



Optimum NaHCO_3 -to-vinasse ratio for *Chlorella pyrenoidosa* cultivation and poly-b-hydroxybutyrate (PHB) production

Gregorius Prima Indra Budianto^{a,*}, Yari Mukti Wibowo^a, H. Hadiyanto^b, W. Widayat^b

^aChemical Engineering Department, Engineering Faculty, Setia Budi University, Jl. LetJend Sutoyo Mojosoongo, Surakarta, Indonesia, Tel. +6287738167428; emails: greg.budianto87@gmail.com (G.P.I. Budianto), yarimukti@gmail.com (Y.M. Wibowo)

^bChemical Engineering Department, Engineering Faculty, Diponegoro University, Jl. Prof. Soedarto, Tembalang, Semarang, Indonesia, emails: hadiyanto@live.undip.ac.id (H. Hadiyanto), widayat@live.undip.ac.id (W. Widayat)

Received 18 December 2019; Accepted 4 August 2020

ABSTRACT

Chlorella pyrenoidosa is a microalga that grows best under mixotrophic conditions. The lag phase reflects how long *C. pyrenoidosa* takes to adapt and grow well in a cultivation medium and also affects the performance of *C. pyrenoidosa* in the next phase. NaHCO_3 is used as an alternative inorganic carbon source during *C. pyrenoidosa* cultivation. In addition, vinasse is wastewater with potential as an organic carbon source during *C. pyrenoidosa* cultivation. To determine the optimum NaHCO_3 -to-vinasse ratio, we performed an experiment over 10 d in batch mode with the following six cultivation medium ratio variations: 100% NaHCO_3 , 80% NaHCO_3 and 20% vinasse, 60% NaHCO_3 and 40% vinasse, 100% vinasse, 80% vinasse and 20% NaHCO_3 , and 60% vinasse and 40% NaHCO_3 . The lag phase of *C. pyrenoidosa* cultivation was determined using nonlinear logistic, modified Gompertz, and Richard equations. The chemical oxygen demand (COD), dry mass, and poly- β -hydroxybutyrate (PHB) content were measured every day. The ratio of 60% vinasse and 40% NaHCO_3 showed the best *C. pyrenoidosa* growth, the highest biomass productivity, and the shortest lag phase during *C. pyrenoidosa* cultivation. The lowest and highest COD efficiency was observed with 100% NaHCO_3 and 100% vinasse, respectively. In addition, to obtain PHB, the optimum ratio is 20% NaHCO_3 and 80% vinasse. A NaHCO_3 and vinasse combination is economically feasible and recommended.

Keywords: *Chlorella pyrenoidosa*; NaHCO_3 ; Vinasse; Poly-b-hydroxybutyrate

1. Introduction

Chlorella pyrenoidosa is a mixotrophic microalga that grows well with neither an organic nor an inorganic carbon source [1]. The only acceptable inorganic carbon source is sodium bicarbonate (NaHCO_3). In an optimum concentration, NaHCO_3 enhances *Chlorella* sp. growth, but excessive NaHCO_3 concentration inhibits growth [2]. NaHCO_3 also acts as a buffer to keep the pH of the system appropriate for algal cultivation [3], enhances chlorophyll formation, and enhances biomass productivity [4]. In addition, NaHCO_3

enhances algal growth in a nonsterile medium [5]. NaHCO_3 also enhances bioproduct (lipid and lutein) formation [6–8].

Organic carbon sources are always associated with organic wastewaters because organic wastewater is abundantly available and is cheap. Using a cheap organic carbon source minimizes the production cost from €3 to 1.8/kg [9]. Vinasse is a bottom product of the ethanol industry that contains simple nutrients such as carbon (represented by the chemical oxygen demand (COD)), nitrogen, and phosphate [10,11]. Adding vinasse to the cultivation medium can enhance *Chlorella* sp. growth [12] and bioproduct formation because of its glucose content [13]. Melo et al. [14] reported

* Corresponding author.

that during cultivation, *Chlorella* sp. minimize vinasse toxicity by decreasing vinasse's genotoxic potential. Vinasse and NaHCO_3 can be used as a cultivation medium, but there is limited information available about their performance with regard to *C. pyrenoidosa* growth, especially the lag phase. The lag phase reflects how long *C. pyrenoidosa* takes to adapt and grow well in a cultivation medium and also affects the performance of *C. pyrenoidosa* in the next phase. Usually, the lag phase of microalgal cultivation can be determined using nonlinear logistic, modified Gompertz, and Richard equations [15,16]. Frunzo et al. [17] used a nonlinear equation to obtain the best-fit lag phase.

This study established lag phase prediction of *C. pyrenoidosa* in different vinasse and NaHCO_3 concentrations using three growth equations. In addition, the effect of the lag phase on biomass productivity, the COD degradation rate, active cell concentration, specific growth rate, and conversion of active cells into poly- β -hydroxybutyrate (PHB) was determined.

2. Materials and methods

2.1. Cultivation medium

In this study, NaHCO_3 and vinasse were used as the cultivation medium. Briefly, we diluted 2 g of NaHCO_3 in 1 L tap water, sterilized 5 mg of vinasse to eliminate bacterial content, and then diluted vinasse in 1 L tap water (COD = 1,435 mg/L); the pH was set to 10. Both were mixed in the following six concentrations: 100% NaHCO_3 , 80% NaHCO_3 and 20% vinasse, 60% NaHCO_3 and 40% vinasse, 100% vinasse, 80% vinasse and 20% NaHCO_3 , and 60% vinasse and 40% NaHCO_3 .

2.2. Stock of *C. pyrenoidosa* culture

C. pyrenoidosa was obtained from CV Algae Park (Sukoharjo, Indonesia). It was cultivated in a synthetic medium [18] and was ready to use when the optical density (OD) reached 1.59.

2.3. Cultivation conditions

We used six 1 L glass flask disks with a fluid capacity of 1,000 mL each as artificial reactors. The artificial reactors were conditioned to each contain 2 mg/L of Guillard nutrient every 5 d and have 54 W neon lighting, and they were aerated (2 L/min) using a conventional aerator. We performed our experiments over 10 d in batch mode. The *C. pyrenoidosa*-to-medium ratio was 1:1 v/v with 1 L fluid volume.

2.4. Measurements

We assayed a sample every day to calculate its OD by UV-visible spectrophotometry ($\lambda = 680$ nm), dry cell mass by autoflocculation [19], COD by open reflux, and PHB content by the Senior method [20].

2.5. Lag phase prediction

We used logistic, Gompertz, and Richard equations to estimate the acclimatization phase during *C. pyrenoidosa* cultivation [15], as shown in Eqs. (1)–(3), respectively:

$$x(t) = \frac{x_\infty}{1 + \exp\left[\frac{4\mu}{x_\infty}(\lambda - t) + 2\right]}, \quad (1)$$

$$x(t) = x_\infty \exp\left\{-\exp\left[\frac{2.718282\mu}{x_\infty}(\lambda - t) + 1\right]\right\}, \quad (2)$$

and

$$x(t) = x_\infty \left\{1 + v \exp\left[1 + v + \frac{\mu}{x_\infty}(1 + v)^{1+1/v}(\lambda - t)\right]\right\}^{(-1/v)}, \quad (3)$$

where $x(t)$ is the active cell concentration at time t (mg/L), x_∞ is the maximum active cell concentration (mg/L), μ is biomass productivity (mg/L d), and λ is the lag phase (d).

2.6. Kinetic parameters

C. pyrenoidosa cultivation is a complex mechanism that includes cellular metabolism. We performed simplification by assuming all of organic matter was represented as a quasi-limited-single substrate (S). On the basis of the substrate mass balance in the artificial reactors, the substrate COD degradation was expressed as Eq. (4):

$$-\frac{dS}{dt} = k_L S^n, \quad (4)$$

where k_L is the COD degradation constant (per day), S is the COD concentration at time t (mg/L), and n is the kinetic order (the best value was obtained by the trial-and-error method).

We calculated the specific growth rate of *C. pyrenoidosa* as previously described [21]:

$$\mu_m = \frac{\ln(\text{OD}) - \ln(\text{OD}_0)}{t - t_0}, \quad (5)$$

where μ_m is the biomass specific growth rate (per day).

Theoretically, the PHB accumulation reaches 80% by dry cell mass [22]. We used the concept of the Y coefficient to quantify the PHB content as follows:

$$\frac{dx}{dt} = \frac{1}{Y_{\text{PHB}/x}} \frac{dP}{dt}, \quad (6)$$

where $Y_{\text{PHB}/x}$ is the PHB yield per unit active cell.

We used the COD degradation constant (k_L), biomass specific growth rate (μ_m), biomass productivity (μ), lag phase (λ), and the PHB-to-COD yield ($Y_{\text{PHB}/\text{COD}}$) to quantify the performance of the artificial reactors.

3. Results and discussion

3.1. *C. pyrenoidosa* growth in vinasse

The mixotrophic growth of *C. pyrenoidosa* in NaHCO_3 is better compared to autotrophic or heterotrophic growth [1].

Fig. 1 shows *C. pyrenoidosa* growth in different concentrations of vinasse and NaHCO_3 .

Fig. 1 shows that the active cell concentration increased over time in all six artificial reactors, indicating that vinasse can be used as a cultivation medium for *C. pyrenoidosa*. In addition, *C. pyrenoidosa* grew better at higher concentrations of vinasse compared to NaHCO_3 . Vinasse contains essential nutrients, such as nitrogen and phosphate, that support *C. pyrenoidosa* growth. The artificial reactor with 60% vinasse and 40% NaHCO_3 showed the best *C. pyrenoidosa* growth.

3.2. Prediction of lag phase and its relationship with biomass productivity

We used quantitative analysis to determine the optimum performance of the six artificial reactors. The lag phase (λ) is a parameter that shows how long *C. pyrenoidosa* cells take to acclimate with their external environment. We predicted λ in the six artificial reactors at different concentrations of vinasse and NaHCO_3 using nonlinear logistic, modified Gompertz, and Richard equations because the growth curve of *C. pyrenoidosa* is similar to the microorganism growth curve (sigmoidal shape). Fig. 2 shows *C. pyrenoidosa* grow exponentially over 10 d, marked by a precipitous slope in the curve in each artificial reactor.

The active cell concentration in each artificial reactor could be optimized by the three equations as well. As shown in Fig. 2, which the curve has coefficient of determination are close one respectively. On the basis of Fig. 2 we predicted biomass productivity (μ) by calculating the slope. In contrast, we predicted λ by taking the straight line on the curve until it intersected with the x axis (time). Quantitatively, Table 1 shows μ and λ .

Using 100% NaHCO_3 caused the shortest λ and the lowest μ . To increase μ , we combined NaHCO_3 and vinasse. The artificial reactors with 80% NaHCO_3 and 20% vinasse, and 60% NaHCO_3 and 40% vinasse showed a significant increase in μ but λ was longer. In contrast, increasing the

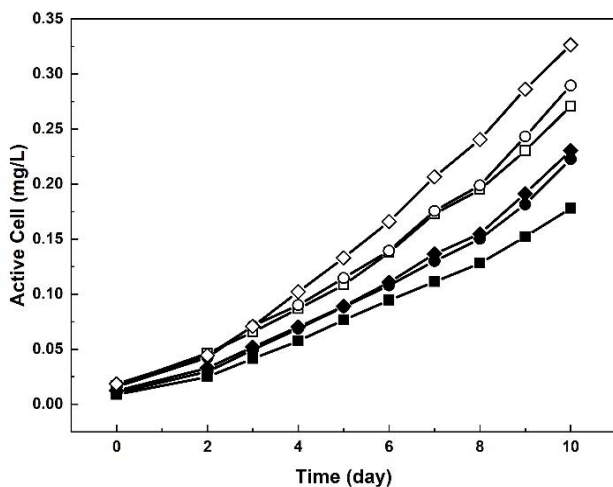


Fig. 1. Active cell concentration (mg/L) profile in 100% NaHCO_3 (■), 80% NaHCO_3 and 20% vinasse (●), 60% NaHCO_3 and 40% vinasse (◆), 100% vinasse (□), 80% vinasse and 20% NaHCO_3 (○), and 60% vinasse and 40% NaHCO_3 (◇) over time.

concentration of vinasse compared to NaHCO_3 decreased λ , with 100% vinasse significantly increasing μ but only slightly increasing λ (Fig. 3).

The maximum active cell concentration (x_∞), or active cells in the stationary phase, represents the maximum concentration of active cells that form until the stationary phase in an artificial reactor. As shown in Table 2, x_∞ depends on λ ; the longer the value of λ , the greater the value of x_∞ . x_∞ was inconsistent compared to λ and μ . In contrast, x_∞ was more sensitive to condition alteration compared to λ and μ . However, to determine the optimum condition, other parameters are required, such as the COD degradation rate (k_1), which represents the rate of organic matter degradation by *C. pyrenoidosa*; the specific growth rate (μ_m), which is the maximum growth rate of *C. pyrenoidosa*; and the PHB yield per active cell ($Y_{\text{PHB}/x}$). PHB is a valuable by-product of *C. pyrenoidosa* cultivation. Table 2 shows the values of kinetic parameters in this study.

3.3. COD degradation rate

In this study, we used NaHCO_3 as the inorganic carbon source, and we regularly added Guillard nutrient every 5 d to fulfill the need for large amounts of nutrients for photosynthesis (autotrophic conditions). Previous studies have shown that light and a large amount of nutrients enhance microalgal growth under autotrophic conditions [23–25]. Previous studies used simple organic carbon sources such as sucrose, fructose, and glycerol under mixotrophic conditions [4,18]. Organic wastewater is a mixture of several simple organic and inorganic constituents (especially vinasse, which contains glucose and phenolic compounds), so its degradation is the same as that of complex material [23]. The COD is a suitable parameter to determine the amount of organic material in the case of wastewater treatment by *C. pyrenoidosa*.

Table 2 shows the rate of COD degradation (k_1), which represents how fast organic material can be degraded in

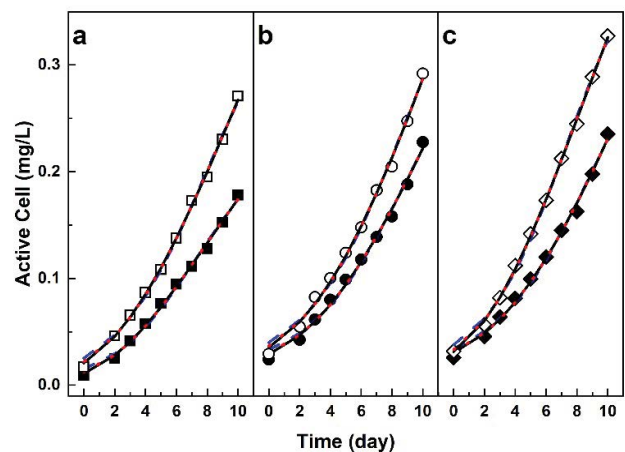


Fig. 2. Active cell concentration (mg/L) comparison (a) between 100% NaHCO_3 (■) and 100% vinasse (□), (b) between 20% vinasse and 80% NaHCO_3 (●) and 80% vinasse and 20% NaHCO_3 (○), and (c) between 40% vinasse and 60% NaHCO_3 (◆) and 60% vinasse and 40% NaHCO_3 (◇) over time, optimized by logistic (---), Gompertz (—), and Richard (· · ·) equations.

Table 1
Lag phase and biomass productivity by optimization data with LOG, modified GOM, and RICH equations

Reactor	Equation	Lag phase (d)	Lag phase average (d)	Biomass productivity (mg/L d)	Biomass productivity average (mg/L d)
I	LOG	1.61	1.7	0.0209	0.0212
	GOM	1.84		0.0218	
	RICH	1.64		0.0210	
II	LOG	3.15	2.9	0.0315	0.0303
	GOM	2.64		0.0294	
	RICH	2.78		0.0300	
III	LOG	3.66	3.2	0.0353	0.0331
	GOM	2.76		0.0312	
	RICH	3.10		0.0327	
IV	LOG	2.17	2.0	0.0341	0.0337
	GOM	1.99		0.0336	
	RICH	1.97		0.0333	
V	LOG	3.20	2.8	0.0417	0.0396
	GOM	2.52		0.0380	
	RICH	2.75		0.0392	
VI	LOG	1.91	2.0	0.0403	0.0409
	GOM	2.07		0.0419	
	RICH	1.91		0.0404	

LOG, logistic; GOM, Gompertz; RICH, Richard.

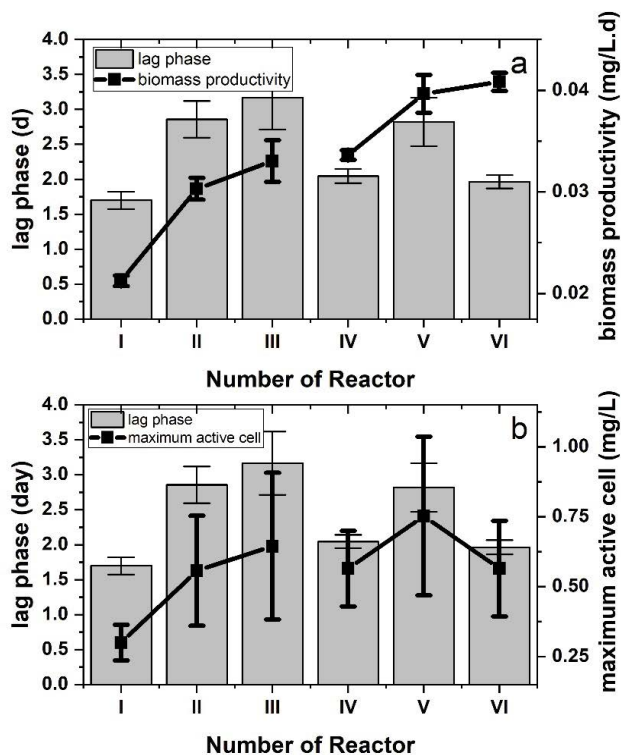


Fig. 3. Comparison between lag phase (days) and (a) biomass productivity (mg/L d) and (b) maximum active cell concentration (mg/L) with (I) 100% NaHCO_3 , (II) 80% NaHCO_3 and 20% vinasse, (III) 60% NaHCO_3 and 40% vinasse, (IV) 100% vinasse, (V) 80% vinasse and 20% NaHCO_3 , and (VI) 60% vinasse and 40% NaHCO_3 .

each artificial reactor. The artificial reactor with 100% vinasse showed a significant increase in k_L . k_L increased linearly with an increase in the vinasse concentration (20%–80% v/v), indicating that the greater the vinasse concentration in the artificial reactor, the greater the k_L . k_L showed no correlation with I (Fig. 4.). However, k_L showed a positive correlation with COD efficiency; the lowest and highest COD efficiency was observed in the artificial reactor with 100% NaHCO_3 and 100% vinasse, respectively. However, artificial reactors with both NaHCO_3 and vinasse showed an insignificant difference in COD efficiency (Table 3).

The lowest COD efficiency was found in the artificial reactor with 100% NaHCO_3 , because of limited organic matter content, *C. pyrenoidosa* only consumes inorganic carbon and the growth is autotrophic. In contrast, artificial reactors with both NaHCO_3 and vinasse have both inorganic and organic carbon sources as well as mixotrophic conditions, and the vinasse concentration can be increased up to 66%. The artificial reactor with 100% vinasse showed the highest COD efficiency. This is because of not only *C. pyrenoidosa* activity but also continuous aeration, which indirectly generates oxygen in large amounts and organic matter degradation occurs in the dark phase (heterotrophic conditions) [26,27]. So aeration functions not only as a homogenizer but also as an oxygen supplier to oxidize organic carbon.

3.4. Specific growth rate

The specific growth rate (μ_m) shows how fast *C. pyrenoidosa* can grow in the cultivation medium. Artificial reactors with both NaHCO_3 and vinasse showed an increase

Table 2

Kinetic parameters with (I) 100% NaHCO_3 , (II) 80% NaHCO_3 and 20% vinasse, (III) 60% NaHCO_3 and 40% vinasse, (IV) 100% vinasse, (V) 80% vinasse and 20% NaHCO_3 , and (VI) 60% vinasse and 40% NaHCO_3

Constants	Reactor					
	I	II	III	IV	V	VI
x_∞	0.3001	0.5569	0.6450	0.5645	0.7526	0.5721
k_L^*	0.0363	0.0857	0.1094	0.1474	0.1050	0.1010
μ_m	0.0676	0.0833	0.1020	0.0338	0.1175	0.1223
$Y_{\text{PHB}/x}$	0.0396	0.0483	0.0380	0.0519	0.0403	0.0373

*Best-adjustable n parameter for all reactors is 1, Eq. (4).

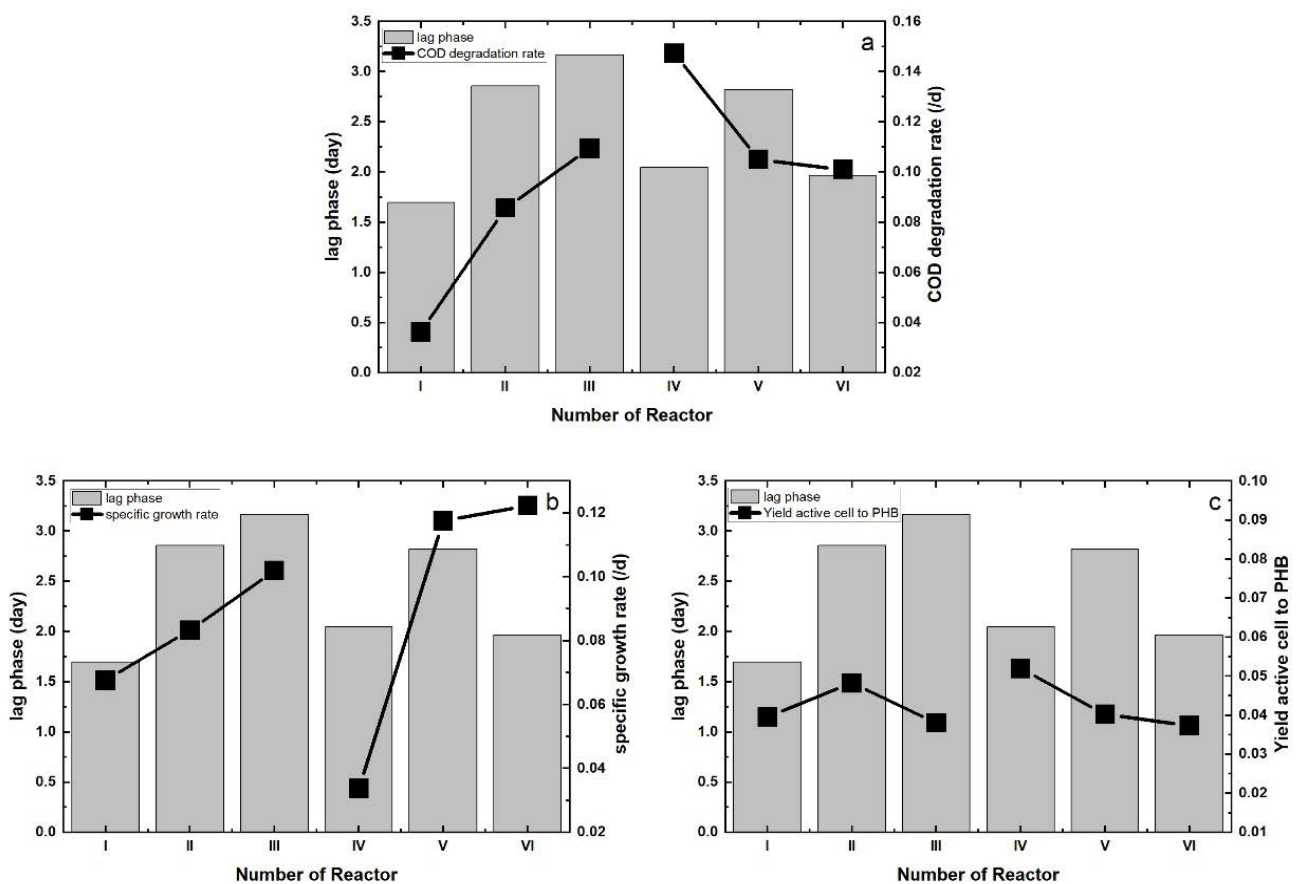


Fig. 4. Comparison between the lag phase (days) and (a) COD degradation rate (per day), (b) specific growth rate (per day), and (c) PHB yield per active cell with (I) 100% NaHCO_3 , (II) 80% NaHCO_3 and 20% vinasse, 60% NaHCO_3 and 40% vinasse, (IV) 100% vinasse, (V) 80% vinasse and 20% NaHCO_3 , and (VI) 60% vinasse and 40% NaHCO_3 . COD, chemical oxygen demand; PHB, poly- β -hydroxybutyrate.

in μ_m with an increase in the vinasse concentration (Table 2). This statement is supported by the correlation between μ_m and biomass productivity (μ), which also increased with an increase in the vinasse concentration. This results showed that artificial reactors with both NaHCO_3 and vinasse had mixotroph conditions affecting μ_m and μ . In correlation with the lag phase (λ), μ_m (Fig. 4) has the same relationship inclination with m (Fig. 3a). The optimum condition was with 60% vinasse and 40% NaHCO_3 , where μ_m and μ were the highest and λ the shortest.

Fig. 5 shows how a higher k_L increases μ_m and μ . The result showed that *C. pyrenoidosa* uses the organic content in vinasse to grow and that utilization of 100% vinasse as the cultivation medium induces nutrient stress, where μ_m falls lower than that in the artificial reactor with 0% vinasse. μ_m decreases because of the interrupted light penetration in the artificial reactor due to the presence of brown dye (melanoidin) in vinasse. This statement also explains about hesitation of involvement *C. pyrenoidosa* in process of COD degradation. In contrast, *C. pyrenoidosa* growth slowed because

of abundant organic carbon (vinasse), so it was unable to properly perform photosynthesis. Vinasse is utilized by cells as an organic carbon source to synthesize biomass and perform cellular maintenance, and excess organic carbon is used to synthesize PHB, especially under nutrient stress [28].

3.5. Yield of active cell to PHB yield per active cell

PHB is a valuable by-product formed by microalgae inside cells biologically. Several microalgae accumulate PHB in their cells (Table 4). The existence of carbon and an external environment affect PHB accumulation, and both can be modified to obtain better PHB accumulation.

We obtained PHB under three conditions. First, PHB was formed under normal growth conditions with an inorganic carbon source. *C. pyrenoidosa* growth was autotrophic with NaHCO_3 as the inorganic carbon source. Second, *C. pyrenoidosa* growth was mixotrophic with two carbon sources, vinasse and NaHCO_3 ; PHB accumulation was compared to autotrophic conditions. Adding an organic carbon source into the artificial reactor affects the carbon/nitrogen ratio. The higher carbon content with limited nitrogen enhances PHB accumulation [33]. Third, PHB accumulation occurred because of interrupted light penetration condition in the artificial reactor due to the presence of melanoidin in vinasse. However, accumulation of PHB instead of increased in this condition so that the light interruption can be made the *C. pyrenoidosa* in stress condition. Microalgae use excess organic carbon to synthesize PHB in heterotrophic growth [28]. Table 4 also shows that *Nostoc muscorum* better accumulates PHB under heterotrophic conditions and the utilization of organic carbon also enhances PHB accumulation in *Chlorella fusca*.

We found no correlation between λ and $Y_{\text{PHB}/x}$ (Fig. 4) because both parameters represent different things; λ represents the acclimatization time based on active cells, while $Y_{\text{PHB}/x}$ represents the PHB content of every stress cell.

We also found a negative correlation between $Y_{\text{PHB}/x}$ and μ_m and between $Y_{\text{PHB}/x}$ and μ (Fig. 6); when μ_m and μ are high, $Y_{\text{PHB}/x}$ is low. The artificial reactor with 100% vinasse had the highest $Y_{\text{PHB}/x}$ when μ_m was the slowest and μ was higher compared to the artificial reactor with 100% NaHCO_3

Table 3

COD degradation efficiency in (I) 100% NaHCO_3 , (II) 80% NaHCO_3 and 20% vinasse, (III) 60% NaHCO_3 and 40% vinasse, (IV) 100% vinasse, (V) 80% vinasse and 20% NaHCO_3 , and (VI) 60% vinasse and 40% NaHCO_3

Reactor	Influent (mg/L)	Effluent (mg/L)	COD efficiency (%)
I	138	96	30%
II	505	202	60%
III	695	238	66%
IV	1,476	390	74%
V	1,221	419	66%
VI	950	326	66%

COD, chemical oxygen demand.

(Fig. 6). This results were consistent with previous studies that showed the highest PHB accumulation under good metabolism and cells productivity with a low μ_m [32].

3.6. Techno-economic approach

The production of alga-based bioplastic (PHB) is technically feasible [34]. Qualitatively, PHB has good tensile strength and Young's modulus, with a lower extension-to-break ratio compared to petrochemical plastic. The biodegradability potential calculated as a percentage of weight loss and as the result of efficient PHB degradation is 24.58% in 60 d [28]. In addition, PHB has similar physical properties as polypropylene (PP) and more resistance to UV light, but PHB is not recommended for use in solvent packaging [35].

From an economic aspect, to determine the effect of the NaHCO_3 -to-vinasse ratio on PHB production during *Chlorella sp.* cultivation, we calculated the direct production

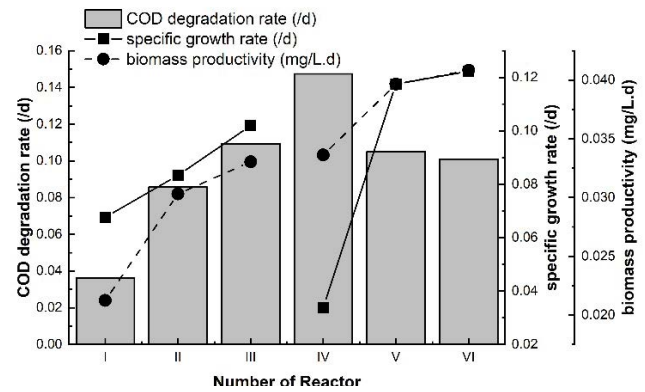


Fig. 5. Correlation between the COD degradation rate (per day) and specific growth rate (per day) and biomass productivity (mg/L d) in (I) 100% NaHCO_3 , (II) 80% NaHCO_3 and 20% vinasse, (III) 60% NaHCO_3 and 40% vinasse, (IV) 100% vinasse, (V) 80% vinasse and 20% NaHCO_3 , and (VI) 60% vinasse and 40% NaHCO_3 , COD, chemical oxygen demand.

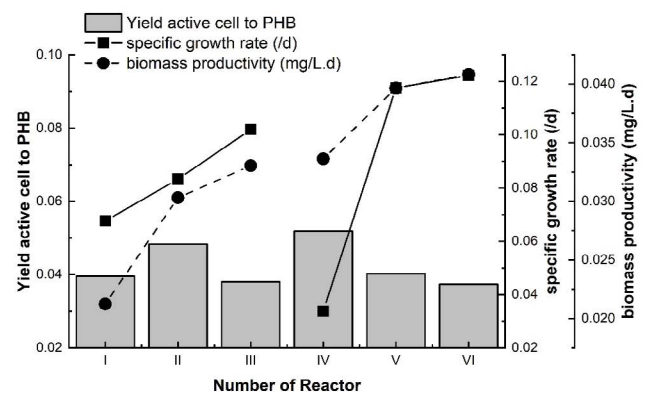


Fig. 6. Correlation between PHB yield per active cell and specific growth rate (per day) and biomass productivity (mg/L d) in (I) 100% NaHCO_3 , (II) 80% NaHCO_3 and 20% vinasse, (III) 60% NaHCO_3 and 40% vinasse, (IV) 100% vinasse, (V) 80% vinasse and 20% NaHCO_3 , and (VI) 60% vinasse and 40% NaHCO_3 . PHB, poly- β -hydroxybutyrate.

cost (DPC) by multiplying the direct manufacturing cost (DMC), indirect manufacturing cost (IMC), and fixed manufacturing cost (FMC) on the basis of the process capacity to produce 1 kg/d of PHB. Raw material, maintenance, and plant supplies are included in the DMC; all components of the IMC are negligible; and the FMC is calculated from depreciation, taxes, and insurance. Maintenance, plant supplies, depreciation, taxes, and insurance depend on the fixed capital investment (FCI). The FCI only focuses on the cost of major processes and utility equipment, their setup, and piping. The major processes and utility equipment in this study included a reactor, a blower, a centrifuge, and two settling tanks. Their costs were adjusted to 2020 using the chemical plant cost index. In addition, to obtain the necessary cost on the basis of size or capacity, we performed

scale-up or scale-down by an exponential law with an exponential factor of 0.85 [9]. The value of the production cost is shown in Table 5.

Depreciation was the highest cost (Table 5). It was calculated as 15% of the FCI, so the installation of processes and utility equipment was the highest and all were included in the FCI. To minimize the FCI, we used an integration strategy with anaerobic digestion. Anaerobic digestion is the process of obtaining biogas with the main constituents being 65% CH₄ and 35% CO₂ [36]. Since the anaerobic process showed good performance, the biogas rate continuity was stable, so it can substitute the air blower. In addition, the effluent slurry and biogas can also substitute vinasse and NaHCO₃ as organic and inorganic carbon sources, respectively. On the basis of the calculation, this strategy can

Table 4
Type of microalgae and the best concentration in their external environment

Microalgae	% PHB	Carbon existence	External environment	Reference
<i>Nostoc muscorum</i>	9%	None	Light dark cycle	[29]
<i>Nostoc muscorum</i>	35%	Acetate	Dark incubation	[30]
<i>Spirulina platensis</i>	6%	CO ₂	Not given	[31]
<i>Spirulina maxima</i>	7%–9%	CO ₂	N and P limitation	[31]
<i>Chlorella fusca</i>	0.5%	NaHCO ₃	Light dark cycle	[32]
<i>Chlorella fusca</i>	3.4%	Xylose	Light dark cycle	[32]
<i>Chlorella pyrenoidosa</i>	4%	NaHCO ₃	Normal growth	This study
<i>Chlorella pyrenoidosa</i>	6%	Vinasse	Interrupted light	This study
<i>Chlorella pyrenoidosa</i>	5%	NaHCO ₃ and vinasse	Normal growth	This study

PHB, poly-β-hydroxybutyrate.

Table 5
Cost of FCI, DMC, and FMC in each artificial reactor

Component	Cost (IDR)					
	R1	R2	R3	R4	R5	R6
Reactor	5,753,068	4,859,430	5,958,327	4,571,389	5,668,017	6,053,240
Air blower	1,141,164	963,905	1,181,879	906,769	1,124,294	1,200,706
Centrifuge	616,423	520,673	638,416	489,810	607,310	648,586
Settling tank	663,128	560,122	686,787	526,921	653,324	697,727
Installation	3,514,727	2,968,776	3,640,126	2,792,802	3,462,766	3,698,111
Piping	2,942,562	2,485,487	3,047,547	2,338,160	2,899,060	3,096,093
Total FIC	14,631,072	12,358,393	15,153,083	11,625,852	14,414,772	15,394,463
NaHCO ₃	505,051	331,263	315,789	0	99,256	214,477
Water	25,253	20,704	26,316	19,268	24,814	26,810
Maintenance	438,932	370,752	454,592	348,776	432,443	461,834
Plant supplies	65,840	55,613	68,189	52,316	64,866	69,275
Total DMC	1,035,075	778,331	864,887	420,360	621,379	772,396
Depreciation	1,463,107	1,235,839	1,515,308	1,162,585	1,441,477	1,539,446
Taxes	292,621	247,168	303,062	232,517	288,295	307,889
Insurance	146,311	123,584	151,531	116,259	144,148	153,945
Total FMC	1,902,039	1,606,591	1,969,901	1,511,361	1,873,920	2,001,280
Total DPC	2,937,114	2,384,922	2,834,787	1,931,721	2,495,299	2,773,676

FCI, fixed capital investment; DMC, direct manufacturing cost; FMC, fixed manufacturing cost; DPC, direct production cost.

decrease the DPC to between 14% until 29%. However, the technical aspect of this integration strategy needs further investigation.

4. Conclusion

To reduce the cost of production, a NaHCO_3 and vinasse combination is used as a *C. pyrenoidosa* cultivation medium. This combination gives a COD efficiency up to a vinasse concentration of 66%. Microscopically, 60% vinasse, and 40% NaHCO_3 give the highest specific growth rate and biomass production with the shortest lag phase. In addition to obtain PHB as valuable by-product, a NaHCO_3 and vinasse combination gives only 5% lower PHB yield compared to 100% vinasse. However, the specific growth rate with 100% vinasse is the lowest, so this is not economically feasible and a NaHCO_3 and vinasse combination is recommended.

Acknowledgments

This research was funded by the national competitive research grant PKPT from the Higher Education Directorate, Ministry of Research, Technology, and Higher Education, Indonesia (number of contract 002/LPPM-USB/Pekerti/V/2019).

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