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Use of Cell Wall Degrading Enzymes to Improve the Recovery of Lipids from *Chlorella sorokiniana*

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Highlights

- Enzyme-assisted extraction of lipids from *C. sorokiniana* was studied.
- Commercial enzyme preparations with different enzyme activities were screened.
- Optimized enzyme cocktails were prepared using the mixture design method.
- High lipid recovery was obtained from the enzymatically treated microalgae.

1. Introduction

The recovery of lipids or other intracellular compounds from microalgae is a challenging and costly operation due to the presence, in these organisms, of a thick multilayered cell wall acting as a barrier to solvent penetration. Therefore, before performing extraction, the cell wall must be broken down. Cell wall disruption is generally accomplished by physical (e.g., ultrasonication, high-pressure homogenization, bead beating) or chemical (e.g., alkali, acid, detergent) methods. Recently, the use of cell wall degrading enzymes has been proposed as a new and sustainable approach to achieve high extraction yields without affecting the properties of the extracted compounds [1].

Despite the potential of enzymatic methods, economic factors and the experimental effort required to find appropriate enzyme systems still hinder their industrial implementation. In a recent study, we used a combination of commercial enzyme preparations of relatively low cost to improve the recovery of lipids from *Nannochloropsis* sp. [2]. Enzyme cocktails with enhanced degradation activity were also prepared using the mixture design method [3]. However, the differences in the compositional and architectural features of microalgal cell walls and the limited number of studies do not allow for definitive conclusions on these methods. Accordingly, the aim of the present study was to evaluate whether the above approach could be applied to other microalgae species. We focused on *Chlorella sorokiniana*, a microalga of industrial interest for its ability to accumulate large amounts of lipids when grown under suitable conditions.

2. Methods

C. sorokiniana (strain 211-8k) was obtained from the Sammlung von Algenkulturen Göttingen (SAG) at the University of Göttingen (Germany). The microalga was grown in a 6-L helical tubular photobioreactor coupled with a degasser, as described elsewhere [4].

Lysozyme was purchased from EPS SPA (Rovigo, Italy), while the other enzyme products (Cellulyve 50LC, Peclyve EXVG and V, Feedlyve AGL, AXC and GMA, Filterlyve AXC and PEM, Superlyve SH) were from Lyven SA (Colombelles, France). All chemicals were of reagent grade and used without further purification.

Experiments were carried out in batch at 50 °C and pH 5 by contacting 0.5 g of dry microalgae with 20 mL of distilled water and 0.25 mL of the enzyme solution. After 5 h, the liquid was removed and the biomass was extracted with n-hexane/2-propanol (3:2, v/v) to recover the lipids.

3. Results and discussion

Preliminary screening of the enzyme preparations showed that some of them were highly effective in improving the recovery of lipids from *C. sorokiniana*, while others had limited or no effect. The most effective preparations (Lysozyme, Peclyve V, Feedlyve AXC and GMA) were taken as basic components for the formulation of enzyme mixtures.

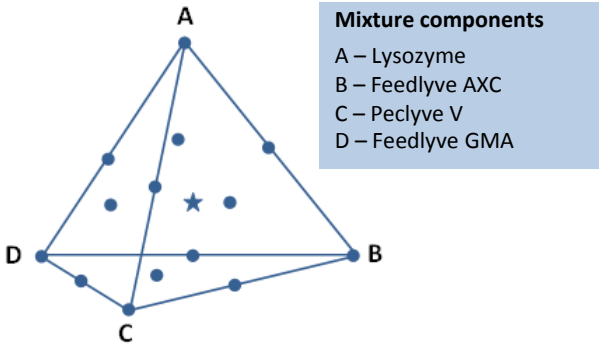

Figure 1. The simplex lattice design for a four-component enzyme mixture.

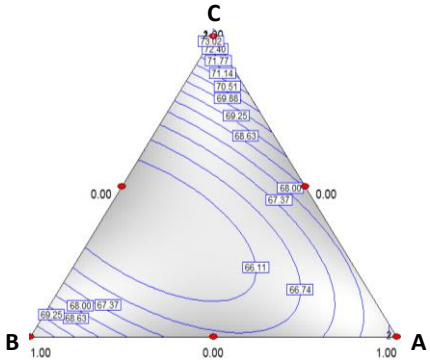

Figure 2. Contour plots showing the influence of mixture components on lipid extraction.

A {4,3} simplex lattice design with replicated vertices and central point (total design points: 20) was used (Figure 1). The amount of extracted lipids, expressed as a percentage of the total lipid content of the biomass, was taken as the response variable. The experimental results were analysed by different Scheffé canonical polynomial models. The two-factor interaction model provided the best result. Model coefficients were estimated by minimizing the sum of squared errors between experimental and calculated values. Analysis of residuals showed no apparent departures from basic ANOVA assumptions. In addition, the lack of fit was not significant, further supporting the adequacy of the model. An example of the contour plots generated from the model equation is shown in Figure 2.

The values of the interaction coefficients and the 3D response surface plots revealed both synergistic and antagonistic effects. The former can be explained in terms of enhanced destructuring of the cellulose–hemicellulose network due to the combined action of cellulase and hemicellulase. Antagonistic effects can arise from competitive non-productive enzyme adsorption. This phenomenon occurs in multi-enzyme systems when enzyme molecules adsorb temporarily on sites that they cannot attack. If the number of sites occupied in this way is sufficiently high, the adsorbed species may cause steric hindrance to the enzymes that are specific for those sites, leading to a decreased degradation rate.

To formulate an optimal enzyme mixture, the extraction yield calculated from the model equation was maximized. A maximum yield close to 76% was estimated. The optimal mixture contained xylanase and galactomannanase as main enzyme components, in addition to cellulase side activities. Validation experiments with this enzyme mixture gave an extraction yield of about 77%, compared to the value of about 35% determined for the untreated biomass.

4. Conclusions

The results of this study indicate that the recovery of lipids from *C. sorokiniana* can be significantly improved by pretreating the biomass with cell wall degrading enzyme mixtures. Optimized enzyme cocktails can be easily prepared using the mixture design method. An optimization of the treatment conditions, such as temperature, pH and enzyme dosage, could likely lead to further enhancement of lipid recovery.

References

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Keywords
Cell wall degrading enzymes; Lipid recovery; Microalgae; Mixture design.