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Halophyte biorefinery for polyhydroxyalkanoates production from *Ulva* sp. Hydrolysate with *Haloferax mediterranei* in pneumatically agitated bioreactors and ultrasound harvesting

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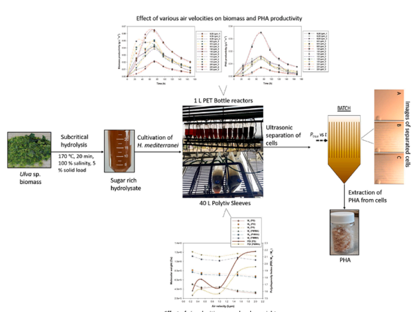
HIGHLIGHTS

- Application of extreme halophilic archaea for PHA production.
- Utilization of pneumatically agitated reactors for outdoor fermentation.
- Application of ultrasonic separation technology for harvesting of archaeal biomass.
- Value addition towards a halophilic biorefinery.

GRAPHICAL ABSTRACT



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ABSTRACT

The present study tested the outdoor cultivation of *Haloferax mediterranei* for PHA production from green macroalgae *Ulva* sp. in pneumatically agitated bioreactors and applied ultrasonic separation for enhanced settling of archaeal cells. Scaled-up cultivation (40 L) yielded maximum biomass productivity of 50.1 $\pm$ 0.11 $mg{\cdot}L^{-1}{\cdot}h^{-1}$ with a PHA productivity of 27 $\pm$ 0.01 $mg{\cdot}L^{-1}{\cdot}h^{-1}$ and conversion yield of 0.107 g PHA per gram $Ulva_{DW}$. The maximum mass fraction of PHA achieved in biomass was calculated to be 56% w/w. Ultrasonic harvesting of *Hfx. mediterranei* cells approached 30% removal at energy inputs around 7.8 $kWh{\cdot}m^{-3}$, and indicated no significant aggregation enhancement by Ca^{2+} addition. Molecular weight analysis showed an increase in Polydispersity Index (PDI) when the corresponding air velocities were increased suggesting that the polymer was more homogeneous at lower mixing velocities. The current study demonstrated scalable processes for PHA production using *Ulva* sp. feedstock providing new technologies for halophilic biorefinery.

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1. Introduction

Global petrochemical plastics production of 335 million tonnes per year in 2016 is raising urgent concerns regarding end-of-life disposal and plastic contamination of the environment. Between 22% and 43% of polymers used worldwide are disposed of in landfills, and 10–20 million tons of plastics end up in oceans each year, causing damage to our ecosystem and an associated cost burden of 13 billion USD per year (Gourmelon, 2015). Governments are now supporting strategies to promote a bio-based and circular economy by enforcing the use of bioplastics and introducing bans on landfills. Amongst biopolymers, polyhydroxyalkanoates (PHAs) are recognized as outstanding sustainable materials due to their tunable co-polymer formulation and mechanical properties and unique degradability in both marine and ambient soil environments (Kalia, 2016). In addition, unlike other biopolymers, PHA polymers are hydrophobic, water-insoluble, indefinitely stable in air, non-toxic, thermoplastic and/or elastomeric, and have very high purity within the cell (Ray and Kalia, 2017). The PHA polymer family has much potential and adaptability for the broad application of biopolymers. Therefore, the search for highly efficient microorganisms for PHA accumulation is the need of the hour. Among wild-type strains, *Hfx. mediterranei*, an extreme halophile, is of utmost significance. The archaea can accumulate high amounts of intracellular PHA utilizing a variety of substrates. Moreover, its extremophilic nature which is characterized by high salt tolerance enable non-sterile cultivation of the organism thereby decreasing the cost and energy required for the process (Koller, 2019).

Despite decades of research, the commercialization of PHA processes is slow. This could be explained by the lack of high-value applications for the initial stages of product penetration to the market, where the cost barriers of petroleum-based plastics are challenging. However, the development of the halophyte biorefinery processes for PHA production from seaweeds is still challenging and there are gaps in the scalability of these processes that will give them a clear advantage over current processes. For example, closed controlled fermenters that are used for PHA production with bacteria today are expensive and require a lot of freshwater and energy for mixing of culture media (Mahler et al., 2018). In addition, bacterial biomass harvesting is usually done with centrifuges, which are barely scalable and are not stable for the corrosive high salt media required for extreme halophiles growth. Moreover, most of the studies regarding extreme halophilic archaea have been performed in shake flask cultures or bioreactors of working volumes of 1 – 10 L (Alsafadi and Al-Mashaqbeh, 2017; Hezayen et al., 2000). Therefore, studies are required which need to be focussed on the scale up of PHA production from halophilic archaea using various carbon substrates. The high salinity of the cultivation medium also poses challenges with regard to the material of construction of reactors. Higher salinities provide more corrosive conditions thereby requiring corrosion resistant reactors (Hezayen et al., 2000). Oxygen is a requisite for the growth of microorganisms and other metabolic activities. This becomes more important in highly saline conditions where solubility of oxygen in the media decreases further. Oxygen limiting conditions have been studied for enhancement of PHA production whereas oxygen replete conditions help in increasing the biomass concentrations thereby leading to high density cultivations (Maheshwari et al., 2018). Therefore, it becomes important to assess the role of oxygen concentration on the growth as well as the PHA production in extreme halophilic archaea.

PHA being an intracellular product requires high cell density cultivations because the restricted availability of cytoplasmic space limits the maximum amount of PHA which can be accumulated in the cells. Recovery of PHA begins after separating and concentrating cells from the fermentation medium. As PHA recovery from halophiles can be easily done by cell lysis using tap-water, separation of cells from the fermentation medium becomes a challenge in the overall recovery of PHA. Generally, separation of cells is performed using centrifugal force at a laboratory scale. Industrial separation of cells from the fermentation medium requires highly efficient continuous centrifuges. These methods are adequate when extracellular products are required but when large quantities of washed bacteria are wanted, the need to scrape or wash out continuous flow centrifuge rotors makes a sterile harvest difficult to achieve. Similarly, filter blockage and difficulty in washing organisms off filters aseptically make simple filtration time-consuming and unreliable (Reid and Adlam, 1976). Harvesting cells in an ultrasonic standing wave provides a potential alternative to problematic conventional separation technologies. There have been various studies that discuss the separation of microorganisms using ultrasound with very high separation efficiencies (Coakley et al., 2000; Cousins et al., 2000). Recently, ultrasonic separation has been utilized for separation of microalgal cells from the cultivation medium (Coons et al., 2014). Sonication produces highly localized cavitation and cell disruption for recovery of PHA from *Hfx. mediterranei* (Hwang et al., 2006)., and is a fundamentally different phenomena from the trapping and concentration of cells in an ultrasonic standing wave. The effectiveness and energetic requirements of harvesting bacteria cells by ultrasonic standing waves need to be explored to determine whether it presents a viable alternative to conventional dewatering technologies.

The goal of this study was to address the challenges of halophiles (oxygen requirement, outdoor fermentation) for PHA production using *Hfx. mediterranei* grown in pneumatically agitated bioreactors in the outdoor fermentation using macroalgal hydrolysates. In addition, to address the challenge of biomass harvesting, we tested harvesting by ultrasound (Coons et al., 2014) for archaea cells removal from the fermentation media. The fermentation was performed in 1 L PET bottles and 40 L plastic sleeves under non-sterile conditions which operated as bubble column reactors. The process conditions (aeration rate, time of fermentation) were optimized for efficient PHA production from the hydrolysate. The structural characteristics of the obtained PHA were determined along with the molecular weight of the final polymer. The energy required for archaea cells harvesting with ultrasonic separation was obtained from experiments and used to forecast biomass concentrations needed for sustainable harvesting. Conditions leading to maximum biomass production coupled with efficient harvesting and dewatering technologies need to be discovered to overcome these challenging barriers and deliver an economically viable and renewable biopolymer industry.

2. Materials and methods

2.1. Macroalgae biomass production

Ulva sp. was cultivated in macroalgae photo-bioreactors (MPBR) (Polytiv, Israel, Length 100 cm, thickness 200 mm, width 40 cm). These were custom made photobioreactors which are used to grow macroalgae under controlled conditions in natural irradiance from September 15 to November 3, 2016. The optimised conditions from our earlier work was utilised for the growth of seaweeds in MPBRs (Chemodanov et al., 2017). The elemental analysis of the *Ulva* sp. biomass was done using a CHNS analyser (Thermo Scientific, USA). The ash and moisture content was determined by burning the biomass at 550 °C in a muffle furnace (M.G. Furnaces, Faridabad, India) and the starch content was analyzed using total starch assay.

2.2. Subcritical hydrolysis of macroalgal biomass

The hydrolysis of seaweed biomass was performed in a batch reactor (0.25 L working volume) equipped with electric heating system (CJF-0.25, Keda Machinery, China). Temperature and pressure inside the reactor were monitored using a digital temperature meter (MRC Ltd., Israel) and a pressure gauge (MRC Ltd., Israel) respectively. In order to mix the hot slurry inside the reactor, it was equipped with a stirrer which was cooled using chilled water from a chiller (Guangzhou Teyu Electromechanical Co. Ltd., China). In order to eliminate residual air from the reactor, a vacuum pump (MRC Ltd., Israel) was utilised. The conditions for hydrolysis were as follows: temperature of 170 ℃, total residence time of 20 min, salinity of 38 g·L^{-1} and a solid load of 5 %. The above conditions were chosen for maximum sugar yield as suggested by previous studies (Greiserman et al., 2019). The hydrolysate was separated into liquid and solid phases by centrifugation at 10,000 rpm for 3 min (Yingtai Instruments TGL-18, China).

2.3. PHA production by Hfx. Mediterranei

PHA production was investigated by utilising the hydrolysate of *Ulva* sp. with the aid of *Hfx. mediterranei* ATCC 33500 (NCIMB 2177). The *Hfx. mediterranei* strain were routinely grown in rich medium (Hv-YPC). The culture was grown in a shaking incubator (MRC Labs, Israel) at 42 °C with a rotational speed of 180 rpm. The media pH was adjusted to 7.2. For the experiment, seaweed hydrolysate (aqueous phase) was supplemented to saline water (144 g NaCl L^{-1}) at a working concentration of 25% v/v as observed in our previous studies (Ghosh et al., 2021, 2019). The experiments were performed in custom-built reactors which were made of 1 L PET bottles (Length 0.285 m, width 0.08 m). A set of 15 PET bottles were utilized for the cultivation of *Hfx. mediterranei* under different aeration rates (0.25 – 2.0 L min^{-1}) in triplicates. Individual reactors were connected with flowmeters to manipulate the airflow inside the reactor. The air was sparged from the bottom of the reactor for uniform flow inside the reactor. On the top of the reactor, an outlet was provided for gases. The cultivation was further scaled up to 10 L using cylindrical, sleeve-like macroalgae photo-bioreactors (MPBR) (Polytiv, Israel, Length 1.55 m, thickness 0.02 m, width 0.4 m) which were used for the cultivation of *Ulva* sp. The reactor configurations used for the cultivation of *Hfx. mediterranei* and their schematic representations are shown in Fig. 1.

For the ultrasonic separation tests, cells were grown as described by Ghosh et al (Ghosh et al., 2019) in 600 mL of rich Hv-YPC medium (pH 7.2) in a 1L baffled Erlenmeyer flask. The flask was maintained at 42 °C in a shaking incubator at 170 rpm for approximately 48 h to late exponential/early stationary phase and removed from the shaker. The optical density of the culture was 7.9 at 520 nm as measured on a benchtop spectrophotometer (ThermoFisher AquaMATE VIS, 1 cm path length), which roughly corresponds to an ash-free dry weight concentration of 3.9 g·L^{-1}. The *Hfx. mediterranei* culture was transferred to a closed container and stored on the benchtop for two weeks as a test for auto-flocculation, which could improve the particle size and ultrasonic response of the culture. Auto-flocculation was not observed in the standing culture, so enhanced aggregation of *Hfx. mediterranei* cells was attempted by Ca^{2+} addition following the method described by Kawakami et al., 2005 (Kawakami et al., 2005). Cells were pelletized by centrifugation at 1000 × g for 15 min and then twice washed in a Tris buffer (20 mM Tris-HCl and 4 M NaCl, pH 7.0). The cells were then suspended at higher concentration in a salt solution (27 mM KCl, 0.24 mM $NaHCO_3$, 0.49 mM NaBr, 3.7 mM NH_4Cl, 4.3 M NaCl, 18.5 μM $FeCl_3·6H_2O$, pH 7.0). A second cell suspension was washed and rinsed following the same procedure, but the final salt solution was modified to include 10 mM $CaCl_2$. The washed suspensions had an optical density of 11.8 at 520 nm, which corresponds to an ash-free dry weight concentration of 5.8 g·L^{-1}.

2.4. Determination of intracellular PHA content

The PHA content of the cells was measured using the Nile Red staining procedure (Spiekermann et al., 1999). Briefly, the cells were washed and resuspended in 10% saline water. Further, the cell suspension was stained with Nile Red (Sigma Aldrich, USA) which had a final

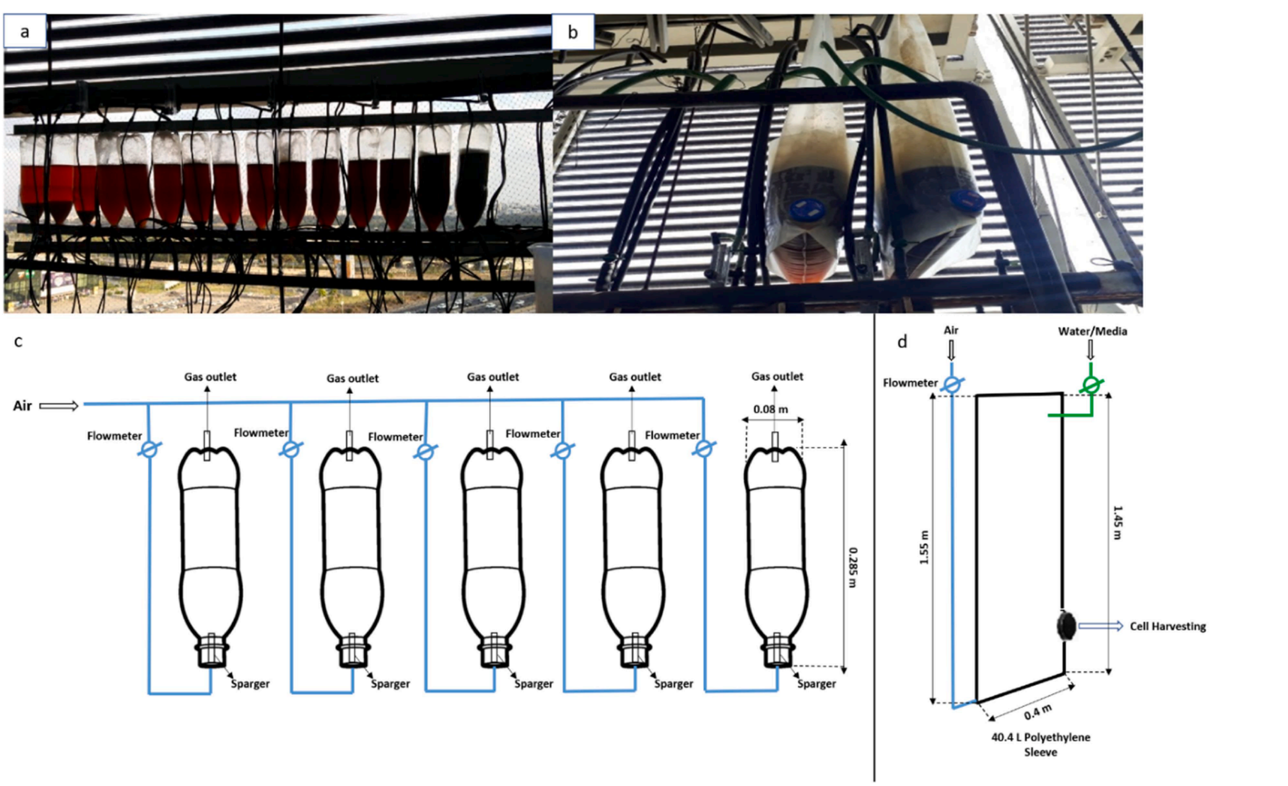

Fig.1. **a)** Experimental setup for optimisation of aeration rates in outdoor cultivation (1 L PET bottles) of *Haloferax mediterranei* **b)** Experimental setup for cultivation of *Hfx. mediterranei* in 40 L sleeves **c)** Schematic representation of the cultivation system in 1 L PET bottles [Experimental conditions: Flow rates: 0.25 to 2.0 L min^{-1}; Fermentation volumes: 800 mL; Temperature: 42 ± 2.5 ℃; Inoculum concentration: 50 g L^{-1}; Initial pH: 7.2] **d)** Schematic representation of a single photobioreactor for cultivation of *Haloferax mediterranei* (40 L) [Experimental conditions: Flow rate: 1.0 L min^{-1}; Fermentation volume: 10 L; Temperature: 42 ± 2.5 ℃; Inoculum concentration: 50 g L^{-1}; Initial pH: 7.2]

concentration of 3.1 μg ml^{-1}. The cells were incubated for 30 mins and further washing and resuspension was performed using 10% saline. The fluorescence of the suspension was measured in a 96 well plate reader (Tecan, Switzerland) at excitation and emission wavelengths of 535 and 605 nm respectively. Commercial PHA (Sigma, USA) was utilised for preparation of standards with varying concentration (0.2–2.0 g L^{-1}). These standards were utilised for preparation of standard curve. A standard curve was plotted and the final concentration of PHA in the cells was determined. The unknown amount of PHA was determined from the standard curve with two repeats per point. The protocol was also verified by using the crotonic acid assay for intracellular PHA content determination. Two ml of concentrated sulphuric acid was added to the dried cell pellet containing the polymer. The mixture was hydrolyzed by heating in water bath at 100 ℃ for 20 min to obtain crotonic acid. The amount of accumulated polymer was quantified by recording the absorbance at 235 nm using concentrated sulphuric acid as blank with multiplate reader (Tecan, Switzerland) (Mahansaria et al., 2018).

2.5. Calculation of biomass and PHA productivities

The following equations were used to determine the volumetric biomass productivity ($X_{Biomass}$, g·L^{-1}·h^{-1}) and volumetric PHA productivity (X_{PHA}, g·L^{-1}·h^{-1}):

$$X_{Biomass} = \frac{B_2 - B_1}{t_2 - t_1} \tag{1}$$

$$X_{PHA} = \frac{C_2 - C_1}{t_2 - t_1} \tag{2}$$

The details of the parameters under consideration are as follows - B_1 and B_2 correspond to the biomass concentrations (g·L^{-1}) at time t_1 (h) and t_2 (h) respectively; whereas, C_1 and C_2 correspond to the PHA content (g·L^{-1}) at time t_1 (h) and t_2 (h) respectively. For determination of PHA productivity, the concentration was determined by PHA extraction.

2.6. Ultrasonic cell harvesting of Hfx. Mediterranei

2.6.1. The viscosity of cell suspensions

The viscosity of the culture and cell suspensions was measured in a Stabinger Viscometer (Model SVM 3001, Anton-Paar, Ashland, VA) at 20 °C and a shear rate of 948 ± 9 s^{-1}.

2.6.2. Settling behavior and cell size

The settling behavior of biomass sheds light on single and multi-particle phenomena that impact and limit the dewatering process. The behavior is monitored as the suspension settles in a centrifuge and the position of the concentrating solids move toward the bottom of the cuvette with time. By repeatedly measuring the transmittance of the suspension along the cuvette axis, the settling behavior over a range of concentrations is visualized. This method also provides a direct measure of settling velocity which is a key property tied to dewatering technologies like centrifugation and ultrasonic separation (Coons et al., 2014). The settling behavior of *Hfx. mediterranei* was assessed using the STEP technology in a LUM analytical centrifuge (LUMiSizer. LUM GmbH, Berlin). Four 1.4 mL samples of the culture and washed cells were placed in 10 mm cuvettes and spun at 4000 rpm for up to 20 min at 12 °C. As shown in Fig. 2a, transmission profiles were measured every 4 s along the length of the cuvette, starting at the air/liquid interface around 109 mm and ending at the bottom of the cuvette at 130 mm. The first 75 profiles were used to measure settling velocity distributions in the upper half of the suspension. Front tracking performed by a Matlab script was used to calculate the velocity of the concentration profiles at constant (midrange) transmittance as solids concentrated 4–5 times that of the starting suspension. Settling velocity at Earth's gravity (v_g) was calculated by dividing the mean settling velocity by the relative centrifugal force (1985 to 2002 g). Cell size range was calculated from the settling velocity using bounding values of cell buoyant density. The relation between the size of a spherical particle and its settling velocity (v_g) was applied as derived from Stoke's law (Coons et al., 2014).

$$d = \sqrt{\frac{18\mu v_g}{g_c \Delta\rho}} \tag{3}$$

where *d* is the diameter of the cell or particle, μ is the viscosity of the medium, g_c is the acceleration due to gravity, and $\Delta\rho$ is the excess density or density difference between the cell and suspension medium. The excess density of *Hfx. mediterranei* cells is unknown, but can be roughly bounded between 0.02 and 0.15 g cm^{-3} based on the survey of bacteria reported by Guerrero et al (Guerrero et al., 1985). The high value is taken from *Chromatium* sp. (purple sulphur bacteria) in natural conditions and is nearly the same as that of *Cupriavidus necator* when presenting PHB inclusions.

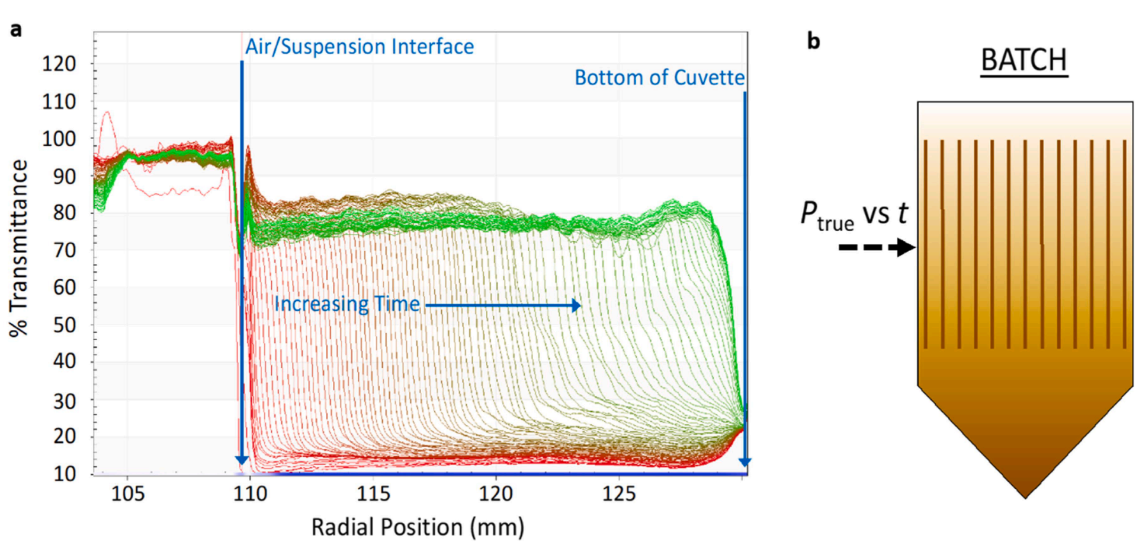

Fig. 2. **a)** Advancing transmittance profiles reveal the settling of *Hfx. mediterranei* cells in the culture media at 12 °C. The transmittance profiles change from red at early times to green at later times **b)** Illustration of the batch ultrasonic separator vessel. Samples were withdrawn at the top of the vessel immediately above the standing wave. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2.6.3. Ultrasonic separation tests

Ultrasonic removal was performed in batch mode using a 20 mL vessel (SonoSep Instruments, Inc., Hinterbrühl Austria) and a LabView control system (National Instruments, Austin TX). Waveforms with frequency around 1.7 MHz were provided to the piezo actuators at 1 W true power following amplification (Electronics & Innovation, LTD, Rochester NY). The vessel had an active volume of approximately 8 mL and was fitted with an open-top as illustrated in Fig. 2b. Samples (50 μL) were withdrawn from the top of the vessel at designated times over a 5-minute period and diluted into 200 μL of DI water in a 96-well plate. Each batch test was repeated three times. Biomass removal was calculated from optical density measurements at 750 nm using a plate reader (BioTek Synergy H4, Winooski, VT) and compared to the initial suspension. Control tests were performed following the same procedure, but without powering the standing wave. True power was measured with a 20 W RF power meter (SinePhase Instruments, Model TPM020, Hinterbrühl Austria) data logged every half second. The cumulative energy density was determined for each sample removed using $\sum P\Delta t/V$, where P is the true power measured at the beginning of a half-second time interval (Δt), V is the active volume of the vessel, and the sum is taken from the start of the test up to the sample time.

2.7. Extraction of PHA from cells

After fermentation, the broth was centrifuged at 10,000 rpm for 10 mins (Yingtai Instruments TGL-18, China). The cell pellet thus formed was dried for 12 h at a temperature of 60 ℃ in a convection oven (MRC Laboratories, Israel). In order to extract the PHA from the cells, the cell pellet was treated with 0.1% SDS in distilled water and incubated at 32 ℃ for 24 h. This induced lysis of the cells thereby releasing the intracellular PHA in the water. The suspension was further centrifuged at 9000 rpm for 15 min for obtaining the PHA as pellet. The pellet was washed with distilled repeatedly until it became white in colour. Further, the cell pellet was dried for further analysis. The PHA obtained from individual fermentations was analysed separately.

2.8. Characterization of PHA

2.8.1. FTIR-ATR analysis

The PHA powder obtained after drying was analysed by a FTIR spectrometer equipped with ATR (Bruker Platinum ATR, USA). The spectrum was recorded in the range of 400 to 4000 cm^{-1}.

2.8.2. TGA/DSC analysis

5 mg of dry PHA powder was weighed in a sealed aluminium pan. The pan was then subject to a linear temperature gradient (30 to 600 ℃) in a differential scanning calorimeter equipped with autoloader (Jupiter STA 449 F5, NETZSCH, Germany). The heating rate was maintained at 10 ℃.min^{-1}.

2.8.3. ^1H NMR analysis

The PHA produced was subject to ^1H NMR analysis. The powdered PHA was dissolved in $CDCl_3$ (concentration of 10 mg.mL^{-1}) and then analyzed in a 400 MHz spectrometer (Brucker, USA).

2.8.4. GC/MS analysis

The butyl esters of PHA was analyzed using GC–MS system equipped with auto-sampler (6890/5977A, G4513A; Agilent, USA) and with HP-5MS UI column (Agilent, USA). The column consisted of a stationary phase of 5% phenyl/methylpolysiloxane which was 30 m in length and an i.d. of 0.25 mm. Helium was used as a carrier gas (99.999 %) at a flow rate of 1.0 mL·min^{-1}. The samples were injected with a split ratio of 1:19 into the injector head at a temperature of 280 ℃. The sample injection volume was 0.2 μL. The conditions for separation of analyte were as follows: initial oven temperature at 70 °C which was held for 5 min and further augmented linearly to 280 °C, at a rate of 15 °C·min^{-1}. This was trailed by a linear temperature increase to 320 °C at a rate of 30 °C·min^{-1}, with a holding time of 5 min at the final temperature. Mass spectra analysis was performed in the EI positive ion mode, using electron energy of 70 eV. Transfer line temperature and ion source temperature were maintained at 280 and 250 °C, respectively. Obtained mass spectra data were collected in full-scan mode (m/z 50–400) and analyzed by using Agilent ChemStation software.

2.8.5. Molecular weight analysis

Gel Permeation Chromatography (GPC) analyses were performed to determine the weighted average molecular weight (M_w), number average molecular weight (M_n), z-average molecular weight (M_z) and poly-dispersity index (PDI) of the PHA biopolymer sample, using a high-performance liquid chromatography (HPLC) system fitted with a refractive index (RI) detector (Agilent 1260, Agilent, USA) and two Phenomenex columns (Phenomenex Inc., USA) at column temperature of 40 °C. Mobile phase used was Tetrahydrofuran (THF) which had a flow rate of 1 mL·min^{-1}. The sample was injected at an amount of 10 μL. Calibration of the GPC system was done with linear polystyrene and poly (methylmethacrylate), as the internal standards.

3. Results and discussion

3.1. Effect of different aeration rates on biomass and PHA productivity

The aeration rates required for mixing in outdoor cultivation of *Hfx. mediterranei* were optimized in the 1 L PET bottle experiments. The PET bottles were used as bubble column reactors for the sequential scale up from 100 mL cultivation volume to 1 L of reactor volume, as well as PHA production studies under outdoor cultivation conditions. The profiles for biomass and PHA concentration over time are shown in Fig. 3a and 3b respectively. After a cultivation time of 72 h, the volumetric productivity was calculated. This was also the maximum time for PHA production. The maximum volumetric biomass and PHA productivity were estimated to be 64.03 ± 0.11 mg·L^{-1}·h^{-1} and 34.07 ± 0.03 mg·L^{-1}·h^{-1} respectively at an aeration rate of 1.0 L min^{-1} or 1.0 vvm. A 10–15 % increase in biomass productivity was observed with an increase of 6–8 % in PHA productivity as compared to volumetric productivities observed in our previous study (Ghosh et al., 2019). Our previous study was performed in 100 mL bottles which were grown in media supplemented with 25% v/v seaweed hydrolysate. The archaea were cultivated aerobically with uniform mixing at 120 rpm and temperature of 42 ℃ in a shaking incubator. Lower aeration rates (up to 1.0 L min^{-1}) were suitable for biomass and PHA production whereas higher aeration rates (above 1.0 L min^{-1}) were suitable for biomass production rather than PHA accumulation. Our studies showed that 1.0 Lpm was the best aeration condition for PHA accumulation. At aeration velocities lower than 1.0 L min^{-1}, the PHA content increases and reaches to a maximum at 1.0 L min^{-1}. Therefore, the proper level of oxygen limitation is 1.0 L min^{-1}. Our studies also demonstrated that PHA production could be obtained along with growth of the organism thereby suggesting that it was a growth-related product formation. Studies on PHB production using glucose and glycerol as a substrate provided and insight on the effect of various aeration rates. They observed that at lower aeration rates, a higher PHB concentration was obtained. A higher aeration rate led to higher biomass production using various substrates but lower aeration rates provided higher PHB accumulation (De Almeida et al., 2010). Aeration rate of 1 vvm was observed to be optimal for maximum biomass and PHA production using *Cupriavidus necator* (Alves et al., 2019). Because oxygen is only slightly soluble in aqueous culture broths, even a short interruption of aeration results in the available oxygen becoming quickly exhausted, causing irreversible damage to the culture. Thus, uninterrupted aeration is necessary. The accumulation of PHA in the cells of *Hfx. mediterranei* might be attributed to the fact that at lower aeration rates there is a limitation of dissolved oxygen in the cultivation medium. Under limited oxygen conditions with an excess of carbon in

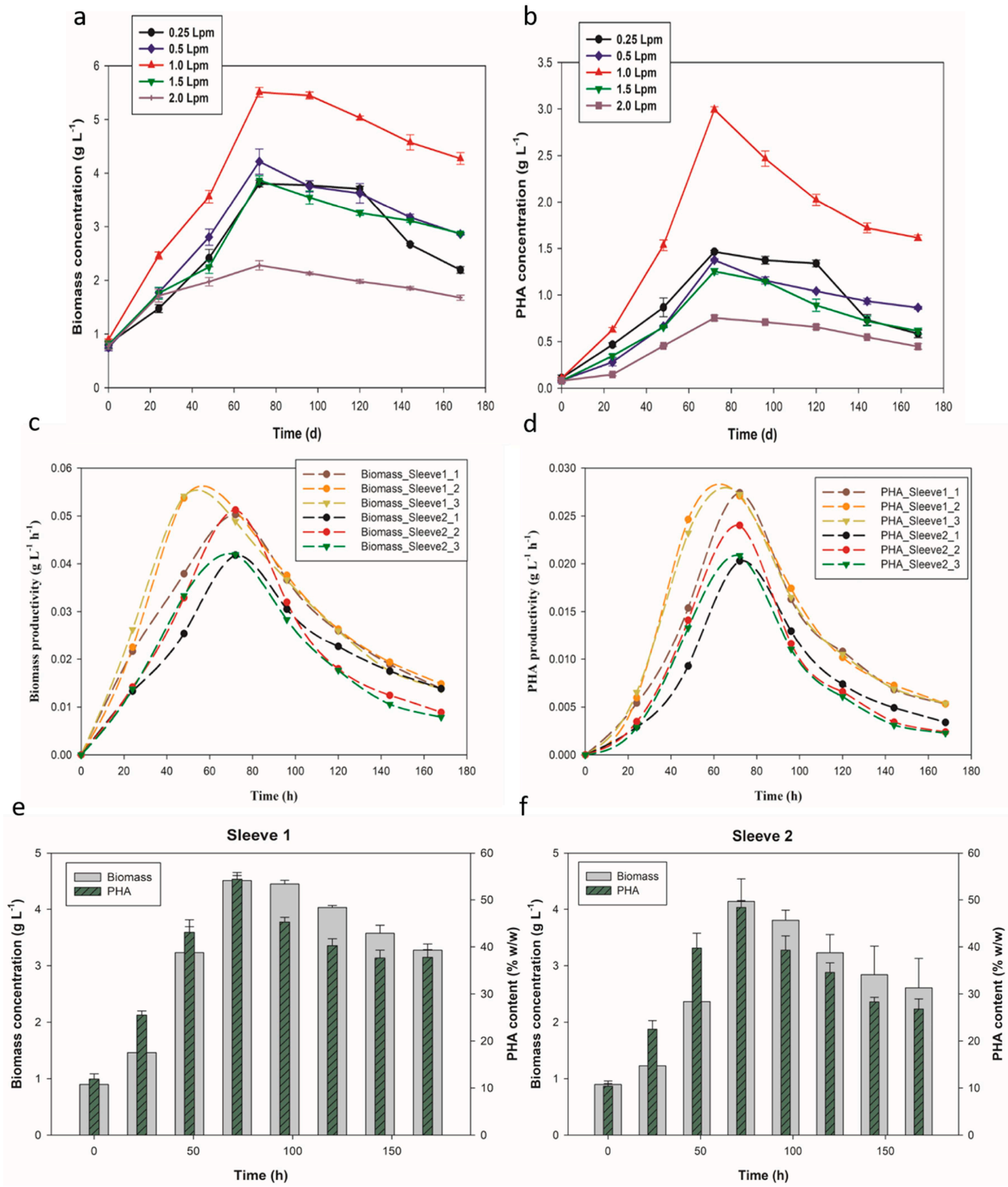

Fig. 3. a) & b) Growth and PHA production curves at different aeration rates for PHA production using *Haloferax mediterranei* [Experimental conditions: Flow rates: 0.25 to 2.0 L min^{-1}; Fermentation volumes: 800 mL; Water temperature: 42 ± 2.5 °C; Air temperature: 37 ± 2.5 °C; Inoculum concentration: 50 g·L^{-1}; Initial pH: 7.2]. **c) & d)** Growth and PHA productivities under outdoor cultivation conditions for PHA production using *Haloferax mediterranei* [Experimental conditions: Flow rates: 1.0 L min^{-1}; Fermentation volumes: 10 L; Water temperature: 42 ± 2.5 °C; Air temperature: 37 ± 2.5 °C; Inoculum concentration: 50 g·L^{-1}; Initial pH: 7.2]. **e) & f)** Change in PHA% in biomass with respect to time in Sleeve 1 and Sleeve 2 respectively.

the medium, NADPH oxidase activity decreases which further leads to an increase in overall NADPH concentration. This would in turn inhibit citrate synthase and isocitrate dehydrogenase thereby increasing the acetyl-CoA concentration of the medium. The excess acetyl CoA would thus be channelised to accumulation of storage products in the cells such as lipids or PHA. The high NADPH/NAD ratio caused by oxygen limitation promotes to PHB synthesis and PHB plays the role as an alternative electron acceptor (Kemavongse et al., 2008).

3.2. Effect of different cultivation time on biomass and PHA productivity

The effect of different cultivation time was studied in 1 L PET bottles at an aeration rate of 1 vvm. The maximum biomass and PHA productivities of 68.01 ± 0.11 mg·L^{-1}·h^{-1} and 28.02 ± 0.03 mg·L^{-1}·h^{-1} respectively were observed at a cultivation time of 72 h (Fig. 3c and 3d). The productivity increases with an increase in time, i.e., up to 72 h after which the productivity started to decrease. The experiments were conducted up to 196 hrs. PHA productivity decreased significantly after 72 hrs. This might be due to the consumption of PHA by the organism during the starvation phase. *Hfx. mediterranei* produces PHA as a storage energy product and have intracellular PHA depolymerases which allows the PHA to be utilized for survival under stress conditions. Cultivation time was supposed to be an important parameter for enhancement of PHA production using waste glycerol as a substrate (de Albuquerque et al., 2018). It was observed that by limiting the growth phase, a nitrogen stress is provided to the cells which in turn enhances the PHA content of the cells. The PHA accumulation starts at the logarithmic phase, increases with the biomass and reaches a peak at the beginning of the stationary phase. PHA synthesis is delayed with respect to biomass development, reaching a maximum rate of synthesis at the end of the exponential phase. This has also been observed in previous studies (Lillo and Rodriguez-Valera, 1990). This also suggests that PHA production in the present study was growth associated. This might be a key characteristic for conversion of the batch culture into a continuos system thereby increasing the PHA production.

The present study presented an alternate strategy for PHA production. Usually, for inexpensive PHA production from mixed microbial consortia (MMC), an aerobic dynamic feeding (ADF) strategy is utilized. The ADF process is often referred to as a feast-famine (F-F) process where the cells undergo an initial stage where they are fed with excess of external substrate followed by a later stage where there is an absence of the same (Cui et al., 2016). The present study utilizes a single step strategy where the organism can utilize external substrate in the growth phase thereby not requiring an additional step for production of PHA. This could be ascribed to the statistic that *Hfx. mediterranei* cultivates quicker than any known affiliate of the *Halobacteriaceae* family and also has an excessive salt tolerance. An additional exciting detail is that it is metabolically very adaptable: it cultivates both on complex media and on simple defined media, using perhaps the major variety of single carbon sources of any haloarchaeon, and it secretes exoenzymes that hydrolyse proteins, polysaccharides and lipids (Oren and Hallsworth, 2014). Moreover, the very high salinities (greater than25%) equips the process with lower levels of contaminants thereby eliminating the requirement for an enrichment step. The PHA content reported for ADF process are in the range of 25–70% DCW (Amulya et al., 2015) which is comparable to the yields observed in the present study. Therefore, the present strategy could provide an alternate for efficient and cost effective PHA production from wastes resources.

3.3. Scale up of PHA production by Hfx. Mediterranei in 40 L sleeves

We further scaled up the production process by cultivation in 40 L plastic sleeves. Initial cultivation volumes attempted were 10 L which were sequentially scaled up to 40 L. The experimental conditions were determined after optimization in our previous experiments – initial culture density of 50 $g{\cdot}L^{-1}$ (Ghosh et al., 2021), aeration rate of 1 vvm, and cultivation time of 72 h. The maximum mass fraction of PHA achieved in biomass was calculated to be 56% w/w. The maximum biomass productivity observed was 50.1 ± 0.11 $mg{\cdot}L^{-1}{\cdot}h^{-1}$ with a PHA productivity of 27.03 ± 0.01 $mg{\cdot}L^{-1}{\cdot}h^{-1}$. The conversion yield was calculated to be 0.107 g PHA·g $Ulva_{DW}^{-1}$. The carbon balance was also calculated from the present study. Out of the input carbon content of *Ulva* sp. biomass (33.4% C, as calculated by CHNS analysis), 32.03% was utilised for PHA production by *Hfx. mediterranei*. The remaining carbon was either utilised for volatile fatty acids (VFAs) production during fermentation or as residual carbon which remains unutilised in the medium. This could be confirmed by further analysis of the spent medium which is out of the scope of the present study. A slight decrease in overall productivities (biomass and PHA) was observed as compared to 1 L PET bottle cultivation. This might be due to inefficient air circulation within the reactor because we attempted a batch fermentation of 10 L in a 40 L bioreactor. Inefficient air circulation leads to unavailability of media components within the reactor. This might lead to decrease in accumulation of cell biomass thereby effectively decreasing the volumetric biomass and PHA productivities. This effect was observed in our studies which might be essential to explain the slight decrease in productivity within sequential steps of scale up for the process. Sequential scale up to 40 L cultivation could further increase the biomass and PHA productivities in the reactor. The biomass and PHA productivities showed a bell-shaped curve with the maximum productivities at 72 h cultivation time. The PHA production also showed similarity with biomass productivity suggesting that the production was a growth-dependent process which decreased with the cultivation time. The decrease in PHA content with time could be explained by the fact that PHA is produced in the archaeal cells as an energy storage product. With the increase in cultivation time, the nutritional stress in the cells increases leading to the utilization of PHA granules for energy generation (Obruca et al., 2020). Various wastes have been utilized for production of PHA. Batch cultivation experiments yielded a PHA productivity of 27 $mg{\cdot}L^{-1}{\cdot}h^{-1}$ where the fermentation experiment utilized tuna condensate as a substrate (Sangkharak et al., 2021).

Technologically, PHA is produced under controlled conditions in a bioreactor or fermenter which is operated in stirred tank mode (STR). The reactor can be operated discontinuously batch (Sangkharak et al., 2021), repeated batch (Gahlawat and Soni, 2017), fed-batch (Kim, 2000; Tan et al., 2011), as a continuous stirred tank reactor (CSTR) (Albuquerque et al., 2018) or CSTR in cascades (Atlić et al., 2011). The reactors are generally made of stainless steel which produces a challenge for the cultivation of *Hfx. mediterranei* at higher salinities (i.e., greater than 22% salinity). This can be overcome by the use of reactors made of polymers and/or ceramics which are non-corrosive (Hezayen et al., 2000). Moreover, pneumatically mixed reactors also could be utilized for the production of PHA with extreme halophiles as their construction and design are very simple. The mixing is done by air bubbles in the reactor which generates lower shear stress on the suspended cells and in turn, utilizes lower energy for mass transfer. Recently, airlift reactors (ALR) have been utilized to produce PHB from *H. boliviensis* from starch hydrolysate in a batch system. The use of an ALR for PHB production from various carbon sources in nitrogen depleted medium by *H. boliviensis* has successfully been demonstrated (Ortiz-Veizán et al., 2020). *Azohydromonas australica* and *C. necator* have reached about 72 % PHB by weight and a biomass concentration of 10 $g{\cdot}L^{-1}$ and 32 $g{\cdot}L^{-1}$, respectively (Gahlawat et al., 2012; Tavares et al., 2004), whilst cultivation of *Burkholderia sacchari* in an ALR has led to 41% by weight PHB and a maximum biomass concentration of 150 g L^{-1} (Pradella et al., 2010). Like airlift reactors, bubble column reactors could also be utilized for PHA production by extreme halophiles due to the various advantages related to pneumatically agitated bioreactors. These advantages include simple design and construction, ease of operation and lower shear stress as compared to stirred tank reactors. However, there are no reports on using the pneumatically agitated bioreactors for PHA production from seaweed hydrolysate using *Hfx. mediterranei*. In the present study, we utilized PET bottles (1 L) as well as sleeve-like macroalgae photobioreactors (40 L) which are non-corrosive and can significantly reduce maintenance costs. The high salinity of the medium also inhibits the growth of contaminants thereby increasing the possibility of outdoor cultivation using extreme halophilic archaea. In our very recent study on revenue assessment and sustainability (Ghosh et al., 2021), we demonstrated that the process was indeed feasible and could generate higher revenues for the seaweed farmer. Also analysing the greenhouse gas emissions to the current feedstocks for bioplastic production, we observed that our emissions were comparable. It was also suggested that efficient designs of the cultivation system (both offshore and onshore) could be a determining factor in the feasibility of the process. Another way we can increase the sustainability of the process is by producing PHA and biochar simultaneously in a biorefinery concept. We observed that when paired with biochar production, the economic feasibility and sustainability improves to a great extent (Ghosh et al., 2021). In the present study, we wanted to provide sustainable solutions to the onshore cultivation of seaweeds in pneumatically agitated reactors which could then be utilized for PHA production in an economically feasible manner. There are challenges related to further scale up of the process. The high salinity of the medium could prove to be an impediment in the whole bioprocess. A further unit for concentration of brine could be utilised where the outlet from the system can be recycled to the cultivation system. In addition to this, as mentioned above, the process can be operated in a biorefinery concept where various products can be achieved from a single biomass substrate thereby increasing the cost efficiency of the process.

3.4. PHA structural analysis (FTIR, TGA/DSC, ^{1}H NMR, GC–MS)

3.4.1. FTIR spectroscopy

FTIR study of the PHA presented several absorption peaks. A peak was detected near 3290 cm^{-1} which could be ascribed to the stretching of hydroxyl (O – H) group. Characteristic bond vibrations for PHA (1720 – 1740 cm^{-1}) were observed and could be accredited to the carbonyl bond (C = O) vibrations. Methyl (CH_3) and Ethyl (CH_2) group stretching were detected at 2914 cm^{-1} and 2879 cm^{-1} respectively. Some other peaks were observed in the range of 1450 – 1000 cm^{-1}. These peaks showed various bond vibrations such as bending of CH_3 group, wagging of CH_2 group and stretching of C – O, C – C and C – O – C. The observations proposed that the polymer was Polyhydroxy (3hydroxybutyrate-co-3hydroxyvalerate) (P(3HB-co-3HV)) in nature. P (3HB-co-3HV) copolyesters production without supplementation of 3HV-structurally related precursor compounds like, e.g., valerate, is a scarce feature and typically found in some haloarchaea like *Hfx. mediterranei* (Koller, 2019).

3.4.2. TGA/DSC thermal analyses

The degradation temperature minima (T_d) of the P (3HB-co-3HV) from *Hfx. mediterranei* was found to be 248 ℃. During the decomposition process, the polymer went through a weight loss which was euivalent to ∼ 70.3%. The melting temperature was observed to be 177.1 ℃.

3.4.3. GC MS and ^{1}H NMR analysis

The GC–MS analysis showed two peaks in the chromatogram which had acquisition times of 9.46 and 10.654 min. After mass spectrum analysis, the peaks suggested that they were butyl esters of 3-hydroxybutyrate (3HB) and 3-hydroxyvalerate (3HV). The GC–MS spectrum suggested that the polymer produced by *Hfx. mediterranei* consisted of the monomers 3HB and 3HV. The ^{1}H NMR spectrum mostly suggested the carbon skeleton of the molecule by ascribing the proton peaks of different groups. According to the spectrum, peaks at 0.83 and 1.26 ppm suggested the presence of CH_3 group which could be related to the presence of 3HB and 3HV group in the polymer repectively. At 1.57 and 2.58 ppm, peaks were observed for CH_2 group of 3HV and at 2.48 ppm for 3HB. CH group peaks were observed at 5.25 ppm. The monomer compostions of the PHA were calculated according to the ^{1}H NMR spectrum. The calculations yielded a compostion of 90.4% 3HB and 10.6% 3HV.

3.4.4. The molecular weight of PHA produced

The molecular weights for PHA produced at different air velocities were determined using Gel Permeation Chromatography (GPC). Table 1 shows the average molecular weight (M_w), number average molecular weight (M_n), Z-average molecular weight (M_z) and PDI of PHA extracted from *Hfx. mediterranei* at various air velocities (0.25 – 2.0 L min^{-1}). The highest average molecular weight (M_w) of PHA (811 kDa using polystyrene standard, 770 kDa using PMMA standard) was obtained at an air velocity of 0.25 L min^{-1} with a PDI of 1.608 (polystyrene)/ 1.544 (PMMA). The obtained values were similar to molecular weights from previous studies of high-quality PHBV production from *Haloferax mediterranei* where they obtained a polymer with a molecular weight of 1057 kDa with a polydispersity index of 1.5 (Koller et al., 2007). In the present study, the average molecular weights (M_w) ranged from 679 to 811 kDa with a PDI range of 1.6 – 2.18 when polystyrene was used as a standard. Using PMMA as a standard, the average molecular weights (M_w) ranged from 656 to 770 kDa with a PDI range of 1.544 – 1.959. An increase in PDI was observed when the corresponding air velocities were increased (Fig. 4). This suggested that the polymer was more homogeneous at lower mixing velocities. The outdoor studies yielded PHA with an average molecular weight of 716 kDa and a PDI of 1.592 which was following our previous results of optimization of mixing velocity. Various studies have been performed to determine the impact of various parameters on the molecular weight of PHA. The dependence of PHA synthase activity on the molecular weight distribution and PDI of PHA was studied by Sim et al., 1997 (Sim et al., 1997). Another study claimed that the molecular weight was a function of the type of substrate used (Quagliano et al., 2001). The dependence of the molecular weight of PHA in different mixing velocities was observed in a study by Kshirsagar et al., 2013 (Kshirsagar et al., 2013). The present study demonstrated that by controlling the agitation and aeration velocity we could alter the molecular weight and PDI of the PHA produced. The ability to control the molecular weight of PHA is an advantage in polymer manufacturing and processing as well as it offers a wide range of industrial applications for PHA in the plastic industry.

3.5. Biomass settling behavior and particle size

Post-cultivation dewatering targets producing a non-soluble solids concentration of around 25 wt% or 250 gdw·L^{-1} as a feed condition for PHA extraction and conversion of other biomaterials to biofuel. Observation of the settling behavior provides insights into single and multiparticle phenomena that impact and limit the dewatering process. All suspensions initially displayed settling characteristics typical of a monodisperse population, with increasing polydispersity and aggregation as the biomass concentration exceeded 2x (Lerche and Sobisch, 2014). Hindered settling was not observed over the 2-5x concentration range investigated, but could be concealed by the effects of aggregation. The cells at the bottom of the cuvette exhibited high compactibility at the centrifuge spin rate, with the height of the pellet less than the measurable minimum of 0.1 mm. This minimum condition is typical of microbe cultures capable of high levels of dewatering, and corresponds to a concentration increase greater than 200-fold relative to the initial suspension.

Low settling velocities associated with small particle diameters were observed. Settling velocity distributions of *Hfx. mediterranei* cells in the culture and washed suspensions are provided in Fig. 5**a**. The culture provided a median settling velocity of 0.0204 ± 0.0006 μm·s^{-1}, and the washed suspensions with and without Ca^{2+} were 0.0244 ± 0.0010 μm·s^{-1} and 0.0273 ± 0.0006 μm·s^{-1}, respectively. The culture and washed suspension viscosities were 1.43 and 1.23 mPa·s at 20 °C, respectively, and assuming the same temperature dependence as seawater (Beaton, 2017), are estimated as 1.74 and 1.50 mPa·s at 12 °C. The lower settling velocity of the culture results from its higher viscosity and lower concentration. The effect of biomass concentration on settling velocity shows a steady increase in settling velocity with concentration as caused by cell–cell interactions and aggregation. The median cell

Table 1
Molecular weight distribution and polydispersity indices (PDI) vs various standards (polystyrene and PMMA) for PHA produced from *Haloferax mediterranei* at different aeration rates.

Aeration rate(L min^{-1})	Polystyrene standard				Poly (methyl methacrylate) standard			
	M_n(kDa)	M_w(kDa)	M_z(kDa)	PDI(M_w/M_n)	M_n(kDa)	M_w(kDa)	M_z(kDa)	PDI(M_w/M_n)
0.25	504	811	1209	1.608	498	770	1114	1.544
0.50	422	750	1144	1.776	442	721	1046	1.630
1.0	445	735	1102	1.652	447	703	1016	1.572
1.5	349	723	1172	2.072	371	697	1082	1.879
2.0	311	679	1117	2.180	335	656	1032	1.959

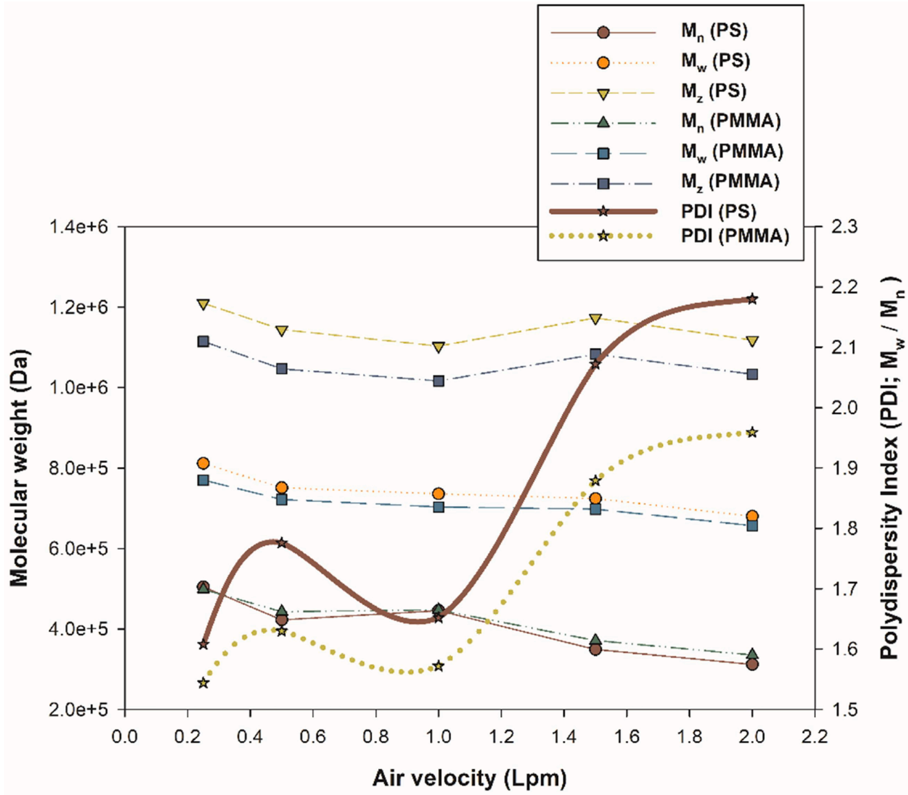

Fig. 4. Molecular weights (M_n, M_w, and M_z) and Polydispersity Index (PDI) distributions of PHA produced by *Hfx. mediterranei* using two standards, polystyrene (PS) and polymethyl methacrylate (PMMA).

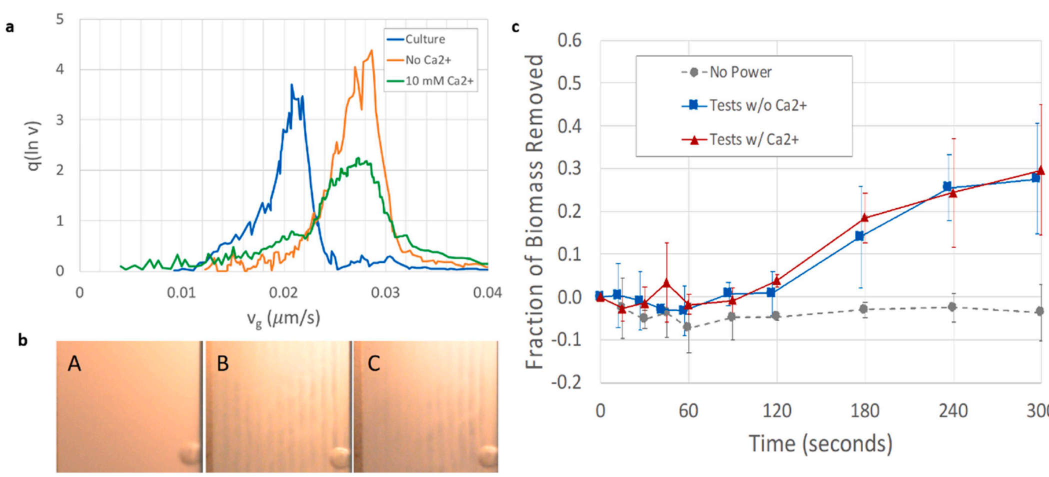

Fig. 5. a) Settling velocity distributions of *Hfx. mediterranei* cells in the original culture and the following washing as measured at 12 °C. The higher viscosity of the culture suspension shifts the velocity distribution to lower values. **b)** Images were taken in the standing wave during ultrasonic separation tests on *Hfx. mediterranei*. The bubble in the lower right is a vessel assembly artifact and serves as a positional reference. No power is applied to the control test (Image A). Concentration lines at 300 s in the suspension with (Image B) and without Ca^{2+} (Image C). **c)** Removal of *Hfx. mediterranei* in batch ultrasonic tests. Sample time in the tests without Ca^{2+} is shifted by 3 s to avoid symbol overlap. Error bars represent the standard deviation obtained from 3 replicates.

diameters calculated from the settling velocity distributions are between 0.7 and 2 μm, and do not differ between the culture and washed suspensions. The consistency in particle size between the 3 suspensions indicates the attempt to enhance cell aggregation by washing, resuspending, and adding Ca^{2+} was unsuccessful.

3.6. *Ultrasonic harvesting of Hfx. Mediterranei biomass*

Concentration lines were not observed upon application of the ultrasonic standing wave to the as-grown culture of *Hfx. Mediterranei*. Biomass removal in low concentration suspensions is difficult to observe and quantify, and is likely an important factor in this result. The washed cells were resuspended at approximately twice the concentration of the culture and responded to the standing wave with visible concentration lines as shown in Fig. 5**b**. Removal in the washed suspensions approached 30% as shown in Fig. 5**c**. Each data point represents the average removal obtained from three tests and the error bars represent the standard deviation. Removals with and without Ca^{2+} ions did not differ significantly, reinforcing the observation from settling velocity measurements that cell aggregation was not enhanced with the addition of Ca^{2+}. The total energy input over the 5-minute treatment period for tests on Ca^{2+}-free and Ca^{2+}-containing suspensions was very similar averaging 7.85 ± 0.10 and 7.77 ± 0.08 kWh·m^{-3}, respectively. Such high energy inputs attest to the difficulties of dewatering suspensions of *Hfx. mediterranei*.

3.7. Inferences from the dewatering of Hfx. Mediterranei

Dewatering operations seek to increase the biomass concentration and its associated energy density well beyond the culture condition to bolster the sustainability of the PHA and biofuel production process. The initial dewatering stage is most challenging as the concentration of biomass energy is at its lowest level and the technology employed can have the highest impact on the overall energetic and economic sustainability. This is more readily apparent by considering the fractional energy cost of dewatering (or FECD).

$$\mathrm{FECD} = \frac{\overline{E}_{\mathrm{Dewater}}}{\mathrm{HHV}_{\mathrm{Biomass}} C_{\mathrm{B,Feed}} F_{\mathrm{BR}}} \tag{4}$$

$\overline{E}_{\mathrm{Dewater}}$ is the energy consumed for dewatering in kWh·m^{-3}, $\mathrm{HHV}_{\mathrm{Biomass}}$ is the higher heating value or energy content of the biomass in kWh·kg^{-1}, $C_{\mathrm{B,Feed}}$ is the concentration of biomass in the feed stream in g·L^{-1} (or kg·m^{-3}), and F_{BR} is the fraction of biomass removed. Equation 5 places the energy consumed for dewatering within the framework of the biomass energy content. Assuming an HHV value of 6.43 kWh·kg^{-1} ash free dry weight (AFDW) (Cordier et al., 1987), the FECD of *Hfx. mediterranei* with ultrasonic harvesting is shown in Fig. 6. The abscissa indicates the biomass removed per volume of culture or suspension, and the ordinate provides the FECD. Energy consumption is noted by the diagonal lines arranged by log scale from 0.1 to 10 kWh·m^{-3} and is technology dependent. Operating between 4 and 8 kWh·m^{-3}, the FECD of the ultrasonic dewatering tests indicates less energy was used than contained in the recovered biomass. However, this result is considerably higher than the suggested target of 0.1 (Ferrell and Sarisky-Reed, 2010). The minimum FECD was between 0.6 and 0.7, which does not position ultrasonic separation as a strong candidate for dewatering at the culture condition or the 2x concentration condition tested. Increasing the responsivity of the biomass via aggregation would improve the recovery and reduce the amount of energy needed for removal. For example, increasing the settling velocity by a factor of 50 would yield complete recovery and meet the FECD target. Alternatively, increasing the biomass concentration to around 30 g·L^{-1} could meet the FECD target, even with a biomass recovery as low as 30%. Ultrasonic dewatering in batch and continuous flow operations yield indistinguishable particle removals when hydrodynamic effects are negligible, which requires additional tests not included in this preliminary study.

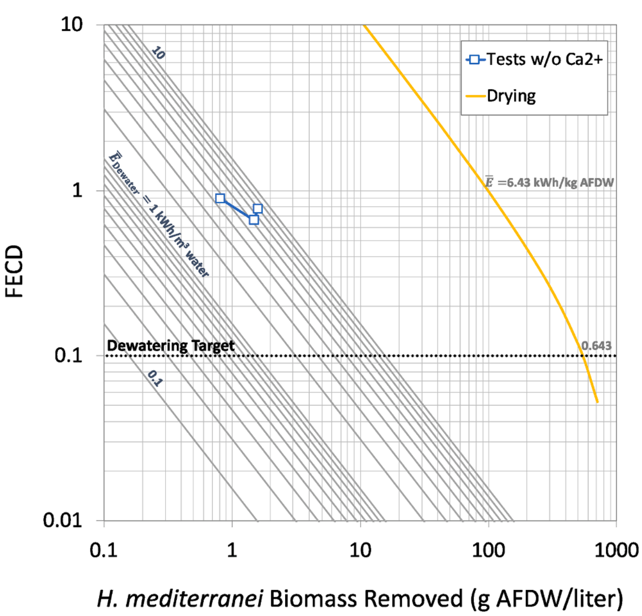

Fig. 6. Cost of dewatering as a fraction of the energy content in the dewatered *Hfx. mediterranei* biomass. Results from the ultrasonic dewatering tests of the washed suspension without Ca^{2+} are shown as symbols. See text for placement of other dewatering technologies.

Other candidate dewatering technologies become apparent when considered within the FECD paradigm. Gravity settlers require a footprint estimated to be around 50 times larger than an ultrasonic dewatering system of similar capacity, but the rake motor consumes less energy than 0.01 kWh m^{-3} (Perry and Chilton, 1973). However, the settling velocity of *Hfx. mediterranei* would have to be increased by a factor of 7000 to exceed the minimum rise rate (135 μm·s^{-1}) needed for biomass recovery. Increasing biomass concentration without increasing the settling rate would result in loss of all biomass in a gravity settler. Dead end filtration can operate in the 0.1 to 1 kWh·m^{-3} energy band (Perry and Chilton, 1973), but requires large membrane areas with a significant footprint (Nagy, 2019). However, frequent backflushing and periodic chemical cleaning to overcome membrane fouling increases the complexity of operation as well as the system footprint (Reid and Adlam, 1976). Operating in this low energy band suggests dead end filtration could meet the dewatering target at the current culture condition. Crossflow filtration reduces the cake build-up on the membrane, but pushes energy consumption to greater than 100 kWh·m^{-3} (Bhattacharjee et al., 2020) without eliminating the need for backflushing and chemical cleaning. While centrifugation operates in a similar energy band as ultrasonic dewatering (1–10 kWh·m^{-3}), the low settling velocity of *Hfx. mediterranei* reduces flow rates. While a centrifuge footprint is relatively small, they introduce safety issues for workers requiring noise abatement and isolation. Increasing the biomass concentration in the culture to greater than 10 g·L^{-1} would be necessary to meet the FECD target with centrifuges. As shown in Fig. 6, drying is even more energy intensive than crossflow filtration and not a practical option except at extreme dry weight concentrations (greater than50 wt%).

4. Conclusions

The current work shows a feasible way to scale up PHA production from inexpensive substrates such as macroalgal hydrolysate. The effect of various cultivation parameters was studied and favourable conditions for enhancement of PHA productivity were established. A novel approach for separation of archaeal cells from the cultivation medium using ultrasound waves was utilized in the present study where separation efficiencies of around 30 % were obtained. The molecular weight analysis suggested that the PHA obtained was more homogeneous at lower aeration velocities. Further studies in a pilot-scale fermentation (e.g., 1000 L) are required for commercial and large scale PHA production.

CRediT authorship contribution statement

Supratim Ghosh: Investigation, Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **Jim Coons:** Investigation, Data curation, Formal analysis, Writing – review & editing. **Chris Yeager:** Investigation, Formal analysis, Writing – review & editing. **Peter Halley:** Investigation, Data curation, Writing – review & editing. **Alexander Chemodanov:** Investigation, Data curation. **Bogdan Belgorodsky:** Investigation, Writing – review & editing. **Michael Gozin:** Investigation, Writing – review & editing. **Guo-Qiang Chen:** Investigation, Writing – review & editing. **Alexander Golberg:** Conceptualization, Methodology, Writing – original draft, Writing – review & editing.

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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