https://icce2019.org/

Mild oxidative organosolv pretreatment of lignocelullosic biomass residues for high added value chemicals and food additives via fermentation processes

Kalogiannis K.[1], Michailof M.[1], Lappas A. [1], Karnaouri A.[2], Chalima A.[2], Topakas E.[2]

[1]Chemical Process & Energy Resources Institute, 6th km Harilaou-Thermi Road, 57001, Thessaloniki, Greece
[2]Biotechnology Laboratory, School of Chemical Engineering, National Technical University of Athens, 5 Iroon Polytechniou Str., Athens 15780, Greece

Abstract

Introduction

Lignocellulosic biomass is an attractive source of fermentable carbohydrates and aromatic compounds, found in the form of lignin, which can be converted to a variety of high added value chemicals. These range from aromatic lignin derived compounds for liquid transportation fuels and phenolic resins to food additives such as Polyunsaturated fatty acids (PUFAs) and prebiotics to novel biocompatible polymers such as poly- lactic acid. Key to successful valorization of biomass is its fractionation towards its 3 main building blocks, cellulose, hemicellulose and lignin. Lignin specifically has been found to be a crucial factor limiting the enzymatic hydrolysis of biomass by acting as a physical barrier between enzymes and holocellulose. Recently, a new pretreatment method, the acetone/water oxidation (AWO), has been developed exhibiting the advantages of wet oxidation such as relatively low temperatures and low yield of degradation products while achieving higher lignin removal (Katsimpouras et al., 2017). In this work, several different process parameters are investigated in an effort to optimize the fractionation process. The derived cellulose is biochemically converted towards PUFAs. PUFAs, especially those with very long chains (LC-PUFAs) such as eicosapentaenoicacid (EPA) and docosahexaenoic acid (DHA), have been widely recognized as important bioactive compounds that can be used in the food and nutraceutical industry. Microalgae are oleaginous microorganisms that have attracted attention, as they are able to accumulate high amounts of LC-PUFAs, when growing in heterotrophic cultures, supported by a carbon source (Santos-Sánchez et al. 2016). At present, industrial production of heterotrophic microalgae is hampered by the high costs of glucose. Using lignocellulosic biomass as a substrate is a promising strategy for developing a sustainable bio-economy. Despite the challenges of the process, concerning the presence of inhibitors and the heterogeneity (hexoses/pentoses) of sugars in the biomass fractions, different substrates, such as rice straw hydrolysates (Joe et al. 2015) have been successfully used in heterotrophic and mixotrophic microalgae cultivations for the production of lipids. The heterotrophic micro alga *Crypthecodinium cohnii* is an interesting source medium for DHA production and for research on DHA biosynthesis due to its unique fatty acid composition.

C. cohnii can accumulate relatively high amounts of lipid (>20%), of which 30–50% can be DHA of the fatty acids while no other polyunsaturated fatty acids is present above 1% (de Swarf et al. 2003).

Experimental

All oxidative organosolv runs took place in a Hastelloy C-276 Parr reactor with a volume of 975 ml. The ratio of biomass to liquid solvents was 1/10. The reactor was heated at temperatures between 150 to 175 ^{o}C and reaction time was 1 or 2 hours. *C. cohnii* was used as microalgae organism towards the study of the efficient production of omega-3 fatty acids from lignocellulosic biomass. Microalgal cells were grown in standing cultures on standard medium containing 9 g/L glucose, 18.7 g/L sea salts and 2 g/L yeast extract with the pH of the medium set at 6.5, at 23°C in the dark. The standing cultures were used as inoculum for precultures. Shaken flask cultivations (precultures and shaken flask experiments), containing 25 ml medium in 50-ml shake flasks, were carried out at 27^{o}C, pH 6.5, 160 rpm in a linear shaker. The cultures were inoculated with 10% (v/v) inoculum and incubated for 184h at 27^{o}C. At the end of cultivation, cells were harvested, washed, lyophilized, and weighed in order to determine the biomass concentration.

Results and Discussion

Mixtures of water with different organic solvents such as Acetone (AC), Ethanol (EtOH) and Tetrahydrofuran (THF) were used. Gas O_2 was used as a mild oxidizing medium that allowed the cleaving of lignin ether bonds and increased the achieved delignification. Delignification degree (DD) and cellulose and hemicellulose recoveries in the solid product are presented in figure 1. The organic solvents proved to be efficient in different temperature regimes. THF was very efficient removing more than 70% of the lignin at the lowest temperature of 150 ^{o}C. AC and EtOH on the other hand required higher temperatures to effectively remove lignin, however at the highest temperature of 175 ^{o}C they achieved more than 95% DD. What was even more important was the fact that 100% of the cellulose was retained in the solid pulp, indicating minimal cellulose degradation.

The other parameters investigated were time, temperature and initial O_2 pressure. Increasing time and temperature had a positive effect on the DD while an enhancement of the hemicellulose depolymerization and hence solubilization was also noted. In all cases almost 100% recovery of the cellulose in the solid was observed. Use of mineral acids such as H_2SO_4 typically used in biomass pretreatment results in acidic wastes, need for corrosive resistant reactors and degradation products that have inhibitory effect in downstream biological conversions. In our work gas O_2 successfully replaced homogeneous acidic catalysis.

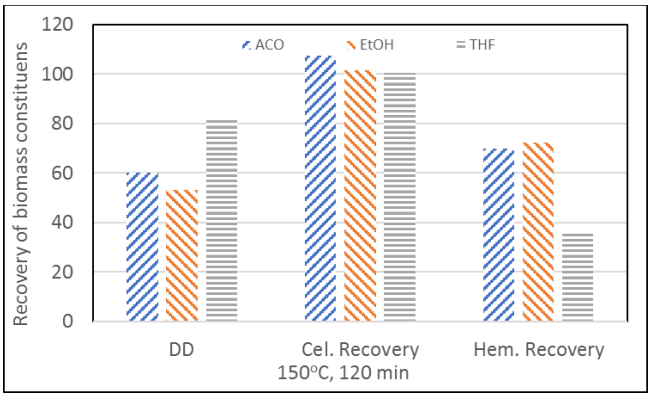

Figure 1: Delignification degree (DD), cellulose and hemicellulose recoveries in the solid pulp after mild oxidative organosolv pretreatment at 150 ^{o}C for 120 min with different solvents.

First, preliminary experiments were conducted using glucose as a carbon source in order to evaluate the effect of different process parameters, such as the initial concentration of carbon and nitrogen source and the feeding strategy, on the growth and the accumulation of fatty acids. At concentrations above 25 g/L, glucose

inhibits growth of *C. cohnii* (de Swaaf et al 1999) as well as lipid accumulation in shake-flask cultures. As shown in Table 1, the lipid content of cells grown on glucose was 41.1% when yeast extract was added at a concentration of 2 g/L, whereas cells grown on 4 g/L nitrogen source contained less, 28%. In general, lipid accumulation in microorganisms is stimulated by an excess of a carbon source and a limitation in one (or more) of the other nutrients, especially nitrogen. The DHA content of the lipids was quite similar (37.1% and 40.4% respectively). The results showed that both growth and lipid accumulation are affected when initial glucose concentration is higher, so a fed-batch strategy was chosen for the case of lignocellulosic biomass-derived sugars.

Table 1: Biomass and total fatty acid (TFA) production by C. cohnii cells in fed-batch cultivation in shake flasks, using either pure glucose (Glc) or biomass-derived sugars (BDS), under different conditions of nitrogen source supply (YE: yeast extract).

Glc (g/L)	YE (g/L)	Additional feeding (96h)	Biomass (g/L)	TFA (g/L)	DHA (g/L)	TFA (% of dry biomass)	DHA (% of TFA)
25	2	25 g/L Glc	15.0 ± 1.3	6.1 ± 0.2	2.3 ± 0.1	41.1 ± 2.3	37.1 ± 1.3
25	4	25 g/lt Glc	20.8 ± 2.6	5.8 ± 0.4	2.3 ± 0.4	28.0 ± 1.6	40.4 ± 3.5
25	2	25 g/L Glc/2g/L YE	16.1 ± 1.4	4.8 ± 0.1	2.1 ± 0.0	30.2 ± 3.6	43.2 ± 1.4
50	4	-	17.7 ± 1.0	5.1 ± 0.2	2.1 ± 0.1	28.8 ± 0.6	40.6 ± 2.3
25*	4*	25 g/L BDS*	9.2 ± 0.7	1.1 ± 0.0	0.5 ± 0.0	22.6 ± 0.2	40.3 ± 0.4

**lignocellulosic biomass-derived sugars were used as carbon source*

The results showed that *C. cohnii* was able to grow on glucose and other sugars produced from the enzymatic hydrolysis of lignocellulosic biomass, although the biomass and the total lipid production was lower compared to that pure glucose feeding. This is an indication that not only the carbon source but also the medium composition is important for the accumulation process in C. cohnii. Among the factors that could possibly affect the growth and metabolism of microalgae cells is the presence of other sugars except for glucose, such as xylose, and the presence of phenolic compounds. Lipid accumulation and DHA content of the lipids was similar to that retained with glucose, reaching 22.6% and 40.31% respectively. Although more experiments need to be conducted in order to optimize the whole process, the overall results of this work demonstrate that there is a potential of lignocellulosic biomass to be utilized for the production for value-added products, such as DHA and other omega-3 fatty acids, through environmentally friendly bioconversion processes.

Acknowledgements

This project has received funding from the Hellenic Foundation for Research and Innovation (HFRI) and the General Secretariat for Research and Technology (GSRT), under grant agreement No 1085.

References

C. Katsimpouras, K.G. Kalogiannis, A. Kalogianni, A.A. Lappas and E. Topakas, Biotechnol Biofuels., 2017, 10, 54.

de Swaaf ME, Sijtsma L, Pronk JT. (2003). High-cell-density fed-batch cultivation of the docosahexaenoic acid producing marine alga Crypthecodinium cohnii. Biotechnol Bioeng. 81(6):666-72.

Joe MH, Kim JY, Lim S, Kim DH, Bai S, Park H et al. (2015). Microalgal lipid production using the hydrolysates of rice straw pretreated with gamma irradiation and alkali solution. Biotechnol Biofuels. 8:125.

Santos-Sánchez NF, Valadez-Blanco R, Hernández-Carlos B, Torres-Ariño A, Guadarrama-Mendoza PC, Salas-Coronado R. (2016). Lipids rich in ω-3 polyunsaturated fatty acids from microalgae. Appl Microbiol Biotechnol. 100(20):8667-84.