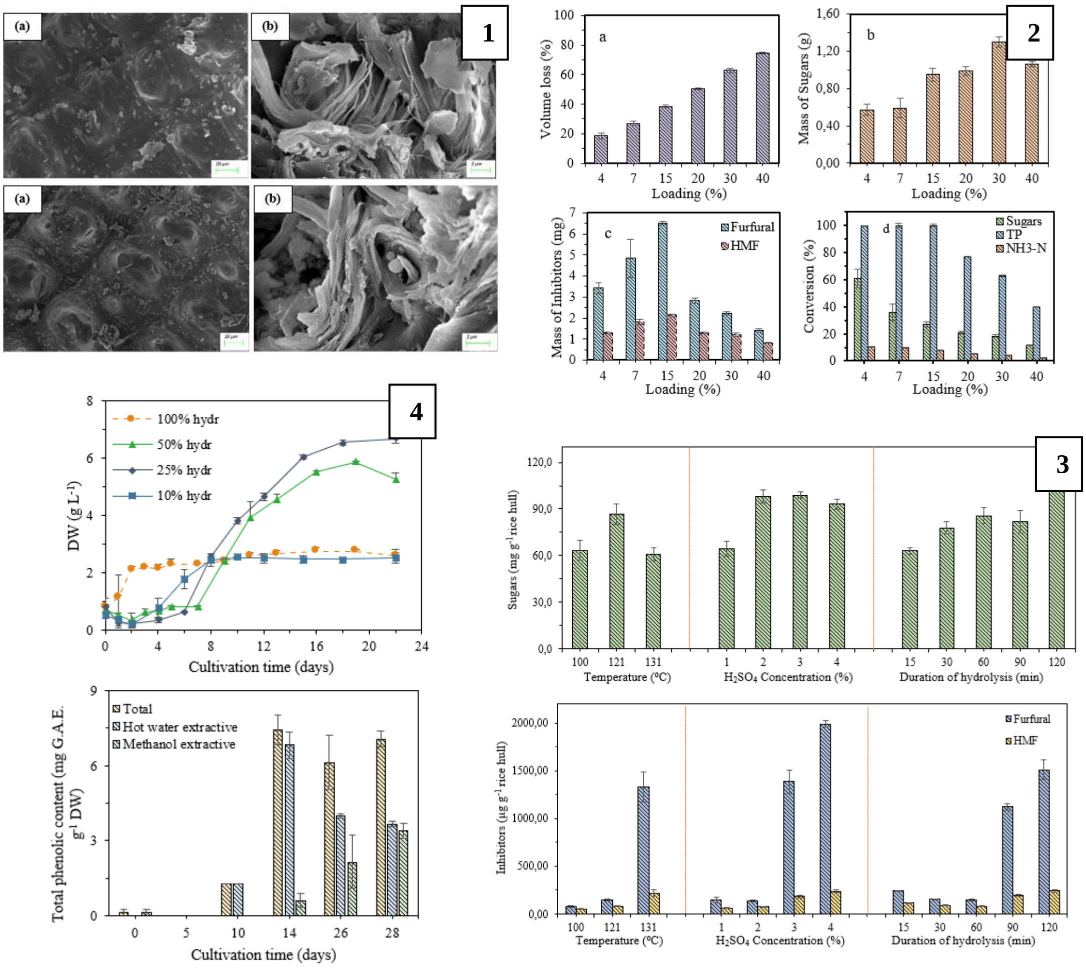

Figure 1: (1) SEM images (various magnifications) of rice hull pre- and post-treatment; (2-3) results from study of rice hull dilute sulfuric acid pretreatment, presenting the effect of tested parameters on sugars an inhibitors-content in hydrolysates; (4) *B. braunii* cultures in rice hull hydrolysate (heterotrophic stage) and phenolics accumulation in two-stage culture (autotrophic stage) are presented.

The research work was supported by the Hellenic Foundation for Research and Innovation (HFRI) under the HFRI PhD Fellowship grant (Fellowship Number: 1426).

Avci, A., Saha, B.C., Kennedy, G.J., Cotta, M.A., 2013. Bioresource Technology Dilute sulfuric acid pretreatment of corn stover for enzymatic hydrolysis and efficient ethanol production by recombinant Escherichia coli FBR5 without detoxification q. Bioresour. Technol. 142, 312–319. https://doi.org/10.1016/j.biortech.2013.05.002

Bilal, M., Wang, Z., Cui, J., Fernando, L., Ferreira, R., Naresh, R., Iqbal, M.N., 2020. Science of the Total Environment Environmental impact of lignocellulosic wastes and their effective exploitation as smart carriers – A drive towards greener and eco-friendlier biocatalytic systems 722. https://doi.org/10.1016/j.scitotenv.2020.137903

Koutra, E., Economou, C.N., Tsafrakidou, P., Kornaros, M., 2018. Bio-Based Products from Microalgae Cultivated in Digestates. Trends Biotechnol. xx. https://doi.org/10.1016/j.tibtech.2018.02.015

Safafar, H., Wagenen, J. Van, Møller, P., Jacobsen, C., 2015. Carotenoids , Phenolic Compounds and Tocopherols Contribute to the Antioxidative Properties of Some Microalgae Species Grown on Industrial Wastewater. Mar. Drugs 7339–7356. https://doi.org/10.3390/md13127069