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Title: Implementation of flocculation and sand filtration in medium recirculation in a closed microalgae production system

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Abstract

Recycling growth medium is a necessity to reduce production cost and ecological foot print of large scale microalgae production systems. To prevent contamination and/or enrichment with particulate matter, medium recycling requires pre-treatment of the centrifuge supernatant (centrate), prior to medium replenishment and re-use. In this study, we investigated the applicability of high pH induced flocculation and/or sand filtration to interface with an existing microfiltration setup in order to prepare recycled growth medium for the mass cultivation of marine microalgae. Sand filtration partly alleviated the burden on subsequent microfiltration, but proved to be particularly useful to remove high pH induced flocs from the centrate. Combination of both techniques resulted in a removal of 78 ± 18% of particles, resulting in an improvement of 'modified fouling indices' by 75 ± 19%. Despite a partial to complete removal of remaining nutrients such as phosphate, calcium and magnesium during treatment, a cost saving of 72% compared to a scenario with fresh medium can be achieved.

Highlights

- Characterisation of flocculation and sand filtration of centrifuge supernatant

- Optimal combination of flocculation and sand filtration was established

- Pre-treatments enable sustainable recirculation of spent microalgae growth medium

Keywords: Microalgae, flocculation, sand filtration, medium recycling

1. Introduction

In order to tap into the enormous potential of microalgae as resource for bulk biofuel, feed/food and chemical applications, substantial reduction in production cost is needed [1]. Recycling culture broth is key to further decrease these costs [2], especially in cases where final applications call for the use of more expensive feed or food grade ingredients. At present, centrifugation is the most commonly used method for harvesting microalgae. Due to the limited separation efficiency inherent to the technique, a fraction of particles present in culture broth, including intact and broken algae cells, bacteria, and other debris are retained in the centrifuge supernatant (centrate). While in principal, this centrate could be reused as growth medium after nutrient replenishment [3–5], applying non-treated centrate as culture medium will contaminate and/or eventually enrich the production system with unwanted algae, protozoan grazers, cell debris and bacteria under normal field operating conditions. Therefore a recycling scheme for culture medium calls for post-treatment of the centrate prior to medium replenishment and re-use. At present, no practical post-treatment process is available that can feed into a microfiltration unit such as the one that is currently operated by us to provide sterile media to Proviron's ProviAPT™ production system. De Baerdemaeker et al. [6] reported that during microfiltration of centrate heavy fouling occurs which results in a severe loss of permeability. Dissolved algogenic organic matter (AOM) including polysaccharides, proteins and humic acid-like organics [7], are reported to cause substantial membrane fouling during microfiltration (MF) [8], ultrafiltration (UF) [9,10] and nanofiltration (NF) [11]. Pre-treatments prior to membrane filtration which decrease the concentration of both suspended solids and dissolved AOM of the centrate are therefore needed to reduce the load on the membrane and thus prolong its lifespan.

Coagulation pre-treatment using metal salts, such as aluminium and iron is commonly applied to control fouling in MF or UF systems [12,13] by removing both particulate and dissolved materials [14]. For our purpose, high pH induced flocculation is an attractive alternative because it is low-cost and is low in energy consumption. Furthermore, it is non-toxic [15], a prerequisite for further use of the cleared medium. The technique was found effective for harvesting microalgae [16,17]. Both NaOH and hydrated lime, $Ca(OH)_2$, are suitable to increase pH in a controlled manner [18]. Presence of AOM, however, interferes with the process [19], resulting in the need of a higher pH to induce flocculation [20]. In this study the applicability of high pH induced flocculation of centrate was investigated.

Sand filtration is frequently used as a cheap, easy and robust technique to remove suspended solids from water. The method has a broad application in the water treatment field, where it has proved its efficacy [21]. A few studies indicated the applicability of sand filtration to separate algae from the liquid broth [22,23]. However, the use of sand filtration as a pre-treatment technique to polish centrate has not been investigated previously. In this study the efficiency of sand filtration to eliminate suspended solids from the centrate prior to membrane filtration was researched.

Rapid sand filtration is usually preceded by coagulation, flocculation and/or sedimentation to increase its removal efficiency. Therefore, implementation of both techniques at optimum settings in a linked setup was also studied in order to assess which (combination of) approaches can be used in a large scale installation to reduce fouling and increase MF performance (shorter filtration times, longer filter lifespan).

2. Materials and methods

2.1. Algae cultivation – centrate collection

The centrate used in this study was obtained from a *Nannochloropsis* sp. culture cultivated semi-continuously in a 12 m³ module of an outdoor production system of the ProviAPT™ photobioreactor [24,25] (Fig 1).

Fig 1: View of a ProviAPT™ microalgae production plant of 48 m³ culture volume

The growth medium consisted of artificial seawater supplemented with nutrients in concentration ratios derived from the *f* formulation [26]. The seawater was prepared using a refined sea salt mixture (Zoutman Industries, Belgium) to obtain a salinity of 26 g L^{-1}. After addition of nutrients, the medium was filtered (0.2 µm, KrosFlo®, Spectrum®, USA) prior to pumping to the reactors. Algae were harvested daily and dewatered using an automated desludging disc centrifuge (SSD 18-06-007, GEA Westfalia, Germany), yielding an algae paste and a supernatant, the centrate. The density of the harvests which provided the used media, ranged from 1.2 to 3.1 g L^{-1}. These centrates were, in turn, characterized by dry weight and optical density. In addition, macronutrient concentrations of the centrates were determined. Magnesium, calcium and trace elements were only measured of selected centrates (2.5 Analysis).

2.2. General setup of flocculation experiments

High pH induced flocculation was investigated using conventional jar test experiments [27]. Centrate was divided in 200 mL portions in 1 L beakers and mixed using a magnetic stirrer. The pH was adjusted to five different levels ranging from 9.5 up to 11.5 with a 0.5 interval by addition of 1 M NaOH or $Ca(OH)_2$. The amount of alkali needed to obtain the desired pH was recorded. Samples were stirred at 300 rpm for 10 minutes following pH adjustment. Next, solutions were allowed to settle for 60 minutes after which a 1 mL sample was collected from the centre of the clarified zone. The flocculation efficiency η_a was determined based on the decrease in optical density of the cleared centrate as suggested by Vandamme et al. [27]:

$$\boldsymbol{\eta_a = \frac{OD_i - OD_f}{OD_i}} \qquad (1)$$

where OD_i is the optical density of the solution after sedimentation without pH adjustment (control), and OD_f is the optical density of the suspension after flocculation and settlement. Next, a 15 mL sample was taken from the cleared upper part of the solutions to investigate nitrate and phosphate removal. Remaining quantities were then stirred again and equivalent samples were taken from the blended mixtures. Both types of samples were neutralised with 2 M HCl and the amounts of acid required to reach their initial pH values were recorded. To assess improvement of filterability, the modified fouling index (MFI) was determined of selected samples from the upper part of flocculated solutions and of blended mixtures. At the end of the flocculation experiment, the morphology of settled flocs was documented using a BX51 Olympus microscope with differential interference contrast (DIC) fitted with an E410 Olympus camera.

Furthermore, calcium and magnesium removal from representative samples was calculated. The removal of trace elements due to flocculation was also investigated to determine the need for replenishment prior to reusing the medium. To this end, samples were taken from the top layer of NaOH flocculated centrate and, after neutralisation, elemental composition was determined. A chemical equilibrium model (Visual MINTEQ 3.0 [28]) was used to predict the theoretical manifestation and speciation of precipitates in standard medium with pH increase.

2.3. General setup of sand filtration experiments

The sand filter was constructed of a polyvinyl chloride column of 1 m with inner diameter of 45.2 mm. At the top and the bottom of the column a 3-way ball valve was installed to allow performing a backflush rinsing. After the top and prior to the bottom valve, a gauge was fixed to observe pressure increase (Fig 2).

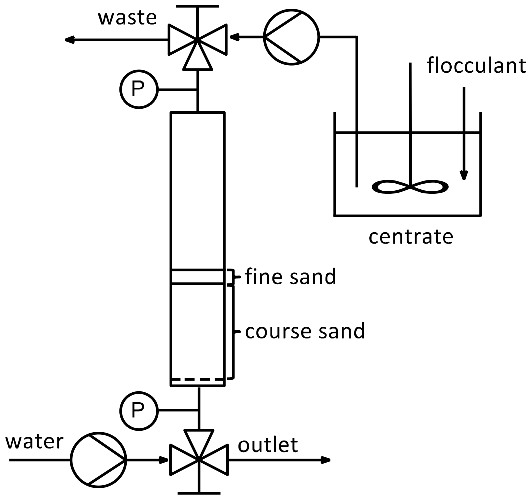

Fig 2: Experimental setup of the sand filtration unit.

Sand with two different grain sizes was used to fill the column. 'Coarse' sand had a diameter between 250 and 500 µm, while 'fine' sand had a median particle size (Dv50) of 170 µm. Sabiri et al. [22] suggested a bed height of 40 cm to obtain a stable removal efficiency. In this study, the bed was packed with 35 cm of coarse sand and 5 cm of fine sand on top to improve separation efficiency. Preliminary experiments indicated that a higher sand bed of fine sand resulted in rapid blockage of the filter, decreasing the flow drastically.

Before filtration, the bed was completely fluidized by backwashing with water to remove air bubbles from the system and to allow sand particles to redistribute according to velocity. Next, particles settled by sedimentation with the finer particles located in the upper section of the bed while larger ones were found at the bottom. Remaining water was evacuated through the bottom valve, resulting in a slight compaction of the sand bed. During filtration, the water flowed in a downward direction through the fixed bed using a demand spray pump (RLF122202, Flojet). The inlet flow was regulated using an adjustable power supply and voltage was maintained constant during filtration. The increase in height of the water column above the sand bed was tracked throughout. At the outlet 50 mL samples were taken every five minutes for further analysis. Between the five minute intervals, the exhaust flow rate was determined. Afterwards, optical density of the permeate samples was measured and filtration efficiency calculated, similar to the flocculation efficiency. Next, as recycling of permeate after sand filtration is the final goal, nitrate and phosphate concentrations were measured to evaluate their retention. Calcium and magnesium were also measured before and after sand filtration. Furthermore, MFI's of selected samples were compared to the control to assess improvement of filterability. After each filtration run, the sand bed was backflushed with tap water until the overflow was clear.

2.4. Centrate clearing by means of combined flocculation – sand filtration

To improve the separation efficiency of the sand filtration, flocculation of the centrate was implemented as a pre-treatment. Both NaOH and $Ca(OH)_2$ were used to alter the pH of 15 L centrate portions to 10.5 and 11.5 prior to sand filtration. After pH adjustment, centrates were mixed on a magnetic stirrer (1200 rpm) for 10 minutes to allow aggregates to form. During filtration over the sand bed, the inlet solution was continuously stirred gently to provide a homogenous mixture (Fig 2). Filtration, sampling and analysis were performed similar to the sand filtration experiments. After filtration of flocculated centrate, the bed was backflushed with neutral or acidified water (pH 2.2).

2.5. Analysis of centrate composition

Centrate was characterised by the amount of suspended solids, which were determined gravimetrically. Aliquots of 20 mL centrate were filtered through pre-weighed, pre-dried A/C glass-fiber filters (pore size 1 µm, Pall, USA). Subsequently, filters were rinsed with an equal amount of 0.5 M ammonium formate to remove interfering salts. Next, the filters were dried overnight at 110 °C until constant weight to calculate the dry weight.

Flocculation and sand filtration efficiency was assessed based on optical density measured as the absorbance at 550 nm in a UV-VIS spectrophotometer (Cary 50 Conc, Agilent Technologies).

Nutrient concentrations were determined following the procedures described in the Standard Methods [29] after filtration of the samples (0.2 µm). The analysis of nitrate is based on the absorbance at 220 and 275 nm in an acid environment ([29], method No. 4500 B Nitrate). The ascorbic acid method was applied to determine the amount of reactive phosphates ([29], method No. 4500-P E). Meanwhile, determination of salt cations (Mg and Ca) was conducted with an atomic absorption spectrophotometer (AAS), while quantification of trace element concentrations was conducted with a high-resolution inductively-coupled plasma mass-spectrophotometer (HR-ICP-MS).

2.5.1. Modified fouling index (MFI) assessment

The modified fouling index (MFI) was used as a tool to assess the fouling potential of the centrate. The MFI is determined by a constant-pressure dead-end filtration process and was originally defined as an index of the fouling potential of reversed osmosis feed water by Schippers and Verdouw [30]. The time required to filter a fixed volume of 500 mL through a standard 0.45 µm microfiltration membrane with a diameter of 47 mm (PES Supor®-450, Pall, USA) under constant pressure (200 kPa) dead-end filtration mode is registered. The samples were diluted 200 times in reversed osmosis water of which the pH was adjusted corresponding to the pH of the sample. The cumulative permeate volume and filtration time is recorded. Next, the obtained data were fit in commonly used models to analyse the membrane filtration process [31], described by equation (2)-(5).

$$\frac{\mathbf{d(V)}}{\mathbf{dt}} = \mathbf{J_0} - \mathbf{k_b} \cdot \mathbf{V} \tag{2}$$

$$\frac{\mathbf{t}}{\mathbf{V}} = \frac{\mathbf{1}}{\mathbf{J_0}} + \frac{\mathbf{k_s}}{\mathbf{2}}\mathbf{t} \tag{3}$$

$$\frac{\mathbf{dt}}{\mathbf{d(V)}} = \frac{\mathbf{1}}{\mathbf{J_0}} + \mathbf{k_i}\mathbf{t} \tag{4}$$

$$\frac{\mathbf{t}}{\mathbf{V}} = \frac{\mathbf{1}}{\mathbf{J_0}} + \frac{\mathbf{k_c}}{\mathbf{2}} \cdot \mathbf{V} \tag{5}$$

In these equations, t is filtration time, V is the cumulative permeate volume, J_0 is the initial flux, while kb, ks, ki and kc (i.e., the above mentioned MFI) are the coefficients of complete blocking, standard blocking, intermediate blocking, and cake filtration models respectively. Modified fouling indices were derived from the model which showed the best fit and used to compare the filterability of the samples. By distinguishing which model showed the best fit, additional information about the reigning filtration modus was obtained.

3. Results and discussion

3.1. Centrate clean-up by flocculation

3.1.1. Efficiency of NaOH and $Ca(OH)_2$ promoted flocculation of centrate

The applicability of flocculation of exhaust medium was investigated using three different centrates (Table 1).

Table 1. Characteristics of three centrates used to test flocculation. Measurements were performed in duplo (n = 2).

Centrate number	Dry weight particulate matter (g L^{-1})	P concentration (µM)	N concentration (mM)	OD (Abs 550nm)
1	0.27 ± 0.03	2.1 ± 0.1	0.42 ± 0.04	0.84 ± 0.02
2	0.23 ± 0.08	59 ± 2	1.29 ± 0.08	0.63 ± 0.02
3	0.15 ± 0.04	706 ± 14	2.56 ± 0.03	0.48 ± 0.02
Fresh medium	-	1700	25	-

Both flocculation efficiency and filterability, determined as MFI's, increased significantly with increasing pH (ANOVA, P<0.01), as illustrated in Fig 3 and Table 2 respectively. Hence, augmentation of pH with either NaOH or $Ca(OH)_2$ can be applied in order to flocculate centrate and improve its filterability. Acquired filtration data fitted best with the standard blocking and cake filtration model [31] (Table 2). Merely based on flocculation efficiency and improvement of filterability, one would be inclined to use $Ca(OH)_2$ flocculation at highest pH of 11.5 to flocculate centrate.

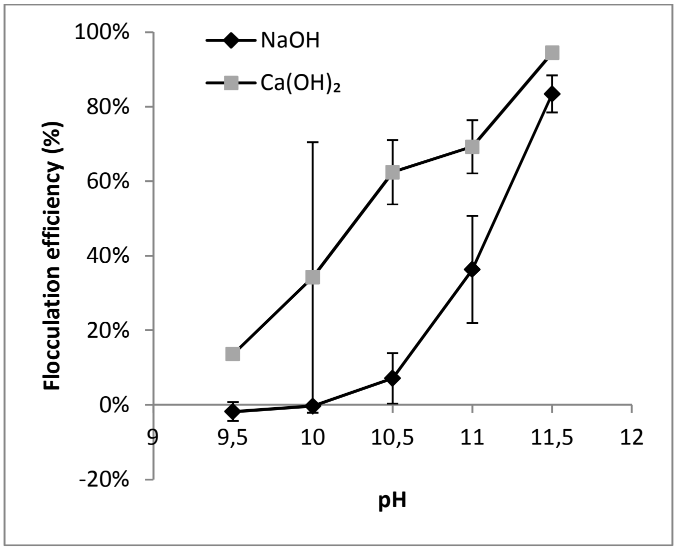

Fig 3: Flocculation efficiency versus pH for two different alkali species. Centrates 1 and 2 were investigated.

Table 2: MFI's ks and kc of flocculated centrate (n=2) using two different alkali species on centrates 1 and 2.

Alkali	pH	ks (m^{-1})	kc ($s\cdot m^{-2}$)
NaOH	control	1.92 ± 0.03	9E+03 ± 1E+03
	9.5	1.91 ± 0.02	7E+03 ± 4E+03
	10.5	1.84 ± 0.06	3E+03 ± 2E+03
	11.5	0.21 ± 0.04	17 ± 4
$Ca(OH)_2$	control	1.91 ± 0.02	5E+03 ± 3E+02
	9.5	1.88 ± 0.02	6E+03 ± 1E+03
	10.5	1.6 ± 0.4	3E+3 ± 4E+03
	11.5	0.3 ± 0.1	3E+01 ± 2E+01

The MFI's of stirred flocculated solutions were also measured. Due to the aggregation of suspended particles, the filtration mechanism changed from standard blocking towards cake filtration. The ks and kc indices of centrates flocculated at pH of 11.5 (ks of 5.0 ± 0.2 and kc of $9\cdot10^3 \pm 5\cdot10^3$) were significantly lower compared to control indices (ks of 6.5 ± 0.4 and kc of $4\cdot10^4 \pm 2\cdot10^4$) (ANOVA, $P<0.01$). Thus, filterability of centrate flocculated at high pH was improved even without removal of flocked particles.

These results suggest the feasibility of flocculation to improve filterability of the centrate. However, flocculation at intermediate alkaline pH did not yield consistent efficiencies when centrate features changed. Furthermore, divergent efficiencies were obtained when different alkalis were used.

3.1.2. Implication of NaOH or $Ca(OH)_2$ utilization

A pH increase induced by addition of $Ca(OH)_2$ resulted in significantly higher flocculation efficiencies (ANOVA, $P<0.01$) compared to NaOH flocculation (Fig 3 and Table 3). This effect was pronounced at slightly to moderately alkaline pH's, where calcium phosphate precipitation is known to induce flocculation [20]. On the one hand, $Ca(OH)_2$ flocculation caused a higher phosphate removal at slightly to moderately alkaline pH values (Table 3). This result suggests that $Ca(OH)_2$ flocculation caused higher calcium phosphate precipitation compared to NaOH flocculation, inducing better removal of organic substances.

Similar results were obtained by Castrillo et al. [32] when comparing the use of $Ca(OH)_2$ and NaOH to flocculate microalgae. Yet, these authors also noted the formation of calcium carbonate precipitates with $Ca(OH)_2$ flocculation which ended up in the flocculated pellet [32]. Similarly, microscopic analysis demonstrated the appearance of rather large calcium carbonate crystals in the flocs at slightly to moderately alkaline pH values (Fig 4). The formation of calcium carbonate crystals is likewise suggested by a much higher calcium removal in relation to phosphate compared to the 5:3 M Ca:P stoichiometry of hydroxyapatite ($Ca_5(PO_4)_3$) which was believed to precipitate during flocculation (modelled with Visual MINTEQ, [33,34]). These crystals can enhance sweep flocculation, contributing to the higher removal rates obtained by $Ca(OH)_2$ flocculation. Since removed phosphate concentrations are three orders of magnitude smaller than the amounts of removed calcium, precipitation of calcium carbonate is likely to have a much higher influence on the flocculation efficiency at slightly to moderately alkaline pH values. A higher $Ca(OH)_2$ requirement to raise the pH to equivalent values compared to NaOH (see section 3.1.3), however, diminishes the advantage of the higher efficiencies achieved.

Table 3. Influence of alkali species, phosphate concentration and pH on flocculation efficiency, calcium removal and phosphate removal. Removals are expressed as changes in molar concentration. Calcium removal is calculated as the difference between the remaining dissolved calcium after flocculation and the initial concentration supplemented with the added $Ca(OH)_2$ (if applied).

Alkali	Phosphate start concentration (µM)	Calcium start concentration (mM)	pH	Flocculation efficiency (%)	Remaining calcium (mM)	Remaining phosphate (µM)
NaOH	3 ± 1	1.97 ± 0.06	9.5	2 ± 2	1.1 ± 0.2	2 ± 2
			10.5	10 ± 8	0.32 ± 0.02	2.5 ± 0.5
			11.5	89 ± 2	0.19 ± 0.06	0.2 ± 0.2
	364 ± 14	1.97 ± 0.06	9.5	10 ± 3	1.5 ± 0.1	193 ± 27
			10.5	40 ± 3	0.39 ± 0.05	28 ± 20
			11.5	90 ± 1	0.23 ± 0.06	3 ± 3
$Ca(OH)_2$	4 ± 4	9.47 ± 0.06	9.5	13 ± 3	1.54 ± 0.02	1 ± 0.6
		16.9 ± 0.06	10.5	43 ± 14	0.8 ± 0.1	0 ± 0.1
		39.47 ± 0.06	11.5	92 ± 4	6.6 ± 0.1	-0.06 ± 0.06
	371 ± 4	8.22 ± 0.06	9.5	24 ± 4	0.99 ± 0.02	71 ± 27
		16.97 ± 0.06	10.5	64 ± 5	0.92 ± 0.03	4 ± 3
		39.47 ± 0.06	11.5	92 ± 4	6.8 ± 0.4	0.2 ± 0.2

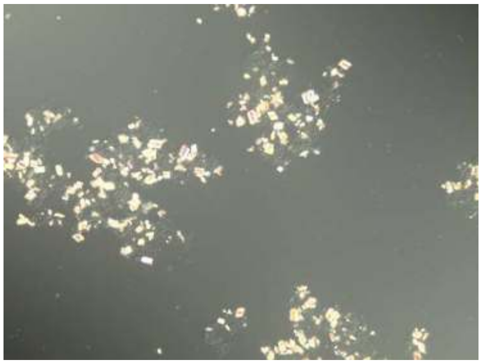

Fig 4. DIC image of $Ca(OH)_2$ flocculated aggregates at pH 10 containing crystals

3.1.3. Chemicals consumption in response to pH increase

Fig 5 shows the pH increase relative to the amount of added hydroxide. The obtained curves resemble titration curves of a weak acid with a strong base. Hence, to obtain a pH value above 11 disproportionally large amounts of alkali are required. Due to the lower dissociation constant of $Ca(OH)_2$, a 1.6 times higher dosage on average is needed to obtain the same pH in comparison with NaOH. Depending on the chemical used, sodium or calcium ions were also added to the centrate. To obtain a pH of 10.5, 15.2 mM sodium is added which is equivalent to 3.8% of the original medium concentration. However, 29.3 mM of calcium is added as $Ca(OH)_2$ to obtain a pH of 10.5 which corresponds to 861% of the medium concentration. During the flocculation process, calcium phosphates and carbonates will precipitate and part of the excess calcium will disappear again from the medium.

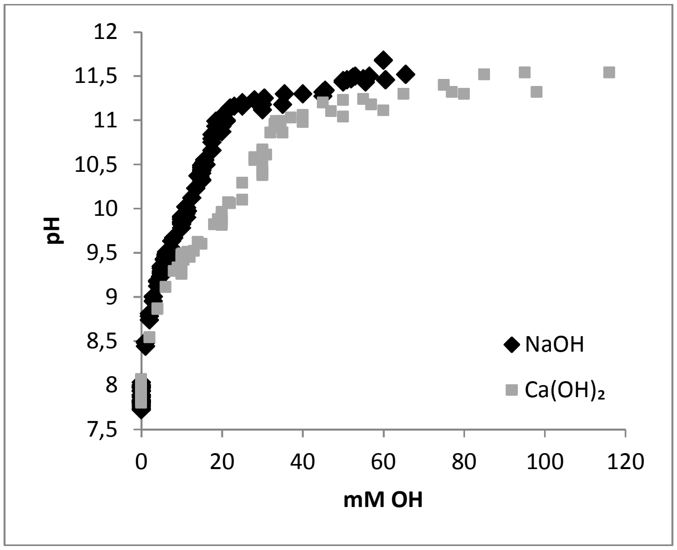

Fig 5. Increase in pH as a function of hydroxide concentration added in the form of NaOH or $Ca(OH)_2$

To enable recycling of the medium, cleared centrate requires neutralisation. Addition of equimolar amounts of H^+ restores the pH value of stirred NaOH flocculated centrate (Fig 6). Since OH^- ions partake in the formation of crystals and flocs, they are removed from the centrate after flocculation. Consequently, the H^+ requirement to neutralise the cleared solution is reduced 4.8 times on average. Neutralisation with HCl adds a maximum relative amount of salt of 14% to the standard concentration. This means that even under the most thorough flocculation conditions a continuous recycle of 87.7% can be maintained without increase in salinity.

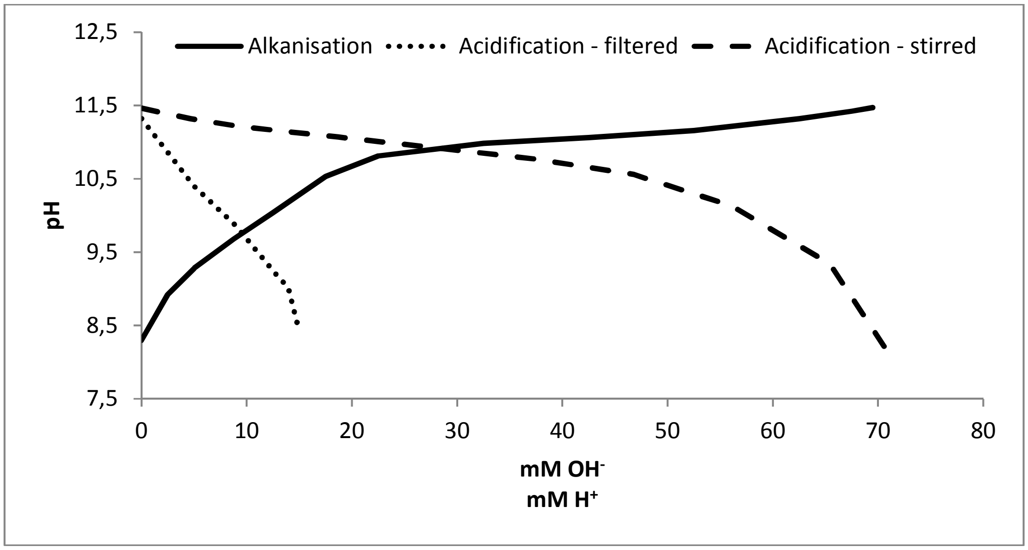

Fig 6: OH^- and H^+ requirement for respective alkalinisation and subsequent acidification of centrate that was either stirred or filtered upon flocculation. OH^- was added as NaOH and H^+ as HCl.

3.1.4. Influence of phosphate concentration on flocculation

Flocculation of microalgae has been shown to depend on the presence of relatively high concentrations of phosphate in the medium [20]. In addition, Bernhardt et al. [35] showed that even low concentrations of AOM (a few mg C L^{-1}) impaired the flocculation process. When AOM is present, flocculation will only occur sufficiently if phosphate concentrations exceed 350 µM and calcium concentrations exceed 500 µM [20]. Due to the marine nature of the growth media used in this study, with a calcium concentration of 3.4 mM, the latter condition is fulfilled. Phosphate, however, is a macronutrient which is almost completely consumed during algae cultivation. Two out of three tested centrates contained less than 350 µM of phosphate (Table 1). Supplementation with KH_2PO_4 up to 364 ± 14 µM phosphate resulted in significantly higher flocculation efficiencies for NaOH flocculation (ANOVA, $P<0.005$, Table 3) and an improved filterability of the centrate at moderately alkaline pH values. Since

$Ca(OH)_2$ flocculation was already more effective at original phosphate concentrations - supposedly due to the additional formation of calcium carbonates - no significantly higher removal rates were reached (α=0.05) by this increase in phosphate concentration.

At a pH above 10.5, precipitation of magnesium hydroxides will occur and contribute to the removal of organic substances. Flocculation at high pH is less affected by the increase of phosphate due to the lower relative contribution of calcium phosphate precipitation at these pH values. Thus, adding surplus phosphate is unnecessary to obtain a sufficient flocculation at high pH.

Due to the variability of algae cultivation, phosphate concentration of obtained centrates may vary. One of the three centrates tested contained an initial phosphate concentration of 706 µM and slightly less organic matter (Table 1). Despite the higher phosphate concentration, similar flocculation efficiencies were attained compared to flocculation of centrates with a phosphate concentration of 350 µM (data not shown). Phosphate concentrations higher than 350µM will therefore not contribute to the flocculation efficiency. Moreover, due to the cost and the declining phosphate reserves, it is not considered sustainable to add additional phosphate to the medium to enhance flocculation. Beukels et al. [20] suggested re-dissolving all precipitated phosphate after flocculation by mild acidification. However, adding additional phosphate is most preferably avoided.

3.1.5. Nutrient removal caused by alkali induced flocculation

Nitrate and phosphate removal

As expected, nitrate was not removed from the medium as it does not participate in the flocculation process (overall removal of 0.6 ± 4.2%). Thus, residual nitrate can be reused entirely. Phosphates, on the other hand, do partake in the flocculation process as mentioned above. Phosphate removal depends on which alkali was used and on initial phosphate concentration (ANOVA, $P<0.05$). $Ca(OH)_2$ induced flocculation results in a significantly higher phosphate removal at the respective pH levels applied. Nevertheless, at pH 11.5 most of the phosphate (99 ± 3% on average) is removed, regardless of the phosphate start concentration, centrate and alkali. Hence, partial or complete replenishment of phosphate is necessary prior to medium reuse.

Magnesium and calcium removal

As magnesium and calcium contribute to the flocculation process [17,36,37] one can expect them to be removed during the course of it. As displayed in Table 4, magnesium removal is significantly dependent on pH and on the alkali species applied (ANOVA, $P<0.001$). $Ca(OH)_2$ flocculation results in a higher removal. At the concentrations tested phosphate hardly affects magnesium withdrawal. At high pH magnesium hydroxides form. When flocculation is executed at high pH, on average 71 ± 6% (or 28 ± 2 mM) of the original magnesium content of fresh medium has to be replenished.

At each pH level, calcium removal was significantly dependent on the applied base (ANOVA, $P<0.005$). These removals, as summarized in Table 4, are calculated by comparing the remaining concentration with the total calcium concentration upon addition of the alkaline chemicals. Especially at a pH of 9.5, the difference in calcium removal between NaOH and $Ca(OH)_2$ flocculation is distinct. It can be attributed to the calcium carbonate formation and higher flocculation efficiency attained with $Ca(OH)_2$. Similar to magnesium, when NaOH is used, on average 93.9 ± 0.8% (or 3.19 ± 0.03 mM) of the original calcium will need to be replenished after flocculation at high pH prior to recycling of the growth medium.

Use of $Ca(OH)_2$ can bring additional calcium to the medium. For instance, despite the high calcium removal, surplus calcium (6.7 ± 0.2 mM) in comparison to the standard medium concentration (3.4 mM) is present after flocculation at pH 11.5. Under these conditions, calcium will accumulate in the medium during repeated medium reuse cycles. At pH 9.5 and 10.5, calcium removal was sufficient to obtain calcium concentrations which were slightly lower than the standard concentration.

Table 4. Influence of alkali species, phosphate concentration and pH on magnesium and calcium removal during flocculation.

Alkali	Phosphate concentration (µM)	pH	Mg removal (%)	Ca removal (%)
NaOH	2	9.5	-1.4 ± 0.02	44.5 ± 0.8
		10.5	3.55 ± 0.06	83.63 ± 0.0
		11.5	64.5 ± 0,1	90.4 ± 0.3
	350	9.5	-0.6 ± 0.1	23.4 ± 0.6
		10.5	5.7 ± 0.3	80.0 ± 0.2
		11.5	62.56 ± 0.06	88.4 ± 0.3
$Ca(OH)_2$	2	9.5	0.9 ± 0.14	83.76 ± 0.07
		10.5	11.1 ± 0,1	95.3 ± 0.4
		11.5	67.3 ± 0.2	83.34 ± 0.02
	350	9.5	5.0 ± 0.5	87.97 ± 0.07
		10.5	14.2 ± 0.2	94.6 ± 0.1
		11.5	77.8 ± 0.4	82.70 ± 0.08

Trace elements removal

Removal of trace elements from the centrate due to NaOH flocculation was also documented. Fig 7 shows that except for molybdenum, all trace elements were removed in a manner dependent on pH level and correlated to flocculation efficiency (ANOVA, P<0.01). Molybdenum is primarily present as the molybdate anion MoO_4^{2-}. Unlike the other micronutrients, the dissolved molybdate concentration increases with increasing pH (modelled with Visual MINTEQ, [33,34]).

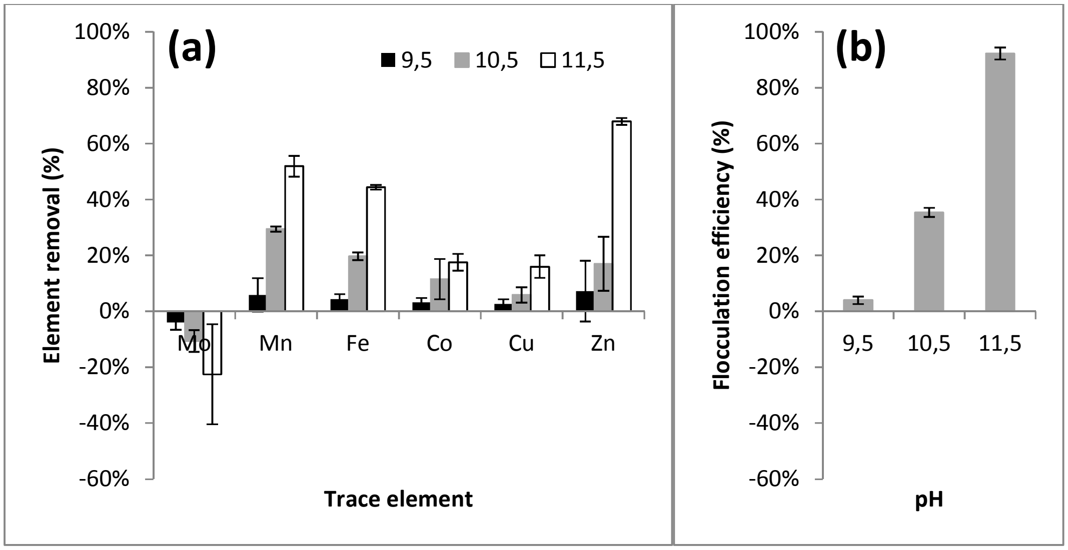

Fig 7. Trace element removal at different pH values (a) and comparison with occurring flocculation efficiencies at corresponding pH (b) (n=2)

3.1.6. Practical implications of high pH induced flocculation

Flocculation by addition of $Ca(OH)_2$ would be favoured to improve filterability of centrate due to the higher flocculation ratio at intermediate pH values. At pH 10.5 at low phosphate concentrations, $Ca(OH)_2$ flocculation was found to be 2.1 times more effective compared to NaOH flocculation. In addition, lime is an economically favourable and less hazardous alkali [38]. However, lime is more difficult to handle because it is used as slurry which has to be constantly agitated. Furthermore, additional calcium is added to the centrate. Even after flocculation, slightly more calcium than the standard calcium concentration may remain dissolved, particularly if appreciable amounts of AOM are present in the centrate. Small excesses will accumulate with repeated medium recirculation, disturbing the broth composition.

NaOH flocculation is an alternative option. To obtain consistent high flocculation efficiencies, pH values up to 11.5 are needed. At this pH, flocculation is 87 ± 5% effective on average while sodium enrichment is moderate (maximum 14% of standard medium concentration). Furthermore, phosphate dependency is insignificant, thus, unsustainable phosphate addition is unnecessary. However, at high pH values, magnesium, calcium, phosphate and trace elements are quantitatively removed. By optimising production, residual phosphate can be minimized, reducing phosphate loss. An advantage of flocculation at high pH is the appreciable degree of disinfection occurring in the process. It was reported that at pH 11.0 – 11.5 and a contact time of 4h, bacterial organisms are completely inactivated [39] and pathogenic microorganisms may be killed [40].

3.2. Centrate clean-up by stand-alone sand filtration

Only minor amounts of suspended solids were retained on a sand filter composed of 35 cm of coarse sand (250 - 500 µm) topped with 5 cm of fine sand (Dv50 of 170 µm). Over six separate runs, an average removal of 7 ± 4% based on optical density was obtained. During filtration, the water height above the sand bed increased steadily with a final increase of 22 ± 4% compared to the initial height. An average outlet flow of 8.2 ± 0.6 $m^3 \cdot m^{-2} \cdot h^{-1}$ was maintained. Furthermore, the fouling index ks (standard blocking) of collected permeate decreased with only 3 ± 2% after 30 minutes and 21 ± 21% after 55 minutes. Removal rates of 90% up to 97.3% as reported by Sabiri et al. [22] and Naghavi et al. [23] for the sand filtration of microalgae were not obtained. Algae investigated in those studies had minimum diameters of 10 and 30 µm respectively. The results of this investigation indicate that some particles present in the centrate were retained on the sand filter but the overall removal is not sufficient to improve the filterability significantly.

3.2.1. Nutrients retained by stand-alone sand filtration

No nitrate and only very small amounts of phosphate were removed from the centrate, -0.2 ± 0.9% and 2.3 ± 1.9% respectively. Likewise, no or only minute amounts of calcium and magnesium were retained on the sand, -1.1 ± 0.1% and 0.81 ± 0.03% respectively. Therefore, no extra cost would be generated to rejuvenate the centrate should one opt to solely use sand filtration for cleansing.

3.3. Centrate clean-up by combination of flocculation and sand filtration

Sand filtration of centrate which was flocculated at pH of 10.5 or higher by addition of $Ca(OH)_2$ resulted in rapid clogging of the sand bed, inhibiting filtration completely. Similar clogging was observed after flocculation at pH of 11.5 with NaOH. Consequently, only the combination of NaOH flocculation at pH 10.5 with sand filtration was further investigated. With a clean sand bed a reduction of 87 ± 2% in optical density was obtained. However, removal rates of subsequent filtration runs decreased rapidly until it was reduced to 61 ± 6% (Fig 8a, run 1B and 2B). This decreased retention may have been caused by the build-up of clumps of sand that disturb the integrity of the sand bed. Retention capacity was completely restored after a cleaning of the sand bed with acidified water to disintegrate these clumps (Fig 8a, run 2A). Over all runs, on average, 78 ± 18% of particles were removed and MFI's were improved with 75 ± 19%. Under 'ideal' circumstances (A runs), 87 ± 2% of particles were removed and MFI's were improved with 84 ± 14%.

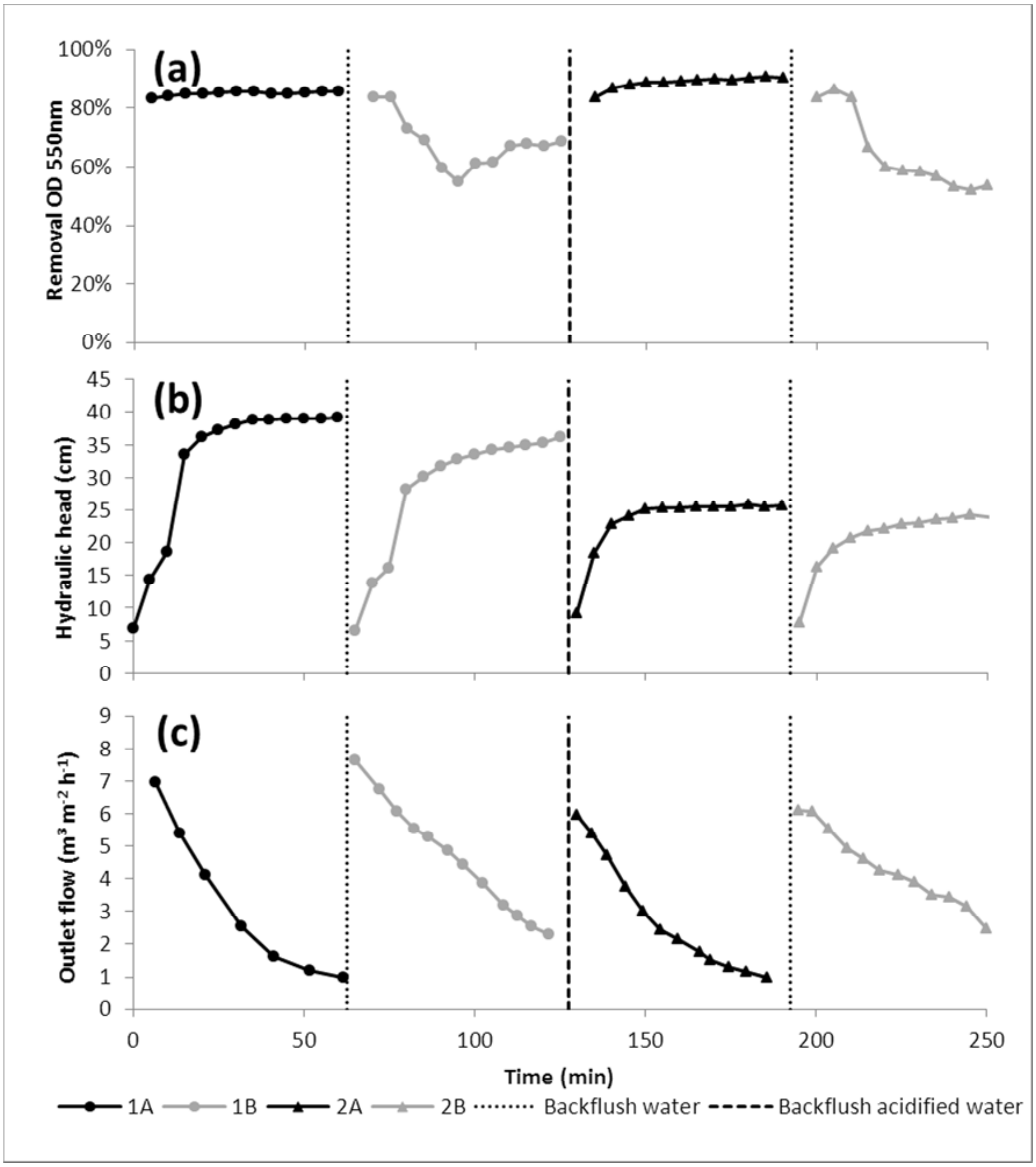

Fig 8. Progression of particle removal (a), hydraulic head (b) and outlet flow (c) of consecutive sand filtration runs of NaOH flocculated centrate at pH 10.5. The sand bed was rinsed with acidified water between run 1B and 2A.

During filtration, the centrate volume and pressure above the filtration surface increased rapidly which indicates clogging of the filter (Fig 8b). The outlet flow decreased accordingly (Fig 8c). This respective increase and decrease was less pronounced in the secondary runs (1B, 2B), indicating a less clogged sand bed with formation of preferential pathways. At the end of the runs, the filtration modus had changed from rapid to slow sand filtration. The enhanced removal was partly due to mechanical straining of the aggregates in the sand bed. In addition, flocs accumulated on the surface of the sand forming an extra filtration layer, increasing the filtration efficiency of the sand filter.

3.3.1. Practical implications of the combination of flocculation and sand filtration

Our results in a lab-scale setup, are counter indicative for the combination of very high pH (>11) induced flocculation with sand filtration. A large scale sand filter can withstand higher pressure while providing a continuous inlet flow, which may sustain longer filtration runs with higher flows, but extensive test runs at pilot scale are needed to verify this.

Based on the results at hand, filtration through a sand bed consisting of 7 parts of coarse sand topped with 1 part of fine sand, preceded by NaOH induced flocculation at pH 10.5 is recommended. If under these conditions pressure still reaches the maximum operating pressure of 1.5 bar adopted in most sand filtration systems, the top layer can be removed. A small loss in filterability of the centrate will however occur. Flocculation with $Ca(OH)_2$ or at higher pH is best combined with a different separation technique or decantation.

The proposed method mainly removes particular matter from the medium. Small particles passing the sand filter are retained on the micro membrane. Dissolved organic material, however, is only partly removed from the medium. Preliminary measurements showed that NaOH flocculation resulted in a removal of dissolved total organic carbon of 25 ± 3 % at a pH of 10.5 and 72 ± 4 % at a pH of 11.5. Remaining organics are recirculated back to the cultivation system. Hence, accumulation of these components may occur with extensive medium recycling. Their effect on productivity requires further investigation.

3.4. Cost calculation

Based on the above, a combination of sand filtration and high pH induced flocculation is proposed as a means of pre-treatment for recycling used algae cultivation media. The additional cost of a sand filtration setup to an existing microfiltration setup, and consumption of chemicals and nutrients should therefore be compared to the replacement cost of fresh medium and the saved cost on filter membrane renewal. Table 5 gives an overview of the cost for a medium filtration setup applicable to a ProviAPT™ pilot production module of 0.288 ha. The installation cost for the membrane unit comprises three micro hollow fibre membranes and a circulation pump. The cost of the sand filtration unit contains a sand filter, sand, a circulation pump and a $1 m^3$ vessel to allow circulation of the centrate over the sand filter. To obtain the total installed cost, equipment costs were multiplied with a typical Lang factor for fluids processing of 4.74. Since the microfiltration unit is already part of the current setup to deliver contaminant-free fresh media, its installation and the associated costs do not add to the cost of its application for medium re-use. Actually, as a result of the suggested pre-treatments, the number of membrane module replacements should become reduced. However, at present, data for an accurate impact estimate are not available.

Total flocculation cost was calculated by taking into account alkali cost to increase pH, cost of acid requirement to neutralise the medium and the replenishment cost of nutrients which were removed from the medium during the course of flocculation. By using NaOH instead of $Ca(OH)_2$ to increase the pH to 10.5 and 11.5 the chemical and nutrient cost is 6% and 21% cheaper respectively. The cost of chemicals and nutrient replenishment for NaOH induced flocculation at pH 10.5 and 11.5 is respectively 1.12 $€\cdot m^{-3}$ and 3.70 $€\cdot m^{-3}$ of treated centrate. Sand filtration adds approximately 0.18 $€\cdot m^{-3}$. These costs are relatively small compared to the current cost of fresh medium of 13.21 $€\cdot m^{-3}$. It should be noted that fresh medium is composed of expensive artificial food grade salts (9.32 $€\cdot m^{-3}$ medium), food grade nutrients (2.12 $€\cdot m^{-3}$ medium) and water (1.77 $€\cdot m^{-3}$ medium). Assuming a recycling rate of 75%, the medium cost decreases to 4.32 $€\cdot m^{-3}$. Complete rejuvenation of all nutrients is included in this cost.

For a 0.288 ha production installation of the ProviAPT™ system, flocculation at a pH of 10.5 and subsequent sand filtration make for an additional running cost of 0.44 $€\cdot kg^{-1}$ DW. Medium costs constitute 1.62 $€\cdot kg^{-1}$ DW or 0.95 $€\cdot kg^{-1}$ DW when respectively 75% or 90% of medium is reused. At 75% recycle the total cost the proposed scheme of 2.08 $€\cdot kg^{-1}$ DW would mean a 58% cut in cost compared to the total fresh medium cost of 4.95 $€\cdot kg^{-1}$ DW. With a cost of 1.39 $€\cdot kg^{-1}$ DW at 90% recycle, a gain of 72% can be accomplished.

Table 5: Summarized cost for installation of filtration equipment, chemical usage for flocculation, nutrient addition after flocculation and medium cost

Installation cost			
Unit			Cost (euro)
Membrane filtration unit (Lang factor 4.74)			67826
Micro membrane filter: 11.2m² 0.2µm (n=3)			13468
Circulation pump			841
Sand filtration unit (Lang factor 4.74)			8107
Sand filter			795
Sand			108
Circulation pump			355
1m³ buffer vessel			452
Energy cost			
Unit			Cost (euro·m^{-3})
Membrane filtration unit: circulation pump			0.025
Sandfiltration unit: circulation pump			0.01
Chemical usage flocculation			
Flocculation pH	Chemical	Consumption (kg·m^{-3})	Cost (euro·m^{-3})
11.5	NaOH	2.3	0.87
	$Ca(OH)_2$	3.4	1.02
	HCl	0.41	0.44
10.5	NaOH	0.8	0.30
	$Ca(OH)_2$	1.3	0.39
	HCl	0.15	0.16
Extra nutrient usage			
Alkali and flocculation pH	Salt	Consumption (kg·m^{-3})	Cost (euro·m^{-3})
NaOH (11.5)	$MgCl_2·6H_2O$	2.70	0.76
	$MgSO_4·7H_2O$	3.28	1.44
	$CaCl_2·2H_2O$	0.47	0.19
$Ca(OH)_2$ (11.5)	$MgCl_2·6H_2O$	3.03	0.85
	$MgSO_4·7H_2O$	3.67	1.61
	$CaCl_2·2H_2O$	0	0
NaOH (10.5)	$MgCl_2·6H_2O$	0.59	0.17
	$MgSO_4·7H_2O$	0.71	0.31
	$CaCl_2·2H_2O$	0.44	0.18

$Ca(OH)_2$ (10.5)	$MgCl_2 \cdot 6H_2O$	0.87	0.24
	$MgSO_4 \cdot 7H_2O$	1.06	0.47
	$CaCl_2 \cdot 2H_2O$	0.37	0.15
Medium cost			
Medium			Cost (euro·m^{-3})
100% fresh			13.21
75% recycle			4.32
90% recycle			2.54

4. Conclusion

The main goal of this study consisted in finding a polishing treatment of centrate originating from harvest operations of high density cultures of *Nannochloropsis* sp. to feed it into a microfiltration setup generating contaminant-free growth medium. A combination of NaOH flocculation at pH 10.5 and subsequent sand filtration removed 78 ± 18% of particles and improved 'modified fouling indices' with 75 $\underline{+}$ 19 %. Our results indicate that there is room for some improvement by optimization of washing regimes. In any case, the treatment will greatly decrease fouling and deterioration of filter membrane, thus allowing prolonged recirculation of growth medium. During such prolonged medium recycling, dissolved organic components may accumulate in the medium, the effect of which on productivity requires further investigation. Yet, at a sustained recycle rate of 90%, a cost saving of 3.56 €·kg^{-1} DW, i.e. 72% of the fresh medium cost, can be achieved.

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Figure and table captions