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Impact of changes in broth composition on *Chlorella vulgaris* cultivation in a membrane photobioreactor (MPBR) with permeate recycle

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HIGHLIGHTS

- A membrane photobioreactor (MPBR) was applied to cultivate *C. vulgaris*.
- Composition of the broth was examined during batch and continuous cultivation.
- The composition of the broth had influence on microalgae growth.
- There is a limit on applicable medium recycle.

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ABSTRACT

A membrane photobioreactor (MPBR) is a proven and very useful concept in which microalgae can be simultaneously cultivated and pre-harvested. However, the behavior with respect to accumulation of algogenic organic matter, including transparent exopolymeric particles (TEPs), counter ions and unassimilated nutrients due to the recycling of the medium is still unclear, even though the understanding of this behavior is essential for the optimization of microalgae processing. Therefore, the dynamics of these compounds, especially TEPs, during coupled cultivation and harvesting of *Chlorella vulgaris* in an MPBR with permeate recycle are addressed in this study. Results show that TEPs are secreted during algae cell growth, and that their presence is thus inevitable. In the system with permeate recycle, substances such as counter ions and unassimilated nutrients get accumulated in the system. This was proven to limit the algae growth, together with the occurrence of bioflocculation due to an increasing broth pH.

1. Introduction

Microalgae have been the subject of research for decades, especially since the 1980s, due to their possible use for biodiesel production. Despite their high production costs and due to the rising prices of conventional fuels and the global warming problems, microalgae keep reappearing as a more promising feedstock option than other bio-based crops (Greenwell et al., 2010). Nevertheless, it seems very unlikely that the process will be developed for biodiesel as the only end-product from microalgal biomass (Walker, 2009; Lam and Lee, 2011). Microalgae can be of interest in other industries too, i.e. as raw material for high-value products (Christenson and Sims, 2011), or in the treatment of wastewater (Park et al., 2011). Still, for long-term sustainability, all processing stages of microalgae should be simplified and energy input should be substantially decreased (Lam and Lee, 2011). The cultivation and the dewatering stage are two of the most critical stages where improvement is needed (Greenwell et al., 2010).

Open raceway ponds and closed photobioreactors (PBRs) are two common ways to cultivate microalgae (Greenwell et al., 2010). Closed PBRs, despite being more expensive in operation, offer several advantages over raceway ponds, such as limited contamination, higher culture densities and better control over physico-chemical conditions. The biggest limitation on productivity in PBRs is the inherent biomass wash-out, which is the disappearance of the microalgae due to a high dilution rate (short residence time), resulting in a harvesting rate (via the outlet) that is higher than the reproduction rate (growth). To prevent this, decoupling of the microalgal biomass retention time (MRT) and the dilution rate (D) is needed. One possible way of doing this is by running the PBR in membrane photobioreactor (MPBR) mode by coupling the cultivation tank to a membrane filtration unit. In the MPBR, the membrane provides complete retention of microalgal cells, thus preventing wash-out and increasing the achievable biomass concentration in the bioreactor, while the medium (water and remaining nutrients) passes as permeate. The biomass concentration can also be better controlled with a separate filtration tank by partly returning the retentate to the MPBR. Recently, the effectiveness of the MPBR system for microalgal biomass cultivation

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and pre-harvesting was proven (Bilad et al., 2013; Honda et al., 2012). Because of the higher flexibility and robustness, the MPBR could operate at both higher dilution and higher growth rates, resulting in a 9× higher biomass productivity compared to the PBR (Bilad et al., 2013). In addition, pre–harvesting could be achieved by applying variable concentration factors. The remaining nutrients in the permeate could be recycled to the reactor as feed medium with minimum effect on the growth. This way, a substantial reduction in the water footprint and in nutrient costs could be achieved (Bilad et al., 2013). Recycling culture media is even considered a key issue for the development of large-scale cultures to minimize water and nutrients consumption (Hadj-Romdhane et al., 2012, 2013), especially considering the depleting sources of a few important nutrients (e.g. phosphorous). Another significant advantage of MPBRs is that they can serve as an effective way of combining wastewater treatment with biomass production (Honda et al., 2012). Although the MPBR shows many advantages, a close monitoring over a prolonged cultivation is necessary to fully assess its behavior, especially when aimed for permeate recycle. Some metabolite products, known as algogenic organic matter (AOM, organic material produced by microalgae), and non-assimilated nutrients are expected to accumulate, which may hinder the prospect of MPBR technology. Understanding and remediating those detrimental effects should be key to applying MPBRs for simultaneous cultivation and pre-harvesting of microalgae.

AOM has been extensively studied (Henderson et al., 2008). It mainly consists of polysaccharides (80–90%) that form dynamic micro-gels, and are known as the main constituents of transparent exopolymeric particles (TEPs). In comparison to the Dubois assay, commonly used as a representative test for AOM, a different carbohydrate fraction is measured by the Alcian blue method for TEP detection. The TEP staining method has several advantages over the Dubois method: the dye is non-toxic and no strong acids are used, so that there are no hazardous residues after the test. No special correction is needed for the presence of nitrate and nitrite, which is necessary for the Dubois assay (Drews, 2010). AOM and TEPs are very important in microalgae production because of four main reasons: they could (1) reduce the potential biomass yield from the assimilated inorganic carbon, (2) become an organic carbon source that allows growth of bacteria, which would also consume the nutrients, (3) increase coagulant/flocculant loading due to their high negative charge during the harvesting process and (4) promote membrane fouling together with the microorganisms present in the broth when membrane filtration is used for harvesting. Especially TEPs have been assumed to have a big impact on membrane fouling and water quality parameters (in the case of water purification), possibly even more than the microalgae cells themselves (Henderson et al., 2008, 2010; Villacorte et al., 2012; Discart et al., 2013a). The excretion of AOM, both the amount and the type, is dependent on several properties of the microalgal broth, such as broth age, microalgae species, concentration, and the occurrence of stress factors (Henderson et al., 2008). Apart from that, medium recycle in an MPBR can also affect the AOM (Hadj-Romdhane et al., 2013), and in this way have an impact on broth characteristics, growth (by high non-limiting nutrient concentrations or high salt concentrations), and the yield of useful products (oil, polysaccharide, protein...).

In this study, *Chlorella vulgaris* was grown for 75 days in a lab-scale MPBR system with permeate recycle in batch and in continuous operation. The latter was the continuation of our previous study (Bilad et al., 2013), now operated at different dilution rates. The batch cultivation was performed to observe the behavior of AOM in absence of any dilution. In the continuous cultivation, the system performance was continuously monitored with main emphasis on nutrients and accumulation of AOM, represented in this study by organic carbon and TEPs (a relatively new parameter in algal research), in particular to their impact on growth. In addition, the influence of the permeate recycle (containing accumulated non-limiting nutrients) was also addressed.

2. Methods

2.1. Microalgae species, growth medium and analysis

C. vulgaris (SAG, Germany, 211–11B) was cultivated in Wright's cryptophytes (WC) medium, prepared from pure chemicals dissolved in demineralized water. The substrate stock solutions were prepared at high concentrations and stored in the dark at 4 °C. *C. vulgaris* is a well-characterized microalgae species that has an excellent potential for CO_2 capture and has a considerably high lipid content. It is one of the few microalgal strains that is considered suitable to be cultivated at large scale (Mallick et al., 2012).

2.1.1. Biomass: dry weight and microscope observations

The biomass concentration was determined by measuring the dry weight of the samples after filtration ($n = 2$) using Whatman glass fiber filters (Sigma–Aldrich) and drying until constant weight at 105 °C. In addition, the optical density was determined at a wavelength of 550 nm. Microscope observations were done to monitor the biomass and make sure that contaminating species were not taking over the broth solution, since the algae were grown as a non-axenic culture.

2.1.2. Conductivity and total organic/inorganic carbon

The conductivity of the feed, retentate and permeate was measured using a conductivity meter. The conductivity measurements were done to evaluate the ion accumulation as a result of permeate reuse from the membrane filtration as medium in the MPBR. The organic and inorganic carbon was measured using a MultiNC2100. In this case, organic carbon can be used to indirectly represent the abundance of AOM in the feed, broth, product and permeate.

2.1.3. TEP concentrations

TEP concentrations were determined according to the method developed by Arruda Fatibello et al. (2004), at pH 4 and at pH 2.5 (Discart et al., 2013b). The measurement at pH 2.5 was performed to enable the comparison with TEP concentrations obtained by other methods, since Alcian Blue specifically stains certain compound at pH 2.5. Usually, staining with Alcian Blue is done at pH 1 or 2.5, depending on the material targeted (Kiernan, 2010; Passow and Alldredge, 1995). In short, 2 ml of sample is stained with 0.5 ml of a 0.06% Alcian Blue solution after addition of a 0.2 mol/L acetate buffer solution or glycine–HCl buffer until a final volume of 10 ml (for pH 4 and pH 2.5, respectively). Afterward, the mixture is stirred for 1 min and centrifuged at 3000 rpm (2160*g*) for 30 min. The absorbance of the supernatant (excess Alcian Blue solution) is measured at 602 nm to determine the amount of Alcian Blue that has formed complexes with TEPs. The absorbance is measured at 602 nm, since this is the maximum absorbance of Alcian Blue in water, as opposed to Alcian Blue in sulfuric acid, of which the maximum absorbance lies at 787 nm.

2.2. Experimental set-ups and system operation

2.2.1. PBR-batch

The experimental set-up of the PBR and MPBR is shown in Fig. 1. Firstly, the 25 L cylindrical PBR was operated batch-wise for one week until the microalgae growth reached the stationary phase. Samples were taken twice a day and analyzed for microalgal biomass, TEP and TOC concentrations, to monitor their dynamics