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Efficient high-concentration dewatering of *Chlorella vulgaris* utilising spiral inertial microfluidics

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ABSTRACT

The aim of this study was to explore an alternative mode of operation for inertial focusing systems and determine the maximum inlet volume fractions at which device performance can be maintained (i.e. cell retention efficiency remains high, here defined as >95%). Microalgae dewatering was selected as a demonstrator application, specifically using *Chlorella*. A spiral inertial focusing device was characterised at a range of inlet concentrations from 0.5–48.8% v/v. The modified method for operation of the spiral device through the separation of a cell free region, with volume removal decreasing as device concentration increases, alongside sample recirculation in 10 x stacked devices, maintained >95% overall recovery efficiency when concentrating from an initial inlet concentration of 1.8% v/v up to a final outlet concentration of 65.3% v/v. Power consumption was calculated to be 1.1 kWh/m³, for a concentration factor of 130, which is comparable to existing dewatering technologies. This work demonstrates the feasibility of the application of inertial focusing technology to a range of bioprocessing cell concentration applications at industrially relevant sample concentrations.

1. Introduction

Concentration of suspended cells or bioparticles from large volumes is a critical step in many bioprocesses, e.g. biomedical research and diagnostics, environmental analysis, biopharmaceutical product recovery or microalgae dewatering. Microalgae, either their biomass or high-value components extracted from the algae, have found a range of applications including bioremediation, water treatment, the production of renewable fuels, animal feed and nutritional supplements and use in cosmetics and pharmaceuticals (Ng et al., 2015; Ahmad et al., 2011; Singh et al., 2005; Barsanti and Gualtieri, 2018). Microalgal cultures are typically highly dilute and therefore one of the major challenges to deliver cost-effective products is the downstream bioprocessing required to harvest and dewater the algae economically (Singh and Patidar, 2018). There are two main steps of harvesting microalgae which are discussed extensively by Barros et al. (2015): first a primary harvesting process which acts to remove the bulk of the volume (100–200 volume reduction) followed by a secondary harvesting process for the formation of a product cake (a further 2–10 volume reduction) (Hønsvall et al., 2016; Barros et al., 2015). This step typically accounts for up to 30% of the total biomass cost at present (Grima et al., 2003).

Currently used harvesting methods include centrifugation,

flocculation, filtration, sedimentation, electrical processes, and mechanical scraping, to varying degrees of success (Singh and Patidar, 2018; Barsanti and Gualtieri, 2018; Barros et al., 2015; Zeng et al., 2016). There is no universal approach adopted, with systems varying in terms of key performance parameters such as cost, energy consumption, biomass recovery and dewatering extent (Singh and Patidar, 2018). Typically, a combination of approaches is adopted for an overall harvesting strategy (Murthy, 2011). Scale-up and operating costs are a particular challenge, with high labour intensity being a most common complaint in a recent industrial survey of 28 EU bio-producers (Fasaei et al., 2018). Furthermore, the processes can be time consuming, during which the algal biochemistry can change, and are energy intensive.

Microfluidic solutions have been proposed as an alternative for cell concentration in a range of bioprocesses, including microalgae; inertial focusing is a passive method capable of supporting high flow rates and as such has been applied to a range of high-throughput bioprocessing operations, e.g. sample processing of waterborne pathogens (Miller et al., 2016; Jimenez and Bridle, 2016), biomedical applications (Rafeie et al., 2016; Wang et al., 2015) or microalgae dewatering (Wang and Dandy, 2017; Xiang et al., 2020). Despite these above examples there has typically been a greater focus on separation and ordering rather than concentration within inertial focusing, as shown in supplementary

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material. Operation of inertial focusing devices for particle separation is typically performed at low concentrations, i.e. less than 0.5% v/v (as shown in supplementary material), normally resulting in a single focused stream of particles which are easily separated from the bulk of the fluid through the device outlets (Di Carlo, 2009; Bhagat et al., 2008; Guan et al., 2013). The theory and applications of inertial focussing have been the subject of a number of recent reviews (Zhou et al., 2019; Gou et al., 2018; Zhang et al., 2016). An upper limit on the particle concentration is determined by the line fraction, i.e. the particle concentration that results in a single stream of particles with an interparticle spacing of 10 times the particle diameter (Lee et al., 2010).

Processing of the initial dilute microalgae culture is not problematic within microfluidics, and microfluidic systems have been utilised in microalgae research across a range of applications at these low concentrations, including cell identification, cell sorting/screening, cell culturing and cell disruption as recently reviewed (Kim et al., 2018; Bodénès et al., 2019). Additionally, microfluidic devices have been trialled as an alternative dewatering approach, with several different systems having been recently investigated. Publications either targeting dewatering, or utilising inertial focusing for the separation of microalgae from contaminating bacteria (Godino et al., 2015; Yuan et al., 2019) or other microalgae (Syed et al., 2018) as well as inertial focusing based microfluidic systems designed to enable study of microalgae morphology (Li et al., 2017; Schaap et al., 2016) are summarised within supplementary material. The maximal inlet volume fraction utilised is 1% v/v (2.4×10^9 cells/mL), for cyanobacterium *Synechocystis* in a serpentine channel with a recovery efficiency of >95% (Wang and Dandy, 2017).

Inertial focusing systems have been scaled-up to demonstrate large volume operation (Miller et al., 2016; Rafeie et al., 2016; Xiang et al., 2020; Xiang et al., 2019; Warkiani et al., 2015). However, one factor which has not often been addressed is the impact of concentration/volume reduction on the cell concentration within the inertial focusing devices. Since a single pass through an inertial focusing system is unlikely to result in a high concentration factor, due to limitations in the number of outlets and the focusing positions of the cells, recirculation is important to maximise dewatering extent. However, as the sample recirculates and becomes more concentrated performance declines. Degradation in device performance at increasing particle concentrations has been attributed to particle-particle interactions disrupting the focused stream of particles which results from the balance of forces within the device (Xiang et al., 2019; Di Carlo, 2009; Reece and Oakey, 2016). In work by Wang and Dandy (2017) on microalgae, with a concentration factor of 3.28, cell recovery dropped from around 90% to less than 80% for the fourth recirculation step at 1×10^6 cells/mL. Similar effects were observed in other concentration applications of inertial focusing, e.g. Martel et al. (2015) achieved >400 times volume reduction in their multiplex inertial focusing design, when the inlet concentration was less than 100,000 WBCs/mL. However, when the inlet concentration is 1×10^6 WBCs/mL only a 50 times volume reduction was possible as successful separation in microfluidic devices is limited by a maximum final outlet concentration.

The aim of this study was to explore an alternative mode of operation for inertial focusing systems and determine the maximum inlet volume fractions at which device performance can be maintained (i.e. cell retention efficiency remains high, here defined as >95%). A spiral channel inertial focusing system was utilised to focus *Chlorella* (size of $6.5 \pm 0.9 \mu\text{m}$) and the alternative operation of inertial focusing enabled use of almost one-hundred-fold higher inlet volume fractions up to 48.8% v/v, allowing high dewatering efficiency across a wide range of inlet cell concentrations. The modified method for operation of the spiral device through the separation of a cell free region, with volume removal decreasing as device concentration increases, enables the application of this technology to a range of bioprocessing cell concentration applications at industrially relevant sample concentrations. Additionally, power consumption was calculated and compared with

current methods for microalgae dewatering, showing that this microfluidic approach offers a feasible alternative.

2. Materials and methods

2.1. Algae used

Chlorella vulgaris (CCAP 211/21A), supplied by The Scottish Association for Marine Science (SAMS) from the Culture Collection of Algae and Protozoa (CCAP), was cultivated in enriched sea water medium F/2 (CCAP 16 May 2000). The F/2 medium was filter sterilised and comprised of: 1 mL 75 g/L NaNO₃, 1 mL 5.65 g/L NaH₂PO₄·2H₂O, 1 mL trace elements (4.16 g/L Na₂EDTA, 3.15 g/L FeCl₃·6H₂O, 0.01 g/L CuSO₄·5H₂O, 0.022 g/L ZnSO₄·7H₂O, 0.01 g/L CoCl₂·6H₂O, 0.18 g/L MnCl₂·4H₂O, 0.006 g/L Na₂MoO₄·2H₂O), 1 mL vitamin mix (0.0005 g/L Vitamin B12, 0.1 g/L Vitamin B1, 0.0005 g/L Biotin), made up to 1 L with artificial seawater (33.6 g/L Tropic Marin – Sea Salt).

Chlorella vulgaris is a species of the *Chlorella* genus and is a unicellular, green microalgae (Baroni et al., 2019). The culture used in this investigation was found to have a cell diameter of $6.5 \pm 0.9 \mu\text{m}$. *Chlorella* is cultured commercially across the globe and has a wide range of applications stemming from the utilisation of produced biomass (Mobin and Alam, 2017), including among others; use as a food supplement, as animal feed, in biodiesel production, pigment production and has even been suggested for use in a spacecraft life support system (Niederwieser et al., 2018). This species was chosen due to the industrial prominence of *Chlorella* and potential scope for use. *C. vulgaris* (CCAP 211/21A) is a marine species and was identified as one of the highest lipid producers of over 100 tested from the CCAP (Slocombe et al., 2015). The use of marine species is also of interest due to the reduction on freshwater supply compared to the use of freshwater species when considering the high volumes required for algae culture in industrial applications.

2.2. Culture method

For fresh cultures 20 vol% inoculations were employed under recommended culturing techniques from the CCAP to give optimum growth. Cultures were grown in 2 L conical flasks, stoppered with cotton wool to allow for gas diffusion, within a light box under constant illumination. Manual agitation of flasks was performed daily via Vortex former (Scientific Industries Inc., Vortex-Genie2 G-560E) with a flat platform head to ensure thorough mixing and production of a homogeneous culture and their position within the light box was also varied daily. The culture was grown to densities of approximately 0.5% v/v, measured by spectrophotometer (see Algal Enumeration). The culture was maintained by 40 vol% harvest of culture and replenishment with fresh media.

As the starting concentration of *C. vulgaris* sample grown under laboratory conditions is approximately 0.5% v/v, to obtain higher concentration samples the initial cultures were centrifuged, and the resulting pellets diluted using fresh media to the required concentration.

2.3. Algal enumeration

Determination of algal concentration was performed by measurement of sample optical density (OD) using a spectrophotometer (Biochrom Ltd., WPA S800+) at a wavelength of 750 nm. The wavelength of 750 nm was chosen to reduce the error in optical density measurement as a method of determining cell concentration, which can result from the variation in algal cells high pigment quantity throughout their life span (Griffiths et al., 2011). This is achieved as the wavelength of 750 nm is outside of the range of algal cells most prominent pigments (carotenoids, chlorophylls) absorbance wavelengths (Griffiths et al., 2011).

To allow for concentration comparison, calibration of optical density with haemocytometer cell counts was performed giving cells/mL. A

calibration curve was completed over the 0.1–1.5 OD measurement range used throughout the experiments by dilution of algal samples. Haemocytometer cell counts were performed with an Improved Neubauer chamber (Bright-Line™ Haemocytometer) and corresponding coverslip, set up under an optical microscope with an attached camera (Allied Vision, Mako U–130B) for imaging. 10 µL of algal sample was pipetted into the counting chamber and an image captured for later counting, a total of three 10 µL samples were imaged per optical density reading. Per image, 4 central squares (0.2 mm × 0.2 mm per square) were counted and an average value per square taken across the 3 images.

2.4. Device design and manufacture

Devices were designed using AutoCAD, and comprised a spiral channel with 6 loops, one inlet, four outlets, with a channel width of 170 µm and a channel height of 30 µm. The layout of this spiral is based on designs utilised for stem cell purification and waterborne pathogens sample processing (Guzniczak et al., 2020; Jimenez and Bridle, 2016).

$$CF = \frac{\text{Concentration of Recovered}}{\text{Concentration of Inlet}} = \frac{\text{Cells Recovered}/\text{Volume Recovered}}{\text{Cells In}/\text{Volume in}} = \frac{RE}{1 - DE}$$

For the stacked devices, 10 layers of the same channel geometry were positioned directly on top of each other to form a single stacked device. Each layer's inlet was fed through the same large inlet bore hole, located at the top of the stack, and each outlet were collected together for all layers. This method of stacking results in an uneven flow rate distribution across the layers, with the top layers having a higher flow rate than the bottom layers. This concept is discussed by B. Miller et al., 2016 in the supplementary information (Miller et al., 2016). Manufacturing in PMMA was undertaken by Epigem Ltd. (Redcar, UK).

2.5. Device operation/experimental method

For experiments the microfluidic system was connected to a syringe pump (CETONI, neMESYS 1000 N) via an external pressure sensor (CETONI, neMESYS External Pressure Sensor) utilising PTFE tubing (Cole-Parmer, 1/16" OD) and ferrules (VICI, CFL-1G). Samples were collected from each of the four outlets directly into cuvettes used for measurement (Fisherbrand, Polystyrene Macro Cuvettes for Visible Wavelengths). Fluid flow was driven through the devices at flow rates ranging from 0.25–1.25 mL/min using a 5 mL stainless steel syringe for single layer device investigation and a 10 mL stainless steel syringe for the 10-layer stacked device investigation, operating at flow rates 10 times higher than the single layer device.

2.6. Imaging

Imaging of the channel was performed using a 1.3 MP Dino-Lite Premier USB Camera positioned to capture the channel just before the expanding outlet section. Intensity plots were obtained using the channel images, given in supplementary material, using a custom MATLAB code.

2.7. Sample analysis

The optical densities of the collected outlets were recorded at 750 nm. Any readings greater than 1.5 OD were diluted with fresh F/2 media and a value calculated from the dilution volumes used. The volume of each outlet was measured using a micropipette in 0.1 mL increments. The optical density, as representative of cell concentration, was used to

determine the cell distribution as a percentage across the outlets for use in calculation of recovery efficiency (RE) and subsequently concentration factor (CF). The recorded volumes were used to determine the percentage volume split across the outlets which in turn could be used in the calculation of actual dewatering extent (DE) and subsequently concentration factor. Recovery efficiency is defined as follows:

$$RE = \frac{\text{Cells Recovered}}{\text{Cells In}}$$

Dewatering extent was calculated as:

$$DE = \frac{\text{Volume in} - \text{Volume Recovered}}{\text{Volume in}}$$

Initially it was assumed that flow would be split equally across the outlets. However, experimental volume splits varied and thus an actual dewatering extent was calculated from the volume splits.

The above RE and actual DE are used to determine concentration factor:

Three replicates were undertaken at each flow rate and concentration increment. The resulting values for outlet concentration were utilised alongside volume split across the outlets to give the distribution of algae in each outlet. The 3 values were averaged, and the corresponding standard deviation calculated which was used to show variation among the results.

2.8. Concentration analysis

The main goal in this investigation is to determine the maximum operational concentration that our device can operate at while still achieving effective recovery and dewatering, defined here as >95% RE with at least 25% DE. To allow for more accurate comparison with other literature, it is beneficial to report concentrations as volume fraction in place of cell concentration (cells/mL) as cell concentration does not take into consideration cell size. For the same cells/mL larger cells will make up a larger fraction of the sample volume than the same cells/mL of a smaller cell size.

Volume fraction (φ) is calculated using cell concentration and average cell size as:

$$\varphi = \text{Volume of a cell} \times \text{Cell Concentration}$$

$$\varphi = \frac{4}{3}\pi \left(\frac{\text{cell diameter}}{2} \right)^3 \times \frac{\text{Cells}}{\text{mL}} = \frac{\text{mL of algae}}{\text{mL of sample}}$$

3. Results and discussion

3.1. Inertial focusing of *Chlorella* at a typical culture concentration (< 0.5% v/v)

Past work, outlined in supplementary material, illustrates that inertial focussing based dewatering systems have typically used cell sizes of greater than 10 µm. However, it is for the concentration of small microalgal species that standard methods of dewatering are unsuccessful or costly. For example, harvesting of small microalgae using filtration results in rapid fouling of membranes from the small cell size (Murthy, 2011) and intensifications such as vacuum filtration result in very high energy requirements while still hindered by fouling. Also harvesting via

centrifugation, though less energy intensive than methods such as vacuum filtration, can result in low efficiencies while resulting in a relatively high energy input (Barros et al., 2015) as well as potential cell damage (Xiang et al., 2020); and harvesting using the passive sedimentation method is ineffective due to the low settling velocities of almost zero for small cells (Zeng et al., 2016). Those systems that have successfully focussed small microalgae have had limited throughput.

The first stage of this work was to demonstrate successful application of spiral inertial microfluidics for the focusing of small microalgae, using *C. vulgaris* (~6.5 μm). The impact of flow rate on microalgae focusing within the device was investigated at typical culture concentrations for microalgae, which is similar to the cell concentrations usually utilised within microfluidic applications (<0.5% v/v). Flow rates from 0.25 to 1.25 mL/min were investigated in increments of 0.25 mL/min. These flow rates are comparable to the majority of other inertial focusing microfluidic dewatering solutions as shown in supplementary material. The upper flow rate limit results from the high pressure within the system reaching operational limits as well as a decline in performance. Data was obtained through sample collection from each of the four outlets which then subsequently underwent absorbance measurements.

As can be seen in Fig. 1A, as flow rate is increased from 0.25 mL/min, recovery efficiency (RE) increases until 0.75 mL/min, reaching its maximum value of $96.8 \pm 0.4\%$ RE at a concentration of 0.5% v/v. When flow rate is increased above 0.75 mL/min device recovery efficiency starts to decrease. The performance decline at higher flow rates is attributed to the shift of focussing position away from the inner wall (Martel and Toner, 2014), an effect previously observed within these systems for waterborne protozoa (Jimenez and Bridle, 2016; Jimenez et al., 2017). Although the optimum flow rate is 0.75 mL/min at 0.5% v/v concentration of *C. vulgaris*, the recovery efficiency at 0.5 mL/min is only 0.5% lower ($96.3 \pm 0.2\%$) therefore yielding similar results which are not significantly different.

Throughout this initial device investigation, at 0.5% v/v with *C. vulgaris*, dewatering extent (DE) is $76.3 \pm 0.6\%$ with the outlet at the inner wall of the device containing the concentrated microalgae and the three remaining outlets being separated. This high DE yields a single pass CF of 4.08. A comparison of other microfluidic dewatering devices shown in supplementary material shows that for other applications of similar concentrations, >0.5% v/v, DE varies from 50 to 70% and RE from 65–100%.

If DE is decreased to 50% (combining outlets 1 and 2 into a concentrated algae outlet and separating outlets 3 and 4) the RE is increased to 98.2% at 0.75 mL/min. This would also result in overlapping errors across the flow rate range 0.5–1 mL/min (Fig. 1B). This RE can be increased further by decreasing the DE to 25% through combining outlets 1–3, only removing outlet 4. The resulting RE is >99% for an increased flow rate range of 0.25–1 mL/min.

3.2. Investigating the cell concentration processing limitations

With the spiral inertial focusing system presented in this paper processing *Chlorella*, the line fraction calculation would result in a maximum concentration for operation of ~0.08% v/v (5.5×10^6 cells/mL) and implies that above this concentration device performance will decline. To address this limitation, and enable application of this system at higher concentrations an alternative mode of device operation, outside of the normal definition of successful focusing in a microfluidic device, is proposed, utilising a cell-free and cell dense region. This cell free region can be separated from an area of high cell density while still achieving high recoveries at varying dewatering extents without the need for a single focused stream. A similar method was used by Martel et al. (2015) in their centrifugation replacement system exploiting inertial forces for siphoning of a cell-free region, rather than single stream focussing, operated successfully up to ~4.18% v/v (8×10^7 cells/mL) (Martel et al., 2015).

To understand how the concept of a cell free region can be exploited for high efficiency separation, imaging and analysis of cell behavior in the system was performed. Images showing algae position at increasing inlet concentration are given in supplementary material. The resulting intensity plots obtained from the MATLAB data are shown in Fig. 2, A–D where the high intensity value corresponds to high cell concentration areas.

It can be seen from the intensity plots (Fig. 2, A–D) that as concentration is increased the cells are not focused into a tight stream which is normally observed when performing inertial focusing at low concentrations (shown in supplementary material). Instead, the cells occupy a high concentration region of the channel, leaving the rest of the channel as a low density, cell-free region. The two regions are not separated by a firm boundary but instead by a decreasing cell gradient, as can be seen in Fig. 2, B–D. This cell free region can then be separated from the high concentration region.

To determine the limitation of cell concentration at which performance is maintained (defined here as >95% RE and >25% DE), device operation was investigated across the inlet concentration range 0.5% - 56.3% v/v and flow rate range of 0.25–1.25 mL/min. The optimum flow rate was found to be 0.5 mL/min as it achieved the highest RE over all concentrations, apart from at the lowest concentrations of 0.5–2.0% v/v where the RE's were < 0.5% higher for 0.75 mL/min at 50% DE. A potential reason for this change in optimum flow rate as cell concentration is increased is that particle-particle interactions become more dominant at the higher concentrations and are further increased by flow disturbances from the increased flow rate. This has previously been observed by Shen et al. (2019). The flow rate of 0.75 mL/min has similar, slightly lower, RE's for all other concentrations with overlapping errors. Therefore, when considering recirculation using this system both 0.5

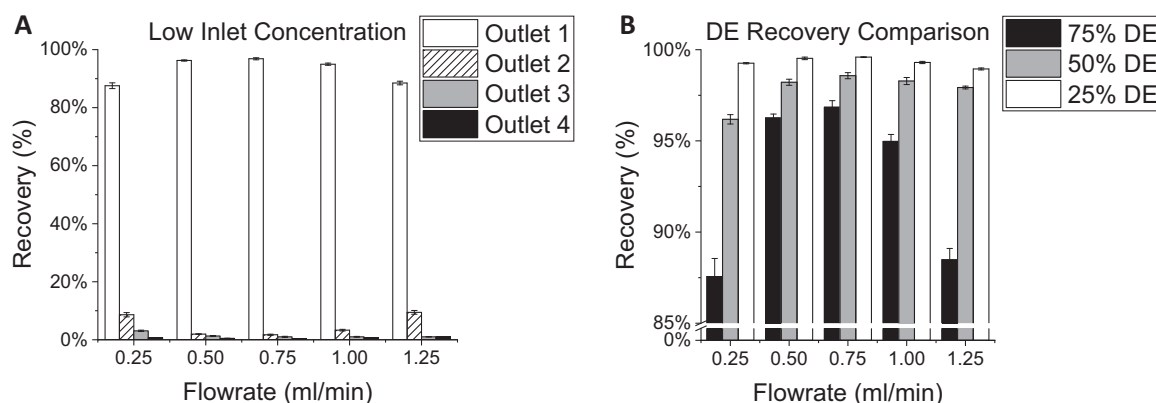


Fig. 1. A) Recovery distribution across device outlets 1–4 at increasing flow rates 0.25–1.25 mL/min for low inlet concentration 0.5% v/v. B) Comparison of recovery when DE is increased by combining outlets.

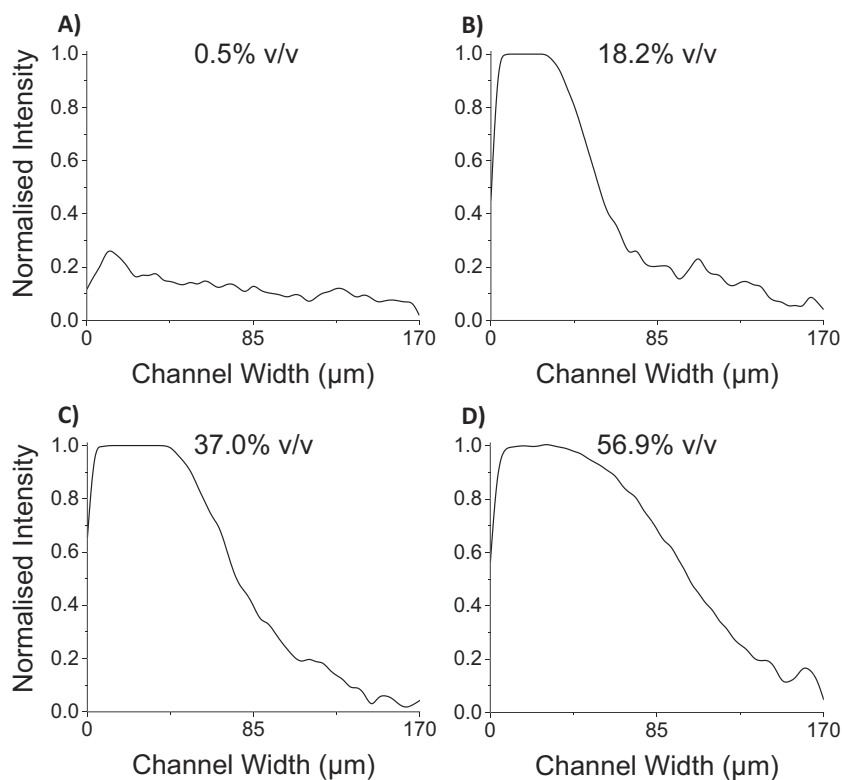


Fig. 2. A-D) Intensity plots at increasing concentrations obtained from Channel Imaging at increasing algal concentration at 0.5 mL/min.

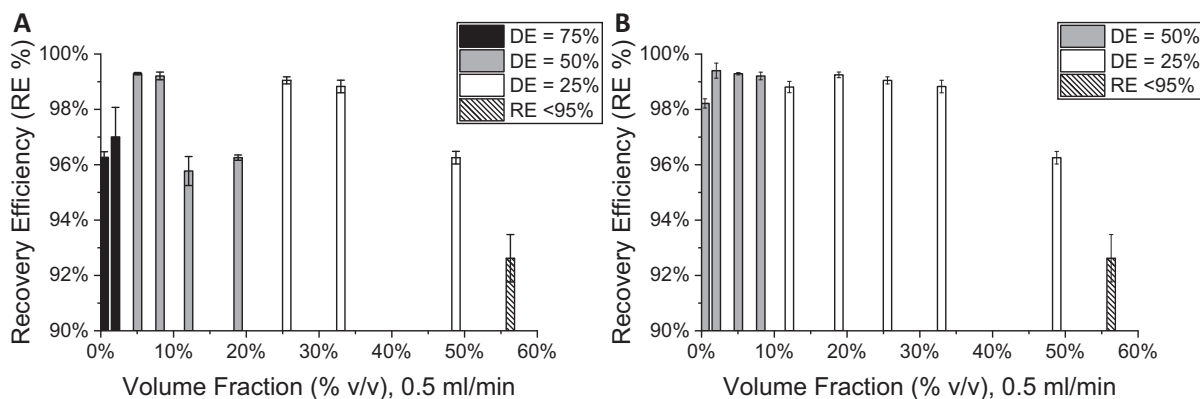


Fig. 3. A) RE of *C. vulgaris* at increasing concentrations with DE changing to maintain a RE >95%, 0.5 mL/min. B) Recovery efficiency vs sample concentration with optimised dewatering extent, 0.5 mL/min.

and 0.75 mL/min were investigated.

The required DE (either 75%, 50% or a minimum of 25% as constrained by the device layout) was determined for each concentration increment to maintain a RE of >95% (this value was chosen to match/exceed previously reported performances as given in supplementary material), these results are shown in Fig. 3A for the optimum flow rate 0.5 mL/min.

The maximum inlet concentration where a RE of >95% could be obtained (at the minimum possible DE of 25%) was found to be at approximately $\varphi = \sim 48.8\%$ v/v, where the actual value lies between 48.8% - 56.3% v/v (Fig. 4). Using the maximum inlet concentration achieving a RE >95% of 48.8% v/v, the theoretical maximum outlet concentration was calculated as 63.9% v/v in a single pass through the device at 0.5 mL/min, only removing outlet 4 (DE = 25%):

$$\text{Max Outlet Concentration } (\varphi) = 48.8\% \times \frac{RE}{(1 - DE)} = \frac{48.8\% \times 96.3\%}{(100\% - 26.45\%)} = 63.9\% \text{ v/v}$$

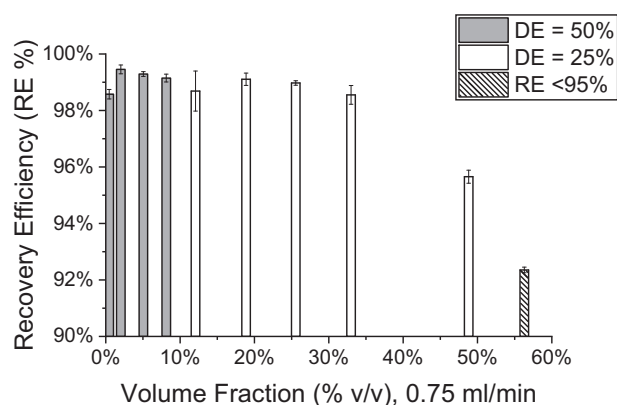


Fig. 4. Recovery Efficiency vs sample concentration with optimised dewatering extent, 0.75 mL/min.

Taking the calculated value for cell size to be $\sim 6.5 \mu\text{m}$ this maximum concentration has a cell concentration of 4.4×10^9 cells/mL. This single pass concentration factor is 1.31.

As mentioned previously, a single pass through the device would be insufficient for most industrial applications where maximisation of concentration is desired, instead recirculation should be considered. Therefore, modification of device operation is recommended to reduce the fluctuation in device RE observed in Fig. 3A as concentration is increased. Although $>95\%$ RE at a DE of 75% can be achieved up to a concentration of 2% v/v (as seen in Fig. 3A) these values are notably lower than those achieved from 2.0–8.1% v/v, where RE is $>99\%$. Therefore, it is not recommended to perform separations at 75% DE and these should be performed at 50% DE instead. Similarly, there is a significant drop in RE at 12.1% - 18.9% with 50% DE. Therefore, it is recommended that a DE of 25% is used from 12.1%. Fig. 3B summarised these results and shows the RE's when the maximum DE is set to 50%.

As can be seen in Fig. 3 there are two potential methods for device operation. The first being maximisation of DE and thus minimisation in processing time as larger percentages of the flow are removed per minute by following the DE for sample concentration found Fig. 3A. Alternatively, device operation can be performed to maximise RE following the DE for sample concentration found in Fig. 3B, which minimises fluctuation in RE as concentration is increased through reduced DE, while still utilising the high DE of 50% initially. The higher DE is useful initially as the sample volume undertakes the largest volume reduction over the concentration from 0.5–2 v/v %, accounting for removal of $\sim 75\%$ of the initial sample volume which can therefore be achieved more quickly while reducing RE fluctuations. It is recommended to operate the device following the trend of Fig. 3B when performing recirculation as maximisation of RE is more important as lower RE's are amplified in a recirculation. The corresponding graph to Fig. 3B for 0.75 mL/min is shown in Fig. 4, which follows the same trend.

3.3. Demonstrating scale-up through recirculation

When applied to the industrial dewatering of microalgae, separation using a single layer inertial focusing system could not be performed in a single pass through the device as even a maximum DE of 75% is not viable for complete dewatering. Thus, application would be performed via recirculation through the system to achieve the required overall dewatering extent. When performing a recirculation in the system there is a balance required between the maximisation of recovery efficiency (this would be achieved through a constant DE of 25%) and minimisation of processing time (this is achieved by utilising the results found in Fig. 3B). Another consideration to minimise sample processing time is system scale-up through multiplexing/parallelisation of the

devices. Here the results from completed actual recirculations from 1.8 $\pm 0.2\%$ v/v using a 10-device stack at 5 and 7.5 mL/min are presented.

The main concern when performing a recirculation to high concentration is the overall recovery efficiency as multiple passes of the same sample through the system increases the overall sample losses (e.g. 2 passes through a system with 90% RE would result in an overall RE of 81%).

Using the device characterisation found in Fig. 3B and Fig. 4 the sample concentrations where the recovering outlets are switched throughout the recirculations are determined. This reduces the overall pumping requirement, by keeping dewatering extent as high as possible while still achieving successful separation. By performing calculations for theoretical recirculations using the data obtained during the high concentration ($>0.5\%$ v/v) investigation, the volume removal required before switching recovering outlets can be calculated in place of sample concentration. This removes the requirement to continuously monitor the sample concentration throughout the recirculation. The maximum dewatering extent used is 50% for both 0.5 mL/min and 0.75 mL/min. Therefore, the recovering outlets are only changed once during the recirculations, when the DE is dropped from 50% to 25%. For a starting concentration and volume of 1.8% v/v and 100 mL, the outlet switch was calculated to be after removal of 79 mL of the total sample volume.

The recirculations were performed until the final sample volume was ~ 2.5 mL, a volume reduction of 97.5%. The results of the recirculation are shown in Table 1. These results show 0.5 mL/min to be the optimum flow rate for recirculation. The final outlet concentration for the 0.5 mL/min recirculation was measured as $65.3 \pm 1.2\%$ v/v. An overall RE of $>95\%$ is achieved due to the fact that for most of the recirculation the single pass RE is $>98.5\%$ (Fig. 3B and Fig. 4), much higher than the previously defined 95% RE cut off for efficient separation.

3.4. Power consumption and comparison with existing technologies

The recirculation results are highly promising when compared with the definition of primary harvesting described earlier, i.e. a volume reduction of 100–200. The normal culture density achieved under laboratory condition for *C. vulgaris* was found to be $\sim 0.5\%$ v/v. The successful operation of this inertial focusing system to $>65\%$ v/v, when considering device scale-up and recirculation, corresponds to a CF of >130 which makes the device a viable primary harvesting alternative for microalgae. However, when considering the application of this technology to industrial algal harvesting one of the main concerns, after proving high recoveries can be met with significant media removal, is the energy requirement. This can be shown as power consumption in kWh/m³ and is given over the optimum flowrates in Table 2. The power consumption is calculated from the pressure required by the pump to overcome the chip backpressure by unit conversion. Multiplication of power consumption by flowrate gives the power requirement for processing (W).

The energy consumption required for different harvesting techniques is summarised in Table 2. The development of lower power consumption techniques, such as hydrocyclones, which have comparatively lower concentration factors has been recommended to aid in the reduction of overall power consumption. This is achievable by using the lower, less effective, dewatering technology as a primary dewatering method acting to remove the bulk of the culture volume, reducing the volumetric load on the higher power consumption harvesting techniques (Amaro et al., 2017).

A summary of the energy use of different harvesting technologies

Table 1
Recirculation results.

Flow rate	Overall recovery efficiency
0.5 mL/min	95.7 \pm 1.7%
0.75 mL/min	91.2 \pm 1.0%

Table 2
Power consumption and dewatering technologies.

Technology		Power consumption	Concentration factor	References
10-Device Stack	0.5 mL/min	0.52 ± 0.03 kWh/m ³	~130	
	0.75 mL/min	0.77 ± 0.03 kWh/m ³	~130	
Disk Stack		1–1.4 kWh/m ³	120	(Amaro et al., 2017)
Nozzle Discharge		0.9 kWh/m ³	20–150	(Murthy, 2011)
Continuous flow		0.8–20 kWh/m ³	17–96	(Barros et al., 2015)
Filtration		0.1–5.9 kWh/m ³	10–245	(Barros et al., 2015)
Hydrocyclones		0.3 kWh/m ³	4	(Grima et al., 2003)

was curated by G. Murthy (2011) and the lowest power consumption, continuous processing technique was hydrocyclones however their reliability has been considered as low (Grima et al., 2003). The hydrocyclone data was achieved using *Coelastrum proboscideum* (Grima et al., 2003) which form large cell clusters and thus cannot be reliably applied to small microalgal harvesting. Syed et al. (2017) expand on the use of hydrocyclones for microalgae harvesting and successfully harvest *Tetraselmis suecica* with a quoted energy use of 0.83 kWh/m³; however, the system struggled to separate particles smaller than 10 µm which also limits application to microalgae harvesting.

Application of microfluidics to microalgae harvesting by Wang et al. (Wang and Dandy, 2017) using a serpentine channel was achieved with a quoted concentration factor of 3 and energy consumption of 1.6 kWh/m³ using a processing flowrate of 0.5 mL/min. In comparison, without recirculation, for a culture concentration of 0.5% v/v, using a dewatering extent of 75%, the spiral microfluidic device used in this investigation achieves a single pass concentration factor of ~4 at 0.5 mL/min with a power consumption of 0.52 kWh/m³.

The key parameter in the effective use of microfluidic technology for industrial application is in the successful scaling and multiplexing of the comparatively low throughput devices. Scale up through parallelisation, as used in this investigation through a 10-device stack, allows for no increase in overall device back pressure as the increased flow is split equally over the corresponding increased number of device layers.

Dewatering using microfluidic devices up to a concentration factor > 130 has been shown as feasible in this work through recirculation. If processed at 0.5 ml/min per device layer, this recirculation would result in an energy requirement of 1.1 kWh/m³ as it would require approximately 10 passes through the device with the volume being recirculated decreasing with each pass. This value is comparative to many standard harvesting techniques in use currently. Some advantages to this technology over existing techniques are; flexibility in design for more targeted separation, reduced equipment complexity with few moving parts and the potential for reduce manufacturing cost (for large volume production) (Gale et al., 2018).

4. Conclusions

While microfluidics has been proposed as an alternative dewatering technology for microalgae harvesting, a key limitation was the degradation in performance as cell concentration is increased. Here, modification of device operation, by decreasing device dewatering extent as concentration increases, has enabled successful operation of a spiral inertial focusing device at inlet concentrations up to 48.8% v/v. With scale-up and recirculation, dewatering *Chlorella* from 1.8%–65.3% v/v with RE >95% was demonstrated. Device operation with a concentration factor of 130 was shown to be feasible, with a calculated power consumption of 1.1 kWh/m³, comparable to existing technologies.

CRedit authorship contribution statement

CH performed all the experiments, data analysis and figure production. CH and HB designed the experiments together and co-wrote the paper.

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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

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