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Exploiting flocculation and membrane filtration synergies for highly energy-efficient, high-yield microalgae harvesting

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Abstract:

High energy consumption during harvesting is one of the main bottlenecks for sustainable microalgae production. Membranes can efficiently separate microalgae from liquids with low energy consumption, but membrane fouling remains an important issue. Flocculation prior to membrane filtration can increase membrane

fluxes and decrease fouling, thus offering a low-cost and efficient solution to harvest microalgae. Biobased cationic cellulose nanocrystals were successfully used as flocculants for microalgae and were effective over a wide pH-range and for both freshwater and marine microalgae. Such flocculation was for the first time combined with vibration-assisted filtration using a charged, surface patterned membrane, enabling operation at very high flux (95 L/m² h) using a vibration frequency of only 1 Hz, and even under sub-optimal flocculation conditions. Intermittent vibration decreased energy consumption further while keeping excellent filtration performance to finally achieve a record-low energy consumption for the membrane filtration of only 6.7 Wh/m³, which is more than 25-times lower than that of normal membrane filtration. Interaction forces revealed that increasing particle size through flocculation prior to membrane filtration can significantly prevent microalgae attachment on the vibrating membrane surface. This work opens a new direction for sustainable microalgae harvesting with an ultra-low energy consumption, combined with a very high microalgae recovery, reduced use of chemicals, and lower membrane investment cost.

Key words: Patterned membrane; Anti-fouling; Vibration system; Membrane bioreactor; Interaction force; Cationic flocculant

1. Introduction

Harvesting microalgae from their growth medium still remains a problem, because of

the small microalgal cell size and the strongly diluted biomass concentration [1]. Centrifugation, flocculation-induced sedimentation, and membrane filtration are commonly used for microalgae harvesting [2]. Centrifugation is the most efficient process, but entails a very high energy consumption (8000 Wh/m³) [3]. Flocculation-induced sedimentation is the most energy-efficient method [4]. However, flocculation, e.g. with metal salts or polyacrylamides, in combination with simple gravity sedimentation is slow, never results in a 100% microalgae recovery and complicates recycling of the culture medium. Moreover, many commonly used flocculants display pH-dependent activity and underperform at the natural pH of microalgal cultures. In addition, flocculation conditions using traditional flocculants need to be optimized per microalgal species, and are more difficult to be used in natural water (especially in seawater) that contains different microalgal species. Considering the negatively charged microalgal surface, cationic flocculants derived from natural biopolymers with positively charged surface have gained attention recently [4, 5]. Cellulose, as one of the most abundant and non-toxic biodegradable materials, has ample OH-groups which can be functionalized easily. Cationic cellulose nanocrystals (CNCs) thus have a large potential to flocculate even multi-species broths without the need for pH adjustment [6] in both fresh and sea water [1].

Membranes can retain 100% of microalgae from a liquid with low energy consumption [7]. However, fouling caused by microalgal cells and their extracellular organic matter (EOM) lowers membrane performance as well as membrane life-time

through frequent cleaning, which also increases costs [8, 9]. Several approaches, like introducing turbulence, membrane surface modification (e.g. increased surface hydrophilicity, charge and patterning), and flocculation-assisted filtration have been used to mitigate membrane fouling [10]. A recent study showed that combining membrane surface charge, patterning and vibration significantly increased membrane permeance [11]. Other studies showed that flocculation can facilitate membrane filtration even under sub-optimal flocculation conditions and at low vibration frequencies [12, 13]. It is now hypothesized that membrane performance could be further improved still by introducing sub-optimal flocculation prior to membrane filtration together with the combination of membrane surface charge, patterning, and vibration at low frequency. A significantly reduced use of energy and flocculants could then be realized.

The universality of the novel cationic CNC-based flocculants was first investigated. Four favorable approaches to improve membrane performance (flocculation prior to filtration, membrane surface charge, patterning, and vibration-assisted filtration) were then combined for the first time to improve membrane performance, maximize microalgal harvesting yield and decrease energy consumption. The permeate drag and inertial lift forces were calculated to analyze the microalgae-membrane interface interactions in the magnetically induced membrane vibration (MMV) system, further unraveling the mechanism of membrane fouling mitigation.

2. Methods

2.1 Cultivation of microalgae

Three freshwater microalgal strains (*Desmodesmus* sp., *Dictyosphaerium* sp. [10] and *C. vulgaris* (SAG, Germany, 211-11B)) were separately cultivated in three 30 L column photobioreactors with 25 L BG-11 medium each under an illumination density of $100 \mu\text{mol photons/m}^2\cdot\text{s}^1$ and continuous aeration of 15 L/min for 10 days at 22 ± 1 °C. The marine microalgal strain (*N. oculata*) was also cultivated under the same conditions with extra 30 g/L sea salt (Sigma-Aldrich, Belgium) in the culture medium. The microalgae were chosen due to their high oil content and fast growth. Dry weight was determined after 10 days of cultivation by filtering a known volume of microalgae culture broth onto a pre-weighed $0.1 \mu\text{m}$ filter to remove medium and inorganic salts. The filter with microalgal paste was then dried at 105 °C to constant weight. The final dry biomass amounts of *Desmodesmus* sp., *Dictyosphaerium* sp., *C. vulgaris*, and *N. oculata* were 0.86 ± 0.03 g/L, 0.81 ± 0.11 g/L, 0.64 ± 0.03 g/L, and 0.41 ± 0.10 g/L.

2.2 Synthesis of cationic CNCs

Two cationic CNCs with different DS (0.34 and 0.28) were modified with pyridinium (PYR) and methylimidazolium (MIM) groups, respectively, following previously published studies [1, 4]. Briefly, 25 g cotton wool (German Pharmacopeia grade, Chem-lab Analytical) was hydrolyzed in 500 mL HCl solution (4 M) at 80 °C for 4 h. The hydrolysate was washed, dialyzed, and ion-exchanged (50 g Amberlite MB-6113 mixed bed ion exchange resin) using deionized water. The hydrolysate was then sonicated and freeze dried for 48 h. The dried hydrolysate was Soxhlet extracted using

ethanol for 48 h and finally dried in a vacuum oven at 40 °C to remove the organic solution and obtain the final CNCs. The dry CNCs, 4-(1-bromomethyl)benzoic acid (Acros organics, Belgium) and *p*-toluene sulfonyl chloride (Sigma-Aldrich, Belgium) were suspended in 250 mL dry pyridine (Acros organics, Belgium) or 1-methylimidazole (Sigma-Aldrich, Belgium) under argon atmosphere. The reaction mixtures were then heated and left to react under continuous stirring at 80 °C for 48 h and at 70 °C for 16 h, respectively. After the reaction, the modified CNCs were filtered through a cellulose Soxhlet thimble, and purified using Soxhlet extraction with dichloromethane and ethanol for 24 h and 48 h, respectively. The solid product was dried under vacuum to obtain white CNCs bearing cationic surface functionalities.

2.3 Synthesis of sulfonated polysulfone

Sulfonated polysulfone (sPSf) was blended with PSf to create membranes with a negative charge and increased hydrophilicity [14]. sPSf was synthesized according to a previous study [11]. Briefly, 0.08 g/L PSf (Solvay, Udel P-1700, Belgium) solution was prepared by dissolving PSf in CHCl₃ (99+%, Fisher, Belgium) for 15 h at room temperature, then 6 M chlorosulfonic acid (CA, 99%, Sigma-Aldrich, Belgium) solution (dissolving in CHCl₃) as a sulfonating agent was added dropwise to the PSf solution at room temperature to form a final CA concentration of 0.2 M. The mixture was vigorously stirred for 1 h at 0 °C. The supernatant was removed, and the precipitate was washed three times using chloroform to remove unreacted CA. After drying, the precipitate was washed using deionized water until neutral pH. Finally, the

sample was dried in a vacuum oven for 4 days at 40 °C to get sPSf.

2.4 Membrane preparation and characterization

Patterned membranes were prepared using a spray-modified non-solvent induced phase separation method [15, 16]. A 3D-printed, wave-like, resin (VeroWhitePlus™ RGD835, Stratasys Ltd, Eden Prairie, USA) patterned knife (pattern height and distance are 700 µm and 900 µm, respectively) was used, and a sprayer was installed on the casting machine (Porometer, Belgium) to allow deionized water to be sprayed on the cast film right after the passage of the casting knife to realize immediate phase inversion and maintain the surface pattern on the freshly shaped membrane surface [15]. PSf (13.5w%) and sPSf (4.5w%) (total polymer concentration of 18w%) were dissolved in N-methyl-2-pyrrolidone (99w%, Acros organics, Belgium) at 60 °C with 28w% polyethylene glycol (PEG, Mw~1000 Da, Fluka, Belgium) as pore-forming agent [10]. After degassing overnight, the solution was cast on a smooth glass plate with a wet thickness of 200 µm (measured from the valley point of the corrugation to the bottom of the polymer film, below referred to as base-layer) at a casting speed of 2.3 cm/s. Finally, the cast film was immersed in deionized water (21 °C) to complete the phase inversion. A flat membrane with 18% PSf (without sPSf) and a flat membrane with 4.5w% sPSf were prepared for comparison. Flat membranes were prepared using a 200 µm gap with a conventional casting knife, and the cast film was directly immersed into deionized water without extra spraying. These patterned (P) and flat membranes (F) were referred to as P-sPSf, F-PSF (i.e. without sPSf), and F-sPSf, respectively.

The surface and cross-section of the membranes were observed using scanning electron microscopy (SEM, JEOL JFC-1300, Tokyo, Japan). The membrane surface chemical composition was characterized using attenuated total reflectance Fourier-transform infrared spectrometer (ATR-FTIR, Bruker Alpha, Germany) with a 4000-400 cm^{-1} scanning range. The zeta potential of the membrane surface was determined by streaming potential measurements using an adjustable-gap measuring cell (SurPASS Electrokinetic Analyzer, AntonPaar), following the procedure described before [17]. Visiolab software was used for data analysis.

2.5 Module preparation and filtration setup

Two membrane sheets with an active area of 100 cm^2 each were glued at the edges (UHU-Plus endfest 300, Germany) to form an envelope-like membrane module with a plastic mesh and a permeate tube in between.

The membrane module was fixed in an efficient and energy-saving MMV system [18, 19] with two dummy plates at both ends to generate the same hydrodynamic conditions for each membrane. The magnetically induced sinusoidal movement is controlled by a computer using vibration driver software (MotionLab 2.13.1.658, Schoten, Belgium). The membrane panel is connected to a multichannel peristaltic pump (Watson-Marlow, Belgium) with a pressure gauge (Wika DG10, Germany) in between to monitor the transmembrane pressure (TMP). The direction of the vibration was vertical, and membranes were mounted with their pattern lines horizontal.

2.6 Flocculation experiments and feed preparation

The flocculation performances of the cationic CNCs in different microalgal broths at

different pH levels (4, 5, 6, 7, 8, 9, 10, and initial pH) were tested in a 50 mL jar test experiments that were carried out in triplicate. Experiments were performed with 4 single-species microalgal broths (*Desmodesmus* sp., *Dictyosphaerium* sp., *C. vulgaris*, and *N. oculata*) as well as a mixture of the 3 freshwater microalgal broths (1:1:1 ratio). HCl (Sigma-Aldrich, Belgium) (0.5 mol/L) and NaOH (ACROS, Belgium) (0.5 mol/L) stock solutions were used for pH adjustment. The CNCs stock solutions (5 g/L) were prepared by suspending the respective cationic CNCs in MiliQ water and sonicating for 10 min. A chitosan solution (5 g/L) was used as comparison by dissolving chitosan in a 0.1 mol/L HCl solution. Microalgae suspensions with initial pH of 7-9 were adjusted to pH 4, 5, 6, 7, 8, 9, and 10 before dosing flocculants, and the flocculation performance at initial pH (i.e. without pH adjustment) was also investigated. After being dosed with different concentrations of flocculants (2, 5, 10, 20, 40, 60, 80, 100, and 150 mg/L) at each pH level, the microalgae suspensions were first stirred vigorously (350 rpm) for 10 min, after which the suspensions were mixed gently at 50 rpm for 20 min to form flocs. The suspensions were allowed to settle for 30 min, and the samples were pipetted from the middle zone of the beaker. The optical density (OD) was measured using a spectrophotometer (UV-1800, Shimadzu, Germany) at 680 nm and flocculation efficiency E was calculated as Eq. (1):

$$E = \frac{OD_{bf} - OD_f}{OD_{bf}} \quad (1)$$

where OD_{bf} is the optical density before and OD_f the optical density after flocculation.

2.7 Filtration experiments

Microalgal broths with (under optimal and sub-optimal flocculation conditions) and

without (untreated microalgal broth) flocculation treatment were used as feeds. Flocculation experiments were performed prior to filtration in the MMV system. The membrane modules were immersed into the microalgal broth to filter microalgae. The TMP was read from the pressure gauge. The membrane permeance in real microalgal broth and the harvesting efficiency were calculated using Eqs. (2) and (3), respectively. The harvesting efficiency (i.e. the retention of microalgae) was determined using the optical density measurement at 680 nm using a spectrophotometer (DR-2800, Hach Lange, USA). The harvesting efficiency was used to decide whether the membrane can be used to harvest microalgae (i.e. with a harvesting efficiency higher than 97%).

$$J \text{ (L/m}^2 \text{ h bar)} = \frac{V}{A \cdot t \cdot \text{TMP}} \quad (2)$$

$$\eta_m (\%) = \frac{OD_{feed} - OD_{permeate}}{OD_{feed}} \quad (3)$$

where J is the membrane permeance; V (L) the volume of permeate; A (m²) the active area of membranes; t (h) the filtration time; η_m the harvest efficiency of microalgae; OD_{feed} and $OD_{permeate}$ are the optical density of feed and permeate at 680 nm, respectively.

An improved flux-step method (IFM) was used to determine the critical flux (CF) according to the previous studies, and the microalgal broth was used as feed [3, 18]. The initial flux, step height, and step length were 5 L/m² h, 5 L/m² h, and 10 min, respectively. Unlike the traditional flux step method, IFM contains an intermediate flux decrease to 5 L/m² h (i.e. releasing process). The reversible fouling is removed during the releasing process and a more accurate CF is obtained due to the decrease in

flux. A TMP growth of 10 Pa/min was seen as the critical point where irreversible fouling started to significantly affect filtration.

The intermittent filtration was investigated for energy-saving purpose, which includes an idle time (i.e. without vibration) and a vibration time to accomplish a cycle in which the ratio of the vibration time to the full cycle time is defined as the vibration ratio (noted as R_v) (Eqs. 4 and 5). To investigate the effect of the vibration ratio on filtration performance, the full cycle time and total filtration time were set as 60 min and 130 min, respectively. The TMP was read every 2 min.

$$t_c = t_v + t_i \quad (t_c = 4, 24, 60 \text{ and } 120 \text{ min}) \quad (4)$$

$$R_v = \frac{t_v}{t_c} \quad (R_v = 0\%, 10\%, 30\%, 50\%, 70\%, 90\% \text{ and } 100\%) \quad (5)$$

where t_c is the cycle time (min), t_v the time of filtration with vibration (min), t_i the time of filtration without vibration (min), R_v the vibration ratio (%).

2.8 Energy consumption calculation

The energy consumptions of the MMV system, peristaltic pump, and computer were monitored using an energy-logger (Voltcraft 4000 FR Datalogger).

To compare the energy consumption of the vibration-assisted filtration method to that of conventional harvesting by means of centrifugation, the evaluation was adapted from a full-scale sub-merged MBR applied in wastewater treatment with a flux of 250 m³/h, which was used as the referenced flux (J_{ref}) [18]. The active membrane per module unit was set as 30 m² in accordance with a pilot microalgae harvesting system [20]. This capacity was used to simulate microalgae production in microalgal industry.

The ratio (R_N) of the J_{ref} to the operational flux (J_{of}) was defined as the theoretical module number, which was calculated according to Eq. (6).

$$R_N = \frac{J_{ref}}{J_{of}} \quad (6)$$

$$E_T = E_p + E_v + E_C \quad (7)$$

where E_T is the total energy consumption (Wh/m³); E_p the energy consumption of peristaltic pump (Wh/m³); E_v the energy consumption of the vibration engine (Wh/m³); E_C the energy consumption of computer (Wh/m³).

2.9 Shear rate calculation

The maximal shear rate of the liquid near the vibrating membrane surface was calculated according to previous studies [11, 19] using Eq. (8). The frequency and amplitude were converted to a shear rate.

$$\gamma_{max} = \frac{a\omega^{1.5}}{2\nu^{0.5}} \quad (8)$$

where γ_{max} is the maximal shear rate (s⁻¹); a the amplitude (m); ω the angular frequency ($=2\pi f$, rad/s), f the frequency (Hz), and ν the kinematic viscosity. In this case, the kinematic viscosity was assumed to be equal to the kinematic viscosity of water (1.3×10^{-6} m²s⁻¹ at 20 °C).

2.10 Interfacial force calculation

To investigate the effect of the MMV system on microalgae separation, the permeate drag force (F_D) and inertial lift force (F_{IL}) experienced by microalgal cells were considered in this study. The gravity and buoyance of microalgae are neglected in the calculation because the density of microalgal broth is similar to that of water [21, 22].

The F_D during the filtration process can be calculated using Eq. (9).

$$F_D = -6\pi\mu r_M v_W f_H \quad (9)$$

where r_M is the microalgal cell radius (m), μ the apparent viscosity (Pa/s, 0.0018 Pa/s) [11, 23], v_W the permeate velocity (m/s), and $f_H(=F/F_\infty)$ the hydrodynamic correction factor which can be further calculated using Eq. (10).

$$f_H = \left(\frac{2R_m r_M}{3} + 1.072^2\right)^{0.5} \quad (10)$$

where R_m is the membrane hydraulic resistance and can be calculated using Eq. (11).

$$R_m = \frac{TMP}{v_W \mu} \quad (11)$$

In the MMV system, the wall shear exerted on the membrane surface generates an F_{IL} which is opposite to the F_D , mitigating microalgal deposition on the membrane surface. The F_{IL} can be calculated as:

$$F_{IL} = 81.2(\rho\mu\gamma_{max}^3)^{0.5} r_M^3 \quad (12)$$

where ρ is the broth density (kg/m³, 1024 kg/m³).

3. Results and discussion

3.1 Effects of flocculant dosage, microalgal species and pH on flocculation performance

Two cationic CNCs with different DS (0.34-PYR-CNCs and 0.28-MIM-CNCs) were used to flocculate three freshwater microalgal strains (*Desmodesmus* sp., *Dictyosphaerium* sp., *C. vulgaris*), one marine microalgal strain (*N. oculata*) and a freshwater microalgal mixture ($V_{Desmodesmus\ sp.}:V_{Dictyosphaerium\ sp.}:V_{C.\ vulgaris}=1:1:1$) under different flocculation conditions (i.e. different pH and flocculant dosages). *Desmodesmus* sp., *Dictyosphaerium* sp., and the microalgal mixture have never been

tested with cationic CNC-based flocculants. A traditional flocculant (chitosan) was used as the benchmark. The universality of cationic CNCs and their flocculation behavior was thus investigated. This is important as many factors (e.g. microalgal cell wall compositions, biomass concentration, salinity etc.) influence the final flocculation behavior, and the flocculation conditions are therefore not universal. Hence, the species-flocculation combinations in a jar test should quasi be conducted for each specific situation to determine the best conditions.

Each microalgal species showed quite different flocculation behavior even under the same flocculant concentrations (Fig. 1). Chitosan-based flocculation is highly influenced by pH and flocculant dosage, showing that the highest flocculation efficiency point shifted from a low flocculant dosage to a high one with increasing pH. Furthermore, the flocculation efficiency increased first and then decreases when further increasing flocculant dosage, in line with previous studies [12, 24]. Chitosan in freshwater (Figs. 1a, 1d, 1g and 1j) requires a low pH to carry a positive charge (i.e. due to protonation of the primary amine group) and interact with the negatively charged surface of microalgal cells, thereby inducing the flocculation by bridging and charge neutralization mechanisms [25]. The decline in flocculation efficiency when further increasing the chitosan dosage occurs due to a reversal of the surface charge of the microalgal cells: when a higher dosage of chitosan is applied, the microalgal cells tend to repel each other because of the excessive positive charge on the chitosan coated microalgae cells, finally leading to the re-stabilization of the suspension of the microalgal cells [12, 26]. A higher pH (>6.5) results in deprotonation of the primary

amine and a loss of the positive charge, therefore requiring a higher dosage. At high pH, a different mechanism (i.e. sweeping) takes over, which does not rely on electrostatic interactions between chitosan and the microalgal cells but acts by creating a network that drags the microalgal cells with upon precipitation of the chitosan polymers [25, 26]. This sweeping mechanism requires a higher dosage.

A significant difference was found for the marine microalgal broth (*N. oculata* in this study) where almost no flocculation occurred in marine microalgal broth at low pH ($\text{pH} < 7$) (Fig. 1m), even at a high chitosan dosage (150 mg/L), in accordance with previous studies [26-28]. Chitosan as a long chain polymer is coiled in marine medium as the cationic charges are shielded by the high ionic strength (i.e. high salinity, 30 g/L in this study), and the bridging effect is therefore not effective in seawater [25, 26]. A high chitosan dosage was required at pH from 7 to 10 to induce flocculation, and a flocculation efficiency $>90\%$ could only be achieved at $\text{pH} > 8$ and a chitosan dosage >60 mg/L. The chitosan-based flocculation in seawater is mainly induced by a sweeping mechanism associated with precipitation of chitosan at high pH. A better flocculation efficiency was found when $\text{pH} > 8$, even without dosing flocculant. The possible reason may be the synergy from pH-induced auto-flocculation caused by $\text{Mg}(\text{OH})_2$ precipitation [29]. A similar result was also found in a previous study [27]. Although chitosan-based flocculation was generally effective for all freshwater microalgae when tested separately, yet for the multi-species mixture (Fig. 1j), the flocculation efficiency was generally much lower and exceeded 90% only at pH 4. The possible reason for this behavior is that when

using the same volume of microalgal broth (50 mL) for the flocculation jar test, the volume of each freshwater microalgal strain in the mixture was one third of 50 mL, and the applied flocculant dosage could not meet the optimal flocculation efficiency for every strain at the same time. This result also indicates that chitosan may not be suitable for treating natural microalgae-contaminated water.

pH-dependent flocculants such as chitosan require not only addition of a flocculant but also of acid to adjust the pH before flocculation and a base to return the pH to the initial level after flocculation, resulting in an increased cost. CNCs modified with quaternary amines have a cationic charge that is pH-independent (Fig. 1), showing similar flocculation behaviors over an extended big pH-range from 4 to 10 in the same microalgal broth. Cationic CNCs also showed better performance in freshwater microalgal mixture (the optimal flocculation efficiencies were all higher than 90%, especially for CNCs-PYR, with an efficiency higher than 95%). These results indicate that cationic CNCs may be suitable for flocculating natural microalgae-contaminated water with complex mixtures of microalgae without pH adjustment. Besides, this is also an attractive property of cationic CNCs for harvesting single-species microalgae, as the pH of culture medium tends to vary over short time-scales due to photosynthesis effects on the CO₂ concentration in the culture medium.

Chitosan-based flocculation showed the expected decline in flocculation efficiency in freshwater microalgae when exceeding the optimal dosage due to the dispersion re-stabilization. Previous studies showed that no dispersion re-stabilization occurred when using cationic CNCs for *C. vulgaris* harvesting [1, 4], and pointed to a different

flocculation mechanism that is patch mechanism (Supplementary Fig. S1). Unlike chitosan with flexible polymeric chain which can wrap itself around the cells and reorients its positive charges toward the cell [30]. CNCs can not coil or bend due to their inherent rigidity. Therefore, they always display positive charges pointing outwards, leading to positive patches on the cell surface [4]. In this study, we did indeed see no dispersion re-stabilization for *C. vulgaris*, but we did see it for the other species of freshwater microalgae (i.e. *Desmodesmus* sp., *Dictyosphaerium* sp.) as well as the mixture, indicating that dispersion re-stabilization may also occur when using cationic CNCs. Therefore care should be taken when generalizing results from experiments with a single model to other species. The possible reason is illustrated in Supplementary Fig. S1b. Since microalgal cell size (microalgal cell size: 3-5 μm) is more than 150 times bigger than CNCs (around 20 nm in diameter [1]. The whole microalgal cell surface therefore got coated by CNCs, and finally leading to the reversal of the surface charge of the microalgal cells.

Both 0.28-MIM-CNCs and 0.34-PYR-CNCs were effective in flocculating the marine microalgae (i.e. *N. oculata*) (Figs. 1n and 1o) especially at high pH, which may also be aided by pH-induced auto-flocculation. Unlike in freshwater microalgal broth where both chitosan and cationic CNCs showed a similar dose for the highest flocculation efficiency at low pH, cationic CNCs (around 60 mg/L to reach relatively high flocculation efficiency) required a much lower dose than chitosan (around 80 mg/L) to flocculate *N. oculata*, in line with a previous work [1]. In contrast to chitosan, cationic CNCs can not get coiled, even at high ionic strength due to their

crystalline structure and high rigidity property [1, 4]. The flocculation efficiency, however, always remained below 95% for both cationic CNCs. Small flocs were observed, remaining in suspension at the end of the flocculation test, in accordance with a previous work [1]. Negatively charged microalgal cells can attract positively charged ions from the culture medium, resulting in an electrostatic double layer around the cells [31]. The balance between repulsive and attractive energies due to the double layer leads to a stable suspension [32]. In addition, the weakened patch mechanism due to the high ionic strength may be another reason for the stabilization of the suspended small flocs [1]. Membrane filtration can thus be seen as a simple solution to this problem.

The bio-based quaternary ammonium flocculant Tanfloc was used to harvest *C. vulgaris* and *N. oculata* under similar flocculation conditions, showing the highest flocculation efficiency for *C. vulgaris* at a dose of 20 mg/L and at pH 5, and requiring dose of 20 mg/L to induce *N. oculata* flocculation [33]. Cationic CNCs also showed a similar dose to induce *N. oculata* flocculation. Although cationic CNCs required 40 mg/L to achieve the highest *C. vulgaris* flocculation efficiency, Tanfloc flocculation efficacy depends strongly on pH, and requires 70 mg/L at pH 9. Previous study used slightly different broths and filtration conditions, with a reported dose of 400 mg/L to achieve the maximum *C. vulgaris* flocculation efficiency obtained with branched cationic starch [34], further prove the advantages of the cationic CNCs used in the current work.

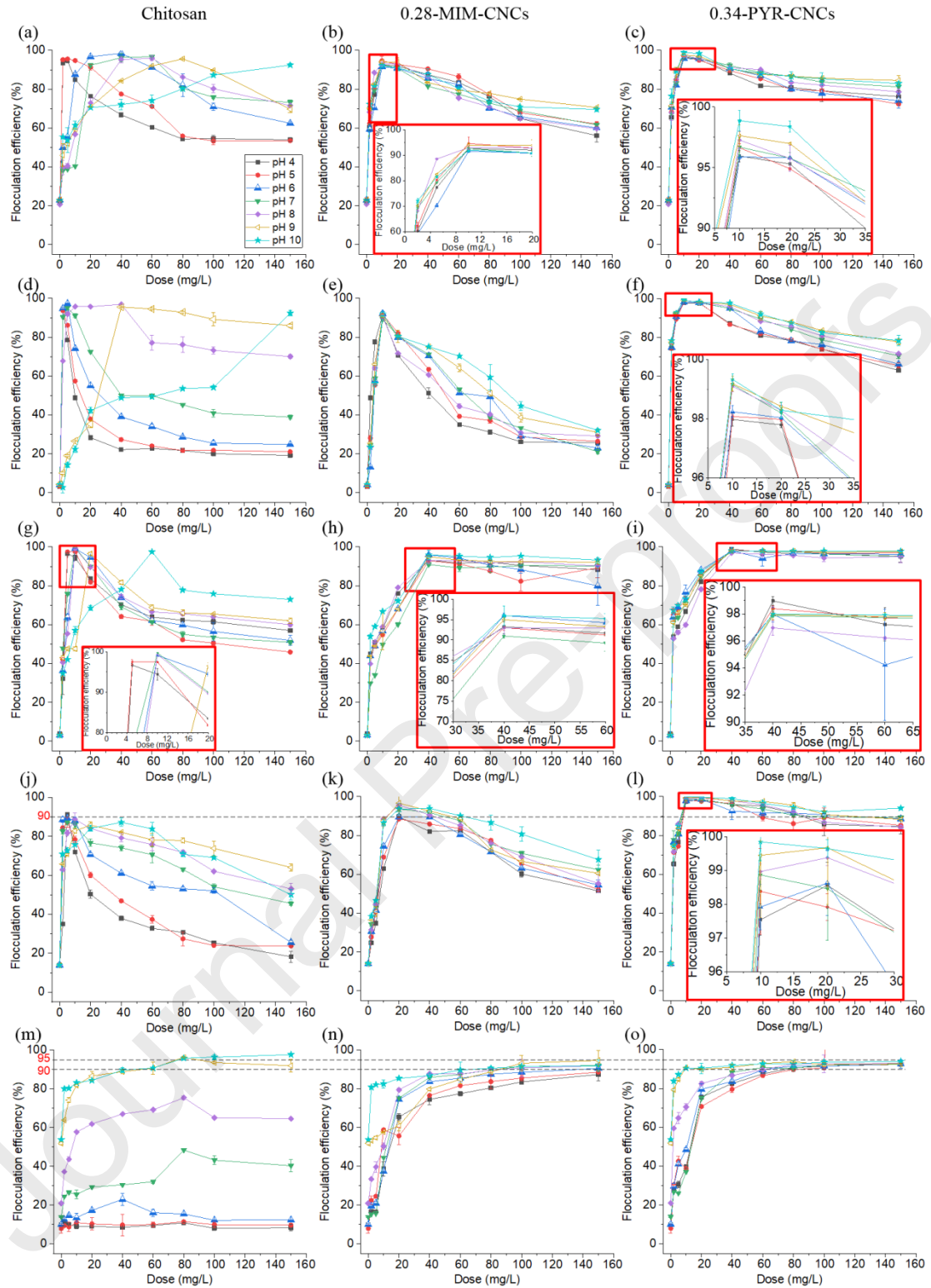


Fig. 1 Effects of pH and flocculant dosage on flocculation efficiencies of (a-c) *Desmodesmus* sp., (d-f) *Dictyosphaerium* sp., (g-i) *C. vulgaris*, (j-l) freshwater microalgal mixture, and (m-o) *N. oculata* using chitosan, 0.28-MIM-CNCs and 0.34-PYR-CNCs.

3.2 Membrane characterization

The surface and cross-sectional morphologies of the patterned and flat membranes are shown in Fig. 2a. The P-sPSf membrane (patterned membrane prepared from a sulfonsPSf/PSf blend) shows a remarkable triple-layer structure: a thick zone with large macrovoids supports a 200-300 μm thick intermediate layer with much smaller macrovoids which is ultimately topped by a spongy skin. This dense skin layer is thicker in the valleys than at the ridges (Fig. 2a close-ups), indicating that the fluxes through the membrane at the ridges may be higher than in the valleys. The possible reason is a different phase inversion process in the valleys than at the ridges as out-diffusing solvent mixes with a smaller amount of non-solvent, which is more stagnant in the valleys than on top of the ridges [14]. The intermediate and the skin layers are probably formed already during the spraying phase of the membrane synthesis process while the large-macrovoid region only forms during full membrane immersion in the coagulation bath. sPSf has a significant impact on the cross-sectional structure of flat membranes. More finger-like macrovoids are found in the flat sPSf/PSf blend membrane (F-sPSf) than in the F-PSf membrane, indicating that water may pass through the sPSf blend membrane.

The existence of a sulfonic acid group and thus a negative charge on sPSf blend membranes was confirmed with Fourier-transform infrared spectroscopy (FTIR) and electrokinetic measurements. The FTIR spectrum (Fig. 2b) shows a peak at $\sim 1041\text{ cm}^{-1}$, attributed to the vibration of the S=O bond in sulfonic acid. Both membranes

displayed a negative charge over a pH from 2 to 10 (Fig. 2c). The sPSf blend membranes showed a much stronger negative charge than the pure PSf membrane. As a result, fewer microalgal cells may finally attach to the sPSf blend membrane due to stronger electrostatic repulsion [14].

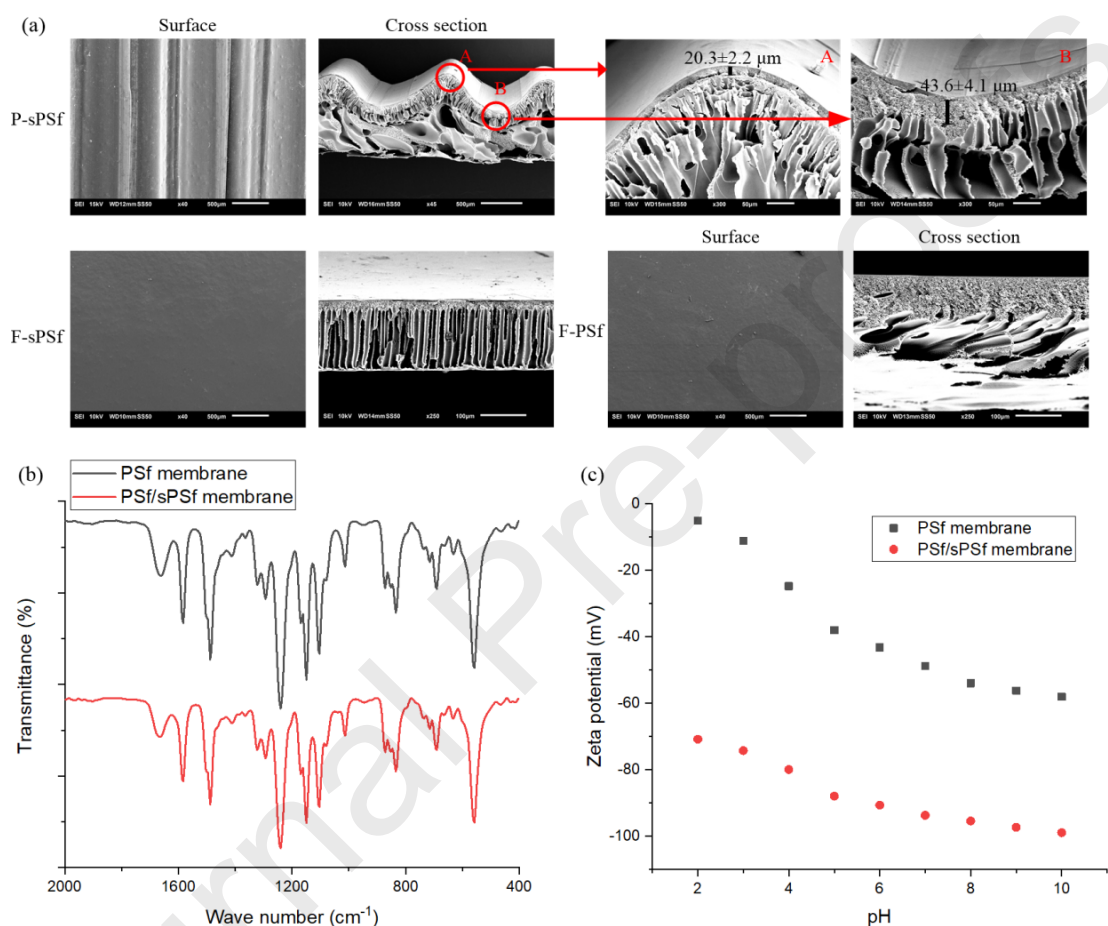


Fig. 2 (a) Surface and cross-sectional images of membranes, (b) FTIR spectrum and (c) zeta potential of PSf and sPSf blend PSf membranes. The close-ups represent the pore structure (A) at the ridges and (B) in the valleys of the patterns.

3.3 Vibration-assisted filtration combined with flocculation

A freshwater and a marine microalgal strain (i.e. *Desmodesmus* sp. and *Nannochloropsis oculata*) were selected for the flocculation-combined membrane

filtration test. Based on the flocculation test in Section 3.1, pyridinium modified CNC (0.34-PYR-CNC) was selected as flocculant. After cultivation, microalgal broths were directly flocculated without pH adjustment under optimal (10 mg/L for *Desmodesmus* sp. and 20 mg/L for *N. oculata*) and sub-optimal (5 mg/L for *Desmodesmus* sp. and 10 mg/L for *N. oculata*) conditions. After 10 days cultivation, the final pH values of *Desmodesmus* sp. and *N. oculata* were 8.62 and 8.05, respectively, and the corresponding flocculation efficiencies were 97% and 89% under optimal conditions, and 86% and 73% under sub-optimal conditions. The feeds were filtered using the MMV configuration, generating vertical shear on P-sPSf, F-sPSf and F-PSf membranes. The filtration of a non-flocculated microalgal broth was applied as benchmark. According to previous studies, frequency strongly affects the required energy in an MMV-system [12]. A low frequency of 1 Hz was thus selected, also to prevent breakdown at high shear of flocs into smaller particles which may block membrane pores more significantly. Additionally, this limited movement helps flocs to settle down more easily, and thus lowers microalgae-membrane contact [12]. The amplitude was fixed at 5 mm (corresponding shear rate is 35 s^{-1}).

Even when using non-flocculated microalgal broths as feed, all membranes already showed 100% harvesting yield (Fig. 3), proving the high retention efficiency of these membranes. The CF was used to evaluate the performance of the vibrating membranes using these 3 different feeds (Fig. 3). When the membrane permeance is higher than the CF, significant fouling occurs, strongly decreasing filtration performance. Membranes with a higher CF thus show less fouling under high flux [7,

11]. Previous studies showed that blending sPSf significantly increases membrane surface hydrophilicity and permeance [11, 14, 35]. In addition, the negative surface charge also repels here microalgal cells from the membrane surface [14, 35]. It was thus somewhat surprising that both flat membranes, irrespective of sPSf blending, showed the same CF-value ($10 \text{ L/m}^2 \text{ h}$) when using a non-flocculated suspension of *Desmodesmus* sp. For *N. oculata*, the F-sPSf membrane ($20 \text{ L/m}^2 \text{ h}$) showed more logically a higher CF-value than the F-PSf membrane ($10 \text{ L/m}^2 \text{ h}$). The microalgal concentration in the feed could explain this (i.e. the CF-value decreases with increasing microalgal concentration) [3, 36]. The dry biomasses of *Desmodesmus* sp. and *N. oculata* after 10 days cultivation were indeed $0.86 \pm 0.03 \text{ g/L}$ and $0.41 \pm 0.10 \text{ g/L}$, respectively, and the IFM only started from $10 \text{ L/m}^2 \text{ h}$. The higher concentration might thus have lowered the CF-value in non-flocculated *Desmodesmus* sp. Suspension to values below the detection limit of $10 \text{ L/m}^2 \text{ h}$.

Patterned membranes systematically showed higher CF-values than the flat membranes in non-flocculated microalgal suspensions (i.e. $25 \text{ L/m}^2 \text{ h}$ and $35 \text{ L/m}^2 \text{ h}$ for *Desmodesmus* sp. and *N. oculata*, respectively). This can be explained by the synergy between vibration-assisted filtration and membrane surface patterning [11]. Patterned membranes offer an extended surface area to achieve higher permeances and unique feed hydrodynamics to realize fouling mitigation [14]. Indeed, vibration can offer surface shear, and the patterns can promote the generation of turbulence eddies in-between the patterns. In addition, a higher wall shear was observed in the valleys and on the apexes of patterned membranes at 1 Hz via computational fluid

dynamics simulation, while no significant wall shear was observed on the flat membrane surface at the same frequency [11, 37].

Upon flocculation, the CF for both microalgae and all membranes significantly increased, irrespective of whether optimal or sub-optimal flocculation conditions were applied. The flat membranes showed better filtration performance for *Desmodesmus* sp. than for *N. oculata*, likely due to the lower flocculation efficiencies for *N. oculata* [12, 38]. Lower *N. oculata* flocculation efficiencies (89% and 73%) were obtained at 0.34-PYR-CNCs dosages of 20 mg/L and 10 mg/L, respectively, compared with *Desmodesmus* sp. (i.e. 97% and 86% at a dosage of 10 mg/L and 5 mg/L, respectively). Small suspended flocs of *N. oculata* were formed during flocculation even under optimal flocculation conditions, explaining why the CF-values of *N. oculata* were always lower than those of *Desmodesmus* sp..

A previous study showed a lower CF-value when flocculating *Dictyosphaerium* sp., and *C. vulgaris* under sub-optimal flocculation conditions than under optimal conditions [12]. However, in the current study, both *Desmodesmus* sp. and *N. oculata* reached ultimate CF-values (95 L/m² h) when using the P-SPSf membrane under both sub-optimal and optimal flocculation conditions. Moreover, these CF-values were more than 9 times higher than for non-flocculated microalgal suspensions. A previous study revealed a synergy between vibration-assisted filtration (to offer shear near the membrane surface), membrane surface pattern (to increase membrane permeance and offer unique hydrodynamics near the membrane surface), and membrane surface charge (to repel more microalgal cells from the membrane surface), showing a more

than doubled CF ($60 \text{ L/m}^2 \text{ h}$) when compared to flat membranes [11]. However, a high frequency (6.9 Hz in that case) was required to achieve that CF-value, which means a significantly higher energy input. In addition, combining flocculation with a vibrating flat membrane could only achieve CF-values of $85 \text{ L/m}^2 \text{ h}$ and $75 \text{ L/m}^2 \text{ h}$ under optimal flocculation conditions for *C. vulgaris* and *Dictyosphaerium* sp., respectively, and $50 \text{ L/m}^2 \text{ h}$ and $65 \text{ L/m}^2 \text{ h}$ under sub-optimal flocculation conditions [12]. In current study, four favorable approaches were now brought together (i.e. vibration-assisted filtration, membrane surface pattern, membrane surface charge, and flocculation), rendering a considerably higher CF at a very low vibration frequency and even under sub-optimal flocculation conditions, hence demonstrating a strong synergy between these four approaches which dramatically reduces energy cost, membrane investment and use of chemicals.

A recent study using flat polyvinylidene fluoride (PVDF) membranes without surface pattern nor charge in a tilted panel and an axial vibration systems to harvest *Euglena* sp. and *Chlorella. pyrenoidosa* showed CF-values of 72 and $60 \text{ L/m}^2 \text{ h}$, respectively [39, 40]. Even with enhanced shear near the membrane surface (and hence higher energy input) and lower microalgal concentration (i.e. 0.6 g/L of *Euglena* sp. and 0.55 g/L of *C. pyrenoidosa*), these CF-values were thus still lower than those reported here. Table 1 shows the membrane permeances which were always low without vibration, irrespective of membrane charges or patterns. Charges or patterns alone thus did not help much against fouling, nor when they were combined. However, flocculation prior to membrane filtration significantly increased membrane permeance even

without vibration-assistance, surface charge or pattern. When these four favorable approaches were combined together, however, a huge improvement in membrane permeance was realized. The order in which each approach separately contributed to enhancing the microalgae harvesting performance is: flocculation prior to membrane filtration > vibration-assistance > surface pattern > surface charge.

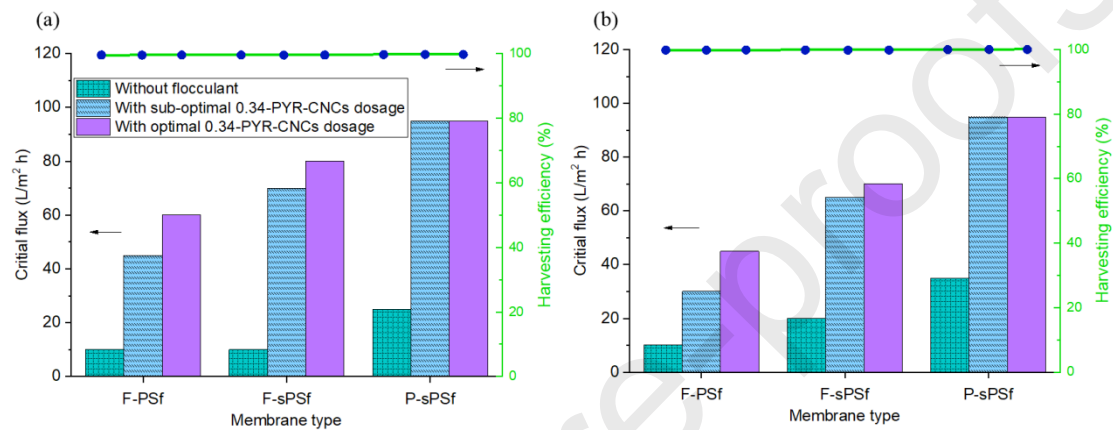


Fig. 3 Critical flux through F-PSf, F-sPSf, and P-sPSf membranes for the three different feeds (i.e. without flocculant, with sub-optimal or with optimal 0.34-PYR-CNCs dosage): (a) for *Desmodesmus* sp. and (b) for *N. oculata*.

Table 1 Comparison of membrane permeances for different membranes and filtration conditions

No.	Surface charge	Surface pattern	Vibration	Flocculation	Membrane permeance (L/m ² h bar)
1	√	√	√	√	5580 ^a
2	√	√	√	-	990 ^b
3	√	√	-	-	115 ^b
4	√	-	-	-	56 ^b
5	-	√	√	-	415 ^b
6	-	√	-	-	48 ^b
7	√	-	√	-	452 ^b
8	-	-	-	√	3750 ^c
9	-	-	√	-	280 ^d
10	-	-	√	-	220 ^e

Note: the experiments were performed using microalgal broth as feed. √ and -

respectively mean in presence or absence of that specific property. ^a data from current study. ^b data from [11] using the same membrane as in current study. ^c data from [12] using polyvinylidene fluoride (PVDF) membrane (pore size 0.013 μm). ^d from [3] using PVDF membrane (pore size 0.1 μm). ^e data from [41] using PVDF membrane (pore size 0.05 μm).

3.4 Filtration strategy

The vibration ratio R_v significantly affects the required energy [7, 18]. Continuous (i.e. R_v 100%) and intermittent vibration-assisted filtrations were therefore compared. The effect of R_v on filtration performance and energy consumption was investigated with a cycle time fixed at 60 min using the P-SPSf membrane. Considering that the highest CF (95 L/m² h) can also be achieved under sub-optimal flocculation conditions, both microalgae were treated with sub-optimal dosage, while MMV frequency, amplitude, and operational membrane flux were set at 1 Hz, 5 mm, and 95 L/m² h, respectively.

TMP was used to evaluate the filtration performance (Figs. 4a and 4b). A higher TMP means more fouling on the membrane surface (i.e. higher filtration resistance) [7]. For both microalgae, the highest filtration resistance was observed without vibration. All intermittent vibration experiments showed a better filtration performance, indicating that this approach can minimize fouling with reduced energy use. The lower fouling rates at higher R_v can be explained by the longer vibration phase, during which aggregation of microalgae on the membrane surface is prevented. It is also noticed that the P-SPSf membrane showed better filtration performance (i.e. lower TMP) for

Desmodesmus sp. than for *N. oculata*. This can be explained by the flocculation efficiencies: *N. oculata* with lower flocculation efficiency (73%) under sub-optimal flocculation conditions has more suspended microalgae in the broth than *Desmodesmus* sp. (86%), leading to more fouling. Although for an R_v of 50% and 70%, the TMP logically increased during the idle time, TMP went back to the lowest value during the vibration time (19 mbar for *Desmodesmus* sp. and 21 mbar for *N. oculata*), while an R_v of only 30% could not reach that lowest TMP anymore. This indicates that no irreversible fouling occurred at $R_v > 50%$ [7, 18], also suggesting that such R_v can be used in a full-scale harvesting process to lower energy use without affecting filtration performance. Unlike previous work [18] where TMP increased dramatically during the idle time, only a relatively small increase was observed in the current study.

The MMV energy consumption was measured at lab scale. A significant impact ($P < 0.05$) of R_v was observed on energy consumption (Fig. 4c). Because the MMV energy consumption is mainly related to the vibration engine and to the membrane flux through the use of the peristaltic pump (fixed at 95 L/m² h), the P-SPSf membrane rendered the same energy consumption for both microalgae. The MMV energy consumption significantly decreased from 4700 Wh/m³ for an R_v of 100% to 3100 Wh/m³ and 2600 Wh/m³, respectively for an R_v of 70% and 50%. A proper R_v and flocculation for the MMV system can thus not only guarantee high-yield harvesting, but also energy- and cost-saving operation.

A recent study using uncharged, non-corrugated PVDF membranes in the MMV

vibration system to harvest *Dictyosphaerium* sp. showed the lowest MMV energy consumption of 5700 Wh/m³ at $R_v=50\%$) [18], thus more than twice the value reported here, again confirming the realised synergy.

The actual energy consumption at larger scale (assumed to correspond for the current MMV-engine to 10 modules with an active area of 0.08 m² each) was estimated theoretically. Because of the very low TMP in submerged filtration, a peristaltic pump is also considered for this larger scale process [3, 7]. The performance of the maximum-scale MMV system was recalculated with an R_v of 50% and an operational flux of 95 L/m² h. From the module number (x), the energy consumption of the MMV can be calculated according to (y , Wh/m³) as $y=65.79/x+0.0003$ (Fig. 5, $R^2=0.99$) to reach a record-low E_T (total energy consumption of membrane filtration) of 6.7 Wh/m³ (energy consumption of 6.6 and 0.094 Wh/m³ for the vibration system and the peristaltic pump, respectively, including computer). This value should be compared with 8000 Wh/m³ for centrifugation [42] at a >90% harvesting efficiency [36]. The energy consumption of conventional microalgae-related membrane filtration normally ranges from 170 to 2000 Wh/m³ [43]. By combining flocculation with filtration using a conventional (i.e. flat, non-corrugated) membrane, a much lower energy consumption of 50 Wh/m³ [44] has been reported. This is still 7 times more than in the current study, confirming the exceptional synergy realized here.

It can be anticipated that fouling may significantly influence the membrane flux when reaching high up-concentration levels [36, 44]. In that respect, this type of membrane filtration can probably be best applied as a pre-concentration step (to reach e.g. a

15-fold pre-concentration) which would not yet significantly influence the membrane flux [36]. It can then be combined with a final dewatering step by centrifugation to achieve an ultimate product concentration of e.g. 20% w dry biomass/v [36, 44-46]. In such a two-stage harvesting process, a dry paste in which only intracellular water remains can be obtained as an end-product with minimal energy use.

When combining the optimized membrane filtration (6.7 Wh/m^3) with centrifugation (8000 Wh/m^3), and assuming an initial microalgae concentration of $0.9 \text{ g dry biomass/L}$ and 15-fold pre-concentration via filtration, the final energy consumption for harvesting would be 540 Wh/m^3 . This value is still far below the energy consumption of single-step centrifugation (8000 Wh/m^3), vacuum filters (5900 Wh/m^3), or the combination of non-vibrating, flat membrane filtration and centrifugation ($900\text{-}2100 \text{ Wh/m}^3$) (Fig. 4d) [36, 44, 47]. The 15-fold pre-concentration can be realized in the current study because, in contrast to conventional high-shear pumping in cross-flow membrane filtration or in air-scoured submerged membrane filtration, microalgal flocs would tend to settle down [12, 14, 48] in the MMV system while agitating only gently (1 Hz vibration over 5 mm amplitude) and with 50% idle moments. This obviously lowers the direct contact between microalgal cells and the membrane surface and reduces cell rupture which could otherwise intensify fouling. Although a lower energy consumption can be found when using the combination of flocculation and centrifugation ($40\text{-}100 \text{ Wh/m}^3$) [44, 49], such flocculation would require more input of chemicals (need to operate under optimal conditions) and could never reach 100% microalgae recovery, causing

significant loss of biomass. In addition, membrane filtration produces a permeate that is free of microalgae and bacteria, allowing it to be reused, which is essential to reduce the water footprint in microalgae cultivation.

Recent studies using PVDF membranes without flocculation prior to membrane filtration, membrane surface charge nor pattern in a tilted panel to harvest *Euglena* sp. and *Spirulina* sp. showed energy consumptions of 0.1 kWh/m³ and 0.03 kWh/m³, respectively [39, 50], which are still significantly larger than those in current study.

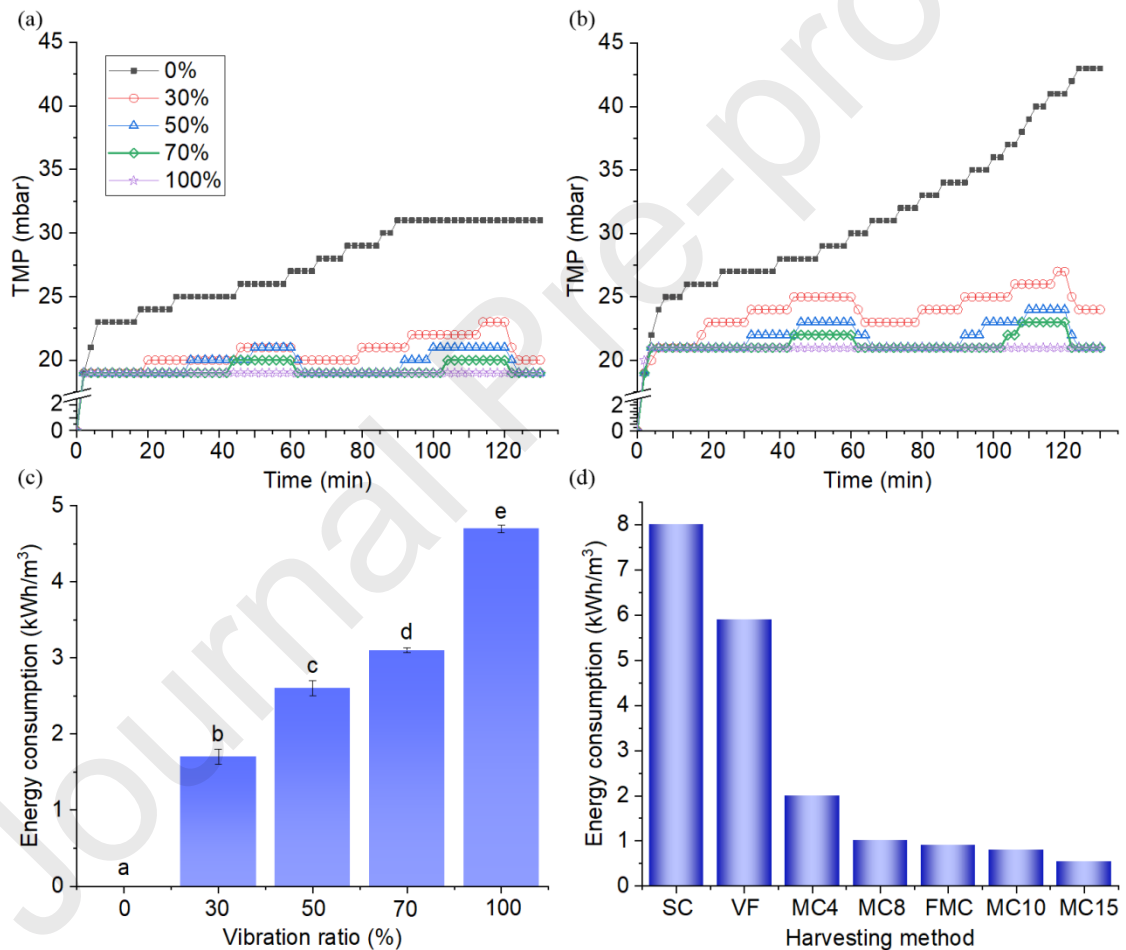


Fig. 4 Effect of filtration strategy (varied vibration ratio (R_v) with a fixed cycle time of 60 min) on filtration performance ((a) for *Desmodesmus* sp. and (b) for *N. oculata*) and (c) MMV energy consumption (1 membrane module with an active area of 200 cm²); (d) Effect of harvesting method on the total harvesting energy consumption (10

membrane modules with an active area of 800 cm² each).

Note: Letters a-e in (c) show the significance; Values marked with the different lower case letters are significantly ($P < 0.05$) different.

SC: single-step centrifugation; VF: vacuum filters [47]; FMC: the combination of conventional flat membrane filtration (15-fold pre-concentration) and centrifugation [36]; MC4-15: the combination of patterned membrane filtration in current study and centrifugation with 4-15-fold pre-concentration.

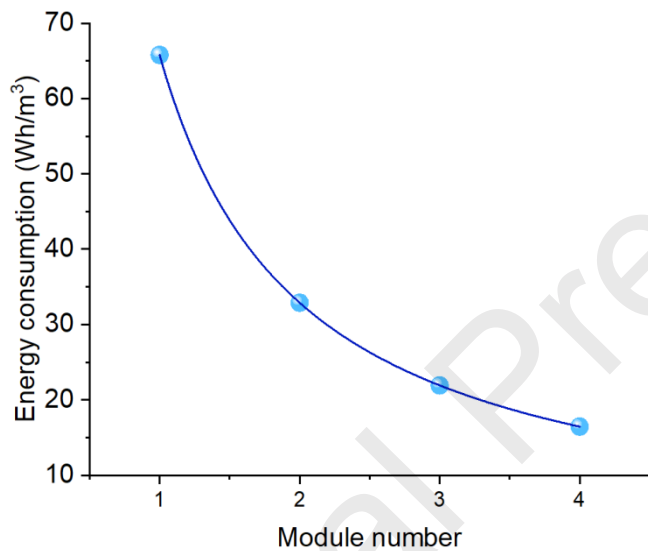


Fig. 5 The modified fitting curve for energy consumption as a function of the number of modules (0.08 m² each) on 1 MMV system at a membrane flux of 95 L/m² h using an R_v of 50%.

3.5 Interaction force analysis

To unravel the mechanism of using an MMV system to separate flocculated microalgae, the F_D and F_{IL} were investigated with the MMV frequency and amplitude set at 1 Hz and 5mm, respectively. F_D is one of the main forces responsible for accelerating microalgae deposition on the membrane surface during the filtration

process, while F_{IL} , as an opposite force, prevents microalgae from attaching to the membrane surface [22]. The profiles of these 2 interaction forces between microalgal cells and the membrane as a function of microalgal radius are shown in Fig. 6. A positive value represents a repulsive energy; a negative value an attractive energy. The microalgal radius strongly influences the F_{IL} , showing that more microalgal cells can be repelled from the membrane surface if the microalgal cell radius is $>20 \mu\text{m}$. However, F_{IL} declines rapidly as the radius decreases, resulting in the deposition of smaller microalgal cells. Similar results were also found in a previous study [22]. Dosing flocculants in the MMV system can thus significantly increase the apparent particle size, resulting in an increased F_{IL} , and can therefore significantly reduce fouling, even at a very low vibration frequency (1 Hz).

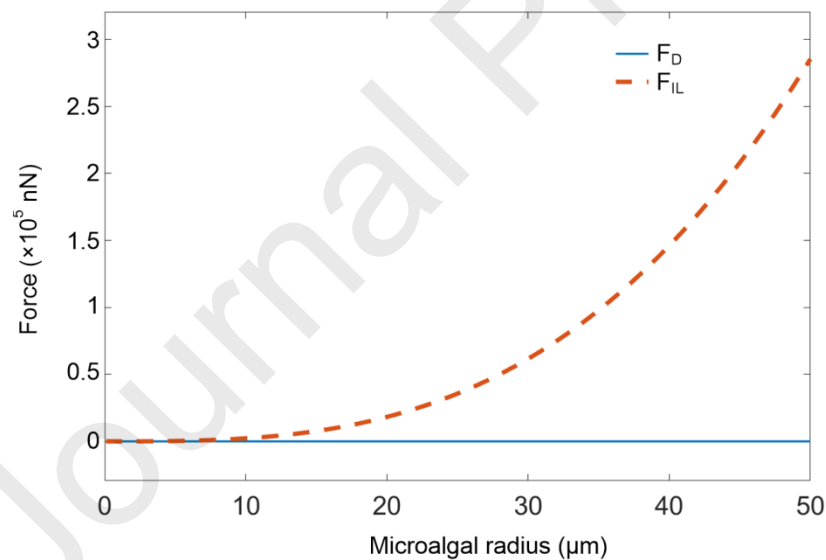


Fig. 6 Profiles of interaction force as a function of the microalgal radius.

4. Conclusions

Flocculation-combined membrane filtration was carried out using cationic CNCs in an

MMV system with minimal agitation and even under sub-optimal flocculation conditions, proving a strong synergy between membrane filtration and flocculation. Four approaches (vibration-assisted filtration, membrane surface patterns, membrane surface charge, and flocculation) were for the first time combined and led to the highest CF (95 L/m² h) with 100% harvesting efficiency (not possible only with flocculation), even already under sub-optimal flocculation conditions, proving that less energy is required and a lower dose of flocculant can be used during the filtration-flocculation process. Intermittent vibration offered an economic operation mode with excellent filtration performance. Simulations for a larger-scale microalgal harvesting plant showed a record-low energy consumption of 6.7 Wh/m³. Due to the extremely low energy consumption and reduced input of chemicals, a much lower microalgae harvesting cost can be anticipated than via traditional centrifugation, thus suggesting a high-yield, cheap and energy-saving process with potential to also lower the water footprint for sustainable microalgal resource development.

The mechanism of alleviating fouling through flocculation in an MMV system was unraveled based on the membrane-cell interaction force by calculating F_D and F_{IL} , showing a higher repulsive force when increasing microalgal radius.

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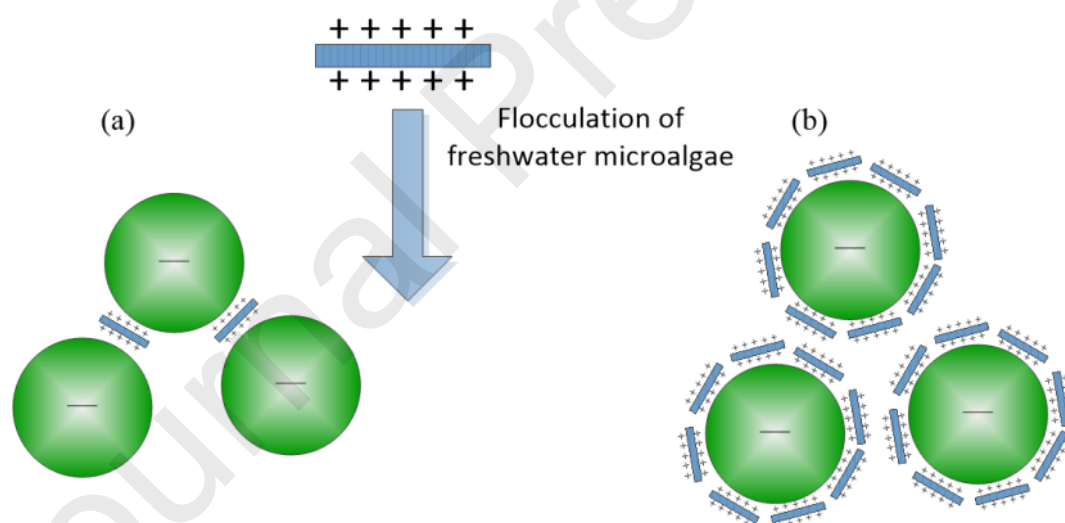
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Supplementary information



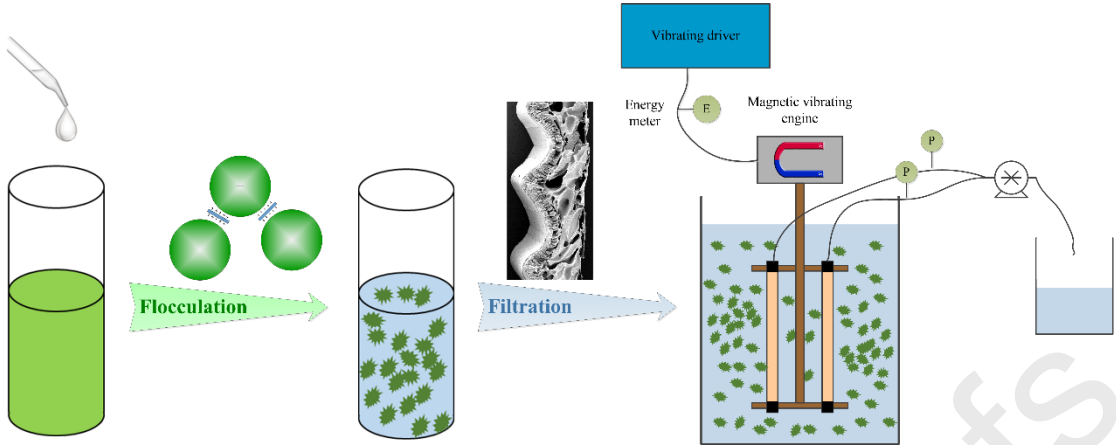
Supplementary Fig. 1 Schematic representations of (a) the possible patch mechanism of cationic modified CNCs, according to a previous study [1], and (b) the possible reason why disperse re-stabilization still occurs in some microalgae.

Supplementary reference

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Highlights

1. Cationic CNCs could achieve >90% microalgae flocculation without pH-adjustment.
2. 0.34-PYR-CNC showed the best performance for both fresh and marine microalgae.
3. A record-low energy consumption of only 6.7 Wh/m³ was achieved.
4. Surface patterning can significantly enhance flux, while reducing energy input.
5. Increasing particle radius can mitigate fouling on the vibrating membrane surface.



Credit Author Statement

Zhenyu Zhao: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data Curation, Writing-Original Draft, Visualization, Funding acquisition. **Jonas Blockx:** Investigation, Writing-Review & Editing. **Koenraad Muylaert:** Writing-Review & Editing, Supervision. **Wim Thielemans:** Writing-Review & Editing. **Anthony Szymczyk:** Writing-Review & Editing. **Ivo F.J. Vankelecom:** Writing-Review & Editing, Supervision, Project administration, Funding acquisition.

Declaration of interests

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.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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