Enzymatic cell wall disruption of *Chlorella sorokiniana* grown under heterotrophic conditions for sustainable biodiesel production

Max Cardenas-Fernandez[1], Nelson H. Caicedo-Ortega[3], Maria F. Villegas-Torres[2], and Frank Baganz[1]*.

[1]Department of Biochemical Engineering, University College London, London, UK. [2]Department of Chemical Science, Universidad ICESI, Cali, Colombia.[3]Departament of Biochemical Engineering, Universidad ICESI, Cali, Colombia.

Introduction

Microalgae cultivation under heterotrophic conditions broadens the possibilities to transform organic residues, which promotes these microorganisms as potential agents for several bioprocesses including the synthesis of lipids.

Microalgae downstream processing is one of the limiting factors for its wider application for biodiesel production, mainly due to the high energy utilisation and cost during the harvesting stage. Here, microalgae recovery by flocculation followed by enzymatic treatment for cell wall disruption, as low energy utilisation method, and lipid extraction is presented.

Materials and Methods

Chlorella sorokiniana (UTEX 1230) was grown heterotrophically in TAP medium supplemented with glucose 10 g L^{-1} at 30 °C for up to 8 days. Culture samples were taken periodically for biomass, pH and acetate, glucose determination as well as lipid content.

Flocculation was carried out with chitosan 22 mg $gdcw^{-1}$ at pH 6. For cell wall disruption, the microalga was treated with hydrolytic enzymes such as chitinase, alkaline protease, lysozyme and viscozyme. Then, intracellular lipids were extracted with n-hexane.

Results

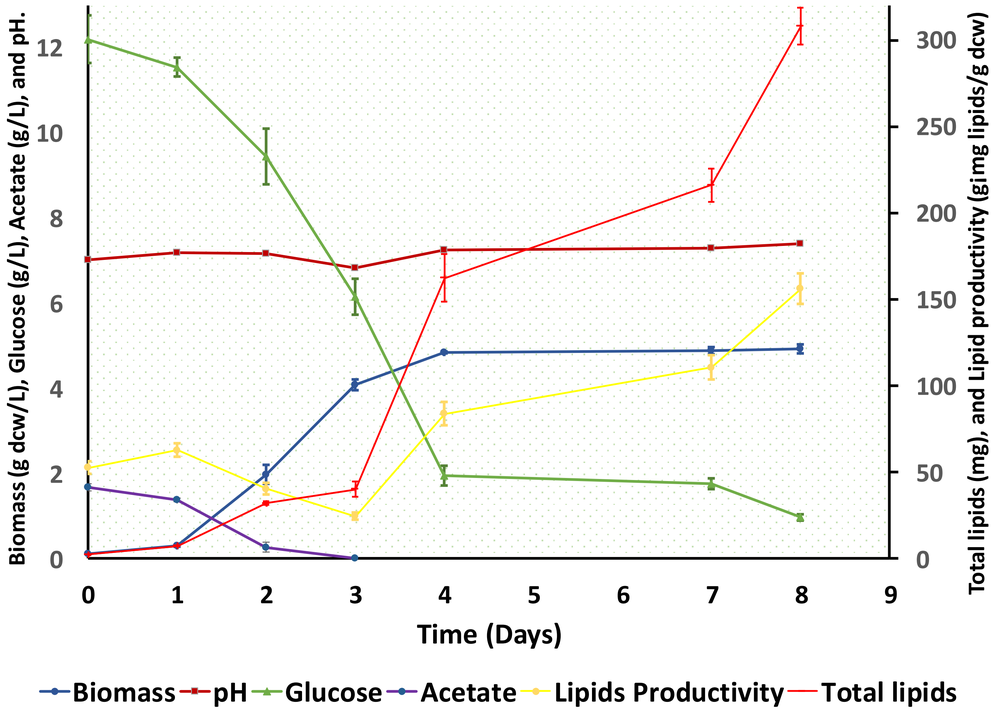

Figure 1. Lipid profile (as fatty acid methyl esters – FAMEs) of microalgal biomass grown heterotrophycally on TAP médium. Bar error: SD

Maximum level of biomass (4.8 g L^{-1}) was reached after 4 days of cultivation whilst lipid content of 770 mg L^{-1} (308,1 mg) was achieved after 8 days. FAMEs profile showed that palmitic, linoleic, oleic and stearic acids are the predominant fatty acids accounting for around 93 % of total FAMEs identified (**Table 1**). Viscozyme showed the best activity for cell wall disruption, achieving monomerisation of polysaccharides present in the microalgae cell wall, releasing mainly glucose, fructose and galactose (**Figure 2**). Double extraction of intracellular lipids with n-hexane after viscozyme treatment allowed recovering 70 % of lipids related to total extractable lipids using sonication and chloroform:methanol extraction (**Figure 3**).

Contact: f.baganz@ucl.ac.uk

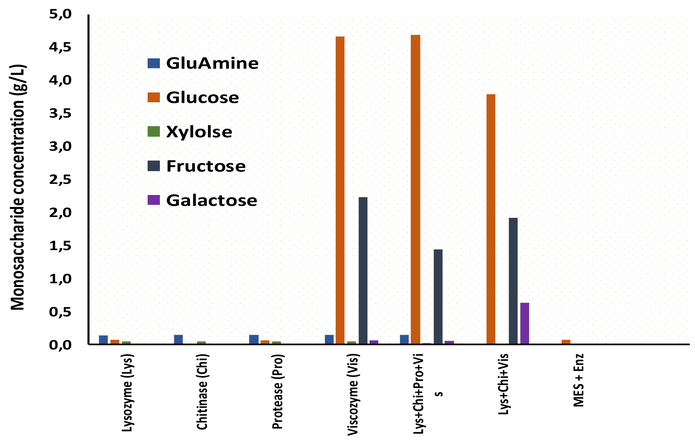

Figure 2. Monosaccharide concentration released after enzymatic treatment of cell of *Chlorella sorokiniana* grown heterotrophycally on TAP medium.

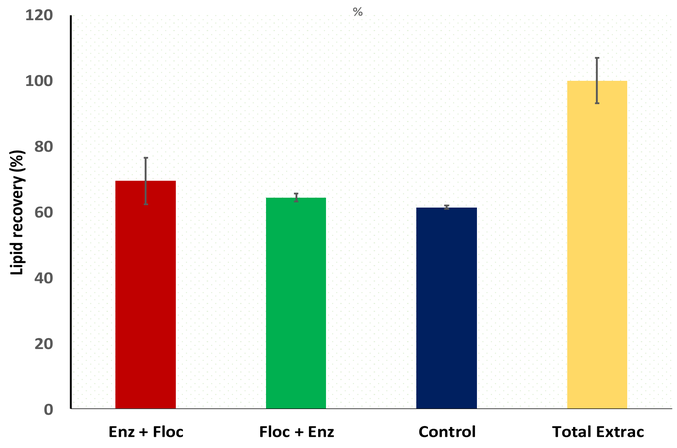

Figure 3. Lipid profile (as fatty acid methyl esters – FAMEs) of microalgal biomass (*Chlorella sorokiniana*) grown heterotrophycally on TAP medium.
Bar error: SD

Enz + Floc: Enzymatic treatment with viscozyme followed by flocculation.
Floc + Enz: Flocculation with chitosan followed by enzymatic treatment with viscozyme.
Control: Direct extraction with hexane (without any type of treatment).
Total Extraction: Extraction by using methanol:chloroform.

Fatty acid as FAMES	(%) ± SD
7,10-Hexadecadienoic acid	2.9 ± 0.3
7,10,13-Hexadecatrienoic acid	1.5 ± 0.4
7-Hexadecenoic acid	2.4 ± 0.5
Hexadecanoic acid (palmitic acid)	38.7 ± 1.5
Heptadecanoic acid	0.4 ± 0.1
9,12-Octadecadienoic acid (Lioleic acid)	23.7 ± 0.8
9/11/16-Octadecenoic acid (Oleic acid)	28.8 ± 2.0
Octadecanoic acid (Stearic acid)	1.6 ± 0.0

Table 1. Lipid profile (as fatty acid methyl esters – FAMEs) of microalgal biomass grown heterotrophycally on TAP medium.

Conclusions

These results demonstrate the feasibility of establishing a wet microalgae downstream process using flocculation for microalgae harvesting followed by enzymatic treatment for cell wall disruption and organic solvent extraction of lipids. Enzymatic transesterification with lipases could potentially be performed as well as the recovery of sugars released to be reused in the cultivation process.