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Intensifying extraction of biomolecules from macroalgae using vortex based hydrodynamic cavitation device

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ABSTRACT

Macroalgae have a tremendous potential to become an important renewable resource for valuable biomolecules and chemicals. New and improved ways of cell disruption and of enhancing rate as well as yield of extraction of valuable products from macroalgae are needed to fully realise this potential. In this work, hydrodynamic cavitation (HC) was used for intensifying rate and yield of extraction of phycoerythrin, proteins and carbohydrates from marine macroalgae *Palmaria palmata*. We use vortex-based HC devices which do not use small restrictions like orifice-based HC devices or moving parts like rotor–stator based HC devices. A bench scale setup with a nominal slurry flow rate of 20 LPM was established. Dried and powdered macroalgae was used. Influence of key operating parameters like pressure drop and number of passes on extraction performance (the rate and yield) was measured. A simple, yet effective model was developed and used for interpreting and describing experimental data. The results indicate that there exists an optimum pressure drop across the device at which extraction performance is maximum. The extraction performance with HC was found to be significantly better than the stirred vessels. HC has resulted in 2 to 20 times improvement in the rate of extraction of phycoerythrin (R-PE), proteins and carbohydrates. Based on the results obtained in this work, pressure drop of 200 kPa and number of passes through the HC devices of about 100 were found to be most effective for HC-assisted intensified extraction from macroalgae. The presented results and model will be useful for harnessing vortex-based HC devices for intensifying the extraction of valuable products from macroalgae.

1. Introduction

Macroalgae are a rich source of valuable phytochemicals such as proteins, pigments and lipids and therefore have tremendous potential to become an important renewable resource for valuable biomolecules and chemicals. These phytochemicals find applications in nutraceuticals, food, cosmetics, energy sector etc. *Palmaria palmata* is one of the important macroalgae known for its high protein and pigment (phycoerythrin) content. It is widely used for food and is also cultivated for its biomolecules [8]. In recent years, strategies of biomass valorisation and biorefinery have gained importance for value addition and complete utilisation of biomass [5].

For realizing maximum valorization of macroalgae, it is essential to develop processing methods for disruption of algal cell walls so as to facilitate extraction of valuable biomolecules from macroalgae. Algal cell walls contain complex interwoven structures of polysaccharides (for instance, agar and cellulose) which offer a major hindrance for cell disruption during the primary extraction of intra-cellular products [22]. Several pre-treatment methods have been developed and are being developed for enhancing cell disruption and thereby effectiveness of extraction of useful products from macroalgae [9,25,19,29,30,32,3,6,17,24]. The pre-treatment methods can be broadly classified under four types: physical, chemical, biological, and physicochemical [3]. Physical methods offer advantages of resulting in smaller particle sizes of biomass. However, they are energy intensive requiring up to ~1 kWh/kg total solids for milling [21]. These physical methods have limitation of not effectively opening up the cell matrix up to the molecular level [22]. As far as enzymatic methods are concerned, they require low energy and are highly specific. However, enzymes are expensive and have limitation of washing out during pre-treatment. On the other hand, the alkali or acid based hydrolysis (chemical pre-treatment methods) and physicochemical pre-treatment methods used for disruption of polysaccharides such as hemicellulose are not suitable options for the extraction of sensitive biomolecules besides being toxic or corrosive or energy intensive in nature. Because of these limitations of existing methods, new and improved ways of cell disruption and enhancing rate, as well as yield of extraction of valuable products from

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Nomenclature

a	Solid-liquid interfacial area (m^2)
α	Mass fraction of biomolecules remaining in the biomass (unitless) and is equal to the product of y_{ps}, m_s and ∅
β	Rate of extraction during stirring (min^{-1})
d_s	Particle diameter of solids (algae) (m^2)
d_t	Throat diameter (m)
δ_P	Rate of extraction during HC at a given pressure drop
δ	Per-pass extraction factor (additional rate of extraction contributing to hydrodynamic cavitation) (min^{-1})
ΔP	Pressure drop (kPa)
E	Energy consumption for HC pre-treatment of slurry (kJ/kg)
Eu	Euler number (unitless); expresses the relationship between a local pressure drop caused by a restriction and the kinetic energy per volume of the flow
M	Mass of slurry in the holding tank (kg)
m_E	Amount of product extracted (kg)
m_{Emax}	Maximum amount of biomolecule that can be extracted (kg)
m_s	Mass fraction of algal biomass (on dry basis) in slurry (unitless)
$\dot{m}_c$	Mass flow rate through cavitation device (kg/min)
n	Number of passes through HC device (unitless)
∅	Empirical factor signifies incomplete extraction
P	Partition coefficient (unitless)
P_E	Productivity (kg/kJ)
P_{Emax}	Maximum productivity (kg/kJ)
Q	Flowrate (kg/min)
ρ_s	Density of solid algae (kg/m^3)
ρ_{sl}	Density of slurry (kg/m^3)
R_i	Recovery of product (%)
k_{SL}	Solid-liquid mass transfer coefficient (m/min)
′	Mass fraction after HC device (unitless)
t	Time (min)
V	Fluid flow velocity (m/s)
X_E	Fraction of biomolecule extracted (unitless)
y_p'	Mass fraction of product at outlet of cavitation device (unitless)
y_p	Mass fraction of product in liquid (unitless)
y_{ps0}	Mass fraction of product in algae at $t = 0$ (unitless)
y_{ps}	Mass fraction of product in algae (unitless)

macroalgae, are needed to fully realise this potential.

In this work, we used hydrodynamic cavitation (HC) for intensifying rate and yield of extraction of useful products from macroalgae. HC involves formation, growth and collapse of vapour bubbles due to pressure changes in the flowing liquid. The cavity collapse leads to formation of localised hotspots with extreme temperatures (>2000 K) and pressures (>100 MPa). It also results in the generation of highly reactive radical species, high-velocity jets and intense shear [37]. These intense physio-chemical effects produced by HC are being increasingly harnessed for a variety of applications ranging from cell disruption, food processing, fatty oil hydrolysis, to waste remediation [35,10,26,16,27,11,33,36]. HC has been used for the pre-treatment of microalgae to enhance the effectiveness of extraction of biomolecules [12,15,39,40,13,43,41]. Unlike the microalgae which are unicellular microscopic small plant-like organisms, macroalgae are large and multicellular with thicker cell walls. The structure and composition of macroalgae cell walls are very different from that of microalgae. The methods used for disrupting cell walls and intensifying extraction of products from microalgae mentioned earlier therefore may or may not work for macroalgae. Despite showing promising applications because of its inherent advantages like low energy consumption and amenability to scale-up, to the best of our knowledge, hydrodynamic cavitation has not been used for intensifying extraction of valuable products from macroalgae. In this work, we have investigated application of hydrodynamic cavitation for intensifying extraction from macroalgae for the first time.

In this work, we used the vortex-based cavitation device [35] for the pre-treatment of macroalgae. The vortex-based cavitation device generates cavitation by harnessing rotational flows and offers many advantages for processing macroalgae such as less risk of clogging since small constrictions are not used, proven scalability up to 50 m^3/h flow rates, keeping cavitation zone away from device walls and thereby reducing risk of erosion and so on [37]. We selected marine macroalgae Palmaria palmata in the present work for extraction of phycoerythrin, proteins and carbohydrates using HC-based pre-treatment. *Palmaria palmata* is a common marine macroalga found in the coastal region of Ireland. A bench scale setup with a nominal flow rate of 20 LPM was established. The influence of key operating parameters like pressure drop across the HC device and number of passes through HC device on the rate of extraction and yield of biomolecule was measured. A simple, yet effective model was developed and used for interpreting and describing experimental data. A brief discussion on energy consumption

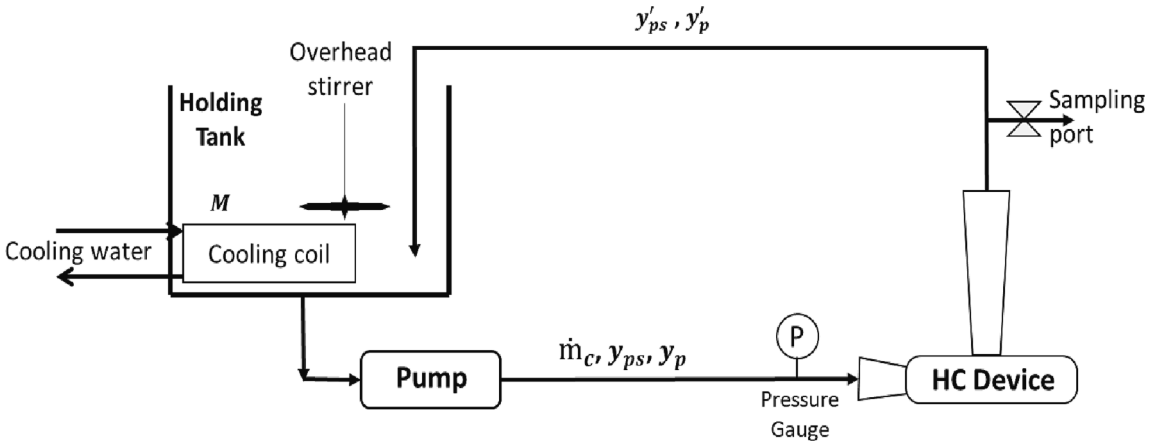

Fig. 1. Schematic of the experimental rig.

needed for the pre-treatment calculations is also included. The presented results and the model will be useful for harnessing HC for enhancing valorisation of macroalgae.

2. Materials and methods

The dried and organic food-grade biomass of macroalgae *Palmaria palmata* was procured from a local supplier (Wild Irish Seaweeds Ltd., Co. Clare, Ireland). The biomass was subjected to sieving and the fraction between 540 and 1000 μm was collected and stored for processing as per the requirement mentioned in the following sections.

2.1. Quantifying maximum extractable content

Serial extraction (repeated extraction) was carried out to estimate the maximum extractable content of phycoerythrin (R-PE), proteins and carbohydrates present in the biomass [25]. A solid–liquid ratio of 1:10 was employed during serial extraction. 2 g biomass (540–1000 μm) was added to 20 ml of solvent (distilled water). The suspension was incubated for 1 h at room temperature (22 ± 2 °C) with stirring at 100 rpm and was centrifuged (Eppendorf 5910R) at 4 °C and 5,000 g for 15 min. The pellet was re-suspended in fresh solvent and incubated for 1 h at room temperature (22 ± 2 °C). This procedure was repeated until no detectable phycobiliproteins were extracted in the buffer. The supernatant of all these primary extraction steps was pooled together for spectrophotometric analysis. The overall steps of identifying maximum extractable content is shown schematically in Fig. S1 (of Supplementary information). This forms the basis (100%) for the estimation of recovery of primary extraction for extraction employing HC for R-PE, proteins, and carbohydrates, and is represented as y_{ps0} (mg of metabolite/kg of algae).

2.2. Pre-treatment using HC

A schematic of hydrodynamic cavitation rig designed for carrying out pre-treatment of macroalgae is shown in Fig. 1 following the previous work on biomass pre-treatment [31,26]. A photograph of the experimental set-up is shown in Fig. S2 of the Supporting Information. It was ensured that with the required operating conditions, cavitation occurred only in the vortex-based device and not in the valves or pump. The HC device used in this work with a nominal capacity of 20 LPH was manufactured using stainless steel and was procured from Vivira Process Technologies, India (https://www.vivira.in).

Pre-treatment experiments were carried out at different pressure drops (50, 200, 300, and 400 kPa) across the HC device for the algal load (10 g/L). A lower algal load was selected to avoid any possibility of clogging of the pump. It is of course possible to use much higher algal load provided that suitable pump is selected. During each experiment, the distilled water is allowed to recirculate through the system from the holding tank and the pre-soaked (for 30 min) algal biomass was then added to the holding tank, making up the final working volume at desired value (5, 10 or 15 L). Samples (suspension: 10 ml) were collected at 50, 150, 250 and 350 passes through HC device. The samples of treated biomass were incubated at room temperature for 5 h, centrifuged at 5000 g for 15 min at 4 °C and the supernatant was analysed for phycoerythrin (R-PE), proteins, and carbohydrates (analysis is discussed in Section 2.3.1).

Experiments at different values of pressure drop across HC device require different batch time to cover a specific number of passes. In order to clearly differentiate contributions of number of passes and processing time, pre-treatment experiments were carried out at different volume of algal suspension in the holding tank (5, 10 and 15 L) at the same algal loading. The samples were drawn at regular interval of time and centrifuged at 5000 g for 15 min at 4 °C and supernatant were analysed for R-PE, proteins, and carbohydrates. The results are processed using the developed model (as discussed in Section 3) for quantitatively understanding the influence of number of passes through HC device.

Table 1
Three stages of extraction from algal biomass.

Stage	Description	Time (min)	Mass fraction of product in biomass (dry basis)	Mass fraction of product in liquid phase
0	Algal biomass	t_0	y_{ps0}	y_{p0}
1	Soaked in water	t_1	y_{ps1}	y_{p1}
2s	Stirred in water	t_{2s}	y_{ps2s}	y_{p2s}
2c	HC treatment	t_{2c}	y_{ps2c}	y_{p2c}
3	Stirred after HC	t_3	y_{ps3}	y_{p3}

2.3. Analyses and characterisation

2.3.1. Metabolites estimation

The samples obtained after centrifugation were evaluated for R-PR, proteins and carbohydrates content. R-PE content was estimated by measuring absorbance at 564 nm, 618 nm and 730 nm using UV–Visible spectrophotometer (Synergy H1 Hybrid Multi-Mode Reader, BioTek). The following equation was used for the estimation of R-PE content [38].

$$R-PE(\mathrm{mg/ml}) = 0.1247[(A_{564}-A_{730}) - 0.4583(A_{618}-A_{730})] \quad (1)$$

where A_{564}, A_{618} and A_{730} are the absorbances at the mentioned wavelengths. Triplicate experiments were carried out for quantifying reproducibility and error bars.

The concentration of proteins was estimated by Bradford method [2]. Briefly, the aqueous solution of sample and the dye reagent were mixed with 1:50 (v/v) ratio. The mixture was incubated at room temperature for 15 min. The absorbance was then measured at 595 nm using a UV–Visible spectrophotometer (Synergy H1 Hybrid Multi-Mode Reader, BioTek). The protein content was calculated using the standard calibration of bovine serum albumin. The measurements were done in triplicate.

Carbohydrates which exist as free sugars and polysaccharides also get extracted. These may interfere with the purification of the target biomolecule. Quantitative estimation of the extracted carbohydrates was carried out by the phenol–sulphuric acid method [4]. In this method, the carbohydrates are first hydrolysed into glucose using dilute hydrochloric acid. The glucose is then dehydrated to hydroxymethylfurfural (HMF) using a hot acidic medium. HMF forms a green-coloured product in the presence of phenol with an absorption maximum at a wavelength of 490 nm. Samples were then analysed using a UV–Visible spectrophotometer (Synergy H1 Hybrid Multi-Mode Reader, BioTek) for the absorbance 490 nm. The carbohydrate content was calculated using the standard calibration obtained from the standard glucose sample.

2.3.2. Fluorimetry, microscopy and particle size analysis

The quantitative analysis of R-PE in samples obtained (as described in Section 2.2) before and after cavitation was carried out via fluorimetry using UV–Visible spectrophotometer (Synergy H1 Hybrid Multi-Mode Reader, BioTek). The fluorimetry is based on the phenomenon of emission of radiation when the molecules are excited by radiation at its excitation wavelength. The emission spectrum was obtained at excitation wavelength scanning ranging from 400 to 700 nm [23].

It will be instructive to examine influence of HC pre-treatment on particle sizes and morphology of algal biomass [25,26]. The images of algal solids before and after HC pre-treatment were therefore captured

using the Olympus IX53 microscope equipped with an Olympus SC100 camera. Particle size distribution measurements of the treated and untreated biomass suspensions were carried out using a laser diffraction particle size analyzer, Malvern Mastersizer 3000 equipped with a Hydro MV accessory for liquid samples (Malvern Instruments, UK). MilliQ water at 25 °C was used as a dispersant. The refractive index and an absorption index were set to 1.46 and 0.01 respectively [14]. The specific surface area and particle size, in terms of surface-weighted mean, D [3,2], and volume-weighted mean, D [4,3], were quantified from these measurements.

3. Mathematical model for interpreting extraction data

Overall extraction of products from macroalgal biomass occurs via three stages (see Table 1). When algal biomass is soaked in water, some amount of products is extracted in water with initial contact with water even without any agitation. This is the first stage. Further extraction may be carried out using either mechanical agitation (Stage 2 s) or hydrodynamic cavitation (Stage 2c). After completing HC, algal slurry may be incubated further in presence of stirring as Stage 3 of extraction process. Notations used to denote mass fraction of desired products at the end of each of these extraction stages and corresponding time duration are listed in Table 1.

Recovery of the product i, R_i, at each stage may be calculated as:

$$R_i = \frac{(1-m_s)y_{pi}}{m_s y_{ps0}} \tag{2}$$

where m_s is mass fraction of algal biomass (on dry basis).

Mass balances of solid and liquid phases during the extraction process may be written as:

$$M(1-m_s)\frac{dy_p}{dt} = \dot{m}_c\left(y_p^{'} - y_p\right) + Mk_{SL}a\left(Py_{ps} - y_p\right) \tag{3}$$

$$Mm_s\frac{dy_{ps}}{dt} = \dot{m}_c m_s\left(y_{ps}^{'} - y_{ps}\right) - Mk_{SL}a\left(Py_{ps} - y_p\right) \tag{4}$$

where, M is the mass of slurry in the holding tank, m_s is the mass fraction of algae in slurry, y_p is the mass fraction of product in liquid, t is the time, $\dot{m}_c$ is the mass flow rate through cavitation device, $y_p^{'}$ mass fraction of product at outlet of cavitation device, k_{SL} is the solid–liquid mass transfer coefficient, a is the solid–liquid interfacial area, P is the partition coefficient, y_{ps} is the mass fraction of product in algae, y_{ps0} is the mass fraction of product in algae at $t = 0$, and y_p is the mass fraction of the product in liquid.

Considering usual extraction processes, the assumptions of $Py_{ps} \gg y_p$, and $m_s \ll 1$ are reasonable. For modelling the influence of HC on extraction, we may use per-pass performance factor approach [34]. If the per-pass extraction in the HC device is independent of y_{ps}, we may write mass balance for the extraction in a single pass as:

$$m_s\left(y_{ps}^{'} - y_{ps}\right) = -(1-m_s)\left(y_p^{'} - y_p\right) = -\delta \tag{5}$$

where δ is per-pass extraction factor and superscript $'$ indicates mass fraction after HC device.

By solving Eqs. (3), (4) and (5), we get,

$$y_p = y_{p0} + m_s\left[y_{ps0} + \frac{\dot{m}_c\delta}{\beta M m_s}\right]\left(1 - e^{-\beta t}\right) \tag{6}$$

If the per-pass extraction in the HC device is dependent of y_{ps}, we may write mass balance for the extraction in a single pass as:

$$m_s\left(y_{ps}^{'} - y_{ps}\right) = -(1-m_s)\left(y_p^{'} - y_p\right) = -\delta y_{ps} \tag{7}$$

With this first-order dependence of per-pass extraction on y_{ps}, we get,

$$y_p = y_{p0} + m_s y_{ps0}\left(1 - e^{-\left(\frac{\dot{m}_c\delta}{Mm_s}+\beta\right)t}\right) \tag{8}$$

Preliminary analysis of the experimental data using Eqs. (6) and (8) indicated that data is consistent with Eq. (8), that is per-pass extraction through HC device is a function of y_{ps}. The preliminary analysis also revealed that it may not be possible to fully recover the products in algal mass even at infinite time. Only fraction of available biomass is recoverable. Therefore, an empirical factor, $\varnothing$ is introduced in Eq. (8) to represent practical extraction process as:

$$y_p = y_{p0} + m_s y_{ps0}\varnothing\left(1 - e^{-\left(\frac{\dot{m}_c\delta}{Mm_s}+\beta\right)t}\right) \tag{9}$$

where, $\varnothing$ signifies incomplete potential for extraction, and $\varnothing$ will always be less than one. The Eq. (9) is used for processing experimental data with and without HC as discussed in the following.

Stage 1 – Soaking in water: The extraction of biomolecules commences as soon as the biomass comes in contact with the solvent. Extraction behaviour of all three biomolecules was studied using the following.

$$y_{ps1} = y_{ps0} - \frac{(1-m_s)}{m_s}\left(y_{p1} - y_{p0}\right) \tag{10}$$

Stage 2s – Extraction with stirring: The biomolecules get released in the solvent during stirring. Hence, to estimate the effect of HC on extraction, it is necessary to estimate the rate of extraction during stirring, so that it can be compared with that of HC. During stirring without HC, the mass flow rate (kg/min) through HC device is zero ($\dot{m}_c = 0$). The Eq. (9) applied to only stirring reduces to:

$$y_{p2s} = y_{p1} + m_s y_{ps1}\varnothing\left(1 - e^{-\beta t}\right) \tag{11}$$

where the y_{p2s} is the mass fraction of the product p in liquid, which was measured as a function of time, t. The time t appearing in Eq. (11) is time from which stirring was started, that is $(t - t_1)$ where t_1 is the end time of soaking stage. The parameter β represents effective rate of extraction during stirring. The known values of y_{p1}, m_s, y_{ps1} and measured values of y_{p2s} were used to obtain best-fit values of parameters β and $\varnothing$.

Stage 2c - Stirring and HC: Influence of HC on extraction rate was evaluated and compared with that of stirring. Mass flow rate ($\dot{m}_c$) at different pressure drops (50, 200, 300 and 400 kPa) across HC device were found to be = 10.7, 21.7, 26.36 and 29.31 kg/min, respectively. Eq. (9) applied to the stirring and HC stage after soaking reduces to:

$$y_{p2c} = y_{p1} + \alpha\left(1 - e^{-\left(\frac{\dot{m}_c\delta}{Mm_s}+\beta\right)t}\right) \tag{12}$$

where $\alpha = m_s y_{ps1}\varnothing$. The values of y_{p2c} were measured as a function of t (time of stirring + HC = $t - t_1$). This experimental data and known values of y_{p1}, m_s, y_{ps1} were used to obtain best fit values of $\left(\frac{\dot{m}_c\delta}{Mm_s}+\beta\right)$ and $\varnothing$.

It should be noted that values of β and $\varnothing$ are expected to remain constant for all stages of extraction since they pertain to stirring (speed of impeller was kept constant for all experiments) and material characteristics. The per-pass extraction factor, δ, may be a function of pressure drop across HC device and other operating parameters like pH, temperature, solid loading and particle size. For the HC device used in the present work, the cavitation inception is expected to occur between 50 kPa and 100 kPa pressure drop [33,36]. Therefore, the value of δ obtained at 50 kPa pressure drop may be used as a reference and use the ratio, $\frac{\delta}{\delta_{50}}$ to understand the influence of pressure drop across HC device

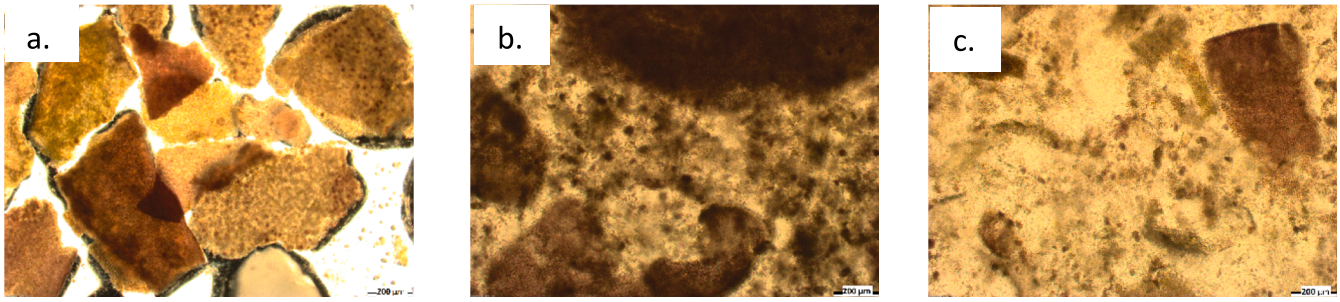

Fig. 2. Microscopic images showing morphological changes in algal biomass due to HC treatment. (a) Untreated, (b) treated at 300 kPa and (c) treated at 400 kPa. Scale indicated at bottom right corner is 200 μm.

on per-pass extraction factors. It may be noted that effective extraction rate factor in presence of HC is $\left(\frac{\dot{m}_c\delta}{M m_s}+\beta\right)$ and therefore the ratio, $\frac{\left(\frac{\dot{m}_c\delta}{M m_s}+\beta\right)}{\beta}$ may used to evaluate net enhancement in extraction rate in presence of HC over simply stirring.

4. Results and discussion

The main goal of the present work was to quantify enhancement in extraction rate caused by HC. Systematic experiments of extraction of cellular metabolites such as R-PE, proteins and carbohydrates from algal biomass of *Palmaria palmata* were carried out. Extraction using simple mechanical agitation via overhead stirrer was used as a reference case. Influence of HC was investigated by carrying out experiments at different pressure drop across HC device (50, 200, 300 and 400 kPa) for up to 350 passes through HC device.

As the extent of cell disruption can be quantified by measuring the release of cellular metabolites, the results expressed in terms of the extracted products are presented and discussed in the following sections, followed by particle size analysis and R-PE fluorescence stability.

Before carrying out the extraction experiments using vortex-based HC devices, the pressure drop across the device as a function of flow rate through the device was measured. This data (see Fig. S3 in the Supporting information) indicates the Euler number (dimensionless pressure drop) of the HC device used in the present work as 42 and 46 for water and algal slurry. Before presenting influence of HC on extraction rate in Section 4.2, preliminary investigations were carried out to understand influence of HC on algal biomass, particle size and potential extraction of products. These results are discussed in the following.

4.1. Influence of HC on algal biomass

The optical microscopic studies were carried out for biomass pre-treated at 300 and 400 kPa and compared with untreated biomass to evaluate the morphological changes and extent of cell disruption. The microscopic images are shown in Fig. 2. Pre-treatment method has a prominent role in extent of cell disruption, which in turn facilitates the release of metabolites into the extraction medium. It can be seen from the figure that HC has resulted in prominent morphological changes between untreated, and HC-treated algal biomass (AB). Both 300 and 400 kPa pressure drop has disturbed the defined structural boundaries of AB, and a disorganized structure is visible when compared with the untreated biomass. There appears to be no significant difference in the appearance of AB treated at 300 kPa and 400 kPa pressure drop. This demonstrates the physical effects caused by cavitation on AB. A loosened and distorted AB particle has a higher surface area compared to a well-defined structure and hence, could be more easily accessed by the extraction media in turn releasing soluble metabolite. Similar observations have been reported earlier for acoustic cavitation treatment on macroalgae biomass [25] and hydrodynamic cavitation of lignocellulose biomass [26].

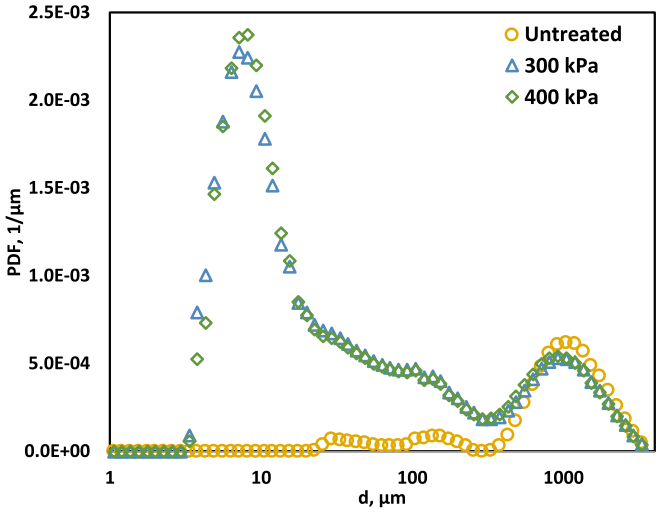

Fig. 3. PSD of untreated and treated biomass of *Palmaria palmata* after 350 passes.

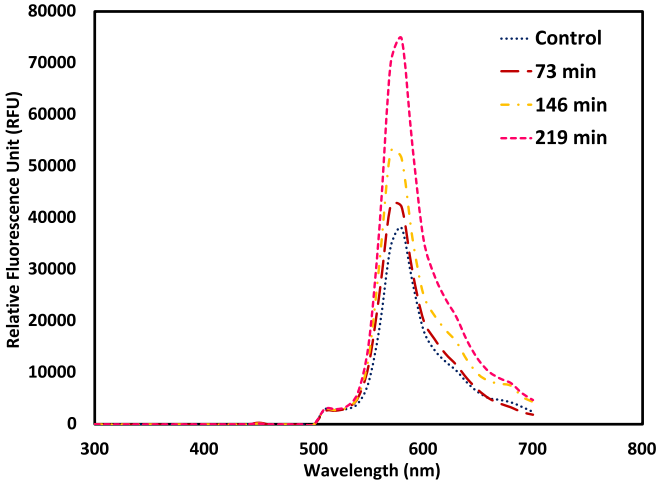

Fig. 4. Fluorescence spectrum of extract from untreated (control – stirring) and treated biomass after 350 passes (ΔP = 300 kPa).

Particle size distribution (PSD) analysis was carried out to evaluate the effect of HC on change in particle size of algal biomass. The measured particle size distribution of untreated and HC-treated biomass is shown in Fig. 3. It can be seen from the figure that HC has significantly

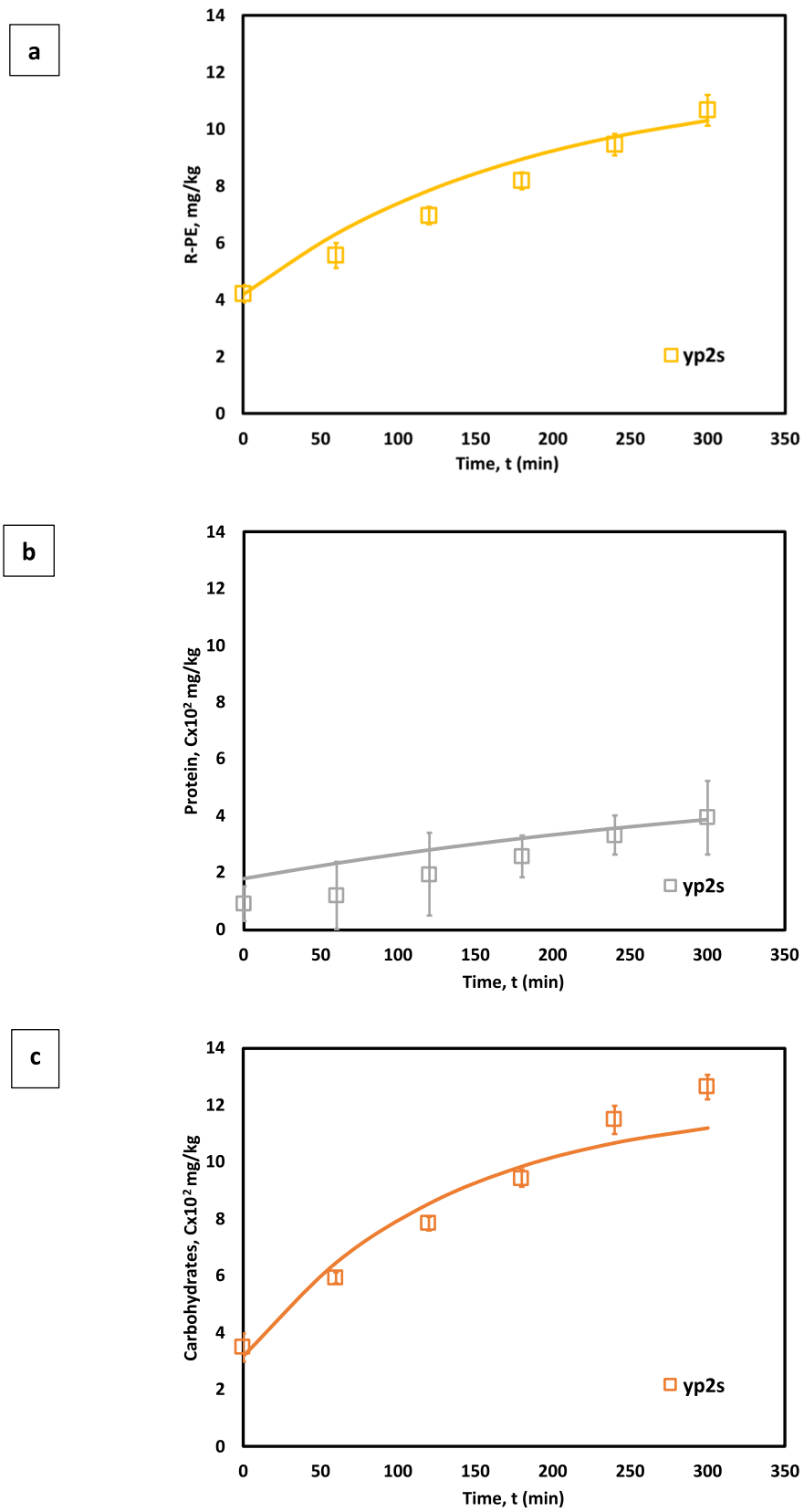

Fig. 5. Extraction of metabolites during stirring a). R-PE b). Proteins and c). Carbohydrates.

Table 2
The values key parameters for only stirring.

Biomolecule	α (mg/kg)	Rate of extraction, β (s^{-1})	φ (–)
R-PE	7.54	5.5×10^{-3}	0.38
Proteins	4.4	2.34×10^{-3}	0.50
Carbohydrates	8.9	7.67×10^{-3}	0.60

reduced particle size of AB. After 350 passes through HC device, the Sauter mean diameter was reduced to around 210 μm from 1100 μm. The reduction in particle size promotes extraction by increasing the surface area. It can be seen from Fig. 3 that the particle size distributions of algal mass treated at 300 kPa and 400 kPa show no significant difference.

Products extracted in liquid phase were analysed using the methods described in Section 2. In order to confirm the intactness of R-PE, fluorimetry of the liquid phase obtained after HC treatment at different slurry volumes (from Section 2.2) was performed and the results are presented in Fig. 4. The excitation wavelength scanning was in the range from 350 to 650 nm. The emission peak was obtained at 575 ± 5 nm for an excitation wavelength of 488 nm. The emission spectrum of the samples was found to be in good accordance with the literature, confirming the intactness of R-PE after processing [42,23].

4.2. Extraction of metabolites

Experimental data on the extraction of R-PE, proteins and carbohydrates as a function of time for only stirring experiments is shown in Fig. 5. The data was interpreted using Eq. (11) and obtained parameters β and φ for the three products are listed in Table 2.

4.2.1. Extraction in presence of HC

Algal biomass was subjected to HC pre-treatment at different pressure drops across HC device. The measured concentrations of extracted R-PE, proteins and carbohydrates are shown in Fig. 6a–c respectively. It can be seen from these figures that the rate of extraction of all the three products observed for the case of ΔP = 50 kPa across the HC device is significantly lower than the cases where operating pressure drop across HC device is higher. It should be noted that the cavitation inception in the vortex-based cavitation device used in this work occurs between 50 kPa and 100 kPa [33,36]. The operation with ΔP = 50 kPa across the HC device can therefore be considered as a reference case without any cavitation. Once the pressure drop across HC device is higher than the cavitation inception threshold, there is a significant enhancement in the observed rate of extraction. It can be seen that extracted R-PE concentration higher than 10 mg/kg, protein concentration higher than 600 mg/kg and carbohydrate concentration up to almost 1000 mg/kg was observed after 350 passes with 1% algal biomass in the slurry. Much higher concentrations may be achieved with a higher solid loading of algal biomass.

The experimental data obtained for R-PE, protein and carbohydrate were interpreted using Eq. (12). It can be seen that the presented model (Eq. (12)) was able to describe the experimental data reasonably well. It should be noted that different pressure drop across HC device will result in correspondingly different flow rates. The time required for completing specific number of passes is therefore different for different operating conditions (pressure drop across HC device). The net extracted product in liquid phase will be a function of both: number of passes through cavitation device as well as time. The effect of time (under stirring conditions) on extraction was independently investigated and corresponding parameters β and φ obtained from only stirring experiments are listed in Table 2. For modelling HC enhanced extraction rate, these same parameter values of β and φ were used to capture influence of time. For each operating condition, per pass extraction factor, δ was obtained from the measured concentration versus time data and Eq. (12). The fitted values of parameters for three products are listed in Table 3. The presented model can therefore provide useful information about HC-enhanced extraction.

This observed increase in the rate and degree of extraction of the three products can be attributed to the disruptive effect of HC on biomass, which is evident from the decrease in Sauter mean diameter and other surface characteristics of HC-treated algal biomass. A similar effect of HC on biomass has been reported previously where HC has resulted in a reduction in particle size of sugarcane bagasse, lignocellulose biomass, grass silage, wood, microalgae etc., in turn exposing the intracellular material [7,1,12,13,20,26,43,11]. A significant enhancement in the rate of extraction was observed between 50 kPa (before inception) and 200–400 kPa (after inception) pressure drop. However, rate of extraction did not vary much with the change in pressure drop across HC device once inception happens. The net enhancement in the rate of extraction with reference to absence of HC, $\frac{\left(\frac{\dot{m}_c\delta}{Mm_s}+\beta\right)}{\beta}$ is shown in Fig. 7.

An alternative way of looking at the influence of HC on extraction is to examine relative values of per pass extraction factor, δ, at operating conditions beyond and before cavitation inception. The relative values of such per-pass extraction factors are listed in Table 4. It can be seen from Fig. 7 and Table 4 that as pressure drop across the HC device increases from 50 kPa (just before the cavitation inception), a significant increase in normalised enhancement in the rate of extraction $\left(\frac{\frac{\dot{m}_c\delta}{Mm_s}+\beta}{\beta}\right)$ for all three biomolecules was observed. The value of $\frac{\delta_P}{\delta_{50}}$ (per pass extraction factor normalised by per pass extraction just before inception) was found to be highest at pressure drop of 200 kPa and decreased with an increase in pressure drop from 200 to 400 kPa. Hence, based on the higher values of $\frac{\delta_P}{\delta_{50}}$, ΔP = 200 kPa may be considered as most suitable pressure drop for the pre-treatment of AB.

In order to verify the applicability of per-pass extraction factor, the extraction experiments were carried out for two different volumes of algal slurry in the holding tank (5 and 15 lit). These experiments were carried out at an operating pressure drop of 300 kPa (circulation flow rate through HC device, $\dot{m}_c$=26.36 kg/min) with 1% solid loading of algal biomass (on dry basis). The experimental results are shown in Fig. 8. It can be seen from the figure that a significant increase in the degree of extraction of the metabolites was observed with an increase in volume of suspension processed. The R-PE, proteins and carbohydrate content in the extract increased from 10.89 to 13.34 mg/kg, 4.69 to 6.59 $\times 10^2$ mg/kg, and 7.43 to 10.63 $\times 10^2$ mg/kg, which reflects into 23, 40 and 43 % increase. These experimental results were also processed using Eq. (12) and the values of fitted parameters are listed in Table 5. It can be seen from the table that the rate of extraction expressed in terms of δ for the given biomolecules (R-PE, proteins and carbohydrates) remains unchanged with an increase in volume in the holding tank. However, the values of α were found to be different from the values given in Table 3. Two different batches of algal biomass were used for these two experimental sets. The observed differences in α and φ values in Table 3 and Table 5 could be due to variations in biomass used during two sets of experiments. These results confirm that per-pass extraction approach is quite appropriate for describing and designing HC-based treatment of algal biomass. It can be seen that the value of δ for R-PE is found to be highest followed by proteins and carbohydrates.

Recovery of R-PE, proteins, and carbohydrates (at 300 kPa, 500 passes with 15 L volume) was calculated employing Eq. (2) and found to be 59.75, 72.3 and 69.8 %, respectively. These values of recovery can be correlated with the values of φ, i.e., value closer to φ, indicates higher recovery of the given metabolite.

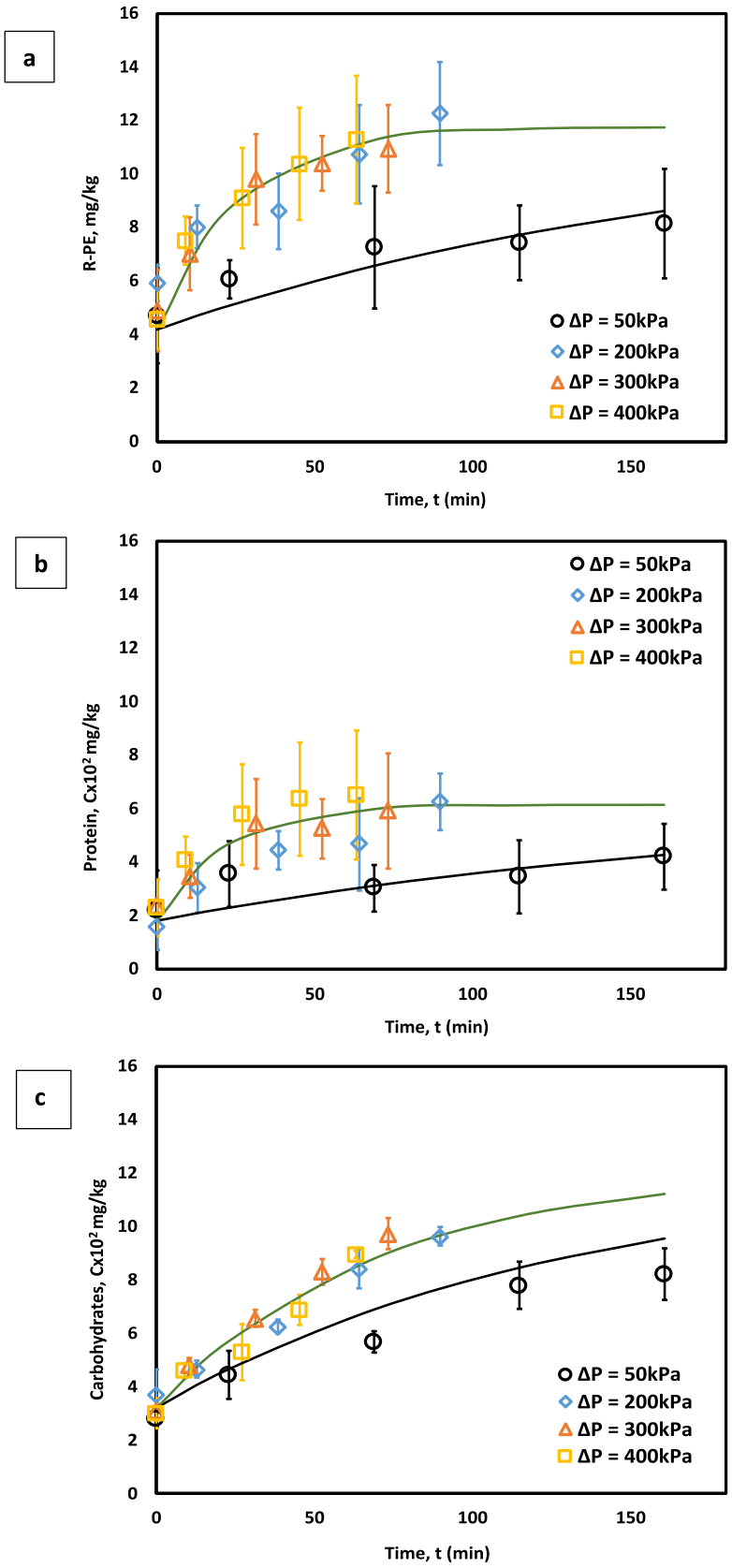

Fig. 6. Extraction in presence of HC (a) R-PE (b) Proteins and (c) Carbohydrates; Values appended to subscript *p2c* indicate pressure drop across HC device in kPa.

Table 3
Values of parameter δ during the pre-treatment for R-PE at different pressure drops.

Pressure drop, kPa	R-PE ($\alpha = 7.5$, $\beta = 5.50E{-}03$, $\varphi = 0.38$)	Proteins ($\alpha = 4.4$, $\beta = 2.34E{-}03$, $\varphi = 0.50$)	Carbohydrates ($\alpha = 8.9$, $\beta = 7.67E{-}03$, $\varphi = 0.60$)
50	1.E−07	1.3E−05	1.3E−06
200	7.2E−05	1.1E−04	1.5E−05
300	6.4E−05	8.6E−05	1.3E−05
400	5.1E−05	7.7E−05	1.2E−05

4.2.2. Energy consumption of HC based pre-treatment

It is evident from the presented results that vortex-based HC pre-treatment of AB was effective in enhancing the extraction of metabolites from macroalgae. The vortex-based cavitation device used in this study has no moving parts and can be scaled up to utilise HC for process intensification. It is worthwhile to examine energy requirements for HC based pre-treatment. The energy consumption for HC treatment can be calculated by Eq. (13) as:

$$\mathrm{E} = \frac{\Delta P Q t}{M} = \frac{\Delta P n}{\rho} \tag{13}$$

where E is the energy consumption for HC pre-treatment of slurry (kJ/kg), ΔP is the pressure drop (kPa), n is number of passes through HC device and ρ is the density of slurry (kg/m^3). It can be seen that at a pressure drop of 300 kPa, energy consumption is about 0.3 kJ/kg of slurry/per pass. Even if a pump efficiency of 0.6 is considered and typical number of passes are considered as 100, the energy consumption to process one kg (~1 lit) of slurry is 0.014 kWh. This is two orders of magnitude lower than typical energy consumption required for ultrasonic cavitation (see for example [28]). The energy consumption of the vortex based HC device used in this work is also much lower (500 kJ/kg compared to up to 5000 kJ/kg) than the energy consumption of conventional HC devices like orifice (see for example [12]). By considering biomass loading of 10%, McHardy et al. [18] have reported energy consumption for high pressure ultrasonic cavitation as 0.66 kWh/ kg of biomass and found it to be of the same order as energy consumption required for pulsed electric field or bead milling (0.45 kWh/kg of biomass). By considering the same biomass loading of 10%, the energy consumption for the pre-treatment using vortex based HC device is less than one third (0.14 kWh/kg of biomass) than these reported values.

Using Eqs. (12) and (13), the fraction of product extracted in liquid and energetic productivity (P_E, kg of extracted product/kJ) of the process can be calculated by Eqs. (14) and (15) respectively (ΔP in kPa):

$$X_E = \left[1 - e^{-\left(\frac{\delta}{m_s} + \frac{\beta M}{\dot{m}_c}\right) n}\right] \tag{14}$$

$$P_E = \left(\frac{m_s y_{ps1} \varnothing \rho}{\Delta P n}\right)\left[1 - e^{-\left(\frac{\delta}{m_s} + \frac{\beta M}{\dot{m}_c}\right) n}\right] \tag{15}$$

It can be deduced from Eq. (15) that energetic productivity decreases with number of passes. The maximum energetic productivity, P_{Emax} is obtained at $n \to 0$ as

$$P_{Emax} = \frac{m_s y_{ps1} \varnothing \rho}{\Delta P}\left(\frac{\delta}{m_s} + \frac{\beta M}{\dot{m}_c}\right) \tag{16}$$

Thus, the energetic productivity can be related to P_{Emax} as:

$$P_E = \left[\frac{P_{Emax}}{\left(\frac{\delta}{m_s} + \frac{\beta M}{\dot{m}_c}\right) n}\right]\left[1 - e^{-\left(\frac{\delta}{m_s} + \frac{\beta M}{\dot{m}_c}\right) n}\right] \tag{17}$$

Table 4
Per pass extraction factor normalised by per pass extraction before the inception.

δ_P/δ_0	R-PE	Proteins	Carbohydrates
δ_{200}/δ_{50}	701.9	7.8	40.3
δ_{300}/δ_{50}	640.4	6.4	33.2
δ_{400}/δ_{50}	575.9	5.8	29.8

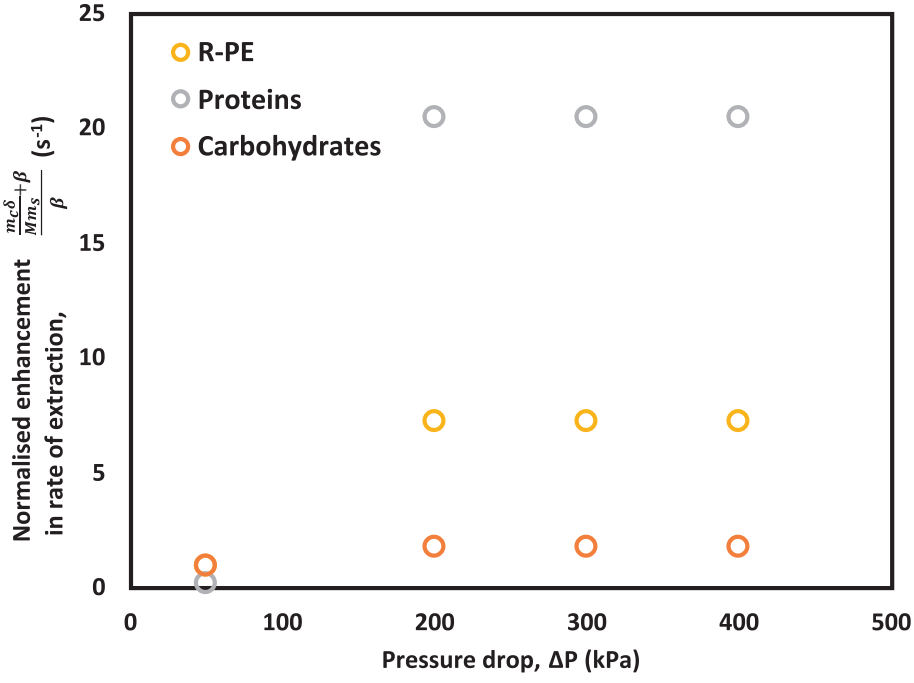

Fig. 7. Ratio of extraction rate constants of given biomolecules at different pressure drops.

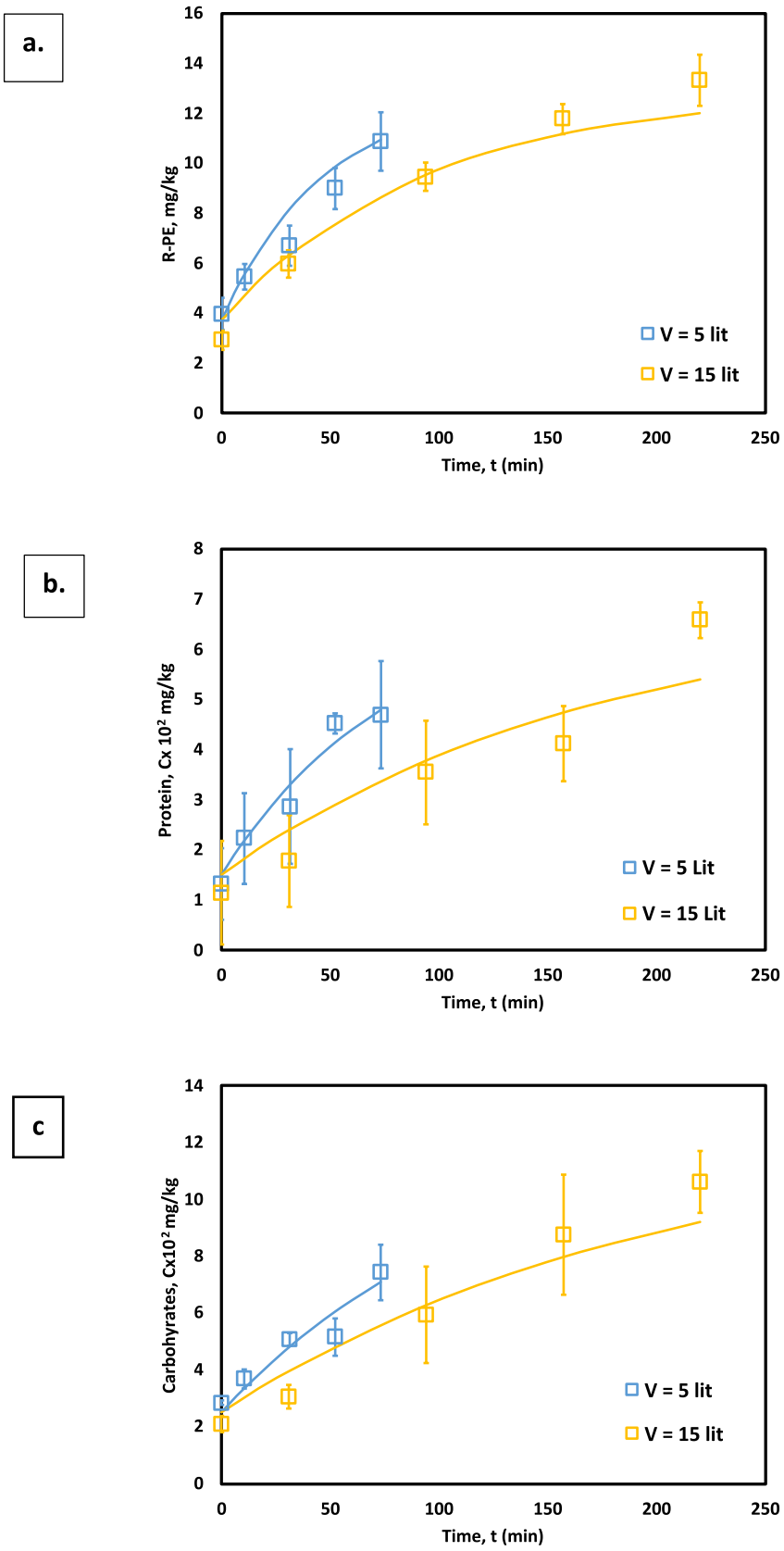

Fig. 8. Extraction with different volumes of slurry in holding tank (a) R-PE; (b) Proteins and (c) Carbohydrates. $\Delta P = 300$ kPa.

Table 5
Parameters during the extraction at different slurry volumes.

Biomolecule	Volume (L)	α	β	φ	δ	$\frac{\dot{m}_c\delta}{Mm_s}+\beta m_c = 26.36$ kg/min	$\frac{\left(\frac{\dot{m}_c\delta}{Mm_s}+\beta\right)}{\beta}$
R-PE	5	9.1	5.50E−03	0.45	3.0E−05	0.02	3.9
	15					0.012	2.0
Proteins	5	7.3	2.34E−03	0.60	2.0E−05	0.013	5.5
	15					0.006	2.5
Carbohydrates	5	10.1	7.67E−03	0.65	1.2E−06	0.008	1.1
	15					0.005	1.0

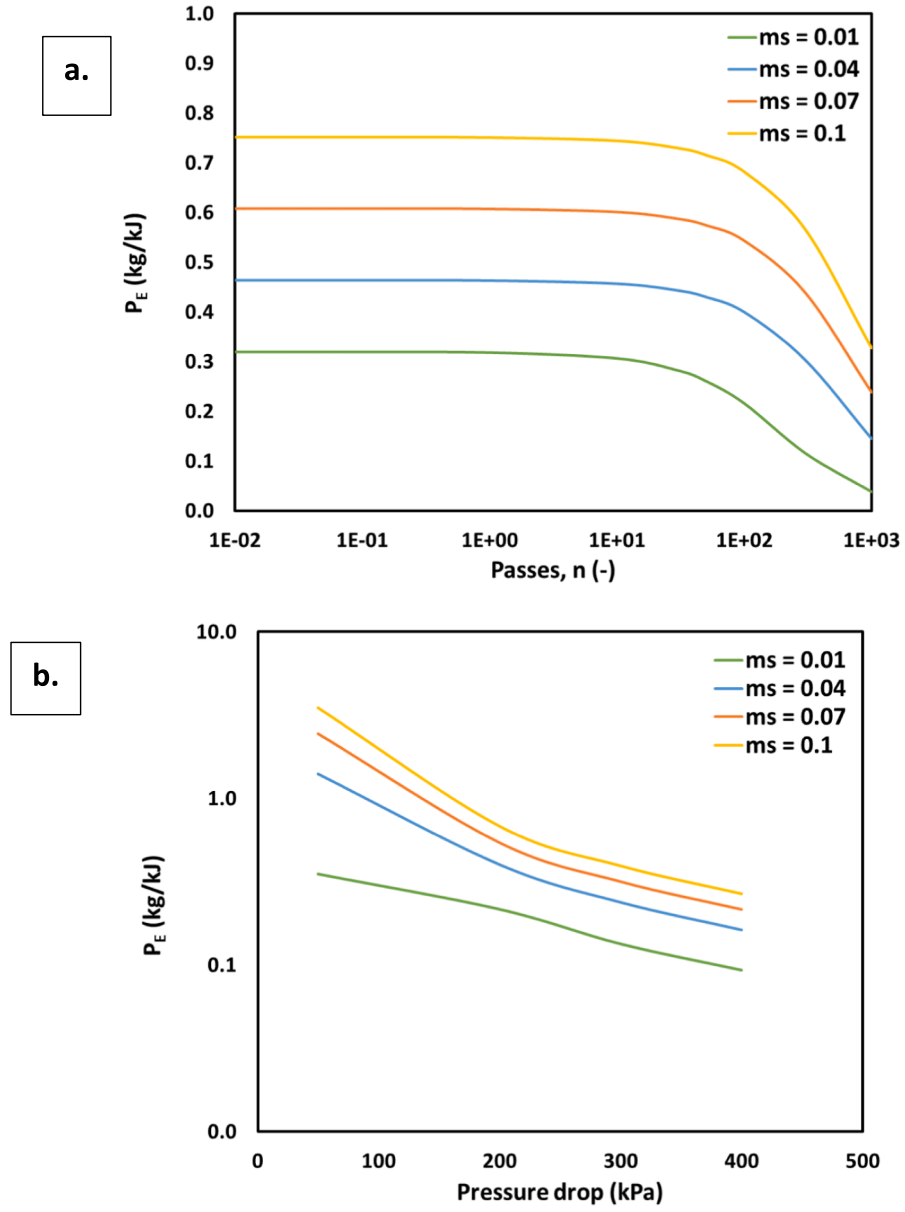

Fig. 9. Productivity at different solid loading for R-PE (a) ΔP = 200 kPa (b) n = 100.

It can be seen that for low values of $\left(\frac{\delta}{m_s}+\frac{\beta M}{m_c}\right)n$, the energetic productivity is independent of number of passes and at high values of $\left(\frac{\delta}{m_s}+\frac{\beta M}{m_c}\right)n$, the energetic productivity is inversely proportional to the number of passes, *n*. The results of productivity values obtained from R-PE as a function of number of passes and pressure drop are shown in Fig. 9a and b respectively for four values of solid loading. Similar figures for other two products are shown in Figs. S4 and S5 of Supporting information.

It can be seen from Fig. 9 that as expected, productivity increases

with an increase in solid loading and decreases with an increase in pressure drop across HC device. The productivity decreases slowly with number of passes up to n = 100. Beyond n = 100, productivity decreases significantly. The number of passes may therefore the kept around 100. The productivity values of about 0.5 kg/kJ look quite attractive considering the value of R-PE, proteins and carbohydrates. The results indicate that pre-treatment of algal biomass using vortex-based hydrodynamic cavitation can significantly enhance effective rate of extraction and can achieve the productivity of about 0.5 kg of product/kJ. The results and analysis presented here will be useful for harnessing hydrodynamic cavitation for deriving value added products from macroalgae.

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5. Conclusions

In this work, we used the vortex-based cavitation device for the pre-treatment of the macroalgal biomass namely, *Palmaria palmata*. The hydrodynamic cavitation (HC) based pre-treatment resulted in structural and morphological changes as revealed by analysis using microscopy, and mastersizer. HC was also found to significantly enhance rate of extraction of valuable products like R-PE, proteins and carbohydrates from *Palmaria palmata*. Specific conclusions drawn from the present work are summarised in the following:

- Pre-treatment using the vortex based cavitation device was found to enhance rate of extraction by an order of magnitude for the three products investigated in this work (R-PE, proteins and carbohydrates).
- The characteristic fluorescence of R-PE was found to remain intact after HC treatment indicating no adverse effect of cavitation on properties of phycoerythrin.
- The developed model (Eq. (12)) was found to describe the experimental data for all the three products reasonably well. The per-pass extraction factor of the HC device used in this work was found to be proportional to the mass fraction of the product in solid algal biomass (y_{ps}).
- The per-pass extraction factor was found to be maximum for pressure drop of 200 kPa within the range of pressure drop considered in this work.
- Energetic productivity (Eqs. (16) and (17)) was found to decrease first slowly with number of passes and then at a faster rate as number of passes exceed 100. The optimal number of passes may therefore be considered as around 100.

The results and model presented in this work will be useful intensifying extraction of valuable products from macroalgae using vortex based HC pre-treatment.

CRediT authorship contribution statement

Rochak Mittal: Investigation, Data curation, Validation. **Vivek V. Ranade:** Conceptualization, Funding acquisition, Investigation, Supervision.

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ultsonch.2023.106347.

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