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RESEARCH ARTICLE

Environmental biotechnology

Poly-β-hydroxybutyrate (PHB) production potential of naturally existing cyanobacterial blooms

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Abstract: Detrimental effects imposed by petrochemical plastics on the environment are among the most often discussed concerns in the current era. Although the scientific community has discovered numerous eco-friendly alternatives, the cost of manufacture has restricted the usage of such material. The present study focused on the direct utilization of naturally existing cyanobacterial blooms to extract biodegradable poly-β-hydroxybutyrate (PHB) aiming to minimize the cost of production by eliminating the need for growing of cyanobacterial monocultures. This attempt provides remedies for plastic pollution and hazardous cyanobacterial blooms simultaneously. Fresh cyanobacterial bloom samples were collected from the hypereutrophic Beira Lake, Colombo. They were maintained under *in vitro* conditions for 7 days, provided with a 12/12 hours light/dark cycle, and deprived of an external supply of nutrients. PHB production was optimized for Nitrogen, Phosphate, and Carbon sources: glucose, sucrose, and lactose. The extracted PHB was quantified by spectrophotometric analysis. Structure confirmation was carried out using FTIR and Raman spectroscopy. The mean percentage weight of PHB yield was 7.13 ± 0.12% w/w. Optimization studies showed that nitrate deficiency and the presence of glucose as an exogenous carbon source imposed a stimulatory effect for PHB accumulation by cyanobacteria. The maximum amount of PHB (9.6% w/w) was found to be accumulated by cyanobacteria on the fourth day following the bloom sample collection. Hence, the present study proposes a sustainable utilization method of cyanobacterial blooms as a promising source for PHB production.

Keywords: Beira Lake, biodegradable plastics, cyanobacteria, poly-β-hydroxybutyrate (PHB), polyhydroxyalkanoates (PHAs).

INTRODUCTION

In the modern era of technology, plastics have become some of the most widely used and indispensable materials worldwide. Plastics are incorporated with home appliances, components of automobiles, computer equipment, packaging, medical tools and devices, and many such essential items that are regularly used (Rahimi & Garcia, 2017). Due to their light weight, stability, durability, and economic feasibility, plastics have replaced wood, glass, and metal components in domestic and industrial applications. Conventional petrochemical plastics are the most extensively used and are generally inexpensive (Geyer *et al*., 2017). Nonetheless, such synthetically produced polymers cause serious environmental problems due to their persistent nature (Chen & Patel, 2012; Muhammadi *et al*., 2015).

These non-biodegradable plastics take a very long time to degenerate and therefore accumulate in the environment creating a large spectrum of environmental impacts (Welden, 2020) as well as animal and human health impacts (Halden *et al*., 2010, Awuchi & Awuchi, 2019). Severe environmental concerns like safe disposal,

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solid waste management, and plastic waste incineration justify the production of materials like green polymers, biodegradable plastics, or bioplastics utilizing renewable resources (Singh *et al*., 2017).

Biodegradable plastics are a class of bioplastics produced from biobased materials and biodegradable into elemental and simple substances (Carpine *et al*., 2015). PHAs are polymers that are entirely of microbial origin and are composed of various hydroxyalkanoic acids as their monomer units (Muhammadi *et al*., 2015). PHA biopolymers are predominantly linear, head-to-tail thermoplastics with hydroxyalkanoic acid as monomer units. The carboxyl group of one monomeric unit forms an ester bond with the hydroxyl group of the adjacent monomeric unit. They are described by the common structural formula as shown in Figure 1. Each PHA monomeric unit possesses a side chain R group which can differ from a hydrogen to a methyl to a tridecyl group (Muhammadi *et al*., 2015).

PHA bioplastics can be subdivided into three groups; short-chain-length-PHAs (SCL-PHAs), medium-chain-length-PHAs (MCL-PHAs), and long-chain length-PHA (LCL-PHAs). Among SCL-PHAs, polyhydroxybutyrate (PHB) is the most widespread in various bacterial and cyanobacterial species. The chemical structure of PHB is illustrated in Figure 2. PHB has gained the scientific community's interest due to its properties such as thermoplastic processability, hydrophobicity, complete biodegradability, biocompatibility, optical purity and piezoelectricity (Singh *et al*., 2017).

Many microorganisms are known to produce PHB as an intracellular energy and carbon storage product (Ansari & Fatma, 2016; Troschl *et al*., 2017). The biosynthesis of this type of polymers is limited to prokaryotic organisms and usually formed as intracellular inclusions under stressed growth conditions; that is, in the presence of an excess of a carbon source and a limited nutrient supply, particularly nitrate and phosphate (Balaji *et al*., 2013; Muhammadi *et al*., 2015). As a result of these unbalanced growth conditions, reduction equivalents created by metabolic oxidation processes are stored in a water-insoluble, chemically and osmotically inert form, which is PHB or any other kind of PHA (Chong *et al*., 2021).

The worldwide progression of the PHB thermoplastic market is powered by its variety of uses in different arenas and the ever-increasing interest in eco-friendly alternatives. Commercial PHB production is based on the pure cultures of natural PHB producing heterotrophic bacteria: *Ralstonia eutropha*, *Alcaligenes* sp., *Azotobacter* sp., *Bacillus* sp., *Nocardia* sp., *Pseudomonas* sp. and *Rhizobium* sp. (Chandani *et al*., 2014).

Successful and viable synthesis of PHB requires the optimal incorporation of technological innovations with economic feasibility for their commercial production and marketing. Therefore presently, to overcome such limitations, advanced and intensified research on the exploitation of photoautotrophic hosts (plant systems and cyanobacteria) is receiving much attention over the heterotrophic bacterial systems with an ultimate aim to produce cost-effective PHB (Sakthiselvan & Madhumathi, 2019).

Cyanobacterial production of PHA thermoplastics was described for the first time in a nitrogen fixing cyanobacterium, *Chlorogloea fritschii*, grown with acetate supplementation (Singh *et al*., 2017). To date, the occurrence of PHB has been reported in many cyanobacterial species, including *Spirulina* sp., *Aphanotheca* sp., *Microcystis* sp., *Nostoc* sp., and *Synechocystis* sp. (Balaji *et al*., 2013). The main advantages of using cyanobacteria for the production of PHB instead of using conventional heterotrophic bacteria and plant sources are, (a) cyanobacteria do not compete for resources with the agro-food market; (b) the contribution of cyanobacteria for the carbon sequestration decreases the burden of the greenhouse gas released to the atmosphere; and (c) the nutrient requirement of cyanobacteria is minimal (Gopi *et al*., 2014; Carpine *et al*., 2015).

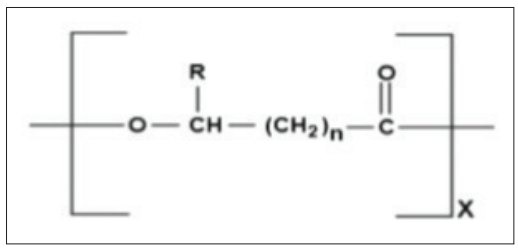

Figure 1: Structure of PHAs (Brandl *et al*., 1990)

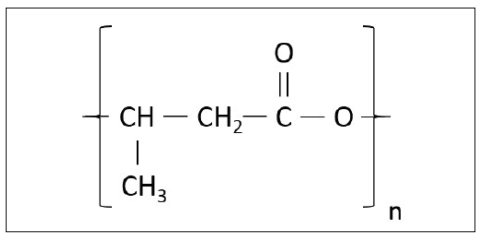

Figure 2: Structure of PHB

Although the cyanobacterial PHB has been the subject of research for many years, it has not reached the market (Kamravamanesh *et al*., 2018). Cyanobacterial monocultures are yet an expensive alternative correlated to the low yield they provide compared to heterotrophic bacteria. Thus, the present study aimed to develop a method to extract PHB from naturally occurring cyanobacterial blooms, which will reduce the cost of culturing the microbes for PHB production and concurrently provides a novel use of problematic algal blooms.

In Sri Lanka, most of the eutrophicated freshwater bodies are conquered by the cyanobacterial genus *Microcystis*. Beira Lake is a hypereutrophic lake situated in Colombo city, where the cyanobacterial species *Microcystis aeruginosa* and *Spirulina* sp. are abundant throughout the year (Idroos & Manage, 2014). Both these genera have been recorded as PHB synthesizers in variable amounts (Balaji *et al*., 2013; Ansari & Fatma, 2016). Thus, the Beira Lake was selected to collect cyanobacteria bloom samples for PHB extraction in the present study.

MATERIALS AND METHODS

Sample collection

Before collecting the bloom samples, the temperature, pH, and electrical conductivity (EC) of the surface water were recorded at the site (GPS location - 6˚55'02" N 79˚51'16" E). Approximately 15 litres of bloom samples were collected into plastic containers and transported to the laboratory. The concentrations of total phosphate and nitrate of the samples were determined using standard methods (Silva *et al*., 1996).

Identification of cyanobacteria

The morphological features of colonies and solitary species in the sample were identified following the standard phytoplankton and cyanobacteria identification keys (Manage, 2012).

Microscopic visualization of PHB

PHB accumulated in cyanobacteria was visualized microscopically using the Sudan Black B staining method (0.3% Sudan Black B in 70% ethanol), following the heat fixed procedure (Wei *et al*., 2011). The slides were immersed in xylene for 5-10 minutes for complete decolorization and then the smear was counterstained with safranine solution (0.5% in water) for 10 seconds followed by gentle rinsing with running tap water. The slides were allowed to dry and then observed under oil immersion using a light microscope (at a magnification of 10×100), and the images were captured by Microscopic Image Projection System (MIPS).

Extraction of PHB

The cyanobacteria bloom samples were stored at room temperature for 7 days under laboratory conditions. Subsequently, the samples were freeze-dried and stored at 4˚C. PHB was extracted using a modification of the method described by Yellore and Desai (1998). A weight of 100 mg of dry biomass was added into an Erlenmeyer flask with 100 mL of analytical grade methanol, followed by sonication for 15 minutes in ice, and stored overnight at 4˚C to remove pigment (Ansari & Fatma, 2016). Subsequently, the samples were centrifuged at 8000 rpm for 20 minutes, and the resulting pellet was dried at 60˚C for 20 minutes to complete the removal of methanol. The polymer was extracted by boiling with chloroform and precipitated with cold diethyl ether. The precipitate was separated by centrifuging at 9000 rpm for 30 minutes and the pellet obtained was washed with acetone and dissolved in hot chloroform, followed by evaporation at 4˚C to obtain the crude extract of PHB.

Determination of the optimum day for yield of PHB

The biosynthesis and utilization of PHB are affected by the intensity of the nutrient stress. Therefore, bloom samples were maintained at a 12/12 light/dark cycle in the laboratory for 7 days without providing any nutrient supplements. Then the production of PHB was determined following the method described. Equal volumes (500 mL each) of bloom samples were stored in 7 similar glass jars, and 100 mL of sub-sample was collected and freeze-dried from one jar per day. PHB was extracted from known weights of dried biomass, and the yield was calculated each day.

Optimization of an exogenous carbon source

PHB production was optimized by changing carbon sources as given in (Table 1).

Nutrient optimization

Optimization was carried out to study the effect of nitrate and phosphate concentrations on PHB yield. The samples were provided with 1 mg/L and 2 mg/L of nitrate (potassium nitrate) and phosphate (anhydrous potassium phosphate), respectively, in excess of their initial concentrations recorded in the field. The samples were maintained in the laboratory for 3 days and the yields of PHB in each sample were quantified.

Table 1: Optimization for the exogenous carbon sources

Jar number	The volume of the algae sample (mL)	Supplement added	The volume of supplement added (mL)
1 (L)	800	Lactose (1g)	200
2 (S)	800	Sucrose (1g)	200
3 (G)	800	Glucose (1g)	200
4 (C)	800	Distilled water	200

Spectrophotometric analysis

Both qualitative and quantitative analyses were performed with Thermo Scientific GENESYS 10S Series UV-Vis spectrophotometer.

The conventional protocol for the determination of PHB is based on the conversion of PHB into crotonic acid (2-butenoic acid) when heated in conc. H_2SO_4 and measuring its absorbance at 235 nm (Ansari & Fatma, 2016). Initially, the UV spectrum was obtained for crotonic acid derived from standard PHB, and the wavelength which gives the highest absorbance (λ_{max}) was confirmed. Then a standard curve was prepared using absorbance measurements for a series of known concentrations.

PHB extracted from 100 mg of dry microalgal biomass was taken into a boiling tube. Subsequently, 10 mL of conc. H_2SO_4 acid was added and heated at 100˚C for 20 minutes.The absorbance was measured at the λ_{max} obtained from the scanning spectrum of PHB. The PHB concentration of the sample was determined using the standard curve. The average weight percentage yield of PHB was calculated using the following equation.

$$\text{average weight \% yield of PHB} = \frac{\text{Mean weight of PHB extracted}}{\text{Weight of dry algal biomass}} \times 100$$

Raman spectroscopy analysis

Raman spectra were obtained for the standard PHB and the crude extract using a DXR2 Smart Raman instrument, and overview spectra were acquired in the range of 60–3300 cm^{-1}.

Statistical analysis

All the tests were conducted in triplicate, and the calculations of statistical mean, standard deviation, standard error and pair-wise comparison of quantitative data were performed using MINITAB 14 software.

RESULTS AND DISCUSSION

Cyanobacterial blooms are a widespread nuisance, abundantly found in eutrophic surface waters worldwide. The mass development of planktonic cyanobacteria forming dense and occasionally toxic blooms in freshwater, brackish, and marine environments threatens ecosystem functioning and human health by causing the water quality for recreation, drinking, and fisheries to deteriorate (Idroos *et al*., 2017). The present study focused on directly utilizing existing cyanobacterial blooms to extract biodegradable PHB, simultaneously providing a considerable remedy to hazardous cyanobacterial blooms availability of other organisms such as heterotrophic bacteria, protozoans, and zooplankton as predators in the ambient environment.

Many species of cyanobacteria are known to perform both autotrophic and heterotrophic production of PHB under illuminated and non-illuminated conditions, respectively. Under non-illuminated environments, both *Microcystis* sp. and *Spirulina* sp. can utilize organic carbon sources available in the environment for their metabolism (Chen *et al*., 1996). The bloom samples were provided with a 12/12-hour light/dark cycle in the present study. Since solar energy is unavailable during non-illuminated hours, it can be assumed that heterotrophic

production of PHB is carried out mostly within the dark 12 hours. Sharma and Mallick (2005) stated that dark periods are required for PHB accumulation in photoautotrophic cultures. A 12/12-hour light/dark cycle has been recognized as the optimum light condition for PHB production in cyanobacterial monocultures (Sharma & Mallick, 2005).

Identification of PHB producing cyanobacteria

Microscopic analysis of the collected bloom samples showed the presence of three species namely *Microcystis incerta*, *Microcystis aeruginosa,* and *Spirulina platensis*. Of these, *M. aeruginosa* was the most dominant and *Spirulina platensis* was observed occasionally.

Microscopic visualization of PHB

PHB is a lipid-like compound that accumulates in granules in the cytoplasm of cyanobacterial cells, which can be visualized under the light microscope by staining with Sudan black B. Sudan black B is a lipophilic stain that stains lipid-rich environments. Since cyanobacteria are visible under the light microscope, owing to the presence of pigments chlorophyll and phycobilin, counterstaining with Safranin is not mandatory. Intracellular vesicles containing PHB were visible in black colour after being stained with Sudan Black B. It was noticeable that the intracellular vesicles containing PHB in *Microcystis* spp. were more numerous on day three than on day one. PHB granules were not observed in *Spirulina* on day one.

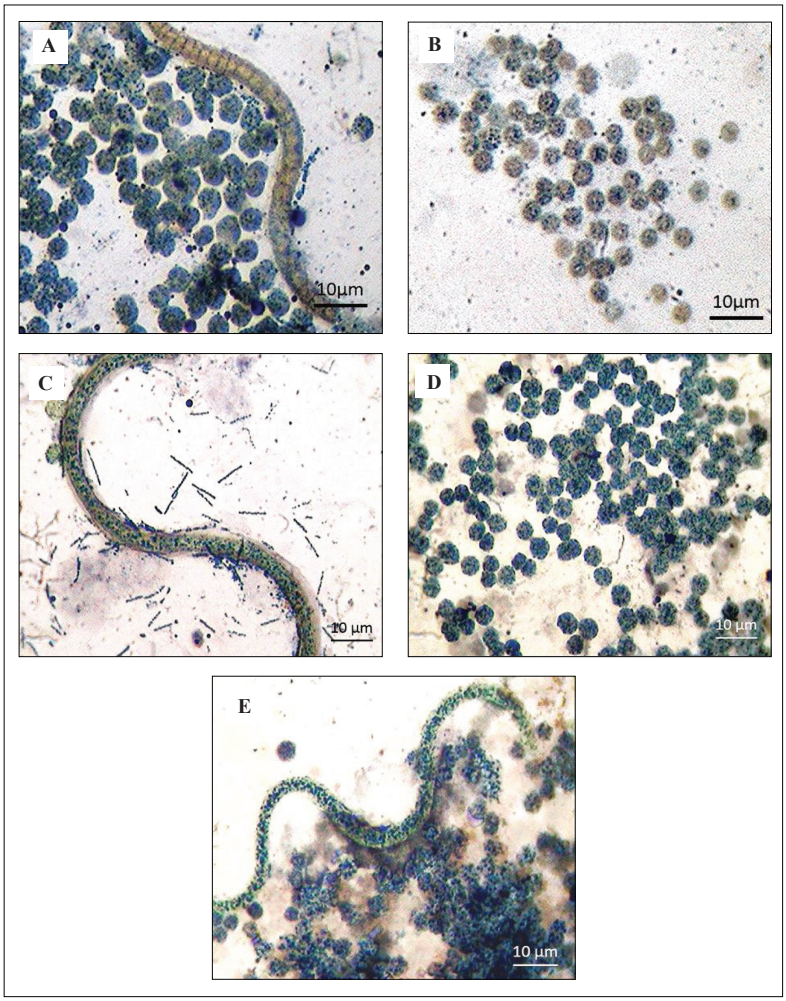

Figure 3: Visualization of PHB by Sudan black B staining. (a) *Spirulina* and *Microcystis* day 1, (b) *Microcystis* day 1, (c) *Spirulina* day 3, (d) *Microcystis* day 3, (e) *Spirulina* and *Microcystis* day 3 at the magnification of 10×100.

Microscopic visualization of bloom samples fixed and stained after one and three days of storing under *in vitro* conditions provides pictorial evidence that synthesis of PHB granules in *Microcystis* spp. commences earlier than in *Spirulina platensis* (Figure 3).

Extraction of PHB

The recovery of PHB requires rupturing of cyanobacterial cells and elimination of the protein layer that wraps PHB granules. Subsequently, PHB has to be selectively dissolved in a suitable solvent. Solvent extraction, used in the present study, was the most extensively adopted procedure to extract PHB from cellular biomass, due to its simplicity and efficiency (Kunasundari & Sudesh, 2011). This method also eliminates endotoxins, causes negligible polymer deterioration and provides high purity (Jacquel *et al*., 2008). Despite the above advantages, the solvent extraction method is not suitable for large-scale recovery of PHB due to the consumption of large amounts of toxic and volatile solvents that pose threats to the environment. Hence, enzymatic digestion methods are well established as alternative recovery methods in the large scale recovery of PHB (Kunasundari & Sudesh, 2011). While solvent extraction methods involve solubilization of PHB, enzymatic digestion involves solubilization of cellular structures surrounding PHB.

Cyanobacterial biomass was pretreated with methanol to remove pigments and cyanotoxins before the extraction. Subsequently, methanol should be removed from the biomass by centrifuging followed by heating at 65˚C for nearly 20-30 minutes since the presence of methanol inhibits solubilization of PHB in chloroform (Carpine *et al*., 2015). Subsequently, PHB was extracted into hot chloroform and precipitated with diethyl ether. Precipitation of the polymer with diethyl ether and washing the precipitate with acetone assists in the removal of contaminating lipids (Sharma & Mallick, 2005). Chloroform was evaporated at a temperature of 4˚C to obtain the final crude extract. This allows gradual precipitation of PHB, enabling crystallization. Evaporation of chloroform at the end of the organic extraction procedure resulted in an off-white coloured crude extract in amorphous form.

Determination of the optimum day for yield of PHB

Under laboratory conditions a continuous supply of nutrients was not provided for the microalgal samples. Henceforth, the growing cyanobacterial community consumes the available nutrients, thus causing progressive nutrient stress. The collected scum samples were maintained in the laboratory for seven days, and the amount of PHB accumulated in the cyanobacteria was measured on each day. The mean PHB yield gradually increased up to day four. The maximum PHB yield, 9.6 % w/w of PHB, was obtained on the fourth day (Figure 4).

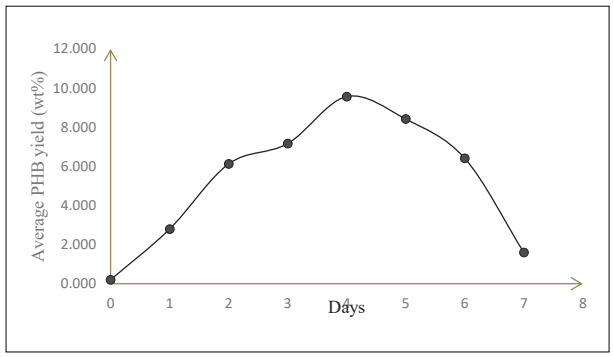

Figure 4: Variation of % yield of PHB yield along with the number of days of maintaining the samples under *in vitro* conditions

In related studies, the results obtained for the optimum day vary within a wide range. This aspect could depend on many variables including the initial concentration of nutrients, the species of cyanobacteria, cell density in the sample, availability of other organisms that consume the same nutrient sources, and bioavailability of nutrients in the solution.

A remarkable reduction in PHB yield was recorded after the 4th day of the sample maintenance. Hence, it could be suggested that produced PHB might be an alternative energy and carbon reserve for cyanobacteria. PHB might have been degraded to be utilized as an energy source to maintain cell viability. Moreover, studies done by Ansari and Fatma (2016) have further confirmed the uptake of accumulated PHB by cyanobacteria.

Optimization of an exogenous carbon source

Carbon dioxide is the major inorganic carbon source used for the synthesis of PHB by cyanobacteria (Wang *et al*., 2013). However, they can also utilize various exogenous organic carbon sources for the heterotrophic accumulation of PHB under dark conditions simultaneously (Francisco *et al*., 2014). In the present study, cyanobacteria bloom samples were provided with glucose, sucrose, and lactose as exogenous carbon sources at a similar concentration (1 g/L).

The percentage mean yields obtained for different carbon sources and the control showed a statistically significant difference (p = 0, One Way ANOVA). The

average PHB yield given by glucose supplementation was significantly higher than that of the control and other carbon sources (Table 2). Although the average PHB yield obtained from the bloom samples provided with sucrose and lactose expressed a higher and a lower value, respectively, than that of the control, the differences were not statistically significant. The samples supplemented with glucose resulted in the highest yield (11.585% w/w) of PHB.

Table 2: Yield of PHB for different exogenous carbon sources

Carbon source	Mean yield (% w/w)
Control*	7.1287^{BC}
Lactose	6.6263^{C}
Glucose	11.585^{A}
Sucrose	8.366^{B}

* No added carbon source. Means followed by the same superscript letter do not differ significantly (Tukey's test)

According to Sharma and Mallick (2005) and Lee *et al*. (1995), higher intracellular concentrations of NADPH and higher ratios of NADPH/NADP stimulate biosynthesis of PHB since NADPH is a prerequisite for the activity of the enzyme acetoacetyle-CoA reductase involved in the PHB biosynthetic pathway. Glucose utilization in cyanobacteria takes place through the pentose phosphate pathway that produces the reduced cofactor NADPH (Lee *et al*.,1995). The enhanced accumulation of PHB in glucose-supplemented samples could be due to high amounts of NADPH generated in the glucose utilization pathway. A similar explanation could be valid for sucrose-supplemented samples (Ansari & Fatma, 2016). The bloom samples supplemented with lactose showed a lower PHB yield compared to the control.

Optimization of nutrients

Nitrogen (as NO_3^-) and phosphorus are the mandatory primary nutrients that are required for cyanobacterial growth and metabolism (Idroos & Manage, 2012; Gunasekara *et al*., 2019). The initial nitrate and phosphate concentrations in the scum samples were 0.011 mg/L and 0.156 mg/L respectively. Nitrate optimization was performed by maintaining scum samples at 0.011 mg/L (initial), 1.011 mg/L, and 2.011 mg/L concentrations. For the optimization of phosphate concentration, samples were provided with 0.156 mg/L (initial), 1.156 mg/L and 2.156 mg/L phosphate concentrations.

The samples treated with three different concentrations of nitrates presented statistically significant differences in PHB yields ($p = 0.002$, One-way ANOVA). The highest average yield of 6.87% w/w was obtained from the cyanobacterial scum treated with the lowest concentration of nitrate. The yield diminishes with the increasing nitrate concentration in the medium. The PHB yields given by scum samples treated with different phosphate concentrations do not show a statistically significant difference ($p = 0.088$, One-way ANOVA) (Figure 5).

The limitation of nitrate appeared to be a suitable stimulant to enhance cyanobacterial PHB production. This corroborates with the findings of Carpine *et al*. (2015), where PHB production was enhanced under nitrogen starvation conditions of cyanobacterial monocultures.

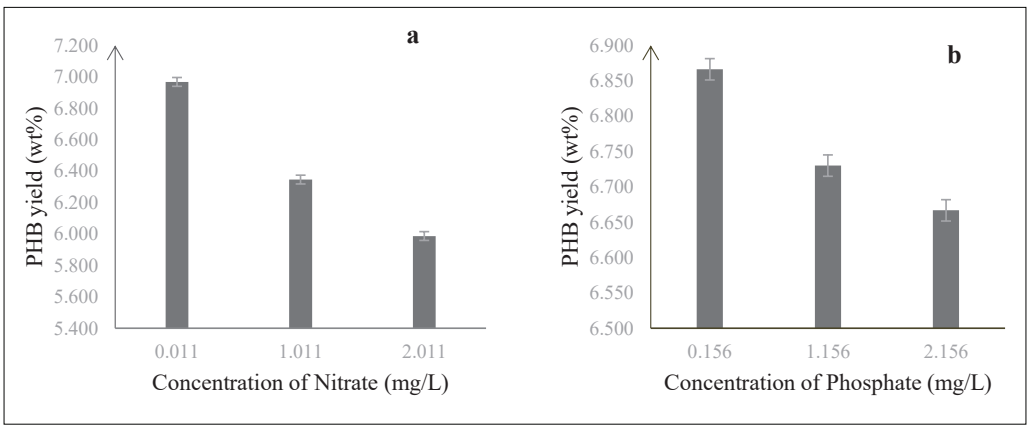

Figure 5: Yields of PHB as percentages in different concentrations of Nitrate (a) and Phosphate (b)

Spectrophotometric analysis

The quantification of PHB was performed by spectrophotometric analysis of acid digested PHB. Under acidic conditions together with high temperatures, PHB is monomerized into crotonic acid (Panda *et al*., 2008). The UV spectrum of the crotonic acid derived from the commercial standard PHB gave the maximum absorbance wavelength (λ_{max}) as 235 nm, corresponding with the literature (Figure 6).

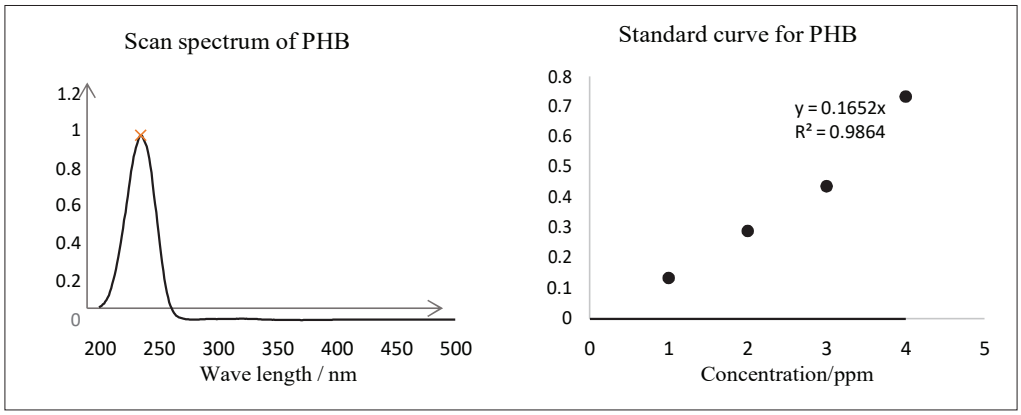

Figure 6: Scanned spectrum and the standard curve for commercial PHB

Raman spectroscopy

Raman spectroscopy has already been proven as a powerful tool for the characterization of PHB as it only requires small quantities of the sample and minimal sample preparation (Hermann *et al*., 2020). Analyses of Raman spectra are precise and less time-consuming.

The Raman spectrum of crude PHB extract conspicuously showed the presence of peaks that are distinctively present in the spectrum of commercial standard PHB (Figure 7). The most prominent bands in the spectrum of standard PHB are located at 433 cm^{-1}, 839 cm^{-1}, and 1725 cm^{-1} and can be assigned to δ(CC) skeletal deformations, ν(CC) skeletal stretches and ν(C=O) stretching vibrations respectively. The regions from 1040 cm^{-1} to 1140 cm^{-1} and from 1250 cm^{-1} to 1460 cm^{-1} show bands caused by ν(CC) skeletal stretches and δ(CH), δ(CH_2), and δ(CH_3) deformations, respectively (De Gelder *et al*., 2008). When recording the spectrum of the crude sample, all Raman-active biomolecules contribute to the resulting range, and their signals overlap. The spectrum of the crude sample shows several Raman peaks provided by PHB (837, 1455, and 1736 cm^{-1}). Among them, the emission line at 1736 cm^{-1} can be considered as the most useful candidate because the vibrations of the other components of crude extract do not considerably interfere with this emission line. The band positions of PHB in the spectrum may differ several wave numbers from the reference spectrum due to differences in crystallinity (De Gelder *et al*., 2008). The dissimilar regions result from the superimposition of signals of PHB and Raman active contaminants.

Although accumulation of PHB in *Spirulina* monocultures has been studied extensively (Costa *et al*., 2018; Da Silva *et al*., 2018; Duangsri *et al*., 2020), the kinetics of biosynthesis of PHB in *Microcystis* sp. has not been widely investigated. This could be due to the relatively low yield given by *Microcystis* sp. in comparison to other cyanobacterial genera such as *Synechocystis*, *Nostoc,* and *Spirulina*. This study is the first record of the potential utilization of *Microcystis* sp. dominated, naturally existing, cyanobacteria blooms for PHB extraction. Since *Microcystis* is one of the most widely prevalent cyanobacterial genera that occur as massive natural blooms in eutrophic waters, the present study has proposed a sustainable application of these blooms in PHB extraction.

Nevertheless, for low PHB yield, the results of the present study are promising with respect to the data reported in the literature. Therefore, the current study can be regarded as a preliminary effort to establish the concept of green innovation via the effective utilization of naturally occurring cyanobacterial blooms as a source for PHB extraction.

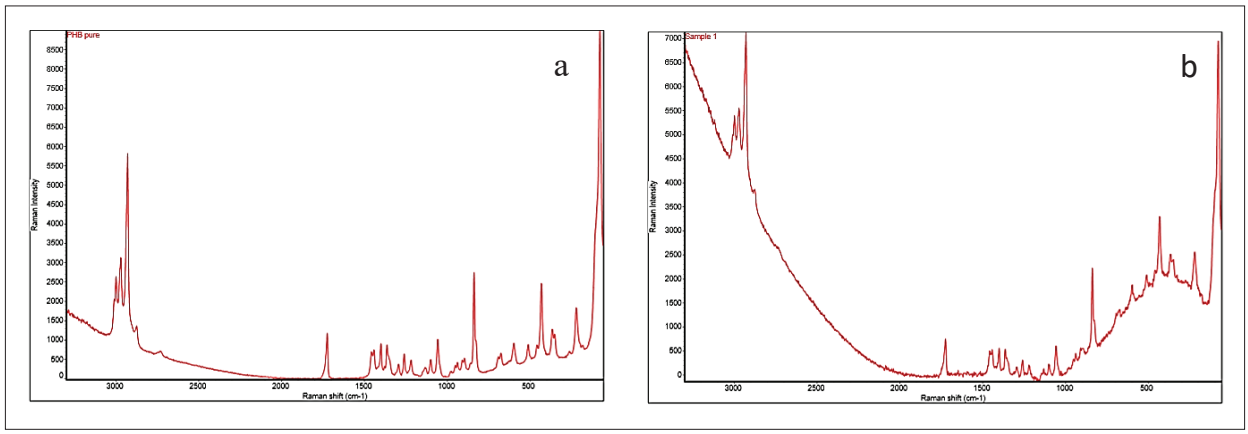

Figure 7: Raman spectrum for commercial standard PHB (a), and the crude extract (b)

CONCLUSION

Plastic pollution has struck the world's oceans and land environment. Hence, explorations on biodegradable plastics are in the limelight. PHB is an intracellular energy and carbon storage product. PHB thermoplastic has conquered the world market mainly due to its variety of uses in different arenas and the ever-increasing interest in eco-friendly alternatives, as a biodegradable plastic material. The current study concludes that naturally occurring cyanobacterial blooms carry a considerable potential to accumulate PHB. The PHB yield can be enhanced by supplementing the bloom sample with exogenous organic carbon sources, mainly glucose. Low nitrate concentration has a significant effect on improving the PHB yield. The mean percentage weight of PHB yield was 7.13± 0.12 % w/w. Optimization studies showed that nitrate deficiency and the presence of glucose as an exogenous carbon source imposed a stimulatory effect for PHB accumulation by cyanobacteria. The maximum amount of PHB (9.6% w/w) was found to be accumulated by cyanobacteria on the fourth day following the bloom sample collection. Hence, *in vitro* maintained cyanobacterial blooms could be used as a promising candidate to extract high concentrations of PHBs.

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