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Highly charged cellulose-based nanocrystals as flocculants for harvesting *Chlorella vulgaris*

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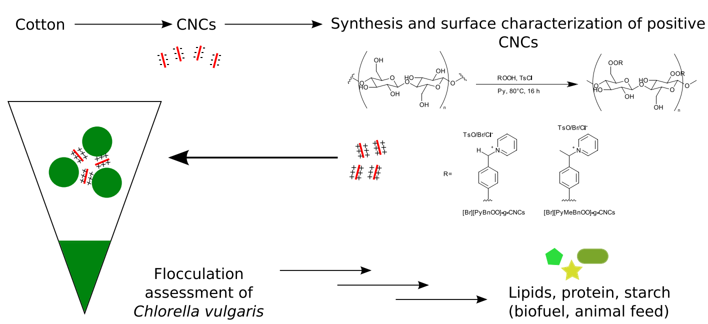
HIGHLIGHTS

- Highly charged CNCs showed great potential as novel flocculant for microalgae.
- The functionalized CNCs had a positive surface charge between pH 4 and 11.
- Flocculation efficiency in terms of dosage was dependent on DS of pyridinium groups.
- Cationic CNCs achieved a flocculation efficiency >95% at a dosage of 0.1 g g^{-1}.
- The CNCs were relatively insensitive to interference by algal organic matter.

GRAPHICAL ABSTRACT



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ABSTRACT

This study presents a novel flocculant for harvesting *Chlorella vulgaris* as model species for freshwater microalgae based on cellulose nanocrystals (CNCs), thus synthesized from a renewable and biodegradable resource. Cationic pyridinium groups were grafted onto CNCs by two separate one-pot simultaneous esterification and nucleophilic substitution reactions. Both types of modified CNCs were positively charged in the pH range 4–11. Both reactions yielded CNCs with a high degree of substitution (up to 0.38). A maximum flocculation efficiency of 100% was achieved at a dosage of 0.1 g g^{-1} biomass. In contrast to conventional polymer flocculants, cationic CNCs were relatively insensitive to inhibition of flocculation by algal organic matter. The present results highlight the potential of these new type of nanocellulose-based flocculants for microalgae harvesting.

1. Introduction

Microalgae are recognized as a promising new source of biomass for biofuels and the production of chemicals. However, successful commercialization is currently hampered by inefficiencies both in processing and product assortment (Šoštarič et al., 2012). The production of high-end co-products has been proposed in several biorefinery concepts to increase economical feasibility of microalgal biomass production (Zhu, 2015). Additionally, the energy input of the production process needs to decrease by an order of magnitude (Baeyens et al., 2015; Wijffels and Barbosa, 2010). Today, microalgae harvesting can consume 30% of the total

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energy input. Lowering the inputs needed to harvest microalgae could contribute significantly to achieving a meaningful decrease in both energy and production costs. Microalgae generally have a negative surface charge which results in a stable cell suspension in the culture medium. A simple, low-cost solution would be to use flocculants that interact with the negative surface charge of the cells, followed by a separation step using simple sedimentation (Vandamme et al., 2013). There are a multitude of inorganic and organic cationic flocculants described in recent literature with varying levels of performance (Barros et al., 2015; Vandamme et al., 2013). Inorganic flocculants generally require a high dosage to be efficient and contaminate the biomass with metal salts. Iron based nanoparticles are reported to be in general more efficient and capable of flocculation followed by magnetically separation but this type of nanoparticles currently lacks chemical stability to be applied in microalgal harvesting (Lim et al., 2012). Alloy-based nanoparticles, such as FePt, FePd and FeCo are more stable but they are currently only used in biomedical applications due to their high production costs (Lee et al., 2015). Biodegradable organic flocculants could offer an alternative, as was recently demonstrated for chitosan, cationic starch and tannins (Vandamme et al., 2010; Roselet et al., 2015). However, the efficiency of these types of flocculants tends to be very sensitive to ionic strength and the presence of algal organic matter (AOM) (Vandamme et al., 2012).

Cellulose is a promising alternative to other biodegradable flocculants since it is the most abundant biomolecule on the planet but it is also goes to waste in the form of agricultural losses in the food processing industry, forestry residues, mill residues and urban waste fractions (Zhao et al., 2007; Ahankari et al., 2011; Chandra et al., 2012; Chen et al., 2012). Targeted modification of cellulose can be used to introduce the desired functionality to prepare the most effective flocculant. In addition, high aspect ratio nanoparticles can be extracted from cellulose as flexible fibers or highly rigid crystals (Klemm et al., 2011). The crystals resulting from hydrolysis of native cellulose are nanosized rods called cellulose nanocrystals (CNCs). Their size and aspect ratio are dependent on the type of cellulose, hydrolysis time and temperature (Beck-Candanedo et al., 2005; Elazzouzi-Hafraoui et al., 2008). CNCs typically have a very large external surface area ($\sim 300\ m^2\ g^{-1}$) covered with hydroxyl groups (2–3 mmol g^{-1}). These unique properties provide an ideal platform for surface modification to develop a material with desired surface characteristics customized for a wide range of applications (Eichhorn et al., 2010; Moon et al., 2011; Klemm et al., 2011; Habibi et al., 2010; Eyley and Thielemans, 2014).

CNCs have been used in diverse applications such as super capacitors, sensors, drug carriers or catalysts (Eichhorn et al., 2010). So far only a few studies have reported synthesis of anionic cellulose nanofibers applied as flocculants (Jin et al., 2014; Suopajärvi et al., 2013). No studies have been published on the synthesis and evaluation of CNCs for the flocculation of microalgal biomass. Ionic CNCs may be very efficient flocculating agents as they are high aspect ratio rigid particles. The aspect ratio gives rise to percolation at low concentrations leading to network (and thus floc) formation. The electrostatic repulsion and rigidity reduces possible gelation as physical and chemical entanglements will not occur, making for easier processing. In this paper, we report the synthesis and characterization of anionic (unmodified) and cationic CNCs with pyridinium groups, grafted by reaction with either 4-(bromomethyl)benzoic acid ([Br][PyBnOO]-g-CNCs) or 4-(1-bromoethyl)benzoic acid ([Br][PyMeBnOO]-g-CNCs) and *p*-toluenesulfonyl chloride in pyridine. The flocculation efficiency of those two types of CNCs versus unmodified CNCs was evaluated for the freshwater green microalgae *Chlorella vulgaris*. Additionally, the influence of algal organic matter (AOM) and biomass concentration on flocculation efficiency were evaluated.

2. Methods

2.1. Materials

Cotton wool, pyridine (99%, for synthesis) and dichloromethane (99.5%, for synthesis) were purchased from Carl Roth. Sulfuric acid (95%, RECTAPUR®), 4-(bromomethyl)benzoic acid (97%, Alfa Aesar), 4-(1-bromoethyl)benzoic acid (98%, Alfa Aesar), *p*-toluenesulfonyl chloride (98%, Alfa Aesar) and ethanol ($\geqslant$99.5%, absolute, EMPLURA®) were purchased from VWR International. Amberlite® MB-6113 (for Ion Chromatography, mixed resin) and orange II (90%, pure, certified) were purchased from Acros Organics.

2.2. Synthesis of cellulose nanocrystals

Cellulose nanocrystals (CNCs) were isolated from cotton wool by hydrolysis with sulfuric acid (10.06 M). The resulting nanocrystals were purified by Soxhlet extraction with ethanol to remove surface contaminants left over from hydrolysis (Labet and Thielemans, 2011). The purified CNCs were modified using a one-pot cationization strategy described previously, whereby CNCs are functionalized with a covalently grafted pyridinium salt (Jasmani et al., 2013). Briefly, CNCs were suspended along with either 4-(bromomethyl)benzoic acid ([Br][PyBnOO]-g-CNCs) or 4-(1-bromoethyl)benzoic acid ([Br][PyMeBnOO]-g-CNCs) and *p*-toluenesulfonyl chloride in pyridine and heated for several hours before isolation and purification by Soxhlet extraction. The strategy was modified slightly from the reported procedure to use a higher dilution and avoid insolubility problems reported previously. Full synthetic and characterization details are described in the electronic Supporting Information.

2.3. Cultivation of C. vulgaris

The green freshwater microalgae *C. vulgaris* 211-11b (SAG) was selected as model species and it was cultivated in dechlorinated deionized water enriched with inorganic nutrients according to the concentration of the Wright's cryptophyte medium (Vandamme et al., 2012). *C. vulgaris* is a promising species for the production of microalgal biomass for food, feed or fuel, and is currently intensively studied (Šoštarič et al., 2012). Bubble column photobioreactors (30 L) were used to cultivate the microalgae. The system was mixed by sparging with 0.2 μm filtered air (5 L min^{-1}) and pH was controlled at 8.5 by addition of 2–3% CO_2 using a pH-stat system. Growth of the microalgae was monitored by measuring the absorbance at 750 nm. Microalgal dry weight was determined gravimetrically by filtration using Whatman glass fiber filters (Sigma–Aldrich) and drying until constant weight at 105 °C. Flocculation experiments were performed in the early stationary phase at a biomass concentration of 0.35 g L^{-1}.

2.4. Flocculation experiments

Flocculation of the microalgal suspensions was investigated in 25 mL jar test experiments ($n = 3$). Prior to CNCs addition, pH of the microalgal suspension was 8. CNCs were suspended in MQ-water at 5 g L^{-1} and adjusted to pH 10.8 using sodium carbonate (1 M). The suspensions were mixed intensively (1000 rpm) for 10 min after CNCs addition. Then, the suspensions were mixed gently (250 rpm) for another 20 min, after which they were allowed to settle for 30 min. Dose–response was evaluated by