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## An energy and resource efficient alkaline flocculation and sedimentation process for harvesting of *Chromochloris zofingiensis* biomass

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### ABSTRACT

Harvesting microalgal cultures is often energetically intensive and costly. To improve efficiencies, a two-step harvesting method utilising alkaline flocculation and sedimentation to pre-concentrate cultures can be used prior to centrifugation. When applied to the microalga *Chromochloris zofingiensis*, high rates of sedimentation (> 90%) were found at low concentrations of base (< 10 mM), with the addition of magnesium to the media (via NaOH/MgSO<sub>4</sub> or Ca(OH)<sub>2</sub>/Mg(OH)<sub>2</sub>) to form Mg(OH)<sub>2</sub>. The process was scaled to 180 L, where sedimentation was as efficient as that achieved at bench scale. Characterisation of the harvested biomass showed comparable composition (following neutralisation of pH) to biomass recovered solely by centrifugation. The alternative two-step processes were assessed for environmental impacts and cost, which indicated that a two-step harvesting generally performs better than centrifugation alone, but that the locally available electricity source is a critical parameter for optimal solution.

### 1. Introduction

Significant advances in microalgal biotechnology have been achieved and e.g. feed production may be close to realisation, yet for large-scale bioenergy production substantial improvements are still necessary (Ruiz et al., 2016; Taelman et al., 2015). Harvesting (dewatering) of biomass from the culture medium is considered one of the costlier aspects of microalgal processing as a consequence of very dilute cultures (< 0.3% dry weight, DW) and handling of large liquid volumes (Gerardo et al., 2015; Ruiz et al., 2016; Vandamme et al., 2013; Weschler et al., 2014). Further challenges are the small sizes of cells (< 50 µm), unfavourable buoyancies (depending on species and biochemical composition) (Hathwaik et al., 2015; Muto et al., 2017), and the negative surface charges of most species which result in electrostatic repulsion and cells remaining suspended (Lama et al., 2016). Thus, effective harvesting needs to be optimised for both algal species and product configuration to minimise energy expenditure.

Centrifugation is the most widely used harvesting technique at commercial scale due to its simplicity, high biomass recovery efficiency and the high solid concentrations achieved; however, the operation of this equipment is energetically costly and the capital investment significant (Dassey and Theegala, 2013; Weschler et al., 2014), e.g. up to

10 kWh m<sup>-3</sup> at demonstration scale (Beal et al., 2015; Huntley et al., 2015). When high biomass containing pastes are required, techniques that can decrease the volume of liquid sent to centrifugation are imperative to enable efficient harvesting. To this end, flocculation, flotation, membrane filtration and other emerging technologies have all been considered as useful options for the pre-concentrating step (Gerardo et al., 2015).

Inducing flocculation of microalgal cells into larger aggregates using chemicals, followed by gravity sedimentation, is considered to be feasible for large scale applications due to its relatively low cost and scalability (Vandamme et al., 2013). The mechanism of flocculation depends on the chemicals used, but involves three principles of forming aggregates (solely or in combination): neutralisation of the negative cell surface; linking/bridging of cells by particular molecules; and larger aggregate/floc formation (often hydroxides, carbonates or phosphates) and cellular entrapment i.e. 'sweeping' (Branyikova et al., 2018). Investigated chemical flocculants include metal salts (alum or ferric chlorides/sulphates), biopolymers (cationic starch or chitosan) and bases. In alkaline flocculation, pH is increased by adding base, often NaOH and occasionally Ca(OH)<sub>2</sub>, and is considered to cause chemical bridges between cells and aggregate formation, leading to sweeping. Drawbacks of using metal salts and biopolymers are that the harvested

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biomass is contaminated, which can limit the possibilities of its use because of toxicity and regulatory restrictions. Additionally, the presence of flocculants can influence downstream processing and they are often associated with higher incurred costs compared to bases (Branyikova et al., 2018; Wan et al., 2015).

The use of alkaline flocculation, therefore, offers a simple and relatively effective harvesting method, which benefits from the reversible removal of flocculant by neutralisation of the recovered biomass (Garcia-Perez et al., 2014; Vandamme et al., 2015; Zhang et al., 2016). Flocculation efficiencies over 90% are achievable by increasing pH to over 9.5. Suggested flocculation mechanisms by increasing pH vary across different studies of *Chlorella* strains (microalgae widely regarded as suitable for large scale biomass production due to their robust growth characteristics), e.g. associated with precipitation of  $Mg(OH)_2$  (Castrillo et al., 2013; Vandamme et al., 2015), or of ferric hydroxides (Yang et al., 2016).  $Ca^{2+}$ , carbonates and phosphates have also been implicated as having roles in sedimentation of algal cultures (Brady et al., 2014; Smith and Davis, 2012). The complexities associated with high nutrient media compositions, diverse algal physiologies, environmental conditions, material availability, local infrastructures and even variations in the pH during cultivation contribute to the variety of mechanisms proposed and reported in the literature.

The aim of this study was to assess alkaline flocculation for 'clean' harvest of the carotenoid producing *Chromochloris zofingiensis* (formerly *Chlorella zofingiensis*). Magnesium was used in the flocculation to enhance sedimentation and biomass recovery in bench scale experiments, prior to 180 L pilot scale trials. The potential mechanism of flocculation and its impact on biomass quality was assessed. Based on the empirical data, a process model was developed to assess the life-cycle impacts and cost of a two-step harvesting processes (flocculation/sedimentation followed by centrifugation) versus just centrifugation and to identify opportunities for further improvements. To our knowledge, this is the first time experimental data from different scales are combined and assessed by life cycle analysis.

## 2. Materials and methods

### 2.1. Strains, stock maintenance and media

*Chromochloris zofingiensis* (SAG 211/14, axenic strain) was obtained from the SAG Culture Collection of Algae (Gottingen, Germany) and transferred to agar plates and later to liquid cultures with Bold's Basal Medium 3N + V (BBM, hereafter) (Andersen, 2005), which was used in all experiments. Master cultures of the strain were maintained at 16 °C and sub-cultured every 4–5 weeks. Upon scaling up, cultures were sub-cultured into 25 mL and then 75 mL tissue culture flasks with hydrophobic coating and sterile 0.2 µm air filter vented caps (Sarstedt AG & Co, Germany), and maintained as described below. The base of the media was 18.2 MΩ deionised water autoclaved (20 min, 121 °C) prior to addition of salts and then adjusted to pH 7.0 using 1 M NaOH.

### 2.2. Cultivation

#### 2.2.1. Bench-scale experiments

Production of biomass for use in bench-scale flocculation and sedimentation experiments was done using 1 L bottles as described previously (Mayers et al., 2018). Cultures were sub-cultured on a weekly basis to maintain rapid growth. Biomass concentration was determined using a standard curve between optical density at 750 nm ( $OD_{750}$ ) and biomass dry weight concentration (DW,  $g L^{-1}$ ) based on twelve separate samples from different batch cultivations representing different physiological states ( $DW = 0.7895 OD_{750} + 0.0026$ ,  $r^2 = 0.999$ ). Optical density was measured in 96-well plates (Sarstedt) using a plate reader (FLUOstar Omega, BMG Labtech GmbH). Dry weight was determined by filtering a known volume of culture onto pre-combusted 47 mm GF/C Whatman glass microfibre filters (in triplicate). Filters

were washed with double the volume of 0.5 M ammonium bicarbonate to remove salts, dried for at least 24 h at 80 °C, before cooling to room temperature in a desiccator (Zhu and Lee, 1997).

#### 2.2.2. Pilot scale cultivation

*C. zofingiensis* was cultivated at 550 L scale in a horizontally stacked tubular bioreactor (Hamilton et al., 2015). Briefly, the photobioreactor (constructed by Bouygues Energies and Services, Manchester, UK) consisted of an array of polycarbonate 50 mm diameter tubes (36 tubes in total, in a 6 tube manifold formation) serving as the photostage, with stainless steel (316S) pipework to a holding tank allowing continuous circulation via a centrifugal pump (at  $30 m^3 h^{-1}$ ). The holding tank (200 L working volume) was continually sparged with air to aid oxygen removal ( $\sim 10 L min^{-1}$ ), while  $CO_2$  delivery was controlled through a pH stat system set to pH 7.0. Culture monitoring was performed via a Proflix 3 interface and included oxygen saturation, pH, conductivity and temperature measurement. The medium used was standard BBM with sodium nitrate replaced by ammonium nitrate at corresponding nitrogen concentrations. The base for the media was tap water collected from the municipal supply in Plymouth, UK, which was sterilised by passing through a UV filter prior to addition of sterile nutrient stocks. The culture was illuminated using Heliospectra LX602G LED panels at approx.  $200 \mu mol photons m^{-2} s^{-1}$  at a 18:6 h light:dark regime (no sinusoidal pattern). Culture temperature was maintained at 23 °C. The culture optical density was measured daily and converted to DW equivalent using the determined standard curve (Section 2.2.1).

### 2.3. Experimental plan and design of flocculation/sedimentation experiments

Flocculation experiments were performed at different biomass concentrations to reflect those obtained by cultures grown in photobioreactors; approx. 0.5, 1.0, 1.5  $g DW L^{-1}$ . Cultures were harvested in linear growth phase by centrifugation (12,000  $\times g$ , 10 min; Beckman Avanti J26-S, JA-20 rotor), washed in BBM lacking Mg, centrifuged, and re-suspended to the desired concentration in fresh BBM lacking Mg, thereby removing any algogenic organic matter.

To identify the optimal material inputs for flocculation, a design of experiment approach was taken utilising a response surface methodology in the software package MODDE Pro (v11. MKS Umetrics AB). For each biomass concentration, a D-optimal design matrix utilising a quadratic model was used, with NaOH (4, 6 and 8 mM) and  $MgSO_4$  (6, 8 and 10 mM) with 2 replicates and 4 central point repeats resulting in 23 experimental runs, including 2 additional controls with no base addition or  $Mg^{2+}$  addition. Suitable concentration ranges of NaOH and  $MgSO_4$  were determined in preliminary experiments with greater ranges. Three bench scale trials were performed, one for each biomass concentration, which were performed on different actively growing cultures. These data informed trials done at pilot scale.

$Ca(OH)_2$  and  $Mg(OH)_2$  were also examined as possible flocculants at bench scale using a design of experiment approach to investigate possible reductions in cost and impact of material inputs. Models were fitted with multiple linear regression for the tested parameters (e.g. Mg, base or dry weight concentration) and flocculation efficiency. The flocculation efficiency data were logistically transformed before used in the model to be constrained between 0 and 100, according to "Flocculation efficiency" =  $\log_{10}(y - 0)/(100 - y)$ . Model validity was examined by ANOVA analysis of the regression model, from which non-significant terms (alpha value = 0.01) were removed.

### 2.4. Flocculation and sedimentation

#### 2.4.1. Bench-scale

For small scale flocculation, 35 mL of culture was decanted into 50 mL Falcon centrifuge tubes. The pH was increased by addition of 0.5 M NaOH or 0.1 M  $Ca(OH)_2$ . Control experiments with no additional

base or Mg were also conducted. The pH of samples was measured after addition of base. Tubes were mixed on an orbital shaker first at 500 rpm for 10 min, then 250 rpm for 20 min, before leaving for 1 h for sedimentation without agitation. Samples were taken after the addition of base ( $X_0$ ) and after sedimentation ( $X_1$ ) from the middle of the clarified zone. Absorbance measurements were blanked against additional samples collected at  $t_0$  that were centrifuged at  $15,000 \times g$  for 10 min (Eppendorf 5417 C/R). The flocculation efficiency was calculated as “Flocculation efficiency” =  $((X_0 - X_1)/t_0) \times 100$ . The concentration factor was determined by transferring 5 mL ( $V_0$ ) of each sample to a 5 mL measuring cylinder with 0.1 mL graduation and allowing to sediment for 1 h before determining the sediment volume ( $V_s$ ). Volumes were rounded up to the nearest 0.1 mL. The concentration factor was calculated as “Concentration Factor” =  $V_0/V_s$ .

Settling velocities were calculated by using 1 L sedimentation cones with 1 mL graduations from 0 to 20 mL and 2 mL graduation from 20 to 60 mL. A 250 mL volume of culture was mixed with the designated concentration of Mg and base, shaken as for those experiments described above. The sample was then decanted to the sedimentation cone and the OD at a depth equal to the 60 mL mark (13.5 cm from the bottom of 23 cm deep sample) assessed at designated time points. The rate of sedimentation was then determined using reported calculations (Wang and Belovich, 2010). The sediment (floculated cells) and supernatant of flocculation samples for these trials were separated via pipetting into separate tubes. Both fractions were titrated with 0.5 M  $H_3PO_4$  to pH 7.0 to determine the acid requirement of neutralisation. This was repeated for cultures ( $1 \text{ g DW L}^{-1}$ ) flocculated with NaOH using HCl as the acid.

#### 2.4.2. Pilot-scale trials

To assess the scalability of this approach, flocculation and sedimentation trials were performed at 180 L scale in an upright settling tank (60° incline, ~40 L inverted cone volume) with a total volume of 200 L. Culture was pumped directly from the bioreactor to the tank with NaOH and  $MgSO_4$  immediately added (5 M and 4 M stocks, respectively). The culture was then mixed using a vertical impeller mixer at 300 rpm for 15 min. Samples were taken from approximately 2/3 of the depth of the tank after mixing and again after 1 h. Biomass was collected from the sample valve at the bottom of the tank and the volume of the sediment recorded. For one of these two trials (both performed on the same day within 2 h of each other), the biomass was centrifuged without neutralising the sediment, while the second was neutralised using 2.5 M HCl to a pH of 7.0 before centrifugation, resulting in two types of harvested biomass. Samples were taken for analysis from the two harvested biomasses, as well as biomass directly from the photobioreactor (recovered by centrifugation). Flocculation efficiency was measured in the same manner as described (Section 2.4.1).

### 2.5. Analytical techniques

#### 2.5.1. Fourier-Transform Infrared Spectroscopy

Briefly, freeze-dried microalgal biomass,  $Mg(OH)_2$  and  $Ca(OH)_2$  were measured on a PerkinElmer Spectrum Frontier instrument equipped with a diamond crystal iATR reflectance cell (Pike GladiATR) with a DTGS detector scanning wavenumbers  $4000\text{--}700 \text{ cm}^{-1}$  at a resolution of  $2 \text{ cm}^{-1}$ . Three replicates (each with an average of 16 scans) for each sample were taken and the results averaged. Background correction scans of ambient air were made prior to each sample scan. Scans were recorded using the PerkinElmer spectroscopic software Spectrum (version 10. PerkinElmer, Germany).

#### 2.5.2. Biochemical determination

Biochemical composition was determined for the two types of harvested biomass and original biomass generated during the pilot scale trial. Protein and carbohydrates were measured as described (Mayers

et al., 2018). Ash was determined by combusting a known volume of freeze-dried biomass at  $550 \text{ }^\circ\text{C}$  for 3 h in pre-weighed and pre-combusted crucibles. Total chlorophyll and carotenoids were extracted in DMSO at  $60 \text{ }^\circ\text{C}$ , and absorbances were converted to concentrations (Wellburn, 1994). Determination of C, N, H and S was done using a Vario MICRO Cube elemental analyser (Elementar) using the manufacturers recommendations.

#### 2.5.3. Inductively-Coupled Plasma Sector-Field Mass Spectrometry (ICP-MS)

ICP-MS analysis was performed to identify the major salts in the sedimented algae and to ascertain if neutralisation of the biomass sediment removed these components. Freeze-dried microalgae were analysed by ALS Scandinavia AB (accredited by the Swedish National Accreditation body and are ISO 17025 compliant). Briefly, duplicates for the samples were digested in 3 M  $HNO_3$  and filtered to  $0.2 \text{ }\mu\text{m}$  before analysis.

### 2.6. Cost and life-cycle impact assessment

Using the empirical flocculation data generated, process models were developed for the different two-step harvesting techniques, for which the following steps were considered: 1) flocculation and sedimentation, 2) neutralisation of both the supernatant and algal sediment, and 3) centrifugation of the algal sediment. The material and energy requirements were calculated for these steps using two cases considering different choices of chemicals; case 1: NaOH,  $MgSO_4$ , HCl; and case 2:  $Ca(OH)_2$ ,  $Mg(OH)_2$ ,  $H_3PO_4$  and compared to just centrifugation. Centrifugation data for all processes were based on a Westfalia clarifier (model 300-96-777) at demonstration scale (Beal et al., 2015; Huntley et al., 2015) with an electricity usage of  $35.5 \text{ MJ m}^{-3}$  of liquid processes, maximum harvesting efficiency of 99% yielding a dry weight content of 20% (Beal et al., 2015). It was assumed that energy requirement for mixing during the flocculation process and pumping are negligible and that flocculated cells had the same harvesting efficiency as non-flocculated cells (no available empirical data).

Processes were compared in terms of their monetary cost (Euros, €), non-renewable energy demand (NRED, MJ), renewable energy demand (RED, MJ), global warming potential (GWP, kg  $CO_2$ -equivalents), eutrophication potential (EP, g  $PO_4$ -equivalents) and ozone depletion potential (ODP, kg CFC-11 equivalents). The functional unit for all processes, was for the harvesting of 1 kg of dry biomass (1 kg DW). No other upstream or downstream processing was considered. The environmental impact of chemicals was taken from EcoInvent version 3.0, while their prices were taken from (Egle et al., 2016). To investigate the impact of different electricity mixes on the harvesting processes, the theoretical production plant was sited in three different locations receiving different electricity mixes: Sweden (mainly nuclear and hydroelectric), Spain (natural gas) and Greece (mainly brown coal and natural gas) (Itten et al., 2014). Impacts of electricity mixes were taken from EcoInvent version 3.0 and a medium voltage power supply was assumed (Weidema et al., 2013). Electricity costs for EU countries were the average industrial electricity prices for medium size consumers (500–2000 MWh) collated by Eurostat (Eurostat statistics explained). Impact values for all chemicals examined in this study are summarised in Supplementary Materials.

### 2.7. Statistical analysis

Differences between multiple treatments were assessed by one-way analysis of variance (ANOVA). If ANOVA results were significant, comparisons between means were made using Tukey's post hoc analysis and the alpha-value was set at 0.01. Statistical analyses were conducted using SPSS version 22.0 (IBM Corp.) or MODDE Pro (see Section 2.3).

### 3. Results and discussion

#### 3.1. Effect of biomass concentration on alkaline and Mg flocculation efficiency

Flocculation of *C. zofingiensis* cultures at NaOH concentrations of 4, 6, and 10 mM and 6, 8 and 10 mM MgSO<sub>4</sub> were conducted to determine the optimal chemical dosing for effective biomass recovery. These were repeated at different biomass concentrations to examine if there is an interaction between biomass concentration and different flocculant levels. Preliminary trials showed these ranges of NaOH and MgSO<sub>4</sub> to be the most effective for flocculation at 1 g DW L<sup>-1</sup> (Supplementary Materials). When not adding base or MgSO<sub>4</sub> (i.e. controls), < 2% of biomass was recovered after 1 h, regardless of biomass concentration. The maximum biomass recoveries at the three biomass concentrations tested (0.5, 1.0 and 1.6 g DW L<sup>-1</sup>) were found at 8 mM NaOH, but with varying Mg<sup>2+</sup> concentrations, and were 82%, 93% and 94%, respectively. The collected empirical data were fitted with a multiple regression model with a logistic transformation of flocculation efficiency to constrain the model data between 0 and 100%. This resulted in the greatest fit, lowest residual standard deviation and highest statistical robustness (Fig. 1, Supplementary Materials). Flocculation efficiency had a significant relationship with all factors ( $p < 0.01$ ). Generally, flocculation efficiency increased as biomass concentration increased (having a significant positive regression coefficient for dry weight,  $p < 0.0001$ ). At the highest base concentration, generally smaller amounts of Mg<sup>2+</sup> were required to achieve > 90% flocculation efficiency. Base concentration accounted for a greater proportion of the variation in flocculation efficiency compared to MgSO<sub>4</sub> concentration and biomass concentration. There were no significant ( $p > 0.05$ ) interactions between MgSO<sub>4</sub> addition and base, between either MgSO<sub>4</sub> addition or base and dry weight or between media pH and flocculation efficiency ( $p > 0.05$ ).

When examining the raw data between different dry weights for a given set of NaOH and MgSO<sub>4</sub> concentrations, subtle interactions can be discerned. For instance, at 4 mM NaOH and 6 mM MgSO<sub>4</sub> (the lowest levels examined), high biomass concentrations perform statistically worse than low biomass concentrations for flocculation efficiency ( $p < 0.01$ ). This finding suggests that at high biomass concentrations, a higher pH or Mg<sup>2+</sup> concentration is required to induce flocculation, in agreement with other studies where Mg<sup>2+</sup> was used to induce flocculation (Garcia-Perez et al., 2014; Wu et al., 2012). However, at the highest dosing level (8 mM NaOH and 10 mM MgSO<sub>4</sub>), the highest biomass experiment had a statistically greater flocculation efficiency than the low biomass experiment ( $p < 0.01$ ), which was also seen at 6 mM NaOH and 10 mM MgSO<sub>4</sub>. These results suggest that there are

other factors or interactions influencing sedimentation efficiency beyond just the concentration of materials added, possibly there are simply too few cells to create large enough flocs in the low biomass treatment. Secreted algal organic matter decreases the efficiency of flocculation, even if alkaline flocculation is less affected compared to other methods (Vandamme et al., 2012b). However, cells were centrifuged and washed to remove spent media to avoid such effects. Differences in the cellular composition between the different biomass concentration experiments may affect the flocculation, but cultures were maintained in nutrient replete conditions to prevent changes associated with nutrient stress.

Knowledge of the interaction between biomass density and flocculant is key in designing bioprocesses that are both resource efficient (i.e. minimal chemical usage) and effective for biomass recovery. If biomass concentrations fluctuate over the course of harvesting periods at scale due to variable ambient conditions or operational parameters, careful monitoring of culture density and physiological state may enable the optimal quantity of materials to be added using the models developed herein. However, in this instance, it appears that a base concentration of 8 mM NaOH and an Mg<sup>2+</sup> concentration between 6 and 10 mM are sufficient to achieve maximum flocculation at all the biomass concentrations tested. These experiments used media containing no additional Mg<sup>2+</sup>; freshwater algal media typically contain  $\leq 0.4$  mM Mg<sup>2+</sup> (Andersen, 2005), so additional Mg is certainly required to achieve high levels of flocculation, whereas in seawater media ( $\sim 50$  mM Mg<sup>2+</sup>) this is not the case (Mayers et al., 2018).

#### 3.2. Application at pilot scale and evaluation of subsequent neutralisation

The process developed at bench scale was examined at pilot scale to ascertain whether this method could be scaled effectively for industrial application. From the flocculation model based on bench-scale data, a dosage of 8 mM NaOH and 10 mM MgSO<sub>4</sub> was predicted to result in the greatest flocculation efficiency at 0.4 g DW L<sup>-1</sup> (0.04% DW). Thus, flocculation at these dosages was evaluated on biomass harvested at approximately 0.4 g DW L<sup>-1</sup>. The pH was observed to be  $11.01 \pm 0.04$  (data from 2 biological replicates) and a flocculation efficiency of  $89.4 \pm 2.2\%$  was achieved with a concentration factor of  $8.1 \pm 0.1$ . The sediment had a biomass concentration of  $3.3 \pm 0.1$  g DW L<sup>-1</sup> (0.33% DW) with a volume that was 12.5% of the volume loaded. These data show that sedimentation efficiency was comparable at pilot scale as for lab scale systems, as the flocculation efficiency at 0.51 g L<sup>-1</sup> for the same dosing was  $88.2 \pm 0.03\%$ .

To allow recycling of the media remaining after sedimentation (supernatant), the acid requirement for neutralisation of the supernatant to pH 7 was determined to be  $2.6 \pm 0.1$  mM of HCl (from a

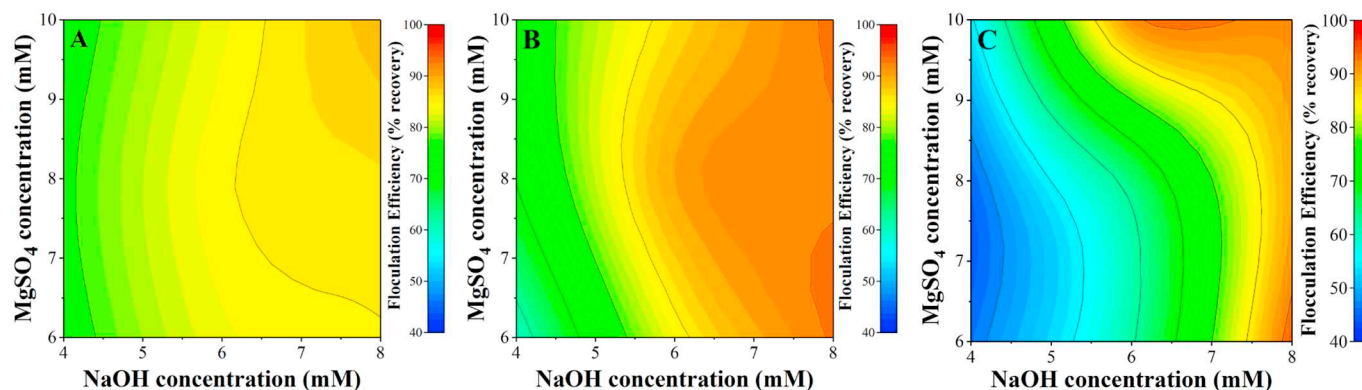


Fig. 1. Contour plots of the flocculation efficiency (% biomass recovery) for sedimentation of *C. zofingiensis*. NaOH and MgSO<sub>4</sub> concentrations (mM) were altered at biomass concentrations: A.  $0.51 \pm 0.003$ , B.  $1.01 \pm 0.03$  and C.  $1.59 \pm 0.14$  g DW L<sup>-1</sup>. Plots were treated by smoothing with 100-point increase factor and a smoothing parameter value of 0.001. Data were analysed by multiple quadratic regression with flocculation efficiency transformed logarithmically (constrained to 0–100%) and non-significant model terms removed from the model (Statistics in Supplementary Material).

**Table 1**  
Biochemical and elemental profile of *C. zofingiensis* biomass (% DW) from different harvesting processes (analytical replicates: 3 for biochemical and 2 for elemental components).

Component (% DW)	Centrifuged	Sedimented, non-neutralised, centrifuged	Sedimented, neutralised, centrifuged
<b>Biochemical components</b>			
Carbohydrates	6.30 ± 0.49 <sup>a</sup>	3.22 ± 0.22 <sup>b</sup>	5.02 ± 0.49 <sup>a</sup>
Protein	47.4 ± 1.70 <sup>a</sup>	30.5 ± 0.69 <sup>b</sup>	48.4 ± 1.41 <sup>a</sup>
Chlorophyll (a + b)	3.81 ± 0.22 <sup>a</sup>	2.09 ± 0.19 <sup>b</sup>	3.12 ± 0.10 <sup>c</sup>
Carotenoids	0.79 ± 0.05 <sup>a</sup>	0.37 ± 0.03 <sup>b</sup>	0.76 ± 0.02 <sup>a</sup>
Ash	6.50 ± 0.70 <sup>a</sup>	31.63 ± 0.19 <sup>b</sup>	6.28 ± 0.16 <sup>a</sup>
<b>Elemental components</b>			
Hydrogen	9.81 ± 0.11 <sup>a</sup>	6.21 ± 0.03 <sup>b</sup>	9.86 ± 0.02 <sup>a</sup>
Carbon	51.67 ± 0.15 <sup>a</sup>	32.04 ± 0.00 <sup>b</sup>	50.80 ± 0.33 <sup>a</sup>
Nitrogen	7.45 ± 0.01 <sup>a</sup>	5.83 ± 0.03 <sup>b</sup>	7.29 ± 0.12 <sup>a</sup>
Oxygen <sup>†,*</sup>	23.79 ± 0.01	23.44 ± 0.15	23.84 ± 0.20
Sulphur <sup>†</sup>	0.79 ± 0.06	0.92 ± 0.09	0.91 ± 0.06
Sodium	0.04 ± 0.01 <sup>a</sup>	0.23 ± 0.03 <sup>b</sup>	0.07 ± 0.01 <sup>a</sup>
Magnesium	0.41 ± 0.11 <sup>a</sup>	9.07 ± 0.03 <sup>b</sup>	0.55 ± 0.02 <sup>a</sup>
Phosphorus	1.72 ± 0.01 <sup>a</sup>	3.15 ± 0.03 <sup>b</sup>	1.53 ± 0.12 <sup>a</sup>
Potassium	1.46 ± 0.03 <sup>a</sup>	0.60 ± 0.18 <sup>b</sup>	1.08 ± 0.04 <sup>a</sup>
Calcium	0.14 ± 0.01 <sup>a</sup>	0.84 ± 0.09 <sup>b</sup>	0.22 ± 0.02 <sup>c</sup>
Iron <sup>†</sup>	0.07 ± 0.01	0.06 ± 0.03	0.10 ± 0.02

Different letters represent statistically significant differences between treatments at an alpha value of 0.01. Tested using one-way ANOVA with Tukey's post-hoc test. <sup>†</sup>Denotes non-significant differences between treatments. \*Oxygen was calculated from the sum of 100-C-N-H-S-ash.

starting pH of 11.1 ± 0.2) and for the biomass sediment 80.0 ± 0.1 mM of HCl. Neutralising to a higher (but still acceptable) pH of the sediment would significantly decrease the acid requirement (e.g. 70 mM HCl to pH 8.0), which would have a clear cost benefit; however, the environmental impacts and the efficacy of the salt precipitation and removal process would need assessment. This work does not empirically consider the reuse of process water for further cultivation; however, several studies have demonstrated its feasibility (Castrillo et al., 2013; Wu et al., 2012).

To evaluate whether neutralisation of the sediment was effective for the removal of precipitants such as Mg, the elemental composition was evaluated (Table 1). Centrifuged, non-neutralised biomass had a significantly greater concentration of Mg, Ca, Na and P, with Mg having the greatest increase (22-fold) compared to directly centrifuged (untreated) biomass. Centrifuged neutralised biomass had approximately the same elemental composition as untreated biomass with regards to Mg, Na, P and other elements ( $p > 0.01$ ), however, Ca remained significantly higher ( $p < 0.01$ ). In contrast, a lower Ca content was previously observed in *Chlorella vulgaris* that was neutralised in comparison to biomass before flocculation and neutralisation, which was attributed to precipitation of calcium carbonates as the pH increased due to photosynthetic activity (Vandamme et al., 2015). Since the processes in this study were performed rapidly and, when appropriate, sediments were stored refrigerated in the dark to avoid changes in composition and growth, we did not observe this phenomenon. We assume another mechanism is responsible for the increase in Ca in this sample, since C content is the same as in the original biomass (Table 1;  $p > 0.01$ ), indicating no increase in carbonates in the biomass.

The biochemical composition of harvested material was evaluated to examine if the sedimentation process had a negative impact on the biomass quality (Table 1). Non-neutralised biomass had an ash content 4.9-fold greater than the control ( $p < 0.01$ ), but neutralisation decreased the ash content to the same level as the control. It is expected that this increase in ash content corresponds to the salts and hydroxides precipitated with the biomass. The concentration of protein, carbohydrates, total carotenoids and all elemental components was also the

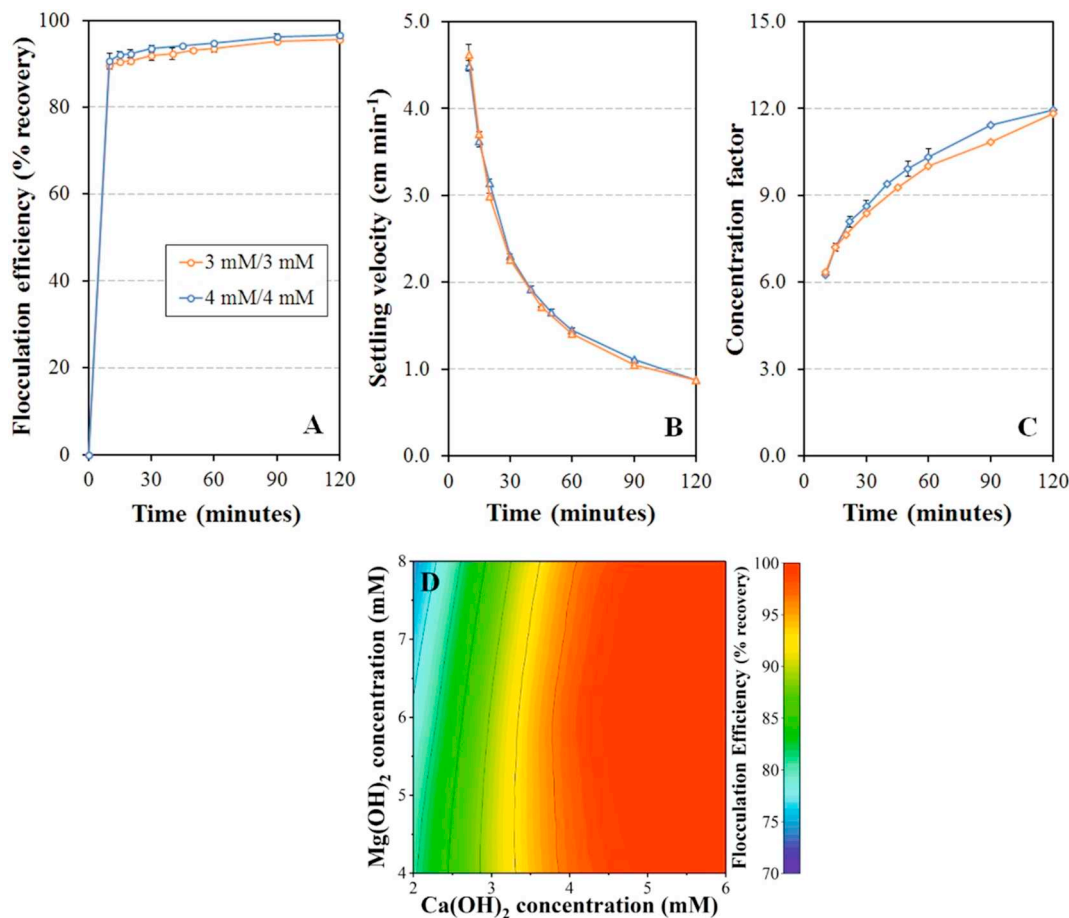
same for control and neutralised biomass ( $p > 0.01$ ). The only significant difference was seen for total chlorophyll content, which was significantly lower in the neutralised biomass compared to the control ( $p < 0.01$ ).

### 3.3. Flocculation mechanism

The flocculation/sedimentation mechanism includes formation of Mg(OH)<sub>2</sub> as supported by an absorption band of the OH stretch at approx. 3700–3695 cm<sup>-1</sup> in the infrared spectra which was present in sedimented biomass that was not neutralised, but absent in neutralised biomass; this peak was still seen after thorough washing (Supplementary Materials). Formation of Mg(OH)<sub>2</sub> is also supported by the increase in Mg in the sedimented biomass (Table 1). Mg(OH)<sub>2</sub> forms at approximate pH 10.5 and is positively charged up to pH 11.5. Both surface charge neutralisation and sweeping can be contributing to the flocculation mechanism and these correlate or are independent of biomass concentration, respectively (Brady et al., 2014; Garcia-Perez et al., 2014). In our trials, the pH was ~11 and there was a strong dependence on biomass concentration (model coefficient of 4.9, Supplementary Materials), and thus we believe that neutralisation of the negative cell surface is the main mechanism in action. Several studies have highlighted the role of Mg(OH)<sub>2</sub> in alkaline flocculation (Vandamme et al., 2015; Vandamme et al., 2012a), but several other studies have also suggested calcium phosphates (formed at > pH 9.0) contributing to surface charge neutralisation and flocculation (Beuckels et al., 2013; Sukenik and Shelef, 1984). Since there was a significant increase in Ca and P in sedimented biomass, precipitation of calcium phosphates may also contribute to the flocculation mechanism. Variations in media ion concentrations and flocculation pH will have an impact on the exact mechanism of flocculation and which salts that co-precipitate with the biomass.

### 3.4. Opportunities for cost saving?

To determine if cheaper input sources of base and Mg<sup>2+</sup> (other than NaOH and MgSO<sub>4</sub>) could be utilised for flocculation and sedimentation of *C. zofingiensis*, biomass recovery was examined using Ca(OH)<sub>2</sub> and Mg(OH)<sub>2</sub> (costs see Supplementary Materials). Additionally, Mg(OH)<sub>2</sub> acts as a base, which potentially decreases base requirements. This combination was examined at approx. 1 g DW L<sup>-1</sup> biomass concentration, 2, 4 and 6 mM Ca(OH)<sub>2</sub> and 4, 6, and 8 mM Mg(OH)<sub>2</sub> in an experimental design (22 individual sedimentations, incl. 5 replicates of the centre point). A maximum flocculation efficiency of ~98% biomass recovery was achieved after 1 h using 4 mM Ca(OH)<sub>2</sub> and 4 mM Mg(OH)<sub>2</sub> (Fig. 2D). The cost of these additives in comparison to the cost of NaOH/MgSO<sub>4</sub> system (93% flocculation efficiency at 8 mM NaOH and 6 mM MgSO<sub>4</sub>) was halved when assessing the necessary flocculant additions to a 10 m<sup>3</sup> tank, i.e. 1.8 € versus 0.95 € (prices used see Supplementary Materials). Potentially, even lower costs can be obtained for the Ca(OH)<sub>2</sub>/Mg(OH)<sub>2</sub> system since for similar efficiency at 93%, less flocculants would be needed. At Ca(OH)<sub>2</sub> concentrations of 2 mM, flocculation efficiency was < 90% regardless of Mg(OH)<sub>2</sub> concentration. The quadratic model generated from these data, showed that Ca(OH)<sub>2</sub> concentration was the major factor influencing flocculation ( $p < 0.0001$ ; Supplementary Materials), whereas Mg(OH)<sub>2</sub> concentration had only a small impact (0.05 <  $p > 0.01$ ). This analysis would indicate that Mg<sup>2+</sup> addition could have been decreased further, however, FTIR scans of biomass flocculated using Ca(OH)<sub>2</sub>/Mg(OH)<sub>2</sub> showed that Mg(OH)<sub>2</sub> is still present in the sediment (Supplementary Materials). For a comparable biomass concentration, when using NaOH/MgSO<sub>4</sub>, a similar phenomenon was seen at 8 mM NaOH, as there was no significant difference in flocculation efficiency at any tested Mg<sup>2+</sup> concentration (Fig. 1B). However, at higher biomass concentrations, higher Mg<sup>2+</sup> concentrations were found to be necessary for the NaOH/MgSO<sub>4</sub> system, but this was not investigated for Ca(OH)<sub>2</sub>/Mg



**Fig. 2.** Detailed temporal profiling of sedimentation, A. floculation efficiency, B. settling velocity, C. concentration factor, of *C. zofingiensis* using  $\text{Ca(OH)}_2$  and  $\text{Mg(OH)}_2$  either with both at 3 mM (blue line) or 4 mM (orange line). Each treatment was performed in duplicate with triplicate absorbance measurements. D. Contour plot of the floculation efficiency using  $\text{Mg(OH)}_2$  and  $\text{Ca(OH)}_2$  at a biomass concentration of  $0.97 \pm 0.04 \text{ g L}^{-1}$ . The treatment of the plot was similar as described in Fig. 1. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

$\text{(OH)}_2$ . Interestingly, for the flocculation of *C. zofingiensis* using  $\text{NaOH/MgSO}_4$  at a comparable biomass concentration, there was found to be no significant correlation between pH and floculation efficiency ( $p > 0.01$ ,  $r^2 = 0.43$ ), whereas for  $\text{Ca(OH)}_2/\text{Mg(OH)}_2$ , pH and floculation efficiency had a significant polynomial relationship ( $p < 0.01$ ,  $r^2 = 0.99$ ), which further strengthens the hypothesis that a different mechanism was responsible in this second case.

The concentration factors achieved for  $\text{Ca(OH)}_2/\text{Mg(OH)}_2$  were comparable to those achieved for  $\text{NaOH/MgSO}_4$  (approx. 7–9 $\times$ ), and previous results for *C. zofingiensis* using  $\text{Mg}^{2+}$  addition and alkaline flocculation (Zhang et al., 2016). However, significantly higher concentration factors were found, 31 for *C. vulgaris* using  $\text{NaOH}$  at 3 mM for induction of flocculation, but this may be ascribed to differences in media salt concentrations resulting in different amounts of co-precipitating salts forming thereby changing sediment volume (Garcia-Perez et al., 2014; Lama et al., 2016). For instance, the media used by Lama et al. contained just  $1.5 \text{ mg P L}^{-1}$ , whereas the BBM media used here contained  $50 \text{ mg P L}^{-1}$ . A greater volume of precipitant in the sediment decreases the concentration factor. Alternatively, it could be the results of species or physiological related differences in floc characteristics and interactions with precipitants.

To further optimise this process for  $\text{Ca(OH)}_2$  and  $\text{Mg(OH)}_2$ , floculation efficiency, settling speed and concentration factor were examined at 4 mM of flocculants in greater temporal detail. Rapid processing speeds and high functional performance are often important to reduce infrastructure costs (Vandamme et al., 2013). In sedimentation cones using 250 mL of culture ( $\sim 1.0 \text{ g DW L}^{-1}$ ), 90% of cells had flocculated

within 10 min and settling velocity was maximal (Fig. 2A–C). The percentage of recovered cells increased to approx. 95% after 1 h and only increased slightly after 2 h (Fig. 2A). However, the concentration factor continued to increase over time, from approx. a 10 $\times$  concentration factor at 1 h, to 12 $\times$  concentration at 2 h, with an indication that it would have further increased with greater time remaining (Fig. 2C). The time cost incurred by increasing concentration factor could increase the likelihood of loss of cellular integrity or metabolite destruction (due to the high pH or contamination), or the requirement for a greater infrastructure processing capacity. However, the decrease in centrifuge volume could result in additional savings downstream in the process. These concentration factors are ultimately quite low and the factors preventing higher concentration factors from being achieved should be investigated. Sedimentation using  $\text{Ca(OH)}_2$  and  $\text{Mg(OH)}_2$  at concentrations of 3 mM was also tested to verify the model prediction of comparable floculation efficiencies, which was found to be the case (no significant difference,  $p > 0.01$ ; Fig. 2A–C).

Neutralisations of the sediment and supernatant for the 4 mM  $\text{Ca(OH)}_2/\text{Mg(OH)}_2$  treatment was performed by titration with 0.5 M  $\text{H}_3\text{PO}_4$ , and it was determined that 8.36 and 2.49 mM  $\text{H}_3\text{PO}_4$  was required to return these fractions to pH 7.0, respectively. This would return 259.9 and 77.2  $\text{mg L}^{-1}$  of P back to the cultivation system if these waters were recycled assuming all  $\text{H}_3\text{PO}_4$  added forms dissolved  $\text{PO}_4$  and is not associated with the biomass (as seen for  $\text{NaOH}$  flocculated biomass neutralised with  $\text{HCl}$ ). However, the concentration of P and Mg in the biomass neutralised using  $\text{H}_3\text{PO}_4$  was not measured and it is possible that at the higher concentrations of P, Mg and Ca in this

system, some of these could be lost due to precipitation as calcium or magnesium salts, or even as struvite with  $\text{NH}_4$ . This could influence the removal of these elements from the biomass and prevent their recycling, emphasising the complex nature of the interaction and the influence of concentration, mineral ratios, temperature and pH. For the benefit of our process model, it was assumed that recovery of P and Mg from the biomass was 95% to reflect loss to precipitation. Furthermore, the biochemical composition of the biomass was assumed to be affected in the same way when either of HCl and  $\text{H}_3\text{PO}_4$  was used as neutralising agents. Further studies should investigate these aspects in greater detail.

### 3.5. Cost and life-cycle impact assessment of a two-step harvesting process

To assess the effectiveness of a two-step harvesting process in decreasing the costs, energy usage and the environmental impact of biomass recovery; operating cost and life cycle analyses were performed. A baseline case of centrifugation was included as well as both chemical choices, with case 1 being: NaOH,  $\text{MgSO}_4$  and HCl, while case 2 was:  $\text{Ca}(\text{OH})_2$ ,  $\text{Mg}(\text{OH})_2$  and  $\text{H}_3\text{PO}_4$  (impacting less than HCl, see Supplementary Materials). These analyses only assessed biomass harvesting and did not include any processes up-stream (i.e. cultivation) or further downstream processing, such as drying. Empirical data from this study for sedimentation of  $1 \text{ g L}^{-1}$  cultures were used. Since centrifugation requires considerable electrical energy it was prudent to evaluate the impact of electricity mix (coal vs. gas vs. nuclear vs. hydroelectric, etc.) on the process and, thus, three representative European countries were selected. These were Sweden, with a low cost and global warming potential (GWP) electricity mix (mainly nuclear and hydroelectric), Greece, with a high reliance on fossil resources with a high GWP (mainly brown coal and natural gas), and Spain, with an intermediate mixture in terms of GWP (mainly natural gas) (Itten et al., 2014).

The result of the cost analysis showed the impact of these different processes varied significantly depending on both configuration and location. Two-step harvesting performed best in terms of cost per kg DW versus centrifugation, especially in Spain and Greece where electricity prices are higher ( $\sim 0.031 \text{ € MJ}^{-1}$ ; Fig. 3A), whereas in Sweden the costs are more comparable since electricity is nearly half the price ( $0.017 \text{ € MJ}^{-1}$ ). Costs for the base case (solely centrifugation) were high for both Spain and Greece Prices of ( $> 1 \text{ € kg DW}^{-1}$ ), whereas all two-step processes had costs of  $< 0.52 \text{ € kg DW}^{-1}$  with only minor differences between the different countries, due to a decreased reliance on electricity (90% less vs. base case). Two-step case 1 was found to be approx. 17–19% cheaper than case 2, due to the higher price of  $\text{H}_3\text{PO}_4$  versus HCl. Overall, the cost of centrifugation accounts for 13–28% of the total cost of the two-step processes cases across the different locations.

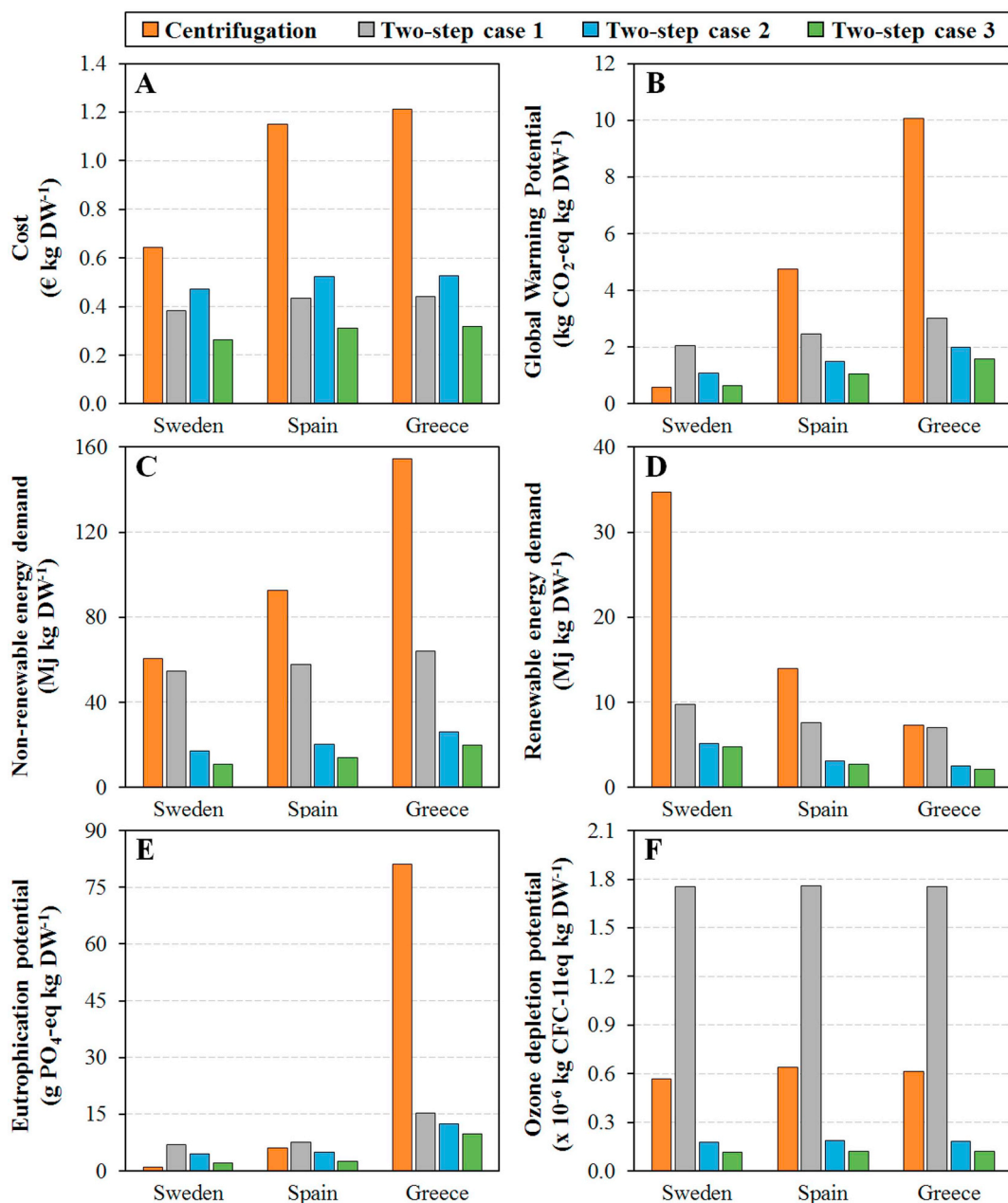
In terms of GWP, Sweden again performed better for all processes than the other locations, with the two-step processes being more impactful than centrifugation alone (Fig. 3B). Chemical production has higher associated GWPs than electricity; and the electricity mix in Sweden has 10–20 times lower GWP than Greece or Spain (Supplementary Materials). However, in Spain and Greece, a two-step process would decrease GHG emissions compared to centrifugation alone. Case 2 performed better (33–47% lower GWP) than case 1 since the production of HCl is highly impactful and double that of  $\text{H}_3\text{PO}_4$  (Supplementary Materials). There is a large spread in the energy usage of the different processes and locations (Fig. 3C, D). Differences in electricity mix resulted in substantial variations across locations for the base case, with an inverse relationship between the three locations with regards to Non-Renewable and Renewable energy Demand (NRED and RED). There are significantly less differences between countries for two-step processes, with Case 2 being particularly efficient, both for NRED and RED, due to production of both  $\text{Ca}(\text{OH})_2$  and  $\text{Mg}(\text{OH})_2$  being less energy intensive per mass compared to NaOH and  $\text{MgSO}_4$ . If it is assumed

that the energy content of algal biomass is approximately  $20 \text{ MJ kg DW}^{-1}$ , the cumulative energy demand (CED) for harvesting using centrifugation and case 1 surpass this in all locations at least 3-fold, while case 2 has a CED slightly larger than the biomass energy content. It is hence imperative that harvesting processes become more efficient to improve the energy return on investment to make bioenergy applications more feasible. There were very large differences in the eutrophication potential of these processes (Fig. 3E). Those located in Greece were especially impactful owing to the significant surface water damage and soil erosion caused by brown coal usage (Theodosiou et al., 2014). Case 1 has a marginally higher impact compared to case 2, which is due to the impact of NaOH use versus  $\text{Ca}(\text{OH})_2$ . Ozone depletion was rather unaffected related to the different locations since the electricity mixes have approx. the same impact ( $1.5\text{--}1.7 \times 10^6 \text{ kg CFC-11 eq MJ}^{-1}$ ). Ozone depletion was significantly worse for case 1 (Fig. 3F), due to the use of NaOH and HCl that have up to 100 times higher ODP than the other chemicals (Supplementary Materials), presumably due to the involvement of chlorine in production.

The material requirements found in this study for efficient sedimentation were comparable to processes presented in other studies (Vandamme et al., 2015; Vandamme et al., 2012a). However, differences in the choice of materials, the quantities required for neutralisation, and costs of the materials used result in significant differences in the estimated costs, e.g. in the total cost of  $\text{€}0.051$  per kg DW estimated for flocculation ( $\text{Ca}(\text{OH})_2$  and HCl), the neutralisation of the supernatant was not included (Vandamme et al., 2015). In comparison, our results predict a higher material cost of  $0.35\text{--}0.38 \text{ € kg DW}^{-1}$  for case 1 and  $0.44\text{--}0.47 \text{ € kg DW}^{-1}$  for case 2 inputs, but these include the neutralisation. Comprehensive LCA of sedimentation processes are somewhat limited for alkaline-induced flocculation techniques, but a two-step process utilising alum followed by centrifugation had process energy costs of  $1.2\text{--}1.4 \text{ MJ kg DW}^{-1}$  depending on the biomass concentration (Weschler et al., 2014), which compares to  $59.3\text{--}62.6$  and  $17.6\text{--}20.8 \text{ MJ kg DW}^{-1}$  for case 1 and 2, respectively, in this study. However, the previous study did not include the energy demand of material or electricity production, used outdated and optimistic energy requirements for centrifugation, and did not consider the removal of alum before utilisation of biomass.

For case 1, if it was assumed that Mg could be recycled, this would meet all Mg requirements for cultivation, but these savings are less significant since  $\text{MgSO}_4$  has comparatively less impact than the other chemicals. In case 2, the use of  $\text{H}_3\text{PO}_4$  for neutralisation enables recycling of P back to cultivation, which could result in significant savings by mitigation of P-fertiliser requirements, which although relatively minor,  $0.1\text{--}2 \text{ g P kg DW}^{-1}$ , (Mayers et al., 2017), decreases reliance on a costly and finite resource. To ascertain the benefit of recycling nutrients (Mg and P) from the harvesting back to cultivation, a case 3 as an expansion of case 2 was established. The percentage of nutrient in the media that could be replaced was calculated for case 1 and 2 and 95% of P from  $\text{H}_3\text{PO}_4$  and Mg from  $\text{Mg}(\text{OH})_2$  was assumed to be recycled back to the culture. This would replace approx. 42% and 63% of media P and Mg requirements, respectively, based the biomass elemental content found in Table 1 and a harvesting rate of  $0.2 \text{ d}^{-1}$ . Exclusion of these percentages of the chemicals (instead effectively assigned to biomass production), i.e. case 3, resulted in 45–48% decrease in cost ( $0.23\text{--}0.25 \text{ € kg DW}^{-1}$ ), a 28–45% decrease in NRED, GWP and ODP, and an 11–18% decrease in NED (Fig. 3). These savings increase the benefits associated with case 3 versus the base case, even versus the Swedish model, for which only EP and GWP are still lower for centrifugation. This strategy results in considerable improvements in the overall process for case 3 if nutrient recycling from two-step harvesting is implemented, highlighting the benefits of smart process design and chemical selection.

Overall, these analyses have shown the benefits of developing a two-step harvesting method utilising alkaline flocculation. We are aware that the energy requirements for centrifugation reported in the



**Fig. 3.** Impact of *C. zofigiengensis* harvesting process configuration on A. cost, B. global warming potential, C. non-renewable energy demand, D. renewable energy demand, E. eutrophication potential, and F. ozone depletion potential. Baseline case assumes the use of centrifugation only, while two different two-step processes were assessed: Case 1 utilised NaOH, MgSO<sub>4</sub> and HCl, while Case 2 utilised Mg(OH)<sub>2</sub>, Ca(OH)<sub>2</sub> and H<sub>3</sub>PO<sub>4</sub>. Case 3 uses the case 2 chemicals but includes nutrient recycling. All analyses were based on bench scale data from Sections 3.1 and 3.4.

literature vary quite significantly and thus our findings for the two-step processes could appear better or worse depending on the value utilised. The values of energy requirement for centrifugation in the literature were reported up to 35.5 MJ m<sup>-3</sup>, with the lowest value of approx. 9 MJ m<sup>-3</sup> reported for Evodos' spiral plate centrifuges (Monte et al., 2018). This study used the higher value of 35.5 MJ m<sup>-3</sup>, which were from trials using commercially available equipment at a large-scale algal production facility (Beal et al., 2015; Huntley et al., 2015) and is thus most appropriate in this context. The use of a less energy intensive centrifugation could decrease the impact of a one-step harvesting process and thus using the value of 9 MJ m<sup>-3</sup>, the cost of the base case would be decreased by 75%, making its impacts more comparable to case 1 (Supplementary Materials). If nutrient recycling is included, case 3 still performed better across all impact categories for Spain and Greece, and on cost, energy usage and ODP against Sweden.

Subsequently, the development of more efficient centrifuges may negate the use of a two-step process in countries with cheap electricity with a low environmental impact.

One final consideration is the processing time required, i.e. from bioreactor to algal paste, and the impact this may have on process choice if this is a critical factor of production (e.g. especially valuable and sensitive biomass). In the case of the two-step method described, at least 90 min are likely required to generate a sediment that has been neutralised and ready for centrifugation (regardless of the volume required). A 10× concentration factor would significantly decrease the centrifugation time, which frees up capacity (i.e. fewer centrifuges). However, for relatively small volumes, the centrifuge considered in this case (3.75 m<sup>3</sup> hr<sup>-1</sup>) would process cultures faster than a two-step process, up to culture volumes of 6–7 m<sup>-3</sup> (data not shown). For greater volumes, a larger capacity centrifuge would be required to

decrease processing time versus a two-step process. Cost of centrifuges most likely far surpasses that of the comparable equipment for sedimentation and hence, there is a careful balance in the selection of process and equipment to match the scale of biomass production with the capacity for downstream processing, both on a capital and operational cost basis. This would require further study to investigate this interplay.

#### 4. Conclusions

This study has found that *C. zofingiensis* can be flocculated effectively by supplementing with Mg and addition of base with similar efficiency both at bench- and pilot scale and that formation of  $Mg(OH)_2$  has a role in the mechanism. Alternative chemicals for flocculating and subsequent neutralisation were evaluated to reduce environmental impact and possibility to recycle nutrients and water. Process modelling of flocculation/neutralisation preceded centrifugation harvesting with cases using alternatives of chemicals was compared to solely centrifugation. The modelling showed that results are sensitive for selection of electricity mix and used chemicals in relation to impact categories evaluated.

#### Declaration of author contributions

JM, MJA and EA conceived and designed the study. JM, ARL and MJA performed growth and flocculation experiments. JM performed analysis and interpretation of the data. JM and EA drafted the manuscript with contributions and critical revisions from ARL and MJA. EA obtained funding to perform the majority of this work. Final approval for submission was decided by EA.

#### Declaration of competing interest

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

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.biteb.2019.100358>.

#### References

- Andersen, R.A., 2005. *Algal Culturing Techniques*. Elsevier Academic Press, London, UK.
- Beal, C.M., Gerber, L.N., Sills, D.L., Huntley, M.E., Machesky, S.C., Walsh, M.J., Tester, J.W., Archibald, I., Granados, J., Greene, C.H., 2015. Algal biofuel production for fuels and feed in a 100-ha facility: a comprehensive techno-economic analysis and life cycle assessment. *Algal Res.* 10, 266–279.
- Beuckels, A., Depraetere, O., Vandamme, D., Foubert, I., Smolders, E., Muylaert, K., 2013. Influence of organic matter on flocculation of *Chlorella vulgaris* by calcium phosphate precipitation. *Biomass Bioenergy* 54, 107–114.
- Brady, P.V., Pohl, P.I., Hewson, J.C., 2014. A coordination chemistry model of algal autoflocculation. *Algal Res.* 5, 226–230.
- Branyikova, I., Prochazkova, G., Potocar, T., Jezkova, Z., Branyik, T., 2018. Harvesting of microalgae by flocculation. *Fermentation* 4, 93.

- Castrillo, M., Lucas-Salas, L.M., Rodriguez-Gil, C., Martinez, D., 2013. High pH-induced flocculation-sedimentation and effect of supernatant reuse on growth rate and lipid productivity of *Scenedesmus obliquus* and *Chlorella vulgaris*. *Bioresour. Technol.* 128, 324–329.
- Dassey, A.J., Theegala, C.S., 2013. Harvesting economics and strategies using centrifugation for cost effective separation of microalgae cells for biodiesel applications. *Bioresour. Technol.* 128, 241–245.
- Egle, L., Rechberger, H., Krampe, J., Zessner, M., 2016. Phosphorus recovery from municipal wastewater: an integrated comparative technological, environmental and economic assessment of P recovery technologies. *Sci. Total Environ.* 571, 522–542.
- Eurostat statistics explained Electricity Prices, Second Half of Year, 2014-2016 (EUR per kWh) YB17. WWW document. [https://ec.europa.eu/eurostat/statistics-explained/index.php/File:Electricity\\_prices,\\_second\\_half\\_of\\_year,\\_2014-2016\\_\(EUR\\_per\\_kWh\)\\_YB17.png](https://ec.europa.eu/eurostat/statistics-explained/index.php/File:Electricity_prices,_second_half_of_year,_2014-2016_(EUR_per_kWh)_YB17.png).
- Garcia-Perez, J.S., Beuckels, A., Vandamme, D., Depraetere, O., Foubert, I., Parra, R., Muylaert, K., 2014. Influence of magnesium concentration, biomass concentration and pH on flocculation of *Chlorella vulgaris*. *Algal Res.* 3, 24–29.
- Gerardo, M.L., Van Den Hende, S., Vervaeren, H., Coward, T., Skill, S.C., 2015. Harvesting of microalgae within a biorefinery approach: a review of the developments and case studies from pilot-plants. *Algal Res.* 11, 248–262.
- Hamilton, M.L., Warwick, J., Terry, A., Allen, M.J., Napier, J.A., Sayanova, O., 2015. Towards the industrial production of omega-3 long chain polyunsaturated fatty acids from a genetically modified diatom *Phaeodactylum tricoratum*. *PLoS One* 10, e0144054.
- Hathwaik, L.T., Redelman, D., Samburova, V., Zielinska, B., Shintani, D.K., Harper, J.F., Cushman, J.C., 2015. Transgressive, reiterative selection by continuous buoyant density gradient centrifugation of *Dunaliella salina* results in enhanced lipid and starch content. *Algal Res.* 9, 194–203.
- Huntley, M.E., Johnson, Z.I., Brown, S.L., Sills, D.L., Gerber, L., Archibald, I., Machesky, S.C., Granados, J., Beal, C., Greene, C.H., 2015. Demonstrated large-scale production of marine microalgae for fuels and feed. *Algal Res.* 10, 249–265.
- Itten, R., Frischknecht, R., Stucki, M., 2014. Life Cycle Inventories of Electricity Mixes and Grid. Paul Scherrer Institut (PSI) Version 1.3, treeze Ltd., fair life cycle thinking. [www.treeze.ch](http://www.treeze.ch).
- Lama, S., Muylaert, K., Karki, T.B., Foubert, I., Henderson, R.K., Vandamme, D., 2016. Flocculation properties of several microalgae and a cyanobacterium species during ferric chloride, chitosan and alkaline flocculation. *Bioresour. Technol.* 220, 464–470.
- Mayers, J.J., Nilsson, A.E., Albers, E., Flynn, K.J., 2017. Nutrients from anaerobic digestion effluents for cultivation of the microalga *Nannochloropsis* sp - impact on growth, biochemical composition and the potential for cost and environmental impact savings. *Algal Res.* 26, 275–286.
- Mayers, J.J., Vaiciulyte, S., Malmhäll-Bah, E., Alcaide-Sancho, J., Ewald, S., Godhe, A., Ekdahl, S., Albers, E., 2018. Identifying a marine microalgae with high carbohydrate productivities under stress and potential for efficient flocculation. *Algal Res.* 31, 430–442.
- Monte, J., Sa, M., Galinha, C.F., Costa, L., Hoekstra, H., Brazinha, C., Crespo, J.G., 2018. Harvesting of *Dunaliella salina* by membrane filtration at pilot scale. *Sep. Purif. Technol.* 190, 252–260.
- Muto, M., Nojima, D., Yue, L., Kanehara, H., Naruse, H., Ujio, A., Yoshino, T., Matsunaga, T., Tanaka, T., 2017. Potential of water surface-floating microalgae for biodiesel production: Floating-biomass and lipid productivities. *J. Biosci. Bioeng.* 123, 314–318.
- Ruiz, J., Olivieri, G., de Vree, J., Bosma, R., Willems, P., Reith, J.H., Eppink, M.H.M., Kleinegriss, D.M.M., Wijffels, R.H., Barbosa, M.J., 2016. Towards industrial products from microalgae. *Energy Environ. Sci.* 9, 3036–3043.
- Smith, B.T., Davis, R.H., 2012. Sedimentation of algae flocculated using naturally-available, magnesium-based flocculants. *Algal Res.* 1, 32–39.
- Sukenik, A., Shelef, G., 1984. Algal autoflocculation - verification and proposed mechanism. *Biotechnol. Bioeng.* 26, 142–147.
- Taelman, S.E., De Meester, S., Van Dijk, W., da Silva, V., Dewulf, J., 2015. Environmental sustainability analysis of a protein-rich livestock feed ingredient in the Netherlands: microalgae production versus soybean import. *Resour. Conserv. Recy.* 101, 61–72.
- Theodosiou, G., Koroneos, C., Stylos, N., 2014. Environmental impacts of the Greek electricity generation sector. *Sustainable Energy Technol. Assess.* 5, 19–27.
- Vandamme, D., Foubert, I., Fraeye, I., Meesschaert, B., Muylaert, K., 2012a. Flocculation of *Chlorella vulgaris* induced by high pH: role of magnesium and calcium and practical implications. *Bioresour. Technol.* 105, 114–119.
- Vandamme, D., Foubert, I., Fraeye, I., Muylaert, K., 2012b. Influence of organic matter generated by *Chlorella vulgaris* on five different modes of flocculation. *Bioresour. Technol.* 124, 508–511.
- Vandamme, D., Foubert, I., Muylaert, K., 2013. Flocculation as a low-cost method for harvesting microalgae for bulk biomass production. *Trends Biotechnol.* 31, 233–239.
- Vandamme, D., Beuckels, A., Markou, G., Foubert, I., Muylaert, K., 2015. Reversible flocculation of microalgae using magnesium hydroxide. *Bioenergy Res.* 8, 716–725.
- Wan, C., Alam, M.A., Zhao, X.-Q., Zhang, X.-Y., Guo, S.-L., Ho, S.-H., Chang, J.-S., Bai, F.-W., 2015. Current progress and future prospect of microalgal biomass harvest using various flocculation technologies. *Bioresour. Technol.* 184, 251–257.
- Wang, Z., Belovich, J.M., 2010. A simple apparatus for measuring cell settling velocity. *Biotechnol. Prog.* 26, 1361–1366. <https://doi.org/10.1002/btpr.432>.
- Weidema, B.P., Bauer, C., Hischer, R., Mutel, C., Nemecek, T., Reinhard, J., Vadenbo, C.O., Wernet, G., 2013. Overview and Methodology. Data Quality Guideline for the EcoInvent Database Version 3. The ecoinvent Centre, St. Gallen (Ecoinvent Report 1 (v3)).
- Wellburn, A.R., 1994. The spectral determination of chlorophylls *a* and *b*, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. *J. Plant Physiol.* 144, 307–313.

- Weschler, M.K., Barr, W.J., Harper, W.F., Landis, A.E., 2014. Process energy comparison for the production and harvesting of algal biomass as a biofuel feedstock. *Bioresour. Technol.* 153, 108–115.
- Wu, Z.C., Zhu, Y., Huang, W.Y., Zhang, C.W., Li, T., Zhang, Y.M., Li, A.F., 2012. Evaluation of flocculation induced by pH increase for harvesting microalgae and reuse of flocculated medium. *Bioresour. Technol.* 110, 496–502.
- Yang, F.F., Xiang, W.Z., Fan, J.W., Wu, H.L., Li, T., Long, L.J., 2016. High pH-induced flocculation of marine *Chlorella* sp for biofuel production. *J. Appl. Phycol.* 28, 747–756.
- Zhang, X.Z., Wang, L., Sommerfeld, M., Hu, Q., 2016. Harvesting microalgal biomass using magnesium coagulation-dissolved air flotation. *Biomass Bioenergy* 93, 43–49.
- Zhu, C.J., Lee, Y.K., 1997. Determination of biomass dry weight of marine microalgae. *J. Appl. Phycol.* 9, 189–194.