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Harvesting microalgal-bacterial biomass from biogas upgrading process and evaluating the impact of flocculants on their growth during repeated recycling of the spent medium

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ABSTRACT

Microalgal-bacterial consortium can be used to upgrade biogas by removing CO_2 and H_2S. Photosynthetic biogas upgrading requires harvesting microalgal-bacterial biomass in order to use the biomass-free cultivation medium as scrubbing liquid in the absorption column. In this study, the efficiency of different flocculants (Zetag 8125, cationically modified cellulose nanocrystals, Tanfloc, chitosan, and $FeCl_3$) to harvest microalgal-bacterial biomass used for biogas upgrading in alkaline medium (inorganic carbon concentration up to 1800 mg L^{-1} and a pH ~10) was evaluated. Zetag and cationic cellulose nanocrystals resulted in maximum flocculation efficiencies of 95% (optimal dose 30 mg g^{-1}) and 93% (optimal dose 20 mg g^{-1}), respectively. Low flocculation was observed with other flocculants at doses as high as 200 mg g^{-1}, which can be ascribed to the high pH of the alkaline medium. Zetag and cationic cellulose nanocrystals were selected for harvesting the biomass during semi-continuous cultivation of the microalgal consortium. Both Zetag and cationic cellulose nanocrystals were effective in flocculating the biomass with efficiencies of over 90% during five successive harvesting cycles. Gravity settling of the flocs formed by Zetag and cationic cellulose nanocrystals resulted in low biomass concentration factors of 7.7 and 2.0, respectively. Screening of flocs using a nylon mesh screen (pore size of 180 μm) resulted in a biomass concentration factor as high as 19.8. Zetag and cationic cellulose nanocrystals could be useful in harvesting biomass under high alkaline conditions without detrimental effects on biomass growth.

1. Introduction

Biogas from the anaerobic digestion of organic waste or wastewater constitutes a promising renewable energy vector able to reduce our current dependence on fossil fuels due to its high CH_4 content (40–75%) [1]. In this context, the removal of biogas pollutants, mainly CO_2 and H_2S, is a mandatory step for its use as a natural gas substitute [2]. Photosynthetic biogas upgrading in high-rate algal ponds coupled with an external absorption column has recently emerged as a low cost (energy consumption of 0.08 kW-h $(Nm^3_{treated\ biogas})^{-1}$) and environmentally friendly (CO_2 emissions of 21 g-CO_2 $(Nm^3_{treated\ biogas})^{-1}$) alternative to conventional physical-chemical technologies to remove CO_2 and H_2S from biogas (energy consumption and CO_2 emissions of 0.30 kWh and 944 g-CO_2 to obtain 1 Nm^3 of treated biogas, respectively, for an activated carbon filter combined with a water scrubbing) [3]. Maintaining a high alkalinity (inorganic carbon concentration > 1500 mg L^{-1}) and pH ~10 of the cultivation medium is essential to increase the mass transfer of acidic gases like CO_2 and H_2S from the biogas to the cultivation medium [4]. Hence, the use of alkaliphilic microalgal-bacterial consortia able to withstand high inorganic carbon concentrations is essential to efficiently remove CO_2 and H_2S from the cultivation medium in high-rate algal ponds [5]. The biogas upgrading process is based on the use of part of the biomass-free cultivation medium as scrubbing liquid in the absorption column. In this sense, separating the microalgal-bacterial biomass generated in high-rate algal ponds from the scrubbing liquid constitutes a critical step. It also allows for control over microalgal productivity under operation with no effluent as a consequence of evaporation losses of water when using digestate as nutrient source (due to its high nutrient concentration,

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which consequently requires low digestate flowrates to sustain algal-bacterial growth) [6].

Several microalgae harvesting methods such as centrifugation, flotation, sedimentation, or filtration have been reported [7]. However, due to low biomass concentration of microalgae in high-rate algal ponds (0.2–1.2 g L^{-1}) and their small cell size (typically in micrometers), some of these technologies do not achieve an efficient solid-liquid separation or they are limited by high-energy requirements with associated increases in operational costs [8,9]. In this regard, flocculation followed by a solid-liquid separation step, such as gravity sedimentation or screening, is considered a rapid and cost-effective alternative for a large-scale harvesting of microalgal biomass [10]. During flocculation, the addition of chemicals leads to the aggregation of microalgal cells forming large flocs [11]. Flocculation can be induced by neutralizing the surface charge of the cells (charge neutralization), by partially reversing the charge of the particle surface, resulting in the connection of particles through patches with opposite charge (electrostatic patch), by precipitation caused by an aggregating polymer network that entangles microalgal cells (sweeping mechanism), or by forming bridges between individual particles (bridging) [12,13].

The optimal dose of the flocculants depends on the characteristics of the microalgal species (i.e. cell size, culture age, and cell wall composition) and the flocculant (e.g. charge, rigidity, and morphology) [14]. Inorganic salts, such as $FeCl_3$, which induce flocculation via charge neutralization, have been widely used as flocculants due to their low cost, in spite of needing higher dose compared to other flocculants [15,16]. Organic polymers such as Zetag, a synthetic copolymer of acrylamide and quaternized cationic monomers, which are able to interact with microalgal cells by charge neutralization and bridging, have been successfully applied in the flocculation of various microalgae [17,18].

Flocculants based on natural biopolymers are attracting interest as flocculants due to their biodegradability. Chitosan from chitin waste is a non-toxic and inexpensive biopolymer composed of linear poly-amino-saccharide chains that can agglomerate individual cells through different mechanisms such as charge neutralization, bridging, sweeping, and adsorption [19–21]. Tanfloc is a commercial biopolymer based on tannins extracted from bark of *Acacia mearnsii* that has also been used as a flocculant for microalgae [18,22]. More recently, cationically modified cellulose nanocrystals (CNCs) have been introduced as a flocculant for microalgae [23–26]. CNCs have a high aspect ratio and high external surface area (~300 m^2 g^{-1}), which is favorable for flocculation. Moreover, they can be readily modified by addition of a wide range of polymer matrices to obtain a flocculant with desired surface characteristics [27,28].

The pH of the culture medium is one of the crucial factors for the performance of the flocculants. Many flocculants get protonated and become cationic only at low pH (<7) [29]. In an alkaline medium, flocculants that carry a pH-independent cationic charge should have a superior performance. Many polymer flocculants experience coiling in high ionic strength conditions and are expected to perform poorly in a medium with a high inorganic carbon concentration [30,31]. Hence, the selection of a flocculant that functions at high pH and at high inorganic carbon concentration is essential for photosynthetic biogas upgrading. Another important feature while applying flocculants in biogas upgrading systems is to obtain a biomass-free medium that can be repeatedly recycled without any detrimental effect on the growth of microalgae and bacteria. Recycling of the spent medium from the absorption column to the photobioreactor is essential for the subsequent removal of CO_2 and H_2S from the medium. While CO_2 will be consumed by microalgae, H_2S will be oxidized to sulphate by sulphur oxidizing bacteria using the oxygen that is generated photosynthetically [32]. In this regard, it is important that accumulation of the flocculant and/or algal organic matter in the recycled culture medium should not lead to microalgal-bacterial growth inhibition [33,34]. Furthermore, the flocculant needs to be versatile in harvesting altogether different microalgal species present in the consortium. Otherwise, those species of microalgae that did not flocculate would eventually alter the microalgal community structure and ultimately make the flocculation process inefficient. So far, no studies have focused on the selection of a suitable flocculant and its dose for efficient use in a repeated recycling of cultivation medium, in spite of the crucial role of this separation step in photosynthetic biogas upgrading.

The aim of this study was to optimize harvesting of a microalgal-bacterial consortium using flocculation, followed by a solid-liquid separation for a photosynthetic biogas upgrading process which requires working under high pH (~10) and alkalinity (inorganic carbon concentration up to 1800 mg L^{-1}), and to evaluate the effect of flocculants on the biomass while recycling the culture medium. For this purpose, different flocculants such as, Zetag® 8125, cationic CNCs, Tanfloc, chitosan, and $FeCl_3$ were tested. Furthermore, the recyclability of the medium after flocculation for the effective flocculants (Zetag and cationic CNCs) was evaluated in a semi-continuous cultivation system. Finally, the feasibility of using screening instead of gravity settling to separate biomass flocs from the culture medium was also assessed.

2. Materials and methods

2.1. Cultivation of microalgal-bacterial consortium

Microalgal-bacterial consortium was obtained from an indoor high-rate algal pond used for biogas upgrading using a high alkalinity synthetic medium as nutrient source located at the Department of Chemical Engineering and Environmental Technology at University of Valladolid. The consortium was grown in 2 L bottles (diameter: 136 mm, working volume: 1.5 L) as fed-batch cultures in a synthetic medium composed of (g L^{-1}): 7.60 $NaHCO_3$, 3.70 Na_2CO_3, 0.58 K_2HPO_4, 1.91 NH_4Cl, 0.10 $MgSO_4{\cdot}7H_2O$, 0.02 $CaCl_2{\cdot}2H_2O$ and 1 mL of a trace metal solution prepared according to the Wright's cryptophyte medium [35]. The cultivation medium was maintained at pH ~10 and fed with 25 mL of fresh medium every day, based on the data on the hydraulic retention time used in the high rate algal pond for biogas upgrading [36]. The flasks were aerated by bubbling with 0.2-μm filtered air and mixed using magnetic stirrers. Cultures were continuously illuminated from front and backside of the flask, each at an intensity of ~100 μmol m^{-2} s^{-1} and maintained at 24 °C in a temperature-controlled room.

2.2. Selection of optimal flocculants for use in alkaline and high pH conditions

Flocculation efficiencies of five flocculants: Zetag® 8125 (BASF, Germany, hereinafter referred as Zetag), in-house developed CNCs grafted with methylimidazolium cationic group (MIM-*g*-CNCs) [25], $FeCl_3{\cdot}6H_2O$ (Chem-lab, >99%), Tanfloc® SG (Tanac, Brazil), and chitosan (Sigma-Aldrich 417963) were tested on the microalgal-bacterial consortium using standard jar tests. For each flocculant a stock solution of 5 g L^{-1} was prepared in distilled water. The stock solution of chitosan (5 g L^{-1}) was prepared in a 0.04 M HCl solution due to its slow dissolution in distilled water [20].

To evaluate harvesting of microalgae-bacterial biomass using different flocculants, conditions for the jar test such as initial stirring speed (300–900 rpm), stirring time (5–30 min), floc settling time (15–120 min), and biomass concentration (0.2–2 g L^{-1}) were initially optimized with 30 mg g^{-1} of Zetag or MIM-*g*-CNCs in order to achieve optimal flocculation efficiency and biomass concentration factor (Supplementary material, Fig. S1).

Dose-response curves for the flocculants were determined by adding different concentrations of flocculants (ranging from 0 to 200 mg g^{-1}

) to 50 mL of microalgae-bacteria suspension (~1 g L^{-1} TSS) while vigorously mixing at 700 rpm with a magnetic stirrer. Following the addition of flocculants, the suspension was gently mixed at 200 rpm for 5 min to promote flocculation. After this, the suspension was decanted in 50 mL plastic tubes and the flocs were allowed to settle for 60 min before measuring the volume and the optical density (750 nm) of the supernatant (Genesis 10S UV–Vis; Thermo Fisher, US). The flocculation efficiency (η_a) was calculated based on measurement of the optical density before flocculants addition (OD_i) and of the supernatant after settling (OD_f) according to the following equation:

$$\eta_a\,(\%) = \frac{OD_i - OD_f}{OD_i} \times 100 \quad (1)$$

In addition, the biomass concentration factor was calculated as:

$$CF = \frac{C_f}{C_i} \quad (2)$$

where C_i and C_f were the initial biomass concentration before addition of flocculants and final biomass concentration in the volume containing the flocculated microalgae, respectively. The jar tests were carried out in duplicate and the results were represented as the average values along with their corresponding standard deviation.

2.3. Repeated recycling of spent medium

Based on the performance of the flocculants, Zetag and MIM-*g*-CNCs were chosen for experiments with repeated recycling of the spent medium in order to check the effectiveness of the flocculants in a semi-continuous cultivation system. In these experiments, three 2 L bottles (working volume 1.5 L) with synthetic medium were inoculated with the microalgal-bacterial consortium (initial biomass concentration of 0.2 g L^{-1}) and incubated under similar conditions as described in Section 2.1. Following 4 days of incubation, 500 mL of the culture from each bottle were harvested either by centrifugation or by Zetag or MIM-*g*-CNCs-based flocculation, and the spent medium was recycled to the culture bottles. The working volume of the cultures was maintained at 1.5 L by addition of fresh medium (NH_4^+ concentration of 100 mg L^{-1} to avoid ammonia inhibition) after harvesting in order to compensate losses in the spent medium. The harvesting of the control cultures was performed by centrifugation at 6000 rpm for 10 min following 30 min settling to test autoflocculation. For Zetag or MIM-*g*-CNCs -based flocculation, the suspensions in a beaker were mixed intensively (250 rpm) with an overhead stirrer for 1 min following the addition of the flocculant. Then, the suspensions were gently mixed (50 rpm) for another 20 min, after which they were allowed to settle for 30 min in a 500 mL Imhoff cone. The recycling experiments were repeated for 5 cycles during 14 days with doses for Zetag and MIM-*g*-CNCs ranging from 25–49 and 20–40 mg L^{-1}, respectively.

The specific growth rate (μ) was calculated as:

$$\mu = \frac{\ln\left(\frac{c_2}{c_1}\right)}{t_2 - t_1} \quad (3)$$

where c_1 and c_2 were the biomass concentration at times t_1 and t_2.

The biomass concentration was measured as total suspended solids (TSS; g L^{-1}). TSS was determined gravimetrically based on GF/C filtration (Whatman, UK) and drying of biomass at 105 °C overnight after washing them 2–3 times with distilled water in order to remove the inorganic salt residue [37]. A linear correlation of optical density values of the culture at 750 nm against TSS (TSS g L^{-1} = 0.7234 × $OD_{750\ nm}$ − 0.0699) was obtained. The pH of the culture medium was monitored every day (Consort C1010; Consort bvba, Belgium) and adjusted to ~10 before the harvesting by adding the necessary volume of 2 M HCl solution. ζ-Potential of the cultivation medium was measured (NanoBrook Omni; Brookhaven Instruments, US) in triplicate before and after flocculation to monitor the flocculant accumulation in the spent medium and the results were represented as the average values along with their corresponding standard deviation. The inorganic carbon concentration was measured before flocculation using a carbonate hardness test (Merck Millipore, Germany).

2.4. Separation of flocs by gravity sedimentation and screening

Screening using a nylon mesh screen with pore size of 180 μm (Elko filtering Co., Switzerland) was evaluated for solid-liquid separation following flocculation to increase the concentration factor. Biomass was flocculated with either Zetag (20 mg g^{-1}) or MIM-*g*-CNCs (40 mg g^{-1}) and allowed to settle for 30 min. Following settling, the entire volume of the suspension was screened through the nylon mesh screen. The flocculation efficiency and the concentration factor were calculated as described in Section 2.2. These experiments were carried out in duplicate and the results were represented as the average values along with their corresponding standard deviation.

3. Results and discussion

3.1. Flocculation of microalgal-bacterial biomass from fed-batch cultures

The microalgal-bacterial consortium was mainly composed of *Chlorella* sp., *Oscillatoria* spp., and uncharacterized bacterial species. Microscopic observation at different time points of fed-batch cultivation confirmed the stable composition of the microalgal consortium.

Among the five different flocculants tested, Zetag and MIM-*g*-CNCs resulted in efficient flocculation of the microalgal-bacterial consortium. While Zetag triggered a maximum flocculation efficiency of 95% with a dose of 30 mg g^{-1} (g flocculant g^{-1} dry matter biomass concentration), MIM-*g*-CNCs resulted in a flocculation efficiency of 93% with 20 mg g^{-1} (Fig. 1). Both are cationic polymeric flocculants carrying respectively quaternary ammonium and methyl imidazolium groups, i.e. cationic charges that are stable over a very wide pH range. Other synthetic cationic polymers have been reported for harvesting marine microalgae, such as Zetag 7557 and Synthofloc 5080H to harvest *Phaeodactylum tricornutum* and *Neochloris oleoabundans* at a pH 7.5 [17], and Magnafloc to harvest *Chaetoceros calcitrans* at a pH 10.2 [38]. With freshwater microalgae *C. vulgaris*, flocculation efficiency of 99% was reported with Zetag 8125 with a dose of 6.4 mg g^{-1}, whereas, with marine microalgae *Nannochloropsis oculata* a flocculation efficiency of ~44% with a dose of 155 mg g^{-1} was reported [18]. In spite of the high pH (~10) and high inorganic carbon concentra-

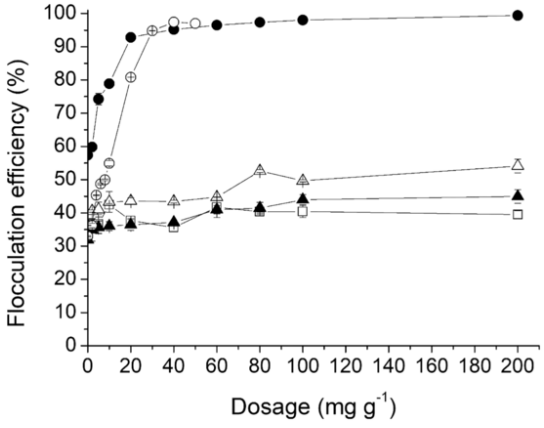

Fig. 1. Flocculation dose-response curves (average values and standard deviation; n = 2) of Zetag (○), cationic cellulose nanocrystals (●), $FeCl_3$ (Δ), Tanfloc (▲) and Chitosan (□).

tion (~1800 mg L^{-1}), a superior flocculation efficiency (95% with 30 mg g^{-1}) was achieved with Zetag 8125 in this study when compared to the flocculation of *Nannochloropsis oculata*. This could be attributed to the relatively low ion concentration in the alkaline medium used in this study compared to the marine culture medium.

In this study, in addition to Zetag, the efficiency of the methyl imidazolium-modified natural cellulose in the form of ribbon-like nanocrystals to harvest microalgal-bacterial consortium at high pH (~10) and inorganic carbon concentrations (up to 1800 mg L^{-1}) was demonstrated. Verfaillie et al. [26] reported a slight decrease in the flocculation efficiency (from 96% to 87%) with the increase of salinity from 0 to 50 g L^{-1} when using 20 mg L^{-1} of cationic CNCs to harvest *Nannochloropsis oculata*. With freshwater microalgae *C. vulgaris*, Blockx et al. [25] reported flocculation efficiencies >80% with 50 mg L^{-1} cationic CNCs at a pH 6 and a biomass concentration of 0.28 g L^{-1}. Reportedly, cationically modified CNCs are efficient and versatile in the sense that they could be used to flocculate microalgae grown under a wide range of cultivation conditions due to their pH independent charge, crystalline nature that provides rigidity to avoid coiling of the polymer under high ionic strength medium, and finally, a high surface cationic charge density that results in high flocculation efficiency at low doses [25,26].

Other flocculants such as $FeCl_3$, Tanfloc, and chitosan resulted in low flocculation efficiencies (maximum values of 54 ± 2, 45 ± 2 and 43 ± 0%, respectively) for doses up to 200 mg g^{-1} (Fig. 1). When compared to organic polymers, inorganic salts such as ferric chloride often requires higher doses to promote flocculation [39]. However, doses higher than 200 mg g^{-1} could result in toxicity of the medium and, moreover, the presence of residual metal ions in the harvested biomass could pose problems during downstream processing [40].

Although Tanfloc has been demonstrated to flocculate marine microalgae [29], low flocculation was observed in this study as a consequence of the high pH (~10) of the medium. Likewise, Selesu et al. [41] achieved a flocculation efficiency of only 30% using Tanfloc for harvesting microalgae *Scenedesmus* sp. at pH 11. Having a point of zero charge of 8.17, Tanfloc assumes a neutral surface charge at higher pH and, consequently, loses its ability to flocculate either through charge neutralization or bridging [29]. Similarly, the conditions of the culture medium did not favor biomass flocculation using chitosan. At pH > 8, the amine groups on the surface of chitosan get deprotonated, which makes it impossible for chitosan to neutralize the microalgal surface charges to induce flocculation by charge neutralization or bridging. Moreover, the high ionic strength of the medium would result in coiling of the polymer [42,43]. Blockx et al. [20] reported that chitosan can also induce flocculation of microalgae at high pH (>7.5) and in seawater medium, but in that case flocculation occurs via sweeping mechanism and much higher doses of chitosan are needed than in freshwater conditions (>75 mg L^{-1}). Similarly, Farid et al. [21] reported higher flocculation efficiencies of chitosan at high pH (9) when compared to neutral pH (7) with marine microalgae *Nannochloropsis* sp. However, no sweeping mechanism was observed in this study with chitosan doses up to 200 mg g^{-1}.

Another important parameter in flocculation is the biomass concentration factor. Less concentrated biomass flocs will require a secondary dewatering process. Maximizing the quantity of culture medium that can be recycled and managing lower volumes of biomass is essential in terms of process economics [44]. Flocculation with Zetag resulted in a maximum biomass concentration factor of 6.5 at a dose of 40 mg g^{-1}, while flocculation with MIM-*g*-CNCs exhibited a concentration factor of only 3.8 at a similar dose (Supplementary material, Fig. S2). Biomass concentration factors in the range of 3.5–14.1 have been reported for different cationic polymers while harvesting marine microalgae by flocculation followed by 2 hour gravity settling [17]. However, concentration factors obtained in this study were less than those reported by Eyley et al. [24] who achieved concentration factor as high as 49 with freshwater microalgae *C. vulgaris*, harvesting by cationic CNCs-based flocculation and 30 min of gravity settling.

3.2. Flocculation during semi-continuous cultivation and repeated recycling of spent medium

In a photosynthetic biogas upgrading process, the spent medium after biomass harvesting is recycled to the photobioreactor through an absorption column to remove the CO_2 and H_2S from the biogas. In this context, it is important to evaluate the impact of flocculation on biomass growth after recycling. Based on the previous results of this study, Zetag and MIM-*g*-CNCs were selected to study their effect during repeated recycling of spent medium. The impact of these flocculants on biomass growth was compared with that of centrifugation.

Spontaneous settling of microalgal-bacterial biomass (after 30 min) without flocculants was negligible, ranging between 1 and 8% over all harvesting cycles tested. Addition of Zetag and MIM-*g*-CNCs resulted in maximum flocculation efficiencies of ~97% at a dose of 23 mg g^{-1} and ~98% at 39 mg g^{-1}, respectively. Different flocculant doses were tested in the subsequent harvesting cycles in order to determine the minimum dose of flocculant. Flocculation with Zetag resulted in a flocculation efficiency of 97% with doses as low as 22 mg g^{-1}, whereas, with MIM-*g*-CNCs, a dose of 20 mg g^{-1} only achieved 55% of flocculation (Fig. 2).

A steady growth of microalgal-bacterial biomass was observed during semi-continuous cultivation using all three harvesting methods (centrifugation, Zetag, and MIM-*g*-CNCs-based flocculation), over 5 cycles of repeated recycling of 500 mL culture medium. Harvesting by centrifugation resulted in a 5–9% increased biomass growth when compared to flocculation-based harvesting (Fig. 2). Specific growth rates differed between the different harvesting treatments and along the time course of cultivation (Fig. S3, Supplementary material). Zetag being a synthetic polyacrylamide polymer and MIM-*g*-CNCs possessing an aromatically dislocated positive charge could be toxic to microalgae at high concentrations. In this regard, although slightly lower growth rates were observed in the last harvesting cycles using Zetag and MIM-*g*-CNCs in comparison with harvesting based on centrifugation, no detrimental effect on microalgae growth was observed along the 5 cycles. Moreover, concentrations of these flocculants were optimized to minimize the dose required to induce flocculation and to avoid the presence of free polymers in the recycled medium. This was verified through ζ-potential analysis of cell free supernatant before and after harvesting at each cycle (Supplementary material, Table S4). The presence of free flocculant in the spent medium should be evident from an increase in ζ-potential in the spent medium. In this study, no significant change in the ζ-potential of the spent medium was observed between centrifugation, Zetag, and MIM-*g*-CNCs-based flocculation, demonstrating that the quantity of flocculant that was returned to the cultivation system was minimal (Supplementary material, Table S4). During the recycling experiments, an increase in the pH of the culture medium (from 10 to 10.8) and a decrease in the inorganic carbon concentration (from 1798 ± 0 to 913 ± 69 mg L^{-1}) were observed as a result of the photosynthetic activity of the microalgae without CO_2 addition (Table S4, Supplementary material). Flocculation did not affect the pH, which is essential for effective biogas upgrading using microalgae.

Moreover, flocculation was uniform and was not selective to particular microalgal species of the consortium. As observed by microscopic analysis, no change in the microalgae community was found during any of the recycling experiments. *Chlorella* sp. and *Oscillatoria* sp. continuously dominated the consortium along with uncharacterized bacterial species.

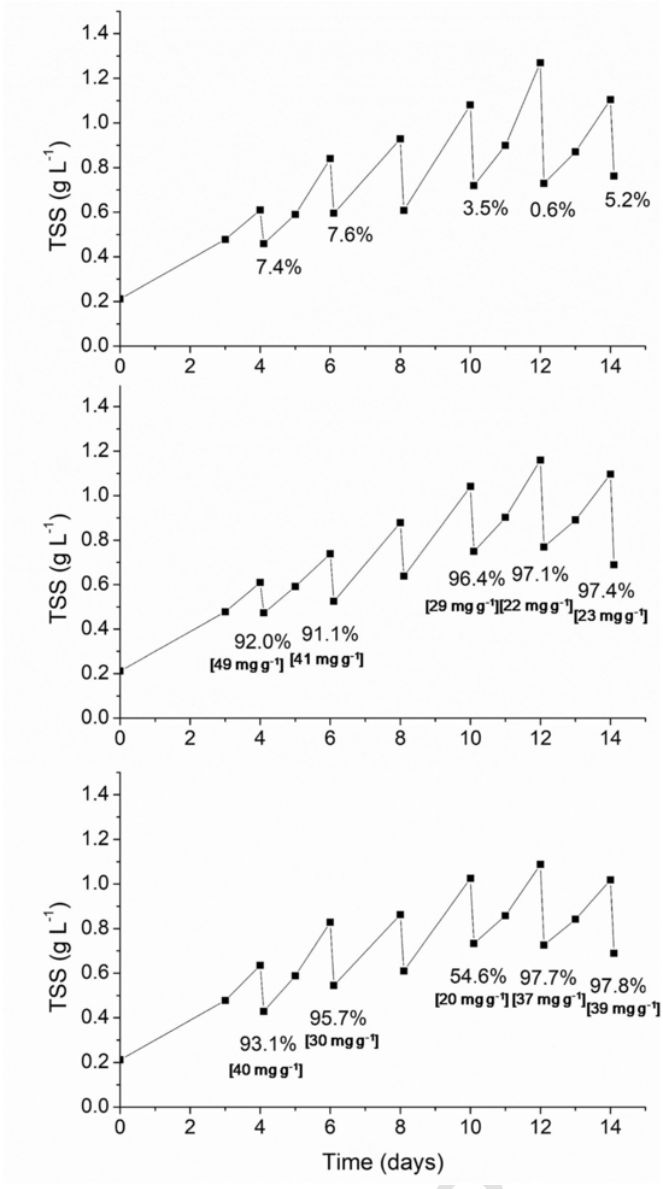

Fig. 2. Growth curve of the microalgal-bacterial consortium in the recycling medium with a) centrifugation (control) and flocculation with b) Zetag and c) cationic cellulose nanocrystals. The values below represent the flocculation efficiencies (%) and dose of flocculants (mg g^{-1}) during each harvesting cycle.

3.3. Biomass separation after flocculation

Following flocculation, separation of biomass flocs from the culture medium is an important process step. The biomass concentration factor is an indicator of the efficiency of biomass separation. Separation was achieved by gravity sedimentation of the flocs for 30 min. The biomass concentration factor during repeated recycling experiments was lower than the ones observed during dose-response experiments (refer to Section 3.2.). Zetag-based flocculation resulted in concentration factors in the range of 3.2–7.7, whereas MIM-*g*-CNCs-based flocculation resulted in a maximum concentration factor of only 2.0 (Fig. 3; Supplementary material, Table S4). The higher concentration factors obtained for Zetag as the flocculant in comparison to MIM-*g*-CNCs could be attributed to a larger floc size and more compact structure as generated with the former (Fig. 3). In this context, Zhang et al. [45] proposed that not only the size of the flocs has influence on the settling velocity and the concentration factor of the microalgal biomass, but also the structure of these flocs, where microalgal flocs with large and compact structure should settle better under gravity.

In order to improve the concentration factor, screening was evaluated as a separation method. The biomass flocs obtained with Zetag and MIM-*g*-CNCs were allowed to settle for 30 min and screened through a nylon mesh screen with a pore size of 180 μm. Microalgal-bacterial culture without flocculants (acting as a control) resulted in harvesting efficiencies of 18% and 24% following 30 min settling and 180 μm screening, respectively. The cell size of microalgae in this consortium varied between 0.5 and 200 μm. Without flocculation, most of the cells crossed the 180 μm screen. In addition, a 30 μm pore size screen was also tested, but this was not efficient due to clogging of the mesh. On the other hand, Zetag-based flocculation resulted in harvesting efficiencies of 97% for both, settling and 180 μm screening. Similarly, MIM-*g*-CNCs-based flocculation resulted in harvesting efficiencies of 98% and 95% for settling and 180 μm screening, respectively (Fig. 4). The slight lower harvesting efficiency for MIM-*g*-CNCs with a 180 μm screen could be due to the fact that some smaller flocs or individual cells that were not flocculated passed through the screen. In this context, Verfaillie et al. [26] reported a low harvesting efficiency when using flocculation with cationically-modified CNCs followed by screening through a mesh with pore size of 180 μm due to unstable structural integrity of the flocs.

Screening resulted in higher biomass concentration factors (up to 19.8; Fig. 4) compared to those for centrifugation (maximum value of 10; Supplementary material, Table S4). With Zetag-based flocculation, concentration factors of 3.7 and 17.7 were obtained for 30 min settling and 180 μm screening, respectively. With MIM-*g*-CNCs-based flocculation, a concentration factor of 19.8 was obtained with screening. This value is ~15 times higher than the concentration factors obtained with gravity settling (1.3; Fig. 4). Hwang et al. [46] reported a maximum concentration factor of 25 using a cross-flow membrane filtration system of polyethylene terephthalate with a pore size of 4 μm using a 3% of polyvinyl alcohol as coating material for harvesting *Chlorella* sp. Monte et al. [47] obtained a concentration factor of 4.8 with a loss of integrity of 10% while harvesting *Dunaliella salina* using a microfiltration membrane with a nominal pore size of 0.1 μm made of polyethersulfone.

In spite of demanding slightly higher energy costs (0.4 kWh/m^3 for screening vs 0.1 kWh/m^3 for gravity settling) [48], considering the advantages of achieving a high biomass concentration in a short time, screening using a 180 μm nylon mesh could be a good alternative to gravity sedimentation after flocculation.

4. Conclusions

In this study, five different flocculants were tested to harvest microalgal-bacterial biomass from a photosynthetic biogas upgrading process. Zetag and MIM-*g*-CNCs resulted in flocculation efficiencies >92% at 30 and 20 mg g^{-1}, respectively. Both flocculants were effective in harvesting biomass under semi-continuous cultivation with repeated recycling of spent medium. Moreover, both Zetag and MIM-*g*-CNCs did not result in any detrimental effect on either microalgal growth or pH of the spent medium during 5 cycles of harvesting. Finally, screening of the biomass flocs with a nylon mesh with 180 μm pore size was demonstrated to achieve high biomass concentration factors. This flocculation-based harvesting is rapid and efficient in solid-liquid separation and hence could be applied in current biogas upgrading processes to replace the traditional gravity settlers-based harvesting.

CRediT authorship contribution statement

María del Rosario Rodero: Conceptualization, Investigation, Formal analysis, Writing - original draft, Writing - review & editing.**Raú**

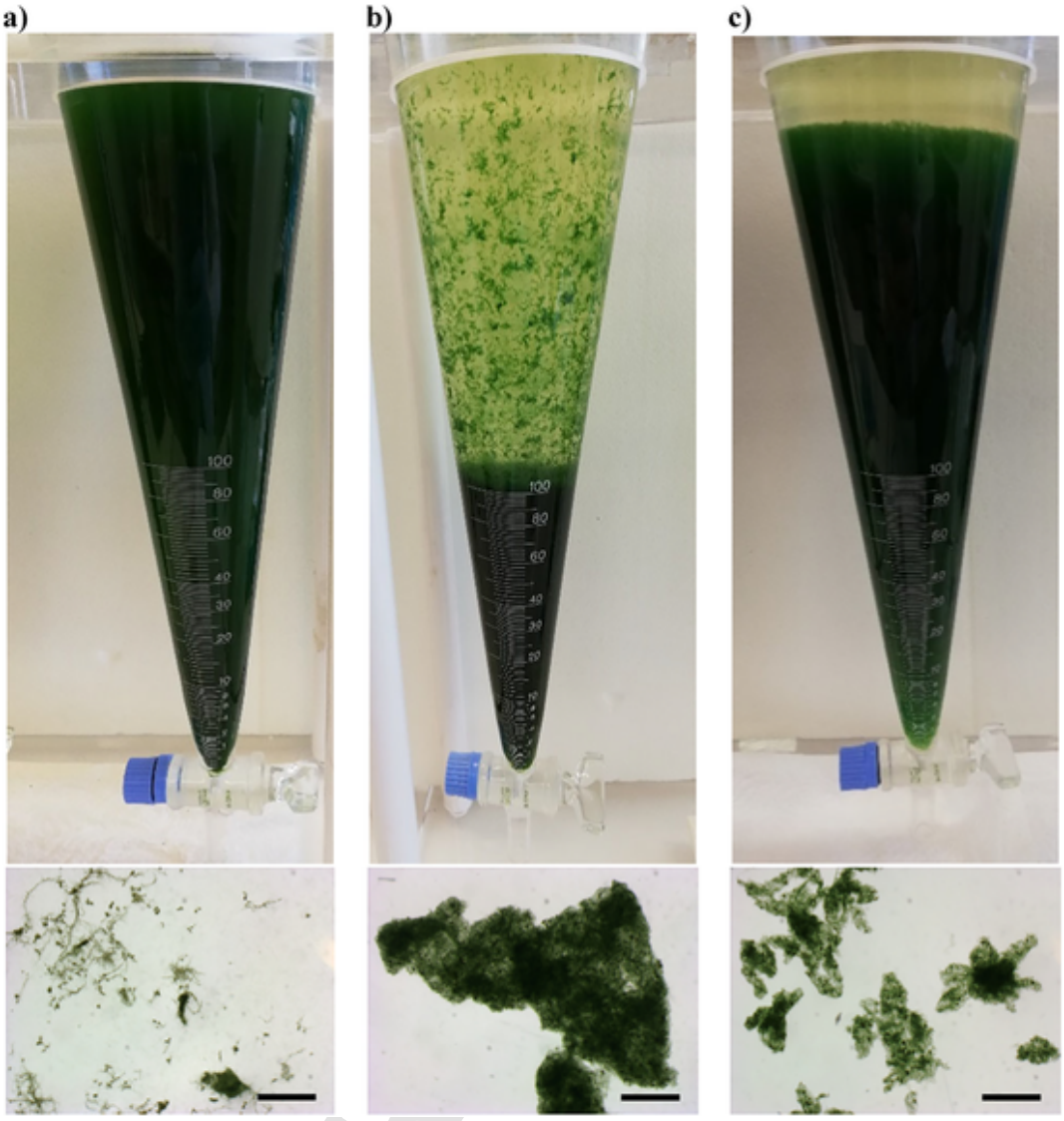

Fig. 3. Concentration of biomass flocs in Imhoff cone after 30 min settling during the repeated recycling experiments and microphotographs of flocs formed during a) gravity settling for 30 min, b) Zetag-based flocculation and c) cationic cellulose nanocrystals-based flocculation. Scale bar represents 250 μm.

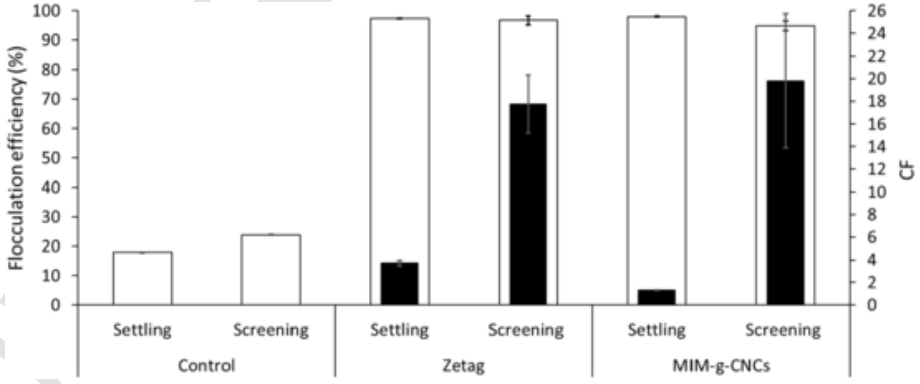

Fig. 4. Comparison of the harvesting efficiency (white bars) and concentration factor (CF; black bar) (average and standard deviation; n = 2) of control (without flocculant), Zetag and cationic cellulose nanocrystals (MIM-*g*-CNCs)-based flocculation under different solid-liquid separation methods (gravity settling and screening with nylon mesh screen of pore size 180 μm).

l Muñoz: Funding acquisition, Conceptualization, Writing - review & editing.**Raquel Lebrero:**Funding acquisition, Conceptualization, Writing - review & editing.**An Verfaillie:**Resources, Writing - review & editing.**Jonas Blockx:**Resources, Writing - review & editing.**Wim Thielemans:**Resources, Writing - review & editing.**Koenraad Muylaert:**Funding acquisition, Conceptualization, Writing - review & editing.**Ra-**

masamy Praveenkumar: Conceptualization, Investigation, Formal analysis, Writing - original draft, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Statement of informed consent, human/animal rights

No conflicts, informed consent, or human or animal rights are applicable to this study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.algal.2020.101915.

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