Journal of Applied Phycology (2021) 33:3697–3708
https://doi.org/10.1007/s10811-021-02578-x

Efficient conversion of acetate or glucose to poly(3-hydroxybutyrate) and glycogen by the single-stage photoheterotrophic cultivation of cyanobacterium *Chroococcus hansgirgi* TISTR 8561

Pragasit Itthirit[1] · Aran Incharoensakdi[1,2] · Tanakarn Monshupanee[1]

Received: 4 January 2021 / Revised and accepted: 21 July 2021 / Published online: 26 August 2021

Abstract
Microbial poly(3-hydroxybutyrate) (PHB) has been used as bioplastic, while the biopolymer of glucose (glycogen) can be used as efficient substrate for the generation of biofuels. Substantial production of PHB and glycogen by heterotrophic microbes has been constrained by the limited resources of organic substrates. Hence, the heterotrophic cultivation that efficiently converts an organic substrate to the two biopolymers is desirable. However, limited research has been conducted to examine the conversion efficiency of an organic substrate to biopolymers by photoheterotrophic microbes. In this study, the single-stage cultivation of a photoheterotrophic cyanobacterium (*Chroococcus hansgirgi* TISTR 8561) which accumulates PHB and glycogen was optimized and evaluated for the mass conversion efficiency (*CE*) of an organic substrate to the two biopolymers. The cells were cultured in BG11 trace element medium with addition of only a single specific organic substrate under light to enable both cellular oxygenic photosynthesis and heterotrophic metabolism, which were evidenced by the oxygen gas evolution and the organic substrate utilization of the cells, respectively. Through optimization of the culture conditions, organic substrate supply, and cultivation time, the mass *CE* of glucose to both PHB and glycogen of 51 ± 15% (w/w) was obtained, while the mass *CE* of acetate to these two biopolymers was 71 ± 16% (w/w). These optimized cultures exhibited *CE* of glucose or acetate to both PHB and glycogen at the higher levels than those of previous reports in photoheterotrophic microbes. This single-stage photoheterotrophic cultivation may be applied for production of biopolymers by other photoheterotrophic microbes.

Keywords Cyanobacteria · Poly(3-hydroxybutyrate) · Glycogen · Conversion · Photoheterotrophy · Mixotrophy

Introduction

Poly(3-hydroxybutyrate) (PHB) and glycogen are two common biopolymers found in heterotrophic bacteria (Lee 1996; Ball and Morell 2003; Chanprateep 2010; Wilson et al. 2010) and phototrophic cyanobacteria (Allen 1984; Drosg et al. 2015; Koller and Maršálek 2015; Luan et al. 2019). Of interest, PHB exhibits a thermostability and polymer hardness comparable to the chemical plastic polypropylene (Lee 1996; Verlinden et al. 2007). Thus, PHB has been used as a bioplastic material for commercial purposes (Abed et al. 2009; Chanprateep 2010). Glycogen is a polymer of glucose, covalently linked through α-1,4- and α-1,6-glycosidic bonds (Ball and Morell 2003; Deschamps et al. 2008), which can be used as an efficient substrate for the production of bioethanol and other high-value carbohydrates (John et al. 2011; Park et al. 2018).

Microbial production of glycogen or PHB using heterotrophic microorganisms is known to yield a much higher production level than those obtained from photoautotrophic microorganisms. However, such heterotrophic culture systems, mainly established in bacteria, rely on an extensive consumption of multiple types of organic substrates. Hence, a heterotrophic approach that requires a lower amount of organic substrate and can efficiently convert this substrate to the bioproduct(s) is desirable.

In cyanobacteria, the two-stage cultivation approach, in which the cells were first pre-grown under normal

✉ Tanakarn Monshupanee
tanakarn.m@chula.ac.th

[1] Department of Biochemistry, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand

[2] Academy of Science, Royal Society of Thailand, Bangkok 10300, Thailand

photoautotrophy to increase the biomass and subsequently transferred to a heterotrophic condition in the dark with a supply of a single organic substrate, mainly glucose (Glu) or acetate (Act) has been demonstrated for the production of PHB (Sharma and Mallick 2005; Drosg et al. 2015; Koller and Maršálek 2015) and glycogen (Singh and Sherman 2005; Gaudana et al. 2013). Because these cyanobacteria were heterotrophically cultured in a trace element medium containing a single organic substrate, the determination of the mass conversion efficiency (*CE*) of the specific organic substrate to the specific bioproduct is possible.

The calculated theoretical value of the biochemical heterotrophic *CE* of Glu or Act to PHB is 48% (w/w) (Yamane 1993). We recently determined the *CE* of the optimized two-stage (photoautotrophy and then heterotrophy in the dark) cultivation of the cyanobacterium *Chlorogloea fritschii* and reported a *CE* of Act to PHB of 51 ± 7% (w/w) (Monshupanee et al. 2016). This obtained *CE* was at a comparable level to the theoretical efficiency, indicating that *C. fritschii* was able to convert a part of its cellular metabolites (accumulated during photoautotrophy) into PHB during the subsequent heterotrophy in the dark (Monshupanee et al. 2016).

However, the drawbacks of such a two-stage approach remain. These include a substantial reduction of the biomass level during the heterotrophic phase in the dark and an extensive time, media, and energy required for two-step cultivation. Such drawbacks can be overcome by using a single-stage photoheterotrophic cultivation approach, in which cyanobacteria are cultured in a trace element medium supplemented with a single organic substrate under light. This photoheterotrophic culture, presumably performing both cellular oxygenic photosynthesis and heterotrophic metabolism, has been conducted for the production of PHB (Monshupanee and Incharoensakdi 2014; Drosg et al. 2015; Koller and Maršálek 2015) and glycogen (De Philippis et al. 1992; Monshupanee and Incharoensakdi 2014). Nevertheless, the *CE* of the consumed specific organic substrate to a specific bioproduct remains to be determined in these photoheterotrophic cultures of cyanobacteria.

Therefore, this study aimed to determine the *CE* of a single organic substrate to the biopolymers (PHB and glycogen) in photoheterotrophic culture of *Chroococcus hansgirgi*. The culture conditions and the organic substrate supplies to obtain maximum *CE* were also determined. In addition, the material properties of PHB polymer produced by *C. hansgirgi* were evaluated in comparison to those of the commercial PHB.

Materials and methods

Strain and culture conditions

The axenic culture of *Chroococcus hansgirgi* TISTR 8561 was obtained from the Thailand Institute of Scientific and Technological Research (TISTR). The strain was previously isolated from a freshwater pond in Bangkok. For routine culture, the cells were grown in the standard BG11 trace element medium containing 17.9 mM nitrate as nitrogen source (Rippka et al. 1979), with the omission of sodium citrate, 20 mM (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid)-NaOH (HEPES–NaOH) was added to the medium, and ferric ammonium citrate was replaced by ferric chloride, as previously described (Monshupanee et al. 2016).

The normal culture condition (NORMAL) was derived by culturing the cells in the standard BG11 medium. The nitrogen deprivation condition (-N) was obtained by culturing the cells in the BG11 medium lacking the nitrogen source (nitrate) as previously described (Monshupanee and Incharoensakdi 2014). The full compositions of BG11 medium used in NORMAL and -N conditions are given in Supplementary Information Table S2. Cells were cultured under an atmospheric carbon dioxide supply (0.04% v/v) with continuous shaking under white light of 70 µmol photons m^{-2} s^{-1} at 28 °C. Sodium acetate, sodium pyruvate, sodium butyrate, sodium citrate, glucose, fructose, or sorbitol were added to the BG11 medium as a substrate when required.

Analysis of the PHB yield

The PHB content was determined using high-performance liquid chromatography (HPLC) as described (Schlebusch and Forchhammer 2010). Approximately 20 mg of dry cells was mixed with 95% (w/w) sulfuric acid and boiled to hydrolyze the PHB into crotonic acid. The obtained sample was diluted with distilled water until reaching a final concentration of 0.014 M sulfuric acid and filtered. The sample solution was analyzed for crotonic acid by HPLC using the UV light detection at 210 nm and an InertSustain 3-µm Carbon-18 column (GL Sciences, Tokyo, Japan). Adipic acid was used as the HPLC internal standard. Commercial PHB (Sigma) was analyzed in parallel, where 82 ± 5% (w/w) conversion of PHB to crotonic acid was obtained.

Analysis of glycogen and glucose (Glu) levels

The glycogen content, as % (w/w DW), was determined as described (Ernst et al. 1984). In brief, glycogen was isolated from 20 mg dry cell biomass by potassium hydroxide extraction, ethanol precipitation, and then the pH was neutralized with Act buffer solution prior to being enzymatically hydrolyzed to Glu by amyloglucosidase and amylase. Oyster glycogen (Sigma) was used as the standard for glycogen quantification.

The Glu solution prepared from the glycogen hydrolysis and the Glu level present in the BG11 culture medium was quantified using the glucose oxidase assay kit (GLUCOSE Liquicolor, Human Gesellschaft fur Biochemica und

Diagnostica mbH, Germany). The obtained product was quantified by measuring the absorbance at 500 nm. Purified Glu (Sigma) was used to form the standard for Glu quantification.

Analysis of total lipids

The total lipids were extracted from 30 mg dry cells, free from PHB, as described (Monshupanee and Incharoensakdi 2014). The total lipids were firstly extracted from dry cells using methanol extraction which did not extract PHB, then followed by 2:1 (v/v) chloroform:methanol extraction. The total lipid amount was quantified using the acidic dichromate method (Monshupanee and Incharoensakdi 2014). The total lipid was determined from the amount of reduced dichromate, as measured by the absorbance at 350 nm. Commercial palmitoleic acid (Sigma) was used as the standard for lipid quantification.

Analysis of acetate (Act) concentration

The Act concentration in the culture medium was measured using the Act colorimetric assay kit MAK086 (Sigma), where the Act concentration was determined by a coupled enzyme assay that results in a colorimetric (450-nm absorption) product proportional to the Act concentration present. Commercial Act (Sigma) dissolved in the BG11 culture medium was used to make the Act standard for quantification.

Determination of the conversion efficiency (CE)

The *CE* proceeded by the photoheterotrophic culture was calculated according to Eq. (1):

$$CE(\%) = 100 \times (BP_s - BP_i)/\left(S_i - S_s\right) \quad (1)$$

where BP_s and BP_i are the amount of biopolymer production (mg L^{-1}) at the specific time point and initial time point, respectively, and S_s and S_i are the concentration (mg L^{-1}) of a substrate (Act or Glu) at the specific time point and initial time point, respectively.

Oxygen evolution rate measurement

Cell samples were collected for determination of the dry cell weight and oxygen evolution. Cell biomass was immediately resuspended in a fresh BG11 medium. The cell sample was incubated under darkness for 30 min. Oxygen evolution was measured by an Oxygraph plus oxygen electrode (Hansatech Instruments, UK). The oxygen evolution measurement was performed using a constant fluorescent light intensity of 70 µmol photons m^{-2} s^{-1} at 28 °C.

Polymer extraction, NMR, and material property analyses

The PHB was extracted from the dry cell mass using chloroform, then precipitated by diethyl ether and washed using acetone as previously described (Yellore and Desai 1998). The natural isotopic ^{13}C (carbon) and ^{1}H (hydrogen) in PHB were determined by nuclear magnetic resonance (NMR) using a Bruker Advance 400 MHz spectrometer (Germany). The polymer thermal properties were analyzed using differential scanning calorimetry equipped with a Netzsch DSC-204-F1 instrument (Germany). The material properties of PHB were examined using a Hounsfield-H10KM machine (UK), while the molecular weight was estimated by gel filtration chromatography using the column (K802.5-K803-K804, Shodex, USA) and chloroform as a running solvent (Monshupanee et al. 2016).

Results and discussion

The supply of glucose (Glu) or acetate (Act) effectively increased PHB accumulation in photoheterotrophic *C. hansgirgi*

We first screened for cyanobacterial strains capable of utilizing organic compounds (Tarawat et al. 2020) and found that the unicellular cyanobacterium *Chroococcus hansgirgi* TISTR 8561 can assimilate acetate (Act) and glucose (Glu) to increase its biomass levels under photoheterotrophy (Fig. 1). Next, we evaluated seven organic compounds, each chemically identical or similar to a metabolite in cyanobacterial carbon metabolism, for their ability to increase PHB accumulation in *C. hansgirgi* (Table 1).

Under normal BG11 nutrient (NORMAL) condition without an organic compound supply, the photoautotrophic cells accumulated PHB at only 0.2% (w/w DW) (Table 1). However, when Act or Glu as a single organic substrate was supplied to the NORMAL medium (photoheterotrophy), it markedly increased the PHB level by up to 32 and 51% (w/w DW), respectively, while the addition of butyrate, citrate, fructose, or sorbitol was inferior to Act or Glu at enhancing the PHB storage level, attaining 8–31% (w/w DW) (Table 1). In contrast, pyruvate slightly increased PHB accumulation to 3% (w/w DW) (Table 1).

Under nitrogen-deprived (-N) condition without an organic substrate supply, *C. hansgirgi* accumulated PHB at 7% (w/w DW) (Table 1). Interestingly, under the -N condition, the supply of the metabolic compounds, i.e., pyruvate, butyrate, or citrate, had little effect on increasing the PHB accumulation level, while the supply of fructose or sorbitol slightly enhanced the PHB level to 15–21% (w/w DW), and the supply of Act or Glu greatly increased

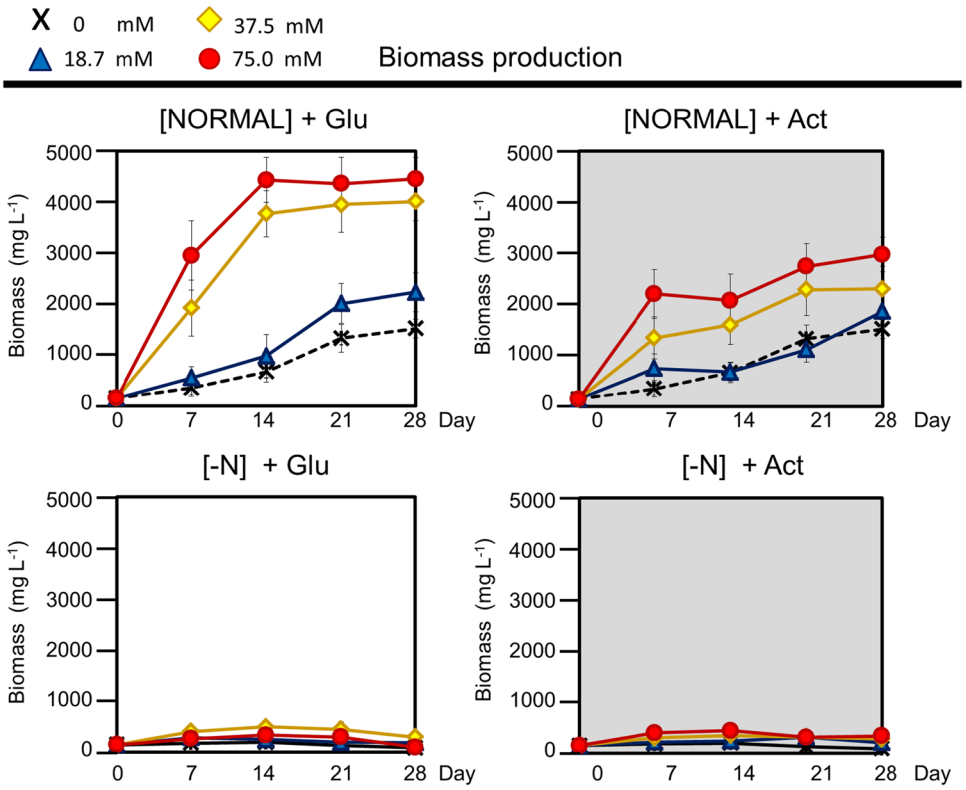

Fig. 1 Biomass levels of *C. hansgirgi* cultured under photoautotrophy and photoheterotrophy supplied with glucose (Glu) or acetate (Act). Fourteen-day photoautotrophically pre-grown cells were diluted and transferred to normal medium (NORMAL) or nitrogen-deprived medium (-N) in the light with atmospheric CO_2 and with or without a Glu or Act supply. Biomass levels of the cell cultures (mg L^{-1}) were determined. Values shown are the mean ± 1SD, derived from 3–5 independent cultures. Broken black lines are the same data of biomass productions obtained from the cultures without Glu and Act supply under the same nutrient condition.

the PHB levels up to 35 and 49% (w/w DW), respectively (Table 1).

Overall, the two most effective organic compounds that yielded the highest PHB accumulations and PHB productivities under both the NORMAL and -N conditions were Glu followed by Act (Table 1). In addition, Glu or Act supply also yielded high levels of biomass production (Fig. 1). Therefore, these two compounds were selected for further determination of the optimal supplied concentration for the production PHB, glycogen, and total lipids.

Table 1 PHB accumulations and productivities under photoheterotrophy supplied with a different organic substrate. The *C. hansgirgi* was cultured in normal medium (NORMAL) or nitrogen-limiting medium (-N) supplied with a single organic substrate in the light for 14 days. Data are the mean ± 1SD from three to six independent experiments. High PHB accumulations and productivities were marked by bold values

Supplied organic substrate		PHB accumulation (% (w/w DW))				PHB productivity (mg L^{-1})			
		NORMAL		-N		NORMAL		-N	
None (cells were supplied with atmospheric CO_2)		0.2 ± 0.0		7 ± 4%		3 ± 1		2 ± 1	
Name	Formula	Supplied concentration (mM)				Supplied concentration (mM)			
		37.5	75.0	37.5	75.0	37.5	75.0	37.5	75.0
Acetate	C_2H_3O	**20 ± 3***	**32 ± 5***	**28 ± 4***	**35 ± 4***	**325 ± 89***	**676 ± 112***	11 ± 4*	15 ± 4*
Pyruvate	$C_3H_4O_3$	3 ± 2	3 ± 1*	10 ± 3	12 ± 4	12 ± 5*	10 ± 4*	3 ± 0	4 ± 2
Butyrate	$C_4H_7O_2$	8 ± 3*	12 ± 1*	8 ± 2	9 ± 2	18 ± 9*	19 ± 6*	3 ± 1	4 ± 1
Citrate	C_6H_8O	12 ± 4*	**31 ± 5***	6 ± 2	11 ± 3	62 ± 15*	109 ± 28*	4 ± 2	5 ± 2
Glucose	$C_6H_{12}O_6$	**51 ± 6***	**43 ± 7***	**42 ± 4***	**49 ± 5***	**1951 ± 284***	**1927 ± 294***	21 ± 3*	20 ± 3*
Fructose	$C_6H_{12}O_6$	19 ± 3*	18 ± 4*	15 ± 3*	17 ± 4*	**280 ± 92***	**316 ± 98***	7 ± 2*	8 ± 3*
Sorbitol	$C_6H_{14}O_6$	10 ± 2*	12 ± 3*	16 ± 4*	21 ± 5*	35 ± 18*	15 ± 5*	7 ± 1*	9 ± 3*

*Significantly higher PHB accumulations or PHB productivities ($P < 0.01$, two-tailed *t*-test) than that derived from the same nutrient condition, but without supplied organic substrate

The supply of glucose (Glu) and acetate (Act) increased biomass production of *C. hansgirgi* under normal nutrient condition

The optimal supply of Act and Glu for biomass production was determined. Under NORMAL nutrient condition without organic substrate (photoautotrophy), the culture yielded a biomass level up to 1.5 g L^{-1} (Fig. 1). The supply of 37.5 mM Glu rapidly increased the biomass level up to 4.0 g L^{-1}, but a twofold higher Glu concentration (75 mM) only slightly enhanced the biomass yield further up to 4.4 g L^{-1} (Fig. 1). The supply of Act at 37.5 and 75 mM moderately enhanced the biomass levels to 2.3 and 2.9 g L^{-1}, respectively (Fig. 1). Overall, under NORMAL condition, Glu supplementation yielded higher biomass levels than did Act.

However, under -N condition, no obvious cell growth was observed regardless of the presence or absence of Glu or Act (Fig. 1). Thus, *C. hansgirgi* TISTR 8561 is likely a non-nitrogen-fixing strain. This was in agreement with previous reports that other *Chroococcus* strains do not fix nitrogen under photoautotrophy (Potts et al. 1983).

Optimal supply of glucose or acetate enhanced the accumulation of PHB and glycogen, but not total lipids

The accumulation of the major cellular bioproducts of PHB, glycogen, and total lipids in *C. hansgirgi* under NORMAL nutrient condition was determined.

At day 0, the PHB concentration of photoautotrophic cells was 0.2% (w/w DW), and then the PHB level slowly increased to 4% (w/w DW) at day 28 of cultivation (Fig. 2). Of particular significance, the optimal Glu supply at 37.5 mM rapidly increased PHB levels up to 51% (w/w DW) within 14 days of cultivation (Fig. 2). The optimal Act supplementation (75 mM) also rapidly raised the PHB accumulation within 14 days, but to the lower PHB yield of 32% (w/w DW) (Fig. 2). Hence, the optimal supply of either Glu or Act stimulated PHB accumulation, but Glu was more effective than Act at enhancing the PHB levels.

For glycogen, photoautotrophic cells slowly increased their glycogen contents from 5 to 38% (w/w DW) during the 28-day cultivation period (Fig. 2). Interestingly, the addition of 37.5 and 75 mM Glu rapidly increased the glycogen levels up to 45 and 50% (w/w DW), respectively, within only 7 days of cultivation (Fig. 2). In contrast, Act at the same concentration only slightly elevated the glycogen contents (Fig. 2). Therefore, Glu was much more effective than Act in enhancing the glycogen level of *C. hansgirgi*.

For the total lipids, there were no obvious differences in the cellular total lipid content regardless of the addition of Glu or Act (Fig. 2). Rather, the total lipid content fell within the narrow range of 6–13% (w/w DW).

Effects of glucose supply on the biosynthesis of PHB and glycogen

Under the NORMAL condition during the early cultivation phase (days 0–7), the optimal Glu addition (37.5 and 75.0 mM) simultaneously and significantly increased both the biomass (Fig. 1) and glycogen levels (Fig. 2), suggesting the co-occurrence of both Glu catabolism for biomass production and Glu anabolism for glycogen accumulation. However, during the next cultivation phase (days 7–14), both the biomass (Fig. 1) and PHB levels (Fig. 2) were sharply increased concurrently, while the glycogen level decreased (Fig. 2), suggesting a potential cellular conversion of glycogen to PHB in *C. hansgirgi*. The metabolic pathway for converting glycogen to PHB has recently been demonstrated in the cyanobacterium *Synechocystis* sp. PCC 6803, where the cellular glycogen content decreased as the PHB content increased (Koch et al. 2019).

In addition, this study showed broad fluctuations in the PHB and glycogen levels of photoheterotrophic *C. hansgirgi* supplied with Glu, whereas the total lipid content was relatively more stable (Fig. 2). Therefore, it is likely that lipid metabolism may not be linked to the metabolism of PHB and glycogen in this photoheterotrophic *C. hansgirgi*.

In the *C. hansgirgi* cultured with the optimal Glu supply (37.5 and 75 mm), glycogen accumulation increased in the early cultivation period (day 7, Fig. 2), which supported the data that Glu was the primary substrate for glycogen biosynthesis, as reported in various bacteria (Wilson et al. 2010) and a number of cyanobacteria (Grundel et al. 2012; Luan et al. 2019). In 37.5 and 75 mM Glu-supplemented cultures, the rapidly increased glycogen levels from days 7 to 28 (Fig. 2) may result from nitrogen depletion since 65–98% of the levels of nitrogen source (nitrate) were depleted from the culture medium (Supplementary Information Table S1) and/or exhaustion of the other trace element nutrients. Nitrogen depletion has been known to stimulate glycogen accumulation in a number of cyanobacterial strains (Luan et al. 2019).

Since the supply of Glu also strongly increased PHB production in *C. hansgirgi*, the cells are likely to be able to metabolize Glu to PHB. In the well-studied cyanobacterial metabolism of *Synechocystis* sp. PCC 6803, Glu can be converted to acetyl-CoA via the glycolytic pathway (Knoop et al. 2010; Monshupanee and Incharoensakdi 2014), and then the acetyl-CoA is subsequently used as the primary substrate for PHB biosynthesis (Taroncher-Oldenburg et al. 2000). In all Glu-supplemented cultures, PHB level peaked at day 14 and declined after that (Fig. 2). The reduced PHB level after reaching a peak was also observed in photoheterotrophic *Synechocystis* sp. PCC 6803 (Panda and

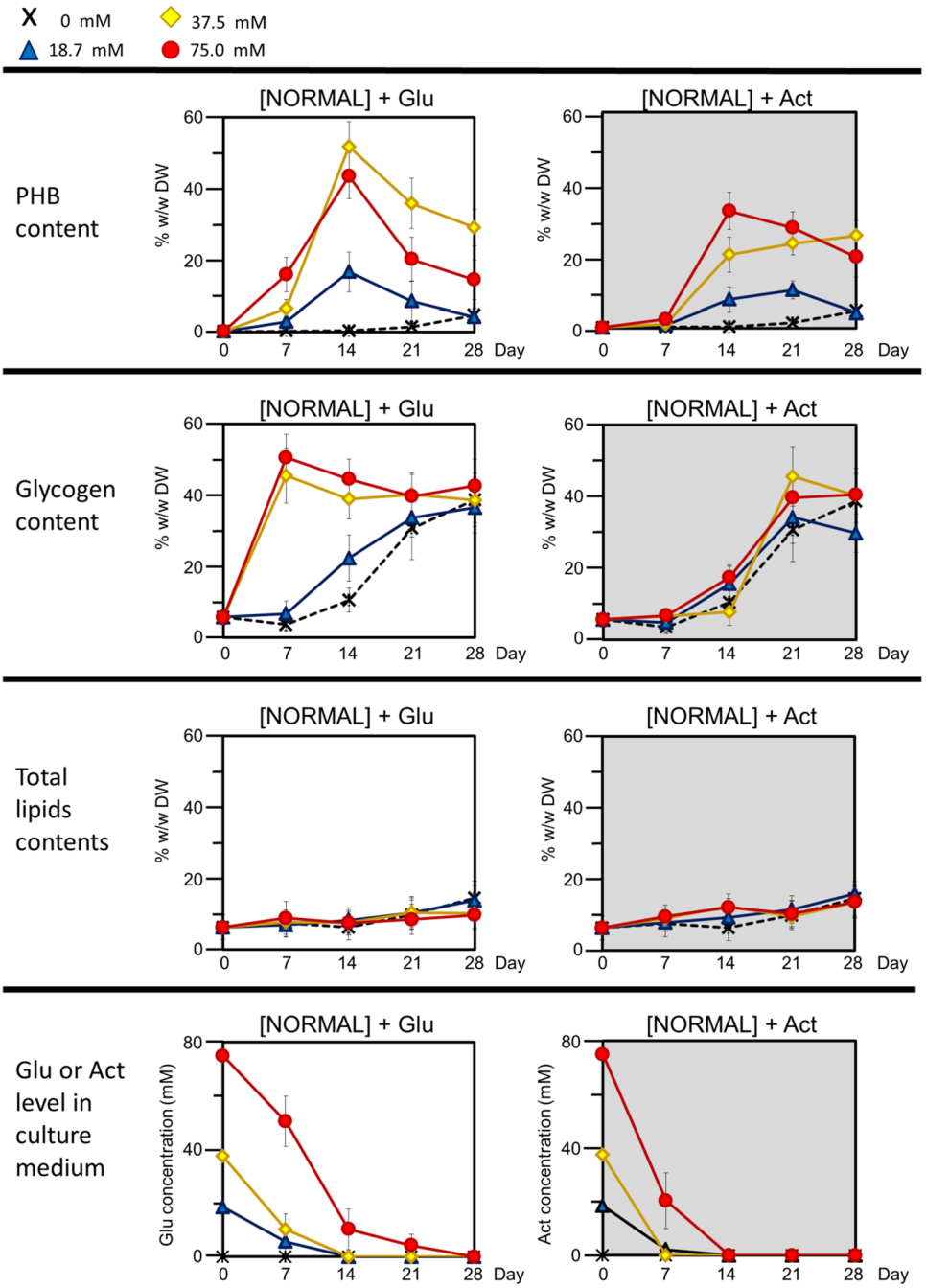

Fig. 2 Accumulation of PHB, glycogen, and total lipids in *C. hansgirgi* cultured under photoautotrophy and photoheterotrophy. Cells were cultured in the normal medium (NORMAL) with or without Glu or Act supply as described in Fig. 1. The concentration of the residual Glu or Act in the culture medium is also presented. Data are shown as the average ± 1SD from three to six independent cultures. Broken black lines are the same data of PHB, glycogen, or total lipids accumulations derived from the cultures without Glu and Act supply

Mallick 2007). We speculated that PHB might be converted to organic acids or fatty acids. Cyanobacterial secretion of organic acids (Wang et al. 2013) and free fatty acids (Eungrasamee et al. 2020) to the culture medium has been reported in *Synechocystis* sp. PCC 6803.

Effects of acetate supply on the biosynthesis of the two biopolymers

Under the NORMAL culture condition, optimal supplementation with Act (37.5 and 75.0 mM) rapidly increased the PHB storages (Fig. 2). This result corresponded to the known cyanobacterial metabolism of *Synechocystis* sp. PCC 6803, where Act can be conjugated with CoA to form acetyl-CoA, which is subsequently used as the primary substrate for PHB biosynthesis (Taroncher-Oldenburg et al. 2000; Knoop et al. 2010; Monshupanee and Incharoensakdi 2014). Due to the fact that the presence of Act did not obviously increase the glycogen accumulation, as found in this study (Fig. 2), *C. hansgirgi* may not have a metabolic pathway for converting Act to glycogen.

In Act-supplied cultures, the rapidly increased glycogen levels after day 14 (Fig. 2) are likely a consequence of nitrogen depletion because 80–93% of the levels of nitrogen source (nitrate) were depleted from the culture medium (Supplementary Information Table S1). It has been also reported in cyanobacterium *Synechocystis* sp. PCC6803 that nitrogen depletion significantly increased glycogen accumulation (Klotz et al. 2016).

Production levels of the PHB and glycogen biopolymers by *C. hansgirgi*

The maximal production (mg L^{-1}) and productivity (mg L^{-1} day^{-1}) of PHB and glycogen derived from *C. hansgirgi* cultures were obtained as follows.

For PHB, the photoautotrophic culture produced up to 71 mg L^{-1}PHB at day 28 (Table 2 in the footnote), but the optimal Glu supplementation of 37.5 and 75 mM markedly increased the production by up to 27-fold to 1951 and 1927 mg L^{-1} PHB, respectively (Table 2). On the other hand, the supply of Act at the same concentrations enhanced PHB production by up to 7- to 10-fold relative to that of the maximal level produced by autotrophic cells without supplementation (Table 2). These results might be due to the data that one molecule of Glu is converted to two molecules of acetyl-CoA (the substrate for PHB synthesis) via universal glycolysis pathway, while one molecule of Act is expected to be converted to only one acetyl-CoA molecule.

For glycogen, the photoautotrophy produced up to 588 mg L^{-1}glycogen at day 28 (Table 2 in the footnote), while the optimal Glu addition (75 mM) increased the production by up to 3.3-fold to 1971 mg L^{-1} glycogen (Table 2). The optimal Act supply at the same concentration only slightly enhanced the glycogen production by 1.8-fold of the level produced by the photoautotrophic cells (Table 2).

For culture productivity, photoautotrophy yielded a low PHB productivity of up to 2 mg L^{-1} day^{-1} at day 28 (Table 2 in the footnote). The supply of 37.5 mM Glu or 75 mM Act strongly increased the PHB productivity up to 139 or 48 mg L^{-1} day^{-1}, respectively (Table 2). For glycogen, the photoautotrophy yielded low glycogen productivity of up to 21 mg L^{-1} day^{-1} at day 28 (Table 2 in the footnote), but the optimal supply of Glu or Act (75 mM) significantly enhanced the productivity up to 213 or 52 mg L^{-1} day^{-1}, respectively (Table 2). Overall, the optimal Glu supply resulted in a higher productivity of both PHB and glycogen than that with the optimal Act supply.

This study obtained a maximal PHB production of 1,951 mg L^{-1} and a maximal PHB productivity of 139 mg L^{-1} day^{-1} from the single-stage photoheterotrophic culture of *C. hansgirgi* supplied with Glu (Table 2). Comparing with the PHB productivity obtained from this study at 139 mg L^{-1} day^{-1}, the slightly higher cyanobacterial PHB productivity of 174 mg L^{-1} day^{-1} has recently been reported in the UV-mutated *Synechocystis* sp. PCC 6714 (Kamravamanesh et al. 2018). The cyanobacterial PHB production (1.95 g L^{-1}) and productivity (0.139 g L^{-1} day^{-1}) obtained from this study are still lower than those obtained from high cell density cultures of heterotrophic bacteria that yielded PHB production of 19–168 g L^{-1} (Favaro et al. 2018) and PHB productivity up to 76.8 g L^{-1} day^{-1} (Wang and Lee 1997).

The *CE* of glucose and acetate to PHB and glycogen

It is known that the supply by Glu or Act can increase accumulations of PHB (Sharma and Mallick 2005; Drosg et al. 2015; Koller and Maršálek 2015) and glycogen (Singh and Sherman 2005; Gaudana et al. 2013) in a number of cyanobacterial strains. Nevertheless, the information on the mass conversion from Glu or Act to the specific biopolymer under photoheterotrophy is lacking. In this study, the mass conversion efficiency (*CE*), as the percent mass of the organic substrate (Glu or Act) that was converted to PHB and glycogen, was then determined in *C. hansgirgi.*

For PHB, the maximal $CE^{Glu->PHB}$ obtained was 29 ± 6% (w/w) from the 37.5 mM Glu-supplied culture, while the maximal $CE^{Act→PHB}_{PHB}$ obtained was 24 ± 4% (w/w) from the 37.5 mM Act supplement (Table 2). A lower or higher concentration of Glu or Act yielded lower *CE* values (Table 2).

For glycogen, the maximal $CE^{Glu->Glycogen}$ obtained was 23 ± 5% (w/w) from the 37.5 mM Glu-supplied culture (Table 2), but a higher maximal $CE^{Act->Glycogen}$ of 47 ± 8% (w/w) was derived from the 37.5 mM Act-supplied cultivation (Table 2).

The *CE* for converting the single substrate to both PHB and glycogen polymers was also determined. The maximal $CE^{Glu->PHB+Glycogen}$ obtained was 51 ± 15% w/w (derived from the sum between 29% of $CE^{Glu->PHB}$ and 22% of $CE^{Glu->Glycogen}$, Table 2) from the 14-day culture with a 37.5 mM Glu supply. Of obvious significance, $CE^{Act->PHB+Glycogen}$ was 71 ± 16% w/w (derived from the sum between 24% of $CE^{Act->PHB}$ and 47% of $CE^{Act->Glycogen}$, Table 2) from the 21-day culture with 37.5 mM Act supply.

For heterotrophic metabolism, the theoretical $CE^{Glu->}PHB$ and $CE^{Act->}PHB$ values calculated based on chemical stoichiometric balances are 48% (w/w) (Yamane 1993), while the theoretical $CE^{Glu->}Glycogen$ and $CE^{Act->}Glycogen$ values have not been reported. Previously, $CE^{Glu->}PHB$ and $CE^{Act->}PHB$ values of 36–42% (w/w) were reported in heterotrophic bacteria consuming composite organic substrates (Wu et al. 2012; Fu et al. 2014; Lin et al. 2015), although these bacterial cultures were supplied with multiple complex organic substrates. In addition, $CE^{Glu->}PHB$ values above the theoretical *CE* have

Table 2 Biopolymer production and *CE* of the organic substrate to the biopolymers. Cells were cultured in the normal medium (NORMAL) alone or with glucose (Glu) or acetate (Act) supplementation as described in Fig. 1. Data are presented as the average ± 1SD from three to six independent cultures. For the maximal productions and productivities values obtained from the 28-day cultures without an organic substrate supply (photoautotrophy), see the footnote below. High levels of production, productivity, and *CE* were marked by bold values

Organic substrate	Polymer	Culture time (day)	Production (mg L^{-1})				Productivity (mg L^{-1} day^{-1})				CE [% (w/w)]		
			Supplied Glu or Act concentration (mM)										
			0.0	18.7	37.5	75.0	0.0	18.7	37.5	75.0	18.7	37.5	75.0
Glu	PHB	**7**	1 ± 0	16 ± 3	125 ± 42	469 ± 87*	0 ± 0	2 ± 0	18 ± 6*	67 ± 12*	1 ± 0	3 ± 1	11 ± 4
		14	3 ± 1	164 ± 32*	**1951 ± 284***	**1927 ± 294***	0 ± 0	12 ± 2*	**139 ± 20***	**138 ± 21***	5 ± 1	**29 ± 6**	17 ± 5
		21	20 ± 4	171 ± 42*	1414 ± 276*	881 ± 142*	1 ± 1	8 ± 2*	67 ± 13*	42 ± 7*	5 ± 2	21 ± 5	7 ± 3
	Glycogen	**7**	13 ± 3	37 ± 10	874 ± 188	1493 ± 241*	2 ± 0	5 ± 1	125 ± 26*	**213 ± 34***	2 ± 1	18 ± 5	34 ± 7
		14	70 ± 21	219 ± 52	1467 ± 213*	**1971 ± 265***	5 ± 1	16 ± 7	105 ± 15*	**141 ± 19***	7 ± 2	**22 ± 4**	17 ± 4
		21	408 ± 69	677 ± 120	1588 ± 254*	1731 ± 187*	19 ± 3	32 ± 5	76 ± 12*	82 ± 9*	21 ± 4	**23 ± 5**	14 ± 3
Act	PHB	7	1 ± 0	7 ± 3	16 ± 3	56 ± 10	0 ± 0	1 ± 0	2 ± 0	8 ± 1*	1 ± 0	1 ± 0	2 ± 0
		14	3 ± 1	54 ± 10	325 ± 89*	676 ± 112*	0 ± 0	4 ± 1	23 ± 6*	48 ± 8*	5 ± 2	15 ± 3	15 ± 5
		21	20 ± 4	118 ± 24	536 ± 111*	765 ± 104*	1 ± 1	6 ± 1*	26 ± 5*	36 ± 5*	11 ± 4	**24 ± 4**	17 ± 4
	Glycogen	**7**	13 ± 3	36 ± 10	90 ± 8	150 ± 12	2 ± 0	5 ± 1	13 ± 1	21 ± 2	4 ± 1	4 ± 1	5 ± 2
		14	70 ± 21	107 ± 13	125 ± 22	364 ± 42	5 ± 1	8 ± 1	9 ± 2	26 ± 3	10 ± 3	6 ± 2	8 ± 3
		21	408 ± 69	383 ± 92	1044 ± 165*	1092 ± 172*	19 ± 3	18 ± 4	50 ± 8*	52 ± 8*	35 ± 6	**47 ± 8**	**25 ± 5**

*Significantly higher levels ($P < 0.01$, two-tailed *t*-test) than the maximal level obtained from the 28-day photoautotrophic culture without organic substrate supply: production: PHB = 71 ± 20 mg L^{-1}, glycogen = 588 ± 158 mg L^{-1}; productivity: PHB = 2 ± 0 mg L^{-1} day^{-1}, glycogen = 21 ± 5 mg L^{-1} day^{-1}

been reported in the heterotrophic bacterium *Halomonas* TD01 at 52% (w/w), which may reflect that the cells also use other organic substrates, rather than just Glu in the composite organic substrate medium, for PHB production (Tan et al. 2011).

We recently reported a $CE^{Act->PHB}$ of 51 ± 7% (w/w) in the two-stage culture of the cyanobacterium *Chlorogloea fritschii* (Monshupanee et al. 2016), which were first cultured photoautotrophically and then heterotrophically with the single organic substrate (Act) supply in the dark. This high $CE^{Act->PHB}$ by *C. fritschii*, at a comparable level to theoretical value, may reflect that the cells can convert some part of their metabolites that had been accumulated during photoautotrophic stage to PHB in the subsequent heterotrophic stage in the dark (Monshupanee et al. 2016). However, such cyanobacterial two-stage cultivations require extensive culture times and resources for the two-step biomass harvesting, two types of media, two culture conditions, and biomass transfer, which reduce its commercial production feasibility. In this study, we demonstrated that the single-stage photoheterotrophy cultivation of *C. hansgirgi*, which requires less resources and time than those of the two-stage cultivation, yielded $CE^{Glu->PHB+Glycogen}$ at 51 ± 15% w/w (derived from the sum between 29% of $CE^{Glu->PHB}$ and 22% of $CE^{Glu->Glycogen}$, Table 2) and $CE^{Act->PHB+Glycogen}$ at 71 ± 16% w/w (derived from the sum between 24% of $CE^{Act->PHB}$ and 47% of $CE^{Act->Glycogen}$, Table 2). These maximum $CE^{Glu->PHB+Glycogen}$ and $CE^{Act->PHB+Glycogen}$ obtained from this work are the highest levels reported in photoheterotrophic microbes so far. It is noted that the determination of *CE* in this study is based only on mass conversion from an organic substrate to PHB and glycogen. Thus, *CE* values of this study must be interpreted with respect to only mass conversion efficiency. The *CE* determination in this study did not make the chemical stoichiometric calculation of different carbon contents of Act and Glu, nor the calculation of the fixed CO_2 amount by the photoheterotrophic cells. Further determination of the conversion efficiency based on such carbon stoichiometry should be made.

The factors that contribute to these high *CE* values of photoheterotrophic *C. hansgirgi*, at 51 and 71% (w/w), were the multiple-fold increase in both the biomass and accumulation levels of PHB and glycogen after the photoheterotrophic cultivation, relative to those levels at the initial cultivation time (Figs. 1 and 2). These obtained high $CE^{Glu->PHB+Glycogen}$ and $CE^{Act->PHB+Glycogen}$ values are not likely to have resulted from only heterotrophic conversion of the substrate to the biopolymers, as observed by the substrate consumption by the cells (Fig. 2), because the cells also performed oxygenic photosynthesis, as evidenced by the cellular release of oxygen (Fig. 3). We speculated that the presence of both phototrophic metabolism (converting CO_2 and light to organic metabolites and energy), and heterotrophic metabolism (converting organic substrate to metabolites and energy) in photoheterotrophic *C. hansgirgi* would contribute to the high *CE* values.

It is noted that photoheterotrophic *C. hansgirgi* had lower photosynthetic activities than the photoautotrophic cells, as determined by the oxygen evolution rate (Fig. 3). The oxygen evolution rates of the Glu- and Act-supplied cells were 26–58% and 36–74%, respectively, of the levels of photoautotrophic cells (Fig. 3). The lower oxygen evolution rates of the photoheterotrophic cells supplied with Glu or Act relative to photoautotrophic cells have been also reported in other cyanobacteria (Wu et al. 2002; Takahashi et al. 2008).

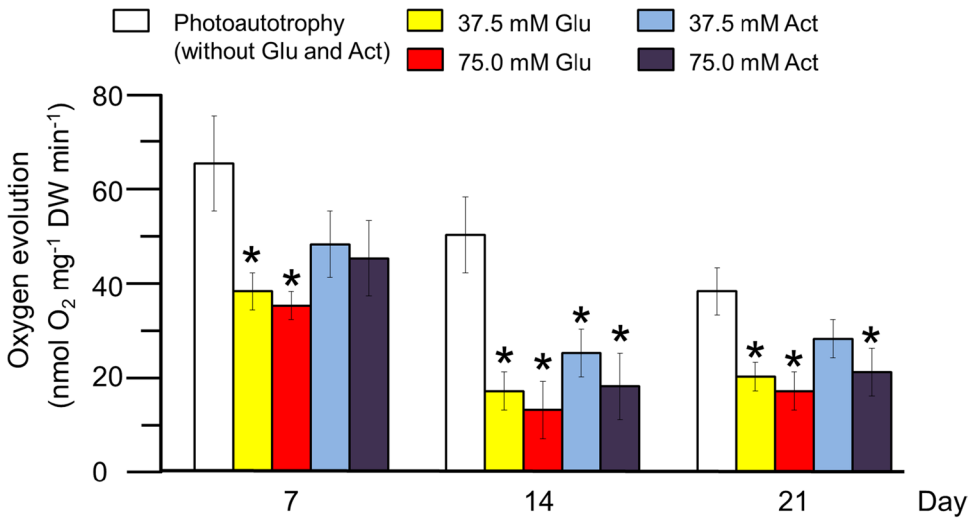

Fig. 3 Oxygen evolution from photoautotrophic and photoheterotrophic *C. hansgirgi*. Cells were cultured in the normal medium (NORMAL) in the presence or absence of Glu or Act as detailed in Fig. 1. The culture in the absence of Glu or Act was marked as photoautotrophy. Data are presented as the average ± 1SD from three to four independent experiments. Asterisks mark significantly different values ($P < 0.01$, two-tailed *t*-test) compared to that obtained from the photoautotrophic cells with the same culture time

Table 3 Material properties of the purified PHB from photoheterotrophic *C. hansgirgi*. T_m, melting temperature (in parentheses is shown the first melting peak); T_g, glass transition temperature; T_{cc}, cold crystallization temperature; ΔH_m, enthalpy of fusion; X_c, % crystallinity; *Mw*, weight-average molecular weight; *Mn*, number-average molecular weight; *Mw/Mn*, polydispersity. Mechanical property values are the average ± 1SD, derived from three independent samples

Source of biopolymer	Thermal properties					Mechanical properties			Molecular weight		
	T_m (°C)	T_g (°C)	T_{cc} (°C)	ΔH_m (J g^{-1})	X_c (%)	Elongation at break (%)	Tensile strength (MPa)	Young's modulus (MPa)	M_w (kDa)	M_n (kDa)	M_w/M_n
Commercial PHB [a]	175 (159)	3	48	99	68	5 ± 1	24 ± 3	820 ± 300	970	330	2.9
C. hansgirgi PHB [b]	172 (150)	5	42	85	58	4 ± 2	32 ± 8	620 ± 212	1215	470	3.3

[a]Heterotrophic bacterial PHB. Data were obtained from Sigma-Aldrich (USA)

[b]This work. PHB was purified from the biomass of photoheterotrophic cyanobacterium *C. hansgirgi* TISTR 8561. Cells were cultured as described in Fig. 1 for 14 days under NORMAL nutrient medium supplied with 37.5 mM Glu and in the light.

Material properties of the PHB produced by photoheterotrophic C. hansgirgi

The ^{1}H- and ^{13}C-NMR spectra of PHB purified from the photoheterotrophic *C. hansgirgi* biomass (Fig. S1, Supplementary Information) matched the respective spectra derived from the commercial PHB (Sigma) and those reported in the literature (Doi et al. 1989). The photoheterotrophic *C. hansgirgi* PHB had comparable thermal and physical properties to those of the commercial PHB, but with the exception that *C. hansgirgi* PHB had a 10% lower crystallinity percentage and 33% stronger tensile strength than those of the commercial PHB (Table 3). It is noted that the PHB from the heterotrophic cyanobacterium *Aulosira fertilissima* was also found to have a 56% stronger tensile strength than the commercial PHB (Samantaray and Mallick 2012). In addition, the *C. hansgirgi* PHB had a 25–42% higher molecular weight than the commercial PHB (Table 3). This makes *C. hansgirgi* PHB a high potential polymer for industrial applications since the plastic polymers with higher molecular weights are more resistant to chemical and physical attack (Mckeen 2013).

Conclusion

This study reported the efficient conversion of Act and Glu to PHB and glycogen using the single-stage photoheterotrophic cultivation of cyanobacterium *C. hansgirgi*. The high substrate *CE* obtained might reflect that the cells can perform both phototrophic and heterotrophic metabolism to produce PHB and glycogen. Further metabolic flux analysis using ^{13}C-labeled acetate/glucose (Yang et al. 2002; Ciggin et al. 2013) for the bioproduction of PHB and glycogen by *C. hansgirgi* would help provide insights into the metabolism of this photoheterotrophic cyanobacterium to create high *CE*. In addition, the culture optimization aiming to achieve a maximal *CE* of a specific substrate to bioproducts, as demonstrated in this study, might be applicable for the production of bioproducts by other photoheterotrophic microbes.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10811-021-02578-x.

Acknowledgements This project is funded by the National Research Council of Thailand (NRCT): NRCT5-RSA63001-21 (to T.M.). P.I. and T.M. were funded by the 90th Anniversary Fund (Ratchadaphiseksomphot Endowment Fund) of Chulalongkorn University grant GCU-GR1125594070M for graduated student research grant. The authors thank Dr. Aparat Mahakhant at TISTR for the *C. hansgirgi* strain and Dr. Robert Butcher for critical proofreading.

Author contribution T.M. developed the concept; T.M. designed the experiments with the input from A.I. and P.I.; P.I. and T.M. conducted the experiments and analyzed the data; T.M. wrote the manuscript. All authors reviewed the manuscript.

Availability of data and material The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests The authors declare no competing interests.

References

Abed RM, Dobretsov S, Sudesh K (2009) Applications of cyanobacteria in biotechnology. J Appl Microbiol 106:1–12

Allen MM (1984) Cyanobacterial cell inclusions. Annu Rev Microbiol 38:1–25

Ball SG, Morell MK (2003) From bacterial glycogen to starch: understanding the biogenesis of the plant starch granule. Annu Rev Plant Biol 54:207–233

Chanprateep S (2010) Current trends in biodegradable polyhydroxyalkanoates. J Biosci Bioeng 110:621–632

Ciggin AS, Orhon D, Capitani D, Miccheli A, Puccetti C, Majone M (2013) Aerobic metabolism of mixed carbon sources in sequencing batch reactor under pulse and continuous feeding. Bioresour Technol 129:118–126

De Philippis R, Sili C, Vincenzini M (1992) Glycogen and poly-β-hydroxybutyrate synthesis in *Spirulina maxima*. J Gen Microbiol 138:1623–1628

Deschamps P, Haferkamp I, d'Hulst C, Neuhaus HE, Ball SG (2008) The relocation of starch metabolism to chloroplasts: when, why and how. Trends Plant Sci 13:574–582

Doi Y, Kawaguchi Y, Nakamura Y, Kunioka M (1989) Nuclear magnetic resonance studies of poly(3-hydroxybutyrate) and polyphosphate metabolism in *Alcaligenes eutrophus*. Appl Environ Microbiol 55:2932–2938

Drosg B, Fritz I, Gattermayr F, Silvestrini L (2015) Photo-autotrophic production of poly(hydroxyalkanoates) in cyanobacteria. Chem Biochem Eng Q 29:145–156

Ernst A, Kirschenlohr H, Diez J, Böger P (1984) Glycogen content and nitrogenase activity in *Anabaena variabilis*. Arch Microbiol 140:120–125

Eungrasamee K, Incharoensakdi A, Lindblad P, Jantaro S (2020) *Synechocystis* sp. PCC 6803 overexpressing genes involved in CBB cycle and free fatty acid cycling enhances the significant levels of intracellular lipids and secreted free fatty acids. Sci Rep 10:4515

Favaro L, Basaglia M, Casella S (2018) Improving polyhydroxyalkanoate production from inexpensive carbon sources by genetic approaches: a review. Biofuel Bioprod Bior 13:208–227

Fu XZ, Tan D, Aibaidula G, Wu Q, Chen JC, Chen GQ (2014) Development of *Halomonas* TD01 as a host for open production of chemicals. Metab Eng 23:78–91

Gaudana SB, Alagesan S, Chetty M, Wangikar PP (2013) Diurnal rhythm of a unicellular diazotrophic cyanobacterium under mixotrophic conditions and elevated carbon dioxide. Photosynth Res 118:51–57

Grundel M, Scheunemann R, Lockau W, Zilliges Y (2012) Impaired glycogen synthesis causes metabolic overflow reactions and affects stress responses in the cyanobacterium *Synechocystis* sp. PCC 6803. Microbiology 158:3032–3043

John RP, Anisha GS, Nampoothiri KM, Pandey A (2011) Micro and macroalgal biomass: a renewable source for bioethanol. Bioresour Technol 102:186–193

Kamravamanesh D, Kovacs T, Pflugl S, Druzhinina I, Kroll P, Lackner M, Herwig C (2018) Increased poly-beta-hydroxybutyrate production from carbon dioxide in randomly mutated cells of cyanobacterial strain *Synechocystis* sp. PCC 6714: mutant generation and characterization. Bioresour Technol 266:34–44

Klotz A, Georg J, Bucinska L, Watanabe S, Reimann V, Januszewski W, Sobotka R, Jendrossek D, Hess WR, Forchhammer K (2016) Awakening of a dormant cyanobacterium from nitrogen chlorosis reveals a genetically determined program. Curr Biol 26:2862–2872

Knoop H, Zilliges Y, Lockau W, Steuer R (2010) The metabolic network of *Synechocystis* sp. PCC 6803: systemic properties of autotrophic growth. Plant Physiol 154:410–422

Koch M, Doello S, Gutekunst K, Forchhammer K (2019) PHB is produced from glycogen turn-over during nitrogen starvation in *Synechocystis* sp. PCC 6803. Int J Mol Sci 20:1942

Koller M, Maršálek L (2015) Cyanobacterial polyhydroxyalkanoate production: status quo and quo vadis? Curr Biotechnol 4:464–480

Lee SY (1996) Bacterial polyhydroxyalkanoates. Biotechnol Bioeng 49:1–14

Lin Z, Zhang Y, Yuan Q, Liu Q, Li Y, Wang Z, Ma H, Chen T, Zhao X (2015) Metabolic engineering of *Escherichia coli* for poly(3-hydroxybutyrate) production via threonine bypass. Microb Cell Fact 14:185

Luan G, Zhang S, Wang M, Lu X (2019) Progress and perspective on cyanobacterial glycogen metabolism engineering. Biotechnol Adv 37:771–786

McKeen LW (2013) Introduction to plastics and polymers compositions. In: McKeen LW (ed) The effect of UV light and weather on plastics and elastomers, 3rd edn. William Andrew, Amsterdam, pp 1–16

Monshupanee T, Incharoensakdi A (2014) Enhanced accumulation of glycogen, lipids and polyhydroxybutyrate under optimal nutrients and light intensities in the cyanobacterium *Synechocystis* sp. PCC 6803. J Appl Microbiol 116:830–838

Monshupanee T, Nimdach P, Incharoensakdi A (2016) Two-stage (photoautotrophy and heterotrophy) cultivation enables efficient production of bioplastic poly-3-hydroxybutyrate in auto-sedimenting cyanobacterium. Sci Rep 6:37121

Panda B, Mallick N (2007) Enhanced poly-beta-hydroxybutyrate accumulation in a unicellular cyanobacterium, *Synechocystis* sp. PCC 6803. Lett Appl Microbiol 44:194–198

Park SH, Na Y, Kim J, Kang SD, Park KH (2018) Properties and applications of starch modifying enzymes for use in the baking industry. Food Sci Biotechnol 27:299–312

Potts M, Ocampo-Friedmann R, Bowman MA, Tözűn B (1983) *Chroococcus* S24 and *Chroococcus* N41 (cyanobacteria): morphological, biochemical and genetic characterization and effects of water stress on ultrastructure. Arch Microbiol 135:81–90

Rippka R, Deruelles J, Waterbury JB, Herdman M, Stanier RY (1979) Generic assignments, strain histories and properties of pure cultures of cyanobacteria. J Gen Microbiol 111:1–61

Samantaray S, Mallick N (2012) Production and characterization of poly-β-hydroxybutyrate (PHB) polymer from *Aulosira fertilissima*. J Appl Phycol 24:803–814

Schlebusch M, Forchhammer K (2010) Requirement of the nitrogen starvation-induced protein Sll0783 for polyhydroxybutyrate accumulation in *Synechocystis* sp. strain PCC 6803. Appl Environ Microbiol 76:6101–6107

Sharma L, Mallick N (2005) Accumulation of poly-beta-hydroxybutyrate in *Nostoc muscorum*: regulation by pH, light-dark

cycles, N and P status and carbon sources. Bioresour Technol 96:1304–1310

Singh AK, Sherman LA (2005) Pleiotropic effect of a histidine kinase on carbohydrate metabolism in *Synechocystis* sp. strain PCC 6803 and its requirement for heterotrophic growth. J Bacteriol 187:2368–2376

Takahashi H, Uchimiya H, Hihara Y (2008) Difference in metabolite levels between photoautotrophic and photomixotrophic cultures of *Synechocystis* sp. PCC 6803 examined by capillary electrophoresis electrospray ionization mass spectrometry. J Exp Bot 59:3009–3018

Tan D, Xue YS, Aibaidula G, Chen GQ (2011) Unsterile and continuous production of polyhydroxybutyrate by *Halomonas* TD01. Bioresour Technol 102:8130–8136

Tarawat S, Incharoensakdi A, Monshupanee T (2020) Cyanobacterial production of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) from carbon dioxide or a single organic substrate: improved polymer elongation with an extremely high 3-hydroxyvalerate mole proportion. J Appl Phycol 32:1095–1102

Taroncher-Oldenburg G, Nishina K, Stephanopoulos G (2000) Identification and analysis of the polyhydroxyalkanoate-specific beta-ketothiolase and acetoacetyl coenzyme A reductase genes in the cyanobacterium *Synechocystis* sp. strain PCC 6803. Appl Environ Microbiol 66:4440–4448

Verlinden RA, Hill DJ, Kenward MA, Williams CD, Radecka I (2007) Bacterial synthesis of biodegradable polyhydroxyalkanoates. J Appl Microbiol 102:1437–1449

Wang B, Pugh S, Nielsen DR, Zhang W, Meldrum DR (2013) Engineering cyanobacteria for photosynthetic production of 3-hydroxybutyrate directly from CO_2. Metab Eng 16:68–77

Wang F, Lee SY (1997) Production of poly(3-hydroxybutyrate) by fed-batch culture of filamentation-suppressed recombinant *Escherichia coli*. Appl Environ Microbiol 63:4765–4769

Wilson WA, Roach PJ, Montero M, Baroja-Fernandez E, Munoz FJ, Eydallin G, Viale AM, Pozueta-Romero J (2010) Regulation of glycogen metabolism in yeast and bacteria. FEMS Microbial Rev 34:952–985

Wu G, Bao T, Shen Z, Wu Q (2002) Sodium acetate stimulates PHB biosynthesis in *Synechocystis* sp. PCC 6803. Tsinghua Sci Technol 7:435–438

Wu SC, Liou SZ, Lee CM (2012) Correlation between bio-hydrogen production and polyhydroxybutyrate (PHB) synthesis by *Rhodopseudomonas palustris* WP3-5. Bioresour Technol 113:44–50

Yamane T (1993) Yield of poly-D(-)-3-hydroxybutyrate from various carbon sources: a theoretical study. Biotechnol Bioeng 41:165–170

Yang C, Hua Q, Shimizu K (2002) Metabolic flux analysis in *Synechocystis* using isotope distribution from ^{13}C-labeled glucose. Metab Eng 4:202–216

Yellore V, Desai A (1998) Production of poly-3-hydroxybutyrate from lactose and whey by *Methylobacterium* sp. ZP24. Lett Appl Microbiol 26:391–394