Environmental Engineering and Management Journal

March 2012, Vol.11, No. 3, Supplement, S64
http://omicron.ch.tuiasi.ro/EEMJ/

"Gheorghe Asachi" Technical University of Iasi, Romania

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EVALUATION OF CAROB PULP AS FERMENTATION SUBSTRATE FOR BIOHYDROGEN AND ORGANIC ACIDS PRODUCTION: SUGARS RICHNESS *VS* TOXICITY POTENTIAL

M. Lima[1], J. Ortigueira[2], L. Alves[2], S.M. Paixão[2], P. Moura[*1,2]

*[1]ISCSEM–Instituto Superior de Ciências da Saúde Egas Moniz, Quinta da Granja, Monte de Caparica, 2829–511, Caparica, Portugal; [2]LNEG-National Laboratory of Energy and Geology, Bioenergy Unit, Estrada do Paço do Lumiar 22-1649-038, Lisboa, Portugal; *e-mail: patricia.moura@lneg.pt*

Abstract

Carob pulp (fruit without seed) represents about 90% of the total dry weight of the fruit containing a very high percentage of readily soluble sugars (up to 54% w/w). This high sugar content renders this raw material very suitable as fermentation substrate, *e.g.* in the production of biofuels. In this study, we tested the potential of carob pulp for the production of biohydrogen and organic acids by *Clostridium butyricum* and two microbial consortia. Since the use of carob pulp may be limited by its content in compounds with antimicrobial properties, a toxicological and chemical characterization was carried out.

Carob pulp was submitted to an aqueous extraction in order to obtain a sugar-rich extract and spent solids. These solids were subsequently washed to recover residual sugars and phenolic compounds. Both carob extract and spent solids were used as carbon sources in fermentation experiments. The specific rate of hydrogen and butyrate production in the fermentations of carob pulp extract by *C. butyricum* attained 0.24 and 0.1 $mmol.h^{-1}.g^{-1}$, respectively, which corresponds to higher values than the ones obtained with glucose. The fermentation by the microbial consortium (LE58) produced mainly acetic acid and generated 0.13 $mmolh^{-1}g^{-1}$ hydrogen. By the contrary, the inclusion of the spent solids in the fermentation media inhibited microbial growth. In order to better understand this antimicrobial potential and its possible correlation with phenols concentration, the spent solids were submitted to different extraction conditions. The resulting liquids were characterized and its toxicity was evaluated. The highest concentration of gallic acid equivalents (GAE) was obtained by using distilled water at 100 ºC, after 30 minutes of extraction. The concentration of total phenols, and sugars and pinitol reached 16.6 ± 0.4 mg GAE and 339 ± 31 mg sugars / g d.w. of spent carob pulp, respectively. All waters obtained from the extraction processes inhibited the growth of *Pseudomonas putida*. Shorter extraction times result in higher sugars:total phenols ratios and lower toxic levels. On the contrary, the water with the lowest sugar concentration, obtained under the severest extraction condition tested, produced the highest toxicity level of 16.54 toxic units. The results showed that the upgrading of carob pulp for the biological production of hydrogen and organic acids is possible. Any fermentation process optimization aimed at upgrading spent carob pulp by the use of residual sugars must consider short extraction times and explore the different solubilisation profiles of carob's sugars and phenolic compounds.