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Pulsed electric field assisted extraction of intracellular valuables from microalgae

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ABSTRACT

This paper discusses the application of pulse electric field (PEF) treatment for the cell disintegration of the fresh water microalgae *Auxenochlorella protothecoides*. The algae were cultivated under controlled conditions in a closed photo-bioreactor. After algae harvest the algae suspensions were concentrated and PEF treated with square pulses with a duration of 1 μs. We investigated the influence of specific treatment energy (52–211 kJ/kg suspension), electric field strength (23–43 kV/cm) and biomass concentration (36–167 g dry weight per kg suspension) on cell disintegration. For all pulse parameters applied, the PEF induced cell disintegration resulted in the release of soluble intracellular matter into the suspension. The disintegration efficiency increased with increasing specific treatment energy, whereas the field strength hardly had any influence. For suspensions with a biomass content of 100 g dry weight per kg suspension the electrical energy input necessary for considerable cell rupture was in the range of 1 MJ/kg dried algae. This is equivalent to 4.8% of the upper heating value of the algae. Although the treated algae contained lipids, PEF treatment only led to the spontaneous release of soluble components. The selectiveness of the process might offer the opportunity to use PEF treatment in a biorefinery concept, where soluble algae ingredients are extracted before solvent extraction of lipids is performed.

1. Introduction

In the past years microalgae gained a lot of attention because they are considered to be a promising, renewable feedstock for food, feed, fine chemicals and biofuel production. For example algae can produce lipids, pigments, unsaturated fatty acids or polysaccharides at comparably high production rates [1]. However, up to now they are mainly used for the production of high value low volume products. Among the reasons for this are the costly scale-up of algae cultivation and the rather difficult downstream processing [2].

Many valuables produced by microalgae are stored intracellular and the extraction of these products involves a cell disintegration step. Algae cells have strong cell walls [3], making cell disintegration and extraction rather difficult and energy intensive. For example, in the case of biodiesel production from microalgae, 30–50% of the production cost is due to the extraction step which includes also cell disruption [2]. Hence, the search for efficient, appropriate cell disintegration methods is vital to increase competitiveness of algae for the production of high volume, low cost substances like biofuels.

An appropriate cell disintegration process has to maximize the yield and the value of the compounds extracted [4]. In other words it disintegrates all the cells precisely without chemical contamination or degradation of the target compounds. For large scale production it is also important that the disintegration process can be scaled up and that it is rapid. In addition the integration of the cell disruption into the downstream processing has to be easy and it should not have a negative impact on subsequent processing steps, e.g. by hampering separation. All these properties influence the overall efficiency of the disintegration process and therefore its overall energy consumption, which is the crucial issue in biofuel production.

Different procedures of algae cell disruption were investigated, e.g. chemical, mechanical, thermal, or enzymatic methods. In most cases solvent extraction alone was less efficient than its combination with cell disruption methods [5–7]. Lee et al. and Prabakaran and Ravindran compared the effectiveness of autoclaving, bead-beating, sonication, osmotic shock and microwaves for the disintegration of different microalgae species [5,6]. Both groups found that the performance of the solvent extraction did not only depend on the disintegration method but also on the algae species. Teixeira who performed the lysis of different microalgae with hydrophilic ionic liquids reported a complete deconstruction of different microalgae species at temperatures between 100 and 140 °C [2]. Another approach is the cell lysis with alkali or acids at ambient temperatures, which has been found to be counterproductive in the case of astaxanthin recovery from

Abbreviations: TOC, Total Organic Carbon; HOC, Hydrophobic Organic Carbon; UHV, Upper Heating Value; CH, Carbohydrates; dw, dry weight; sus, suspension; R, Release-factor; 0, control untreated suspension; P, PEF treated suspension; σ_0, conductivity of untreated suspension; σ_{PEF}, conductivity of PEF treated suspension; c_x, biomass concentration; MO, Microorganism.

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Haematococcus pluvialis due to product degradation [8]. Seibert and Halim et al. performed cell disruption of microalgae using high-pressure homogenization and ball milling. According these studies, high-pressure homogenization was the more effective disintegration method for microalgae [9,10]. In most of the studies carried out, the scale up, the integration in the overall process and the energy consumption were not discussed. The studies revealed that further research is needed, in order to find other cell disruption methods that fulfill the above mentioned criteria.

Pulsed electric field (PEF) treatment may be a promising alternative to conventional cell disintegration methods. The exposure of biological cells to high intensity electric field pulses can alter the structure of the cell membrane. The external field provokes a charging of the membrane. At a sufficiently high transmembrane voltage (~0.5–1 V) the arrangement of the phospholipid molecules changes [11,12]. As a result the membrane looses its barrier function and becomes permeable, a phenomenon often referred to as "electroporation" or "electropermeabilization" [11,13]. According to literature, the major electrical parameters that influence the electropermeabilization efficiency are field strength of the external field, pulse shape, pulse duration, number of pulses applied, and specific treatment energy [11,14].

Depending on the electrical conditions, this membrane permeabilization can be reversible or irreversible. For an effective extraction of intracellular valuables, an irreversible cell membrane permeabilization is necessary. This membrane rupture promotes the release of intracellular matter and facilitates solvent access into the cell. PEF is a comparably gentle cell disintegration process, since it is usually performed at ambient temperatures and does not introduce additional impurities into the process. Hence, it helps to prevent undesirable changes in the target material [15]. PEF can be performed in batch and continuous mode and it is scalable. For example Sack et al. realized an industrial scale PEF device for the continuous treatment of sugar beets that can process 10 t beets per hour [12]. PEF has not only been used to foster the extraction of soluble compounds from plant tissue, it has also been employed to accelerate the extraction of vegetal oils, such as olive oil and maize oil [16].

Although PEF has been used for the enhancement of the extraction of lipids and other cell ingredients from terrestrial plants its application to algae is relatively new [17]. This work aimed to investigate the efficiency of the PEF treatment for the disintegration of microalgae cells. The fresh water microalgae *Auxenochlorella protothecoides* were PEF treated in a continuous process at different pulse parameters. The influence of electric field strength, specific energy input, algae concentration and diffusion time on the extraction efficiency is discussed. In addition the study addressed issues of the energy consumption and the integration of the PEF treatment in the downstream processing.

2. Materials and methods

2.1. Microalgae and cultivation

The microalgae strain used throughout this work was *A. protothecoides* obtained from EPSAG Göttingen, SAG strain number: 211-7a (Fig. 1). The algae were grown mixotrophically and axenically in TAP-medium [18], exhibiting an initial conductivity of 1.4 mS/cm at 25 °C. The batch-cultivations were carried out in a 26 l annular bubble column at 25 °C. The photo-bioreactor was illuminated continuously at 600 μE/s/m^2 by 8 fluorescence lamps (Osram fluora, 36 W). The culture was aerated at a rate of 1000 cm^3/min with an air carbohydrate mixture containing 2.5% carbon dioxide. For maximizing the amount of biomass available for PEF parameter studies, the algae were harvested after 29–35 days during the stationary growth-phase at a biomass concentration of 4.5 ± 0.5 g_{dw}/kg_{sus}. The algal suspensions from the photo-bioreactor were concentrated 10–35 times using a centrifuge (swinging-bucket rotor, 3000 *g*, 20 °C). In this way, algae suspensions with biomass concentrations of 36 to 167 g_{dw}/kg_{sus} were obtained. The conductivity of the concentrated suspensions was not adjusted and ranged between 0.9 and 1.0 mS/cm. The concentrated suspensions were stored at ambient temperature. The PEF treatment was performed within 3 h after the concentration step.

2.2. Pulsed electric field treatment

The algae suspensions were treated in the treatment chamber, shown in Fig. 2. The chamber was constructed for continuous flow treatment of biomass suspensions and manufactured at the Institute of Pulsed Power and Microwave Technology (Karlsruhe Institute of Technology, Germany). It consists of two stainless steel electrodes with a diameter of 60 mm paired in parallel and separated to a gap distance of d = 4 mm by a transparent polycarbonate housing. The treatment volume, length = 47 mm and width = 11 mm, was perfused from bottom to top allowing gas bubbles to exit the chamber. The field distribution in the treatment volume is uniform. The treatment chamber was connected to the output line of a transmission line pulse generator that delivered square pulses with a voltage amplitude between 8 kV and 20 kV, corresponding to field strengths of 20 kV/cm to 50 kV/cm. Fig. 3 shows a typical waveform of the output pulse measured across the treatment chamber by a 40 kV high voltage probe (P6015, Tektronix) during continuous treatment of pre-concentrated microalgae suspension. A pulse amplitude of 14 kV represents an electric field strength of 35 kV/cm in the treatment volume. The pulse rise time is less than 20 ns. The details on the transmission line pulse generator and the acquisition system were reported in our previous work [19].

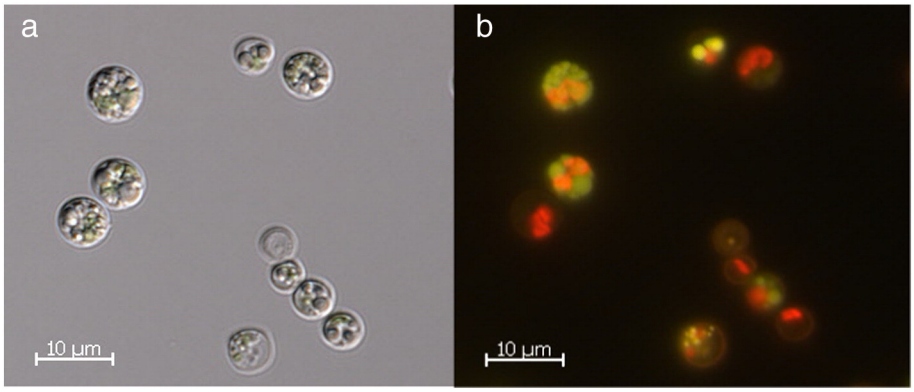

Fig. 1. *Auxenochlorella protothecoides*; 630 times magnified: (a) light-microscope; (b) lipid droplets dyed with Nile-Red (yellow fluorescence). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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