Bioresource Technology 129 (2013) 18–25

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Role of transparent exopolymeric particles in membrane fouling: *Chlorella vulgaris* broth filtration

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HIGHLIGHTS

- The direct role of TEPs on membrane fouling was investigated.
- Fresh and fractionated broths were filtered using 2 MF and 1 UF membrane.
- Sample and filtration parameter correlations were assessed by Pearson coefficients.
- No single dominant sample variable affects fouling.

ARTICLE INFO

Article history:
Received 21 August 2012
Received in revised form 5 November 2012
Accepted 6 November 2012
Available online 16 November 2012

Keywords:
Algae harvesting
Transparent exopolymer particles
Dead-end membrane filtration
Chlorella vulgaris
Micro- and ultrafiltration

ABSTRACT

Recent reports show strong evidence for the involvement of transparent exopolymer particles (TEPs), mainly produced by microalgae in natural environments, in membrane fouling in a wide range of membrane filtration processes. The objective of this study is to fundamentally investigate the direct role of TEPs on membrane fouling by using different *Chlorella vulgaris* broth solutions and different fractions of such broth (the soluble and bound fractions, the cells separated from these fractions and the cells with their bound sugars, separated from the soluble fraction) as filtration feed. The relation between the feed properties and their filterability over three membranes was determined. Scanning electron microscopy and light microscopy showed that the foulant types differed for each broth fraction and confirmed the role of TEPs in the fouling of microfiltration membranes. In addition, this study contributes to the role of TEPs in the filtration of microalgae cultivated for commercial reasons.

1. Introduction

Recent reports show strong evidence for the involvement of transparent exopolymer particles (TEPs) in membrane fouling in a wide range of membrane filtration processes (de la Torre et al., 2010; Villacorte et al., 2009a; Kennedy et al., 2009). Because of the transparent nature of these TEPs, their role in membrane fouling was in the past often overlooked. In addition, they often escaped from standard pretreatments applied prior to membrane filtration because of their gel-like compressibility (Kennedy et al., 2009; Villacorte et al., 2009b). TEPs have natural properties of variable size (0.4–200 μm), a gel-like structure and a high negative charge.

Early indications of the involvement of TEPs in membrane fouling, possibly by inducing colloidal fouling or biofilm formation, or a combination of both, led to a significant research interest in this area. The influence of TEPs was studied in a wide variety of set-ups, in reverse osmosis (Villacorte et al., 2009b) and ultrafiltration (Berman et al., 2011), as well as in membrane bioreactors (de la Torre et al., 2010). In all these systems, TEPs seemed to play at least a partial role in the fouling process.

In natural environments and in membrane systems, TEPs and TEP precursors can originate from human debris, bacteria or multicellular organisms like macroalgae, oysters or sea snails

Abbreviations: TEPs, transparent exopolymer particles; SEM, scanning electron microscopy; Chla, chlorophyll a; SMP_{CH}, carbohydrate fraction of soluble microbial products; SMP_{PR}, protein fraction of soluble microbial products; EPS_{CH}, carbohydrate fraction of extracellular polymeric substances; EPS_{PR}, protein fraction of extracellular polymeric substances; bTEP, bound transparent exopolymer particles; sTEP, soluble transparent exopolymer particles; $Cells^{EPS}$, algae cells associated with their bound polymeric substances; $PC^{0.1}$, polycarbonate filter with pore size of 0.1 μm; $PC^{0.4}$, polycarbonate filter with pore size of 0.4 μm; PES^{5kDa}, polyethersulfone filter with molecular weight cut-off of 5 kDa; spTEP, soluble particulate transparent exopolymer particles; scTEP, soluble colloidal exopolymer particles; $Total_{CH}$, total carbohydrates; L, permeance; L_{CW}, clean water permeance; SFV, specific filtration volume; TTF, time to filter.

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http://dx.doi.org/10.1016/j.biortech.2012.11.034

(Heinonen et al., 2007; McKee et al., 2005), but the majority of the TEP precursors is produced by microalgae in natural environments (Passow, 2001; Engel and Passow, 2001; Bar-Zeev et al., 2009). Surprisingly, no reports have been made so far about the influence of TEPs on the filtration of microalgae cultivated for commercial reasons. Microalgae are photosynthetic organisms with an enormous potential for cultivation as energy crops, but there are still major challenges for the large-scale cultivation of these organisms. One of them is the development of an energetically favorable method to harvest the produced biomass (Bilad et al., 2012).

Membrane techniques are an effective method of harvesting microalgae, with advantages such as almost complete retention of biomass, potential disinfection via removal of protozoa and viruses, no or little need to add chemicals to the system and a relatively low energy consumption (Bilad et al., 2012; Lee et al., 2012; Bhave et al., 2012). The efficiency of the process is however compromised by fouling of the membrane by microalgae and their residues. Also, because of the difficulty of working with pure microalgae cultures, biofilm formation could become a problem in the long run (Ríos et al., 2012). Furthermore, microalgae are abundant and diverse in drinking water supplies including lakes, reservoirs, rivers, and streams. Occasional algal blooms cause significant challenges in drinking water treatment due to the extracellular release of organic compounds into water, or upon cell lysis. The release rates of these compounds are quite variable depending on the algal growth phase, microalgal species, and their physiological and environmental conditions.

The first objective of this study is to investigate fundamentally the direct role of TEPs on membrane fouling by using five different broth solutions of *Chlorella vulgaris* as filtration feed (Bilad et al., 2012). *C. vulgaris* is a well characterized species of microalgae which is often involved in unfavorable algal blooms (Jacquet et al., 2005), but also has an excellent potential for large-scale commercial CO_2 capture and lipid production (Mallick et al., 2012). The microalgal broth samples were taken from different stages of growth in a batch cultivation culture, i.e., five samples were taken over a 21 days cultivation. Factors that are expected to play an important role in membrane fouling were identified and measured, namely the concentration of biomass, soluble microbial products (SMP), extracellular polymeric substances (EPS) (both the proteins, carbohydrates and TEPs), and relevant multivalent cations. The filterability of the five feed samples was screened using three different membranes. The relation between sample properties and their membrane filterability was made using the Pearson correlation coefficients. To fully assess the results, a *C. vulgaris* broth solution was additionally fractionated into different solutions containing specific soluble, bound and biomass fractions that were used subsequently as filtration feed to investigate in detail their individual effect on membrane fouling. In addition, scanning electron microscopy (SEM) and light microscopy were also used to analyze membrane samples. As a second objective, this study contributes to the further development of membrane filtration in commercial-scale algae harvesting (Bilad et al., 2012).

2. Methods

2.1. Cultivation and determination of microalgae concentration

C. vulgaris (SAG, Germany, 211-11B) was cultured in Wright's cryptophyte medium prepared from pure chemicals dissolved in demineralized water (Guillard and Lorenzen, 1975). The algae culture was grown in a plexiglas bubble column photobioreactor, with a working volume of 25 L and diameter of 20 cm. Degassing was carried out with filtered air at a constant flow rate of 4.5 L/min. The composition of the cultivation medium is given in Vandamme et al. (2011). The samples were taken at day 2 (T_2), 4 (T_4), 6 (T_6), 8 (T_8) and after 21 days of continuous cultivation (T_{21}), all stored at 4 °C in the dark until being used for analysis or as filtration feed. Sample T_{21} was additionally fractionated according to the method suggested by Judd (2006). Hereby, four different fractions were obtained:

- The soluble fraction: was obtained as the supernatant of the culture after centrifugation at 4000 g for 5 min.
- The fraction containing the cells with their bound substances (further referred to as 'Cells^{EPS}-fraction'): was obtained by resuspending the pellet, obtained by centrifugation at 4000 g for 5 min, in Ringer's solution (consisting of 0.12 g/L calcium chloride, 0.105 g/L potassium chloride, 0.05 g/L sodium bicarbonate and 2.25 g/L sodium chloride)
- The bound fraction: was extracted from the biomass as the supernatant after resuspending the pellet in Ringer's solution, heating for 10 min at 80 °C and centrifuging again at 5000 g for 10 min.
- The fraction containing the cells (further referred to as 'Cells-fraction'): the pellet obtained after the centrifugation-heating-centrifugation procedure was re-suspended again using Ringer's solution to obtain this fraction

Before filtration, the biomass concentration of every sample was determined by several methods. A Coulter counter was used to determine the amount of particles between 2.45 and 10 μm. The dry weight (DW) of the samples was determined gravimetrically by filtration ($n = 3$) using Whatman glass fiber filters (Sigma–Aldrich) and drying until constant weight at 105 °C. The optical density was determined at a wavelength of 550 nm, while chlorophyll a (Chla) concentrations were obtained using fluorometry (460 nm excitation, 685 nm emission).

2.2. Characterization of microalgae cultures

The microalgae cultures and the different fractions were characterized by determining the amount of carbohydrates, proteins and TEPs in the bound fraction (referred to as EPS_{CH}, EPS_{PR} and bTEP respectively) and soluble fraction (referred to as SMP_{CH}, SMP_{PR} and sTEP respectively). The carbohydrate concentration was determined by the phenol–sulfuric acid method (Dubois et al., 1956) and the protein concentrations by the Bio-Rad protein assay. Nitrite and nitrate concentrations were not detected by ion chromatography and thus assumed to be too low to interfere with the obtained carbohydrate concentrations (Drews, 2010). The concentrations of the multivalent cations Fe and Mg were determined with inductively coupled plasma atomic emission spectroscopy and the calcium concentration with atomic absorption spectroscopy.

The TEP concentrations (both in particulate and colloidal forms) were determined in each fraction by the Alcian blue method described by Villacorte et al. (2009b). Hereby, between 10 and 50 ml of TEP-containing solution is filtered over a polycarbonate (PC) filter (0.4 μm for particulate TEPs and 0.1 μm for colloidal TEPs), after which the filter is stained with 1 ml of a pre-filtered solution containing Alcian blue and acetic acid at pH 2.5. As also mentioned by Villacorte et al., the calibration procedure is vulnerable to several inaccuracies such as weighing very small quantities of Gum Xanthan on PC filters and preparing solutions of Gum Xanthan (suspended/colloidal) with uniform properties. Therefore, the calibration procedure was also not applied in this study (Villacorte et al., 2009b). However, the Alcian blue concentration of the solution was always determined before staining by measuring the absorbance at 787 nm and adjusting it to a value of 0.150 in a multiwell plate reader. This was done for two reasons. Firstly, a