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A 3D-printed mini-hydrocyclone for high throughput particle separation: Application to primary harvesting of microalgae

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Abstract

The separation of micro-sized particles in a continuous flow is crucial part of many industrial processes, from biopharmaceutical manufacturing to water treatment. Conventional separation techniques such as centrifugation and membrane filtration are largely limited by factors such as clogging, processing time and operation efficiency. Microfluidic based techniques have been gaining great attention in recent years as efficient and powerful approaches for particle-liquid separation. Yet the production of such systems using standard micro-fabrication techniques is proven to be tedious, costly and have cumbersome user interfaces, which all render commercialization difficult. Here, we demonstrate the design, fabrication and evaluation based on CFD simulation as well as experimentation of 3D printed miniaturized hydrocyclones with smaller cut-size for high-throughput particle/cell sorting. The characteristics of the mini-cyclones were numerically investigated using computational fluid dynamics (CFD) techniques previously revealing that reduction in the size of the cyclone results in smaller cut-size of the particles. To showcase its utility, high-throughput algae harvesting from the medium with low energy input is demonstrated for the marine microalgae *Tetraselmis suecica*. Final microalgal biomass concentration was increased by 7.13 times in 11 minutes of operation time using our designed hydrocyclone (HC-1). We expect that this elegant approach can surmount the shortcomings of other microfluidic technologies such as clogging, low-throughput, cost and difficulty in operation. By moving away from production of planar microfluidic systems using conventional microfabrication techniques and embracing 3D printing technology for construction of discrete elements, we envision 3D-printed mini-cyclones can be part of a library of standardized active and passive microfluidic components, suitable for particle-liquid separation.

Keywords: *Microfluidics; Hydrocyclone; Algae; 3D Printing; Computational Fluid Dynamics; Separation*

Introduction:

The separation of micro-sized particulate matter in a continuous flow is required for a wide variety of applications that include mineral processing,¹ chemical syntheses,² environmental assessments³ and biomedical analyses.^{4, 5} With the evolution of precision engineering, micro-fabrication, and rapid prototyping techniques the development of microfluidics technology has accelerated to established numerous particle sorting techniques.⁶ These techniques are based on the unique characteristics of microscale flow phenomena, for the continuous separation and sorting of micro-particles.

The separation techniques are broadly classified into two classes: a) Active separation that involve an external field for particle separation encompassing dielectrophoresis,⁷⁻⁹ acoustophoresis,¹⁰⁻¹² magnetophoresis.^{13, 14} These techniques offer relatively high particle sorting efficiency and throughput along with their high operating cost. b) Passive separation in which the particles are sorted due to effect of interaction between the particles, device structure and the flow profiles. These majorly sort the particles using two methods that are membrane-microfiltration¹⁵ and hydrodynamic sorting.¹⁶ The largest portion of microfluidic cell-sorting approaches falls under the heading of hydrodynamic-based sorting methods, which includes pinched flow fractionation (PFF),^{17, 18} deterministic lateral displacement (DLD),¹⁹⁻²¹ hydrodynamic filtration,²²⁻²⁴ inertial separation²⁵⁻²⁹ and disc microfluidics.^{30, 31}

Despite successful development of various microfluidic systems for particle/cell separation, most of these configurations suffer from several drawbacks such as prolonged sample processing time (up to several hours), due to the relatively high fluidic resistance of their lateral fluidic structures,^{32, 33} clogging, high cost and low recovery, complicated integration of external force fields (for active systems) and possible loss of cell viability.³⁴ In addition, production of such systems using standard

microfabrication techniques is proven to be tedious which all render commercialization difficult.³⁵ 3D printing has already been used for direct maskless fabrication of microfluidic devices from a variety of polymeric materials.^{36, 37} A new paradigm in the lab-on-a-chip (LOC) community is to develop modular, low-cost and reconfigurable components (e.g., valves, mixers, sensors, etc.) containing fluidic elements which are adaptable to many different microfluidic circuits. Given the fact that miniaturized liquid-particle separators are essential part of LOC systems, we decided to explore the possibility of developing a simple yet versatile particle-liquid separator using additive manufacturing.

Hydrocyclones are simple and robust separation devices with no moving parts. The hydrocyclone is generally a macroscale scale device that has been employed in a wide range of industries that include oil industry,³⁸⁻⁴⁰ chemical and mineral industry,⁴¹⁻⁴³ food processing,⁴⁴ irrigation⁴⁵ and biotechnology,⁴⁶⁻⁴⁸ for decades. These have been widely investigated using theoretical analysis, simulations as well as experiments.^{42, 45, 49} It has also been investigated that the separation efficiency of the hydrocyclone greatly depends on the overall size of the hydrocyclone such that smaller the size of the cyclone better would be its separation efficiency.^{50, 51} However, due to the limitation of conventional manufacturing approaches, development of a miniaturized cyclone for microfluidic applications has very rarely been reported and to authors' knowledge, this work is the first attempt in developing miniaturized cyclone using 3D printing technology.

High throughput separation of single cell organisms, such as microalgae, could be a very promising application of 3D printed mini-hydrocyclones. Microalgae are fast growing source of biomass with a great potential for the production of bio-based chemicals, food, feed and biofuels.^{52, 53} However, when cultivated in open ponds or enclosed photo-bioreactors their biomass concentration is very much diluted roughly 0.1- 4 g dry biomass/L⁵⁴ which makes cost effective thickening/dewatering

necessary for further processing. This is the major bottleneck for the lucrative employment of microalgae in industry as the dewatering can typically make up to 20–30% of the total biomass production cost, using the existing techniques.⁵⁵ The process of harvesting and dewatering is usually done in multi-stages so that most appropriate and economical method is employed at each stage and each preceding stage reduces the load on each following stage.⁵⁶ Macro-hydrocyclones can be continuously operated to provide primary concentration of microalgae, but have been proven to be unreliable even for microalgae species *Coelastrum proboscideum* that grow in large aggregates.⁵⁵ The application of mini-hydrocyclones for reliable microalgae concentration has never been reported before to date. The aim of this study was to utilize the simple additive manufacturing technique for the fabrication of mini-sized hydrocyclones that could be used commercially for the efficient particle-liquid separation. The effect of the design parameters on the separation efficiency was evaluated both by simulation and experimentation by testing four mini-hydrocyclone designs with varying overflow and underflow diameters and number of inlets. Furthermore, the concentration of the marine microalgae *Tetraselmis suecica* has been showcased as a promising novel application.

1. Theoretical analysis

The hydrocyclone utilizes the fluid pressure energy to create rotational fluid motion. The rotation is produced by tangential injection of the fluid into the cyclone, causing the relative movement of particles suspended in the fluid and consequently allowing separation of these particles from the fluid or from one another. The important criterion which distinguishes a hydrocyclone is the use of fluid pressure to cause rotation.⁵⁰ The rotation/swirl creates a vortex flow inside the hydrocyclone that gives rise to a low pressure zone along the vertical axis. The vortex flow in the cyclone consists of two vortices; one outer downward vortex that is called the primary vortex and the other inner upward vortex that is the secondary vortex (shown in red and blue respectively in figure 1 (A)). Particles are

separated by the accelerating centrifugal force based on size, shape, and density, while the drag force moves slower settling particles to the low-pressure zone along the inner vortex where they are carried upward through the vortex finder to the overflow. Thus, a particle having a diameter D_p and density ρ_p at a radial distance of r will have three forces acting on it: 1) The centrifugal force F_C in an outward radial direction due to the tangential velocity v_t , 2) the buoyant force F_b in an inward radial direction that is due to the density difference of the fluid ρ_f and the particle ρ_p and 3) the drag force F_d having the direction inward or outward, depending upon the direction of the radial velocity v_r of the particle, so that it always opposes the particle movement due to the fluid viscosity μ . The drag force depend on the particle shape and size as well as the turbulence intensity of the flow ⁵⁷.

$$F_c = m \frac{v_t^2}{r} = \frac{\pi D_p^3}{6} \frac{v_t^2}{r} \rho_p \quad (1)$$

$$F_b = -\frac{\pi D_p^3}{6} \frac{v_t^2}{r} \rho_f \quad (2)$$

$$F_d = -3\pi D_p \mu v_r \quad (3)$$

Based on the forces (eq. 1-3), radial movement of the particle is dictated by the size of the particle as well as the density difference between the particle and the fluid such that if the particle is denser than the fluid, the motion is towards the cyclone wall and vice versa. Thus the large and heavy, fast settling particles move towards the hydrocyclone wall and follow the flow out through the underflow due to the influence of high centrifugal forces. On the other hand, for the very fine and slower settling particles, the centrifugal force is eliminated by drag forces and turbulent diffusion and hence they remain dispersed inside the cyclone and may exit through both outlets entrained by the fluid or the bigger particles ⁵⁷⁻⁵⁹. The smaller particles near the center of the hydrocyclone move into the low pressure zone at the center, and follow the overflow up in the secondary vortex whereas the ones near

the hydrocyclone wall flow following the primary vortex to underflow being entrapped by the bigger particles⁵⁹.

1.1 Description of hydrocyclone geometry

The performance and practical use of a hydrocyclone is subjected to its design parameters^{60, 61}. The diameter of the cylindrical part D_c is the key parameter on which other dimensions are based. All the geometrical dimensions have been shown in the figure 1 (A). Two of the most commonly used design ratios for hydrocyclones are Bradley and Reitema's ratios, the former one being more suitable for lower cut-size of particles.⁶² Therefore, in this study, Bradley's geometrical proportions were considered as a reference but with few alterations in order to modify the design and enhance the separation efficiency. The Bradley's design ratios have been given in the Table 1.

Four hydrocyclones have been numerically and experimentally tested and compared in this study. The cylindrical diameter (D_c), cylindrical and conical lengths (L_1 and L_2), inlet diameter (D_i), height of vortex finder (h) have been kept constant for all the hydrocyclones that are 5 mm, 3.6 mm and 38.6 mm, 0.71 mm and 1.67 mm, respectively. The diameters of both outlets play a significant role in the separation efficiency.⁶³⁻⁶⁵ In order to investigate the optimized ratios, three devices with variation of overflow and underflow diameters (D_o and D_u respectively) have been developed. In table 2 these devices are named as HC-1, HC-2 and HC-3. Whereas, another design HC-1A as given in the table 2 is a single feed inlet hydrocyclone with identical dimensions as of HC-1.

2. Methodology

2.1 Numerical analysis

Despite of the simple geometry, the flow inside the hydrocyclone is quite complex owing to the high velocity turbulent swirl flow. For analyzing the particle-liquid behavior of the designed

hydrocyclone, the solid model is first discretized using ICEM 15.0 (ANSYS Inc.). The optimum size of the mesh was chosen based on the grid convergence index, with simple laminar conditions using FLUENT 15.0 (ANSYS Inc.). As the fluid flow inside the hydrocyclone is highly anisotropic turbulent flow, therefore, turbulence models have to be employed with the Reynolds Stress Model (RSM) being the most appropriate one for the purpose.^{47, 66}

In this model the particle-liquid behavior is simulated in a steady flow. The continuous phase is simulated using Eulerian approach and the particle trajectories in the Lagrangian framework. Uniform velocity boundary condition was used for the inlets and both outlets were set as atmospheric pressure outlets. Due to high pressure gradients and double-vortex flow inside the hydrocyclone, it requires a suitable algorithm for the pressure computation. For this purpose, PRESTO (Pressure Staggered Option) pressure interpolation scheme was used. Furthermore, for the pressure velocity coupling and momentum equations the SIMPLE (Semi-Implicit Method Pressure-Linked Equations) algorithm scheme was employed. The optimum choice is the second order for turbulent kinetic energy and the first order for Reynolds stresses. To ensure the accuracy of the solution the criterion for the convergence of the scaled residuals was set at 1×10^{-4} .

Afterwards, particle injection was simulated and trajectories were calculated using the discrete phase model (DPM) as the volume fraction of particles was less than 10%. With the computation of the particle trajectory, the program keeps track of the momentum exchange between the particle and the surrounding continuous phase. For the two-way coupled multi-phase flow, calculations of the continuous phase and discrete phase flows were alternated and a converged solution was achieved. The computation of continuous as well as discrete phase calculations was completed in a total of 12 hours in a parallel 4 nodes system.

Particles reaching the overflow were set to ‘escape’ while those reaching underflow were ‘trapped’. The particle hydrodynamic profiles and velocity and pressure contours on different planes were observed. The separation efficiency was calculated by the equation (6):

$$\text{Separation efficiency (\%)} = \frac{\text{No.of particles trapped}}{(\text{No.of particles trapped} + \text{No.of particles escaped})} \times 100 \quad (6)$$

The numerical model was first validated by modelling the 20 mm hydrocyclone by Hwang *et al.*⁶⁷ Acquiring the similar results as reported in that paper, one of the proposed hydrocyclone designs (HC-1) was simulated using the same procedure. The simulation results were also compared with the experimental results of the 3D printed hydrocyclone under similar operating conditions. The hydrocyclone manufacturing, experimental setup and results have been described on the following sections.

2.2 Mini-hydrocyclone fabrication

The CAD model of the mini-hydrocyclone developed using SolidWorks 2014, was fed to a 3D printer (ProJet® 3500 HD Max, 3D Systems) for the fabrication of the device. The design file of one of the mini-hydrocyclone designs can be found in the supplementary material. The printer has 16 µm print resolution that enables it to print small models with high surface quality using the multi-jet print technology.⁶⁸ The hydrocyclone were built using the Visijet M3 Crystal material that is suitable for functional testing with great plastic performance along with durability and stability. Another advantage of using this material is its biocompatibility, allowing using it for blood cells or other biomedical applications.

After the fabrication, removal of the support material within the 3D printed hydrocyclones was required for their proper functioning. The printed models were kept in oven at temperature around

60-65°C till the wax melted, followed by sonication in vegetable oil bath at 60°-65°C for 2-3 hours till there was no wax inside. Afterwards, wax and oil remnants were removed by sonication in a water bath for 30 mins as well as pumping bleach through the hydrocyclone. These steps are illustrated in figure 2 (A). Figure 2 (B) shows an optical image of the final device after cleaning process. The 3D printed hydrocyclone along with its base is shown in the figure 2 (C).

2.3 Experimental setup and procedure

The 3D printed hydrocyclone along with its base is shown in the figure 2 (C). The feed mixture comprised of PMMA microspheres by Magsphere suspended in MACS buffer which was prepared using phosphate buffered saline (PBS), and 2mM EDTA supplemented with 0.5% bovine serum albumin (BSA) (MiltenyiBiotec, Germany). BSA was used to prevent the nonspecific adhesion of the particles to the tubing and cyclone walls. The low density PMMA particles (1150kg/m^3) of size ranging from 5-20 μm had been used for the experiments, being good surrogate (within the same size range) for most of biological samples such as blood cells, animal cells (e.g., CHO), bacterial, yeast and pathogens or microalgae. The concentration of the feed samples was kept constant at $\sim 200,000$ cells/ml. The feed concentration, as well as the concentration of the samples obtained from the overflow and underflow was calculated using a hemocytometer.

To ensure the homogeneity of the mixture, it was continuously stirred on the magnetic stirrer and hot plate, and was kept at room temperature. The mixture was fed to hydrocyclone using a peristaltic pump (Longer pump with YZ II 25 pump head) and it was connected to the hydrocyclone inlets using silicon tubing as depicted in figure 2(C).

After passing through the hydrocyclone, the discharge from the underflow and overflow was timed to calculate flow rate and collected in falcon tubes and observed under an optical microscope with the

help of the hemocytometer. The separation efficiency (E) of hydrocyclone is the mass fraction of the particles recovered in the underflow, determined from the relation:

$$E = \frac{Q_u X_u}{Q X} \tag{7}$$

Where Q_u and Q are the flow rates of underflow and feed respectively, X_u and X are the concentrations of underflow and feed respectively. However, it is considered that some the particles are captured in the underflow because of bare entrainment and not due to the action of forces⁴⁷.

Reduced separation efficiency or centrifugal efficiency (E') is preferred parameter to evaluate the performance of hydrocyclone as it takes into account only those particles that are separated due to action of forces⁴⁷. The reduced efficiency E' is calculated by

$$E' = \frac{E - R_f}{1 - R_f} \cong 1 - \frac{X_o}{X} \tag{8}$$

$$R_f = \frac{Q_u}{Q} \tag{9}$$

R_f is the flow ratio which is the fraction of fluid discharged in the underflow where it carries solid particles entrained with the fluid and it is regarded as the minimum efficiency of hydrocyclone without action of any forces. X_i and X_o are the concentrations of the feed and overflow mixture in cells/ml. The particle size at which a hydrocyclone has 50% separation efficiency is generally called particle cut-size (d_{50}). The efficiency for the particle sizes greater than d_{50} would be more than 50% and vice versa for smaller ones. Lower E' indicate that the particles are more dispersed to exit from both outlets.

2.4 Concentration of microalgae as primary harvesting method

1 The marine algae strain *Tetraselmis suecica* CS-56/7, obtained from Australian National Algae
2 Culture Collection (ANACC), was used as model microalgae species to demonstrate the potential of
3 micro-hydrocyclones for microalgae concentration as primary harvesting method. The *Tetraselmis*
4 *suecica* cells are motile and prolate spheroid with an average maximum linear dimension of roughly
5 10 μm .⁶⁹ This economically interesting microalgae species is widely used in aquaculture feed
6 because of its high protein, essential fatty acids, sterols, and pigment content.⁷⁰ The *Tetraselmis* was
7 cultured in F/2 medium in 2L bottles incubated in a 16/8 h light/dark cycle at temperature of 23°C.
8 The bottles were irradiated with daylight fluorescent tubes (light intensity, 100 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$)
9 and were bubbled with sterile- filtered air at a rate of approximately 200 mL min^{-1} to create
10 turbulence and avoid CO_2 limitation. Upon the onset of the stationary growth phase (10-12 days), as
11 determined by cell counting, cells were obtained for hydrocyclone testing. The experiments were
12 carried out with the hydrocyclone HC-1 at the flow rate of approximately 4ml/s per inlet. 1300ml of
13 *Tetraselmis suecica* culture was subsequently transferred in a glass tank having concentration of
14 45×10^4 cells/ml and pumped into the hydrocyclone through peristaltic pump similar to as described in
15 section 2.3 for surrogate particles. In order to obtain higher concentration of microalgae, the
16 underflow stream was recirculated to the feed tank while the overflow was collected in a separate
17 tank and samples were collected from both feed and overflow tanks after every 60 sec. Separate
18 samples were additionally collected from the overflow stream to verify how many cells are being lost
19 at the overflow outlet before it gets diluted in the overflow tank. The results obtained from the
20 experiments are described in section 3.2.

21 3 Results and Discussion

22 The designed hydrocyclones were discretised using ICEM 15.0 and grid convergence was performed,
23 for which the mesh was refined till there was no further significant changes in the solution with

changing the grid size. For this purpose, three grid sizes were tested having approximately 0.8 million, 1.5 million and 3.5 million mesh elements. The magnitude maximum velocity and pressure were used as the monitoring parameters. No significant difference in these parameters were observed as the grid size was doubled from 1.5 million to 3.5 million elements. The plot of maximum velocity against number of mesh elements is shown in the figure 3 (C), indicating the results obtained using the mesh with 1.5 million elements were independent of the mesh size.

Figure 3 (A) shows the simulated contours of velocity magnitude along the horizontal and vertical planes. The dual inlet and single inlet hydrocyclones HC-1 and HC-1A are compared at same feed flow rate of 3.8 ml/s. The velocity is maximum at the point fluid enters the cyclone body and then decreases as the fluid swirls. The velocity reduction is augmented by the second inlet which can be observed in the velocity contours of hydrocyclone on the horizontal plane. The feed velocity is retained after entering the cyclone body which deteriorates faster in HC-1A. Consequently, the high velocity in the cyclone body would result in higher centrifugal force on the particles which is required for better separation. This can be seen in the figure 3 (B) as well, which show the distribution of particles along the vertical plane and the particle trajectories through the HC-1. Most of the larger particles shown in yellow and green are pushed towards the hydrocyclone wall to ultimately exit through the underflow; however, some of the 5 μ m particles shown in cyan are found near the centre of the cyclone from where they can exit directly through the overflow.

The separation performance of the four 3-D printed hydrocyclone designs HC-1, HC-2, HC-3 and HC-1A was experimentally validated at variable flow rates and over a range of particle sizes (5-20 μ m) (figure 4). The simulated and experimental results for separation efficiency of HC-1 were compared as function of particle size at a constant flowrate of 3.8 ml/s through each inlet (figure 4(A)). It was observed that the simulation results somewhat overestimated the separation efficiency

of HC-1 and the difference was larger for the smaller particle sizes and was maximum of 20%. The possible reasons for this difference could be because of the key assumptions of no slip theory and neglecting the lift forces, Brownian forces, fine particles entrainment or surface roughness in the simulation, which might play into action due to small scale of the device and particles.⁶⁶ For the larger particle sizes, on the other hand, the percentage difference reduced to less than 5% and experimental results were in fair agreement with the simulation. Therefore, the comparable results obtained through simulation and experiments validate the simulation approach.

The three dual inlet hydrocyclones HC-1, HC-2 and HC-3 were compared on the basis of their separation efficiency and HC-1 was found to have the higher separation efficiency and lower particle cut size as compared to other designs (figure 4(b)). This was because of the larger underflow diameter of HC-1, which allowed more of the particles to be collected at the underflow. However, the difference between reduced separation efficiency of the HC-1 and HC-2 was small. But on the other hand, the hydrocyclone design HC-3 had significantly lesser efficiency owing to the greater overflow diameter that might have caused the particles to short circuit to the vortex finder being directed towards the conical section for separation.⁵⁰ Consequently, HC-3 could offer the particle cut-size of 13 μm , whereas for HC-1 and HC-2 this was found to be 6 μm and 7 μm , respectively. Zhu *et al.* reported a 5mm mini-hydrocyclone having a cut-size of 8 μm for silica beach sand (density: 2600 kg/m^3).⁷¹ Therefore, by modifying the conical length and diameters of inlets and outlets, our designs were found to be capable of showing better separation even for lower density particles (1150 kg/m^3).

The separation performance of each hydrocyclone increased with increasing particle size; however, it was also found that there was a big increase in E' between 5 μm and 10 μm particle size, but on the other hand, the further increase of E' reduced for larger particles (figure 4 (A & B)). One of the

potential reasons for this could be that for larger particles the buoyant force becomes higher and thus results in reduction in the sharpness of efficiency (see table S1 in supplementary).

These hydrocyclones were also tested at different feed flow rates- that is one of the key variables that greatly affect the flow inside the hydrocyclone as well as its performance.^{72, 73} There was a marked increase in E' with increase in feed flow rate per inlet for different particle sizes, for HC-1 Figure 4 (C). The rise in E' was because of higher centrifugal forces acting on the particles as centrifugal force is directly proportional to square of feed tangential velocity as given in equation (1) in section 1. However, it is also worth noting in the figure 4 (C) that the curves somewhat flattened at higher feed flow rates, which is because of short-circuiting of flow in vortex finder, as well as due to the higher degree of turbulence inside the hydrocyclone that disturbs the fluid motion, which is also in accordance with previous studies.⁷³

The results of the comparison of single inlet HC-1A and dual inlet HC-1 for different particle sizes at the constant total flow rate for both the hydrocyclones are demonstrated in figure 4(D). The particle cut-size of HC-1A was found to be approximately 10 μm while this value for the dual inlet hydrocyclone HC-1 was 6 μm . This indicates that the additional tangential inlets resulted in lower cut-sizes in combination with lower required feed flow rates to achieve high separation efficiencies, which means a reduction of power consumption as well.

3.2 Concentration of microalgae as primary harvesting method

We validated our 3D printed hydrocyclone HC-1 for the primary harvesting of microalgae. The initial and final concentrations of the culture in the feed tank and the overflow has been reported in the figure 5(A). 1300ml of *Tetraselmis* culture with an initial concentration of 45×10^4 cells/ml was pumped through the mini-hydrocyclone from the feed tank with recirculation of underflow in 11

minutes till 125ml of culture was left in the feed tank with the final concentration increased by more than 7 folds (see table S2 in supplementary). This concentration factor is higher than what has been reported for *Coelastrum proboscideum* using the macro-hydrocyclone,⁵⁵ despite the fact that the cell diameter of *Coelastrum* is double the cell size of *Tetraselmis*.

The reduced separation efficiency for the *Tetraselmis* cells was 68-70%, which is comparable to the 10 μ m standardized particles as shown in figure 4(B). There was a loss of 30% of the cells to the overflow tank figure 5 (A). As the concentration of the feed tank increased due to the recirculation of the underflow, the number of cells lost to the overflow increased gradually over the course of the experiment. A sudden increase in cell concentration at the overflow outlet was observed after 9 mins where the concentration almost doubled from 49×10^4 to 100×10^4 cells/ml. Although the increase in overall concentration of overflow tank was not very substantial. The loss of these cells could presumably be reduced for species with larger cell size. Further, these cells can be recaptured by the addition of more mini-hydrocyclone in series for multistaging which could consequently result in higher concentration factor.

In this study, the experiment for algae harvesting was performed on a bench scale, however this system has the capability to be used continuously for larger volumes of microalgae broth as well. In our bench scale experiments, a peristaltic pump was used which consumed about 0.83 kWh/m³ to get the concentration factor of around 7, while the energy requirement of macro-hydrocyclones was around 0.3 kWh/m³ to get 4 times the initial concentration, along with poor reliability.⁵⁵ For pilot-scale experiments, appropriate type of pump could be employed alongwith parallel arrangements of mini-hydrocyclones for minimizing the power consumption and harvesting cost. Other primary harvesting technologies such as flocculation-sedimentation (0.1-0.5kWh/m³) and have comparable energy requirements to obtain similar concentration factors. However, filtration and centrifugation

are generally reported to have higher energy consumption up to 10 kWh/m³^{74, 75} (refer to supplementary table S3 for comparison of conventional techniques with current study).

The use of 3D printing for the fabrication of mini-hydrocyclones creates flexibility for numerous design modifications to optimize the overall performance of the hydrocyclone. Further miniaturization of the hydrocyclone can improve performance significantly and it could be transferred to several chemical or biomedical applications. However, pressure drop can be very significant inside small features which restricts operation parameters. In addition, sample concentration plays a key role in the operation efficiency. Parallelization of mini-cyclones, which can be easily obtained through 3D printing, can be a good option for boosting the overall throughput. Further advancement in the additive manufacturing will enable us to build better devices in the future. In addition, combination of wax printing with soft lithography can be another option for creation of smaller cyclones.^{76,77}

Our proposed mini-hydrocyclone has the capability to be employed for primary harvesting of algae, with decreasing operation time and energy requirements, greatly reduced areal footprint and easy installation as compared to other conventional harvesting methodologies. In addition, it could separate marine species without any corrosion risks. Moreover, because of the simplicity of its design further development of 3D printed miniaturized hydrocyclones would minimize the risks of clogging or fouling along with high throughput contrasting to other microfluidic devices.

Conclusions

In this study, four miniaturized hydrocyclones were fabricated using the multijet 3D printing technique and were tested for particle-liquid separation application. The hydrocyclone design designated as HC-1 worked the best for the clarification of finer PMMA standard particles having the

cut-size down to approximately 6 μm ; however, for the particles larger than 10 μm , separation efficiency was higher for HC-2. The application of our designed mini- hydrocyclone for the primary harvesting of microalgae was demonstrated for *Tetraselmis suecica*. The final biomass cell concentration was increased by 7.13 times in 11 minutes of operation time using HC-1 with estimated power consumption of 0.83 kWh/m³. Higher concentration factors can be achieved by working with larger volumes of algae cultures and by implementing multi-staging of the hydrocyclones or by integration with other microfluidic devices. Generally, a number of design modifications can be made to apply this type of mini-hydrocyclones for a various biomedical and chemical applications, such as blood sample preparation, point of care, vaccine preparation, etc.

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Tables:

Table 1 Bradley's geometrical proportions ⁵⁰

	D_i/D_c	D_o/D_c	h/D_c	L_2/D_c
Bradley's ratio	0.133	0.20	0.33	6.85

Table 2 Dimensions of the designed hydrocyclones: overflow (D_o) and underflow diameters (D_u), and number of inlets.

Cyclone designation	D_o (mm)	D_u (mm)	No. of inlets
HC-1	0.75	1	2
HC-2	0.75	0.75	2
HC-3	1	0.75	2
HC-1A	0.75	1	1

Figures:

Figure 1: **A)** Flow inside hydrocyclone: Particle-liquid mixture entering the hydrocyclone from two tangential feed inlets, most of the particles are pushed towards the wall and follow the primary vortex in red to the bottom outlet underflow. Whereas a part of the fluid or fraction of finer particles is captured by the low pressure zone at center of the hydrocyclone and exit following the blue spiral from the top outlet that is the overflow, **B)** Force balance on a particle inside the hydrocyclone. **C)** Schematic overview with description of dimensional parameters of a mini-hydrocyclone **D)** Model of mini-hydrocyclone with stand designed in this study for 3D printing

Figure 2. **A)** 3D printing and post processing procedure **B)** Photo of the mini-hydrocyclones printed with Visijet M3 crystal. **C)** The experimental setup

Figure 3 A) Velocity contours on horizontal and vertical planes for dual (HC-1) and single inlets (HC-1A) respectively, at same feed flow rates B) Contour of DPM particle diameters on the vertical plane and particle trajectories through HC-1 cyclone body. C) Graph between max velocity as monitor point and number of mesh elements.

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Figure 4 A) Comparison of experimental and simulation results for HC-1, B) comparison of the performance of different hydrocyclone designs, C) Effect of feed flow rate on reduced efficiency for HC-1, D) Comparison of the performance of single inlet HC-1A and dual inlet HC-1 hydrocyclones.

Figure 5 (A) Line plots showing the variation of *Tetraselmis suecica* cell concentration for the feed tank (with recirculation of the underflow) and overflow tank as a function of operation time. The blue histograms show the concentrations of samples obtained instantaneously from the overflow outlet stream. (B) Photo showing the feed culture (left), concentrated algae after being pumped through HC-1 (middle) and algae cells lost to the overflow (right). (C) Micrograph of the *Tetraselmis suecica* cells (D) Micrographs showing the cell counts for feed, overflow and underflow respectively on the hemocytometer.