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Optimization of tapioca wastewater treatment in sequencing batch reactor (SBR) using alkaline pre-chlorination

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ABSTRACT

This study was conducted to determine alkaline pre-chlorination effects on chemical oxygen demand (COD) removal and poly-β-hydroxybutyrate (PHB) production in tapioca wastewater treatment. The alkaline pre-chlorination effect was evaluated by applying various chlorine (Cl_2) dosages (0, 2, 4, 6, and 8 mg/L) to the wastewater with a pH of 8 before being treated in a sequencing batch reactor (SBR). The cycle time of the SBR consisted of 1 h of filling and 8 h of aeration. COD and mixed liquor suspended solids (MLSS) of the effluent were measured at 2 h intervals during the aeration period. Both parameters were to estimate the optimum Cl_2 dosage corresponding to the organic removal kinetics, the maximum specific growth rate, and the relationship between substrate utilization and microorganism's growth rate. The effluent of tapioca wastewater pre-chlorination under optimum Cl_2 dosage was treated using the SBR with the cycle period: filling (1 h), aeration (8 h), and settling (8 h). PHB, COD, and MLSS of effluent were measured at 2 h intervals of aeration and settling period for estimating PHB formation kinetics parameter values. For the Cl_2 dosage of 6 mg/L, SBR shows the best performance in terms of COD removal rate constant (k) and maximum specific growth rate (μ_{max}) with values of 0.327 and 2.681 h^{-1}, respectively. First-order kinetics, Contois equation, substrate utilization rate based on Monod equation considers cell death, and non-growth associated product formation was being appropriate models to describe organic removal rate, specific growth rate, kinetic of COD conversion to PHB, and PHB production rate, respectively. Another result showed excessive aeration time and settling time application decreased the level of microbial conversion of COD to PHB and PHB production rate per cell mass formed. Maximum yield coefficient of COD to PHB ($Y_{P/S}$) of 0.01 mg PHB/mg COD and non-growth associated PHB yield coefficient (β) of 9.07×10^{-4} mg PHB/mg MLSS/h was achieved when the aeration time was about 6 h. These results suggest that the alkaline pre-chlorination can effectively enhance the performance of the SBR system, especially treating the tapioca wastewater. The optimum process of this treatment is when SBR operated with a cycle consisted of 1 h of filling, 6 h of aeration, and 0.5 h of settling with a Cl_2 dosage of 6 mg/L (pH 8) as wastewater pretreatment.

Keywords: Alkaline pre-chlorination; Chemical oxygen demand (COD); Cycle time; Poly-β-hydroxybutyrate (PHB); Sequencing batch reactor (SBR); Tapioca wastewater

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1. Introduction

Polyhydroxyalkanoates (PHA) are biopolymers produced by various microorganisms as energy storage materials under limited nutrients with excess carbon sources. Among the PHA family, poly-β-hydroxybutyrate (PHB) is the most common natural microbial PHA due to its properties. PHB is an excellent candidate to replace petroleum-based plastics because of its characteristics such as excellent thermal stability and high biocompatibility [1]. Unfortunately, the production cost of PHB is still about 10 times higher than that of synthetic plastic [2]. Therefore, it needs a great effort from both researcher academia and industry to achieve the economic feasibility of PHB production. One of the efforts is by exploring cheap and abundant available carbon sources.

Oghenejoboh [3] reported that water bodies contaminated by untreated tapioca wastewater had high levels of cyanide, suspended solids, biological oxygen demand (BOD), and chemical oxygen demand (COD). These parameters showed the poor quality of water. One of the promising methods is by converting tapioca wastewater into PHB. Every 1 kg of soluble COD in tapioca wastewater can be potentially converted to 0.19 kg of PHB. Along with the production of PHB from tapioca wastewater, about 73.82% of influent COD could be removed [4].

The activated sludge process is a recognized mixed culture system for reducing the cost of PHA production [5]. The sequencing batch reactor (SBR) process is known to save more than 60% of the operating cost compared to a conventional activated sludge system [6]. A significant advantage associated with SBR is the space-saving by combining the aeration and settling phases in a single tank. Furthermore, the SBR system has a more exceptional ability to remove BOD and total nitrogen concentration than other conventional activated sludge processes [7]. Also, the SBR process has been successfully applied to increase PHB production in activated sludge processes [8].

Dohare and Kesharwani [9] confirmed that the performance of SBR depends on cycle time. The filling phase is required to promote the growth of floc-forming bacteria and to provide good sludge settling characteristics. Sufficient aeration time is essential to ensure complete biodegradation of organic substrates [6] and to increase the microorganism's growth rate [10]. The removal efficiency of COD increases simultaneously with the aeration time until the maximum COD removal is achieved [11]. The maximum PHB production rate was reached during the exponential phase [12]. However, hydrolysis induced the depletion of PHB content that can be observed in the stationary phase [13]. Hence, the settling time in the SBR process is a key parameter for ensuring the efficiency of PHB production [14].

Our previous research has found that a rise in filling time until a certain level can improve COD removal efficiency and microorganism's growth rate in tapioca wastewater treatment using SBR. Excessive filling time should be avoided, especially related to its detrimental effects on effluent COD concentration, *k*, $\mu_{\max}$ [15], and PHA yield [16]. Setyawaty et al. [17] reported that the average of PHA yield increased from 0.043 to 0.102 g PHA/g MLSS (MLSS – mixed liquor suspended solids) by lengthening aeration/non-aeration period in the reaction phase of an SBR from 1/3 h to 2/2 h. On the contrary, the COD removal efficiency decreased from 37% to 26%. Nevertheless, too much aeration may result in a decrease in PHA concentration and yield. Chookietwattana and Khonsarn [18] showed that the SBR system with anoxic/aerobic steps of 4/18 h produced higher PHA than the system with anoxic/aerobic steps of 2/20 h.

It was also proved that an increase of influent pH from 4.91 to 8 had positive impacts on the microorganism's growth and substrate utilization in SBR [19]. Truong et al. [20] have demonstrated that the settleability sludge can be improved by increasing gradually of organic loading rate to 7.5 kg COD/m^3/d. Chaleomrum et al. [21] investigated PHA production and COD removal using the SBR system inoculated by *Bacillus tequilensis* MSU 112. They carried out in a reaction phase which consists of anoxic/aerobic steps of 4/18 h for treatment of tapioca wastewater having an influent COD in the range of 3,000–5,000 mg/L. The tapioca wastewater with influent COD at a concentration of 4,000 mg/L gave the highest PHA production, while the tapioca wastewater with influent COD concentration of 5,000 mg/L provided the highest COD removal efficiency.

The detected cyanide (CN^-) in tapioca wastewater can reach a high level at 200 mg/L [22]. Unfortunately, poisoning of activated sludge may occur after exposure to more than 20 mg/L CN^- [23]. Alkaline pre-chlorination has been proven to be an effective method for the removal of CN^-. The application of chlorine (Cl_2) at pH 8 was successfully applied to decrease CN^- until the standard quality level is achieved. Also, no organochlorine has been detected in the treated effluent of tapioca wastewater [24]. Alkaline pre-chlorination can also dramatically increase the COD removal efficiency in tapioca wastewater treatment using the activated sludge processes [25].

The addition of 0.023 g Cl_2 per g of MLSS in 1 L of the returned sludge to SBR with an influent COD concentration of 600 mg/L has been successfully applied to reduce 45% of excess sludge production and 89% of COD. Nevertheless, the application of Cl_2 to return activated sludge is not feasible to enhance SBR performance for the treatment of tapioca wastewater. It may be explained that mainly due to the detrimental effect of COD removal efficiency in the SBR process [26].

Based on the above explanation, the determination of applied Cl_2 dosage in alkaline pre-chlorination and the recommendation of cycle time for COD removal and PHB production is interesting to be investigated. The optimum of Cl_2 dosage and cycle time was determined by comparing many kinetics models to describe the organic matter degradation, microorganism's growth, substrate utilization, and PHB formation.

The innovation potential of this project is the development of the SBR process for simultaneous COD removal and PHB production from tapioca wastewater through the application of alkaline pre-chlorination and cycle time adjustment. The expected contribution to knowledge from this study is the establishment of new eco-friendly tapioca wastewater treatment especially in the formulation of the PHB production process by combining alkaline pre-chlorination and SBR method. Moreover, the application of alkaline pre-chlorination to the wastewater before being fed to SBR could potentially enhance the competitiveness of PHB production and significantly prevent environmental pollution by untreated wastewater discharge.

2. Materials and methods

2.1. Preparation of raw materials

The wastewater, supernatant liquid from 24 h final settling in tapioca starch production, was obtained from PT Bumi Karya Tapioka, Selogiri, Central Java (Indonesia). The tapioca wastewater characteristics before any treatment are summarized in Table 1.

Activated sludge from PT Bumi Karya Tapioka was preserved in a styrofoam box with dry ice to maintain the temperature at 4°C for further use. Calcium hydroxide ($Ca(OH)_2$) and calcium hypochlorite ($Ca(OCl)_2$) powder at 60% levels of active Cl_2 was kindly supplied by PT Tjiwi Kimia brand as alkaline prechlorination. All chemicals were technical grades and used without any pre-treatment.

Table 1
Characteristics of tapioca wastewater

Parameters	Values
pH	4.92
COD (mg/L)	16,845
Total suspended solids (mg/L)	4,290

2.2. Studies of SBR process

Experimental devices consist of two identical HDPE drums having height and external diameter of 51.5 cm and 31.5 mm connected in series for pre-chlorination tank and SBR. Each drum was equipped with a 3.3 mm diameter hole at one-third height of the drum calculated from the base for effluent discharge. For aeration, SBR was supplied by three aquarium air pumps.

The pre-chlorination process was carried out by mixing tapioca wastewater with 1% $Ca(OH)_2$ solution and 0.1% $Ca(OCl)_2$ solution. $Ca(OCl)_2$ dosage was varied at 0, 2, 4, 6, and 8 mg of Cl_2/L. To adjust the pH value of 8, $Ca(OH)_2$ solution was added to the mixture of tapioca wastewater and $Ca(OCl)_2$. The mixture was then allowed for 24 h for separating the formed precipitate and liquid effluent of alkaline prechlorination. The process was to ensure the liquid was not containing the residual Cl_2 before being fed to SBR.

SBR system was first conducted under five different substrate conditions with the cycle period: filling (1 h), aeration (8 h), and settling (8 h). This step was to evaluate the effect of alkaline pre-chlorination. The liquid effluent of alkaline pre-chlorination with the best values of COD removal rate, maximum specific growth rate, and substrate utilization considering inhibition and decay rate was treated once again in the second SBR system. The cycle period, consists: filling (1 h), aeration (8 h), and settling (8 h), was to determine the effect of cycle time on COD conversion to PHB and the rate of PHB production. In the first SBR system, mixed liquor samples were taken every 2 h during the aeration phase for COD and MLSS analysis. This step was to specify the optimum Cl_2 dosage. For optimization of cycle time, the mixed liquor samples in the second SBR system were also taken at 2 h intervals of aeration and settling period for COD and PHB analysis.

COD concentrations were determined indirectly using a DR 2800 UV-VIS spectrophotometer (Hach, USA). MLSS was analyzed using a drying oven (Thermo Fischer Scientific, USA) based on Method 2540 D of APHA Standard Methods for the Examination of Water and Wastewater. The determination of PHB was carried out by the spectrophotometric method as described by Senior et al. [27].

3. Results and discussion

3.1. Optimization of Cl_2 dosage

3.1.1. Effect of adding Cl_2 dosage towards COD removal rate

The addition of Cl_2 dosage in prechlorination was observed its influence by evaluating the value of COD removal rate constant (*k*). This parameter was chosen as the indicator due to its direct proportion to the reaction rate. The *k*-value was evaluated in two different rate equations as shown in Table 2. These rate equations were then compared to determine the *k*-value. The appropriate equation for each Cl_2 dosage was addressed by the highest correlation coefficient (R^2).

Table 3 shows the *k*-values of various reaction orders for all applied Cl_2 dosages. It can be highlighted that the COD removal rate is significantly influenced by the prechlorination. The optimum Cl_2 dosage is 6 mg/L exhibiting the best SBR performance in terms of the organic removal kinetics. The COD removal rate in the varied process tends to follow the first-order kinetics, with a *k* of 0.327 h^{-1} and R^2 of 0.99. It indicates that the COD removal proceeds at a rate that depends linearly on COD concentration in the reactor [29]. Moreover, it implies that the COD concentration decreased with time following a logarithmic pattern [30]. Some researchers also confirmed that the COD removal rate in wastewater using mixed culture was well explained by the first-order kinetic model [30–32].

An increase of COD removal rate due to prechlorination is probably caused by a decrease in the influent COD concentration in SBR as seen in Table 4. It is well known that prechlorination can effectively remove COD in wastewater. Theoretically, each kg of Cl_2 will deliver 0.225 kg of O_2 [33]. Riyanti et al. [34] demonstrated that 89.02% COD could be removed using the prechlorination of tapioca wastewater with $Ca(OCl)_2$ dosage of 5 mg/100 ml for 1 h (pH 8).

Table 2
The rate equation for various reaction orders [28]

Order	Differential form	Linear regression
1	$-\frac{dS}{dt} = k \cdot S$	$\ln S = -kt + \ln So$
2	$-\frac{dS}{dt} = k \cdot S^2$	$\frac{1}{S} = -kt + \frac{1}{S_0}$

Table 3
COD removal rate constant (k) at all reaction orders (n)

n	Cl_2 dosage = 0 mg/L		Cl_2 dosage = 2 mg/L		Cl_2 dosage = 4 mg/L		Cl_2 dosage = 6 mg/L		Cl_2 dosage = 8 mg/L	
	k	R^2	k	R^2	k	R^2	k	R^2	k	R^2
1	0.088 h^{-1}	0.947	0.088 h^{-1}	0.949	0.11 h^{-1}	0.867	0.327 h^{-1}	0.990	0.019 h^{-1}	0.427
2	2×10^{-5} L/mg/h	0.953	2×10^{-5} L/mg/h	0.914	3×10^{-5} L/mg/h	0.822	4.15×10^{-3} L/mg/h	0.872	2×10^{-5} L mg/h	0.435

Table 4
Comparison of influent COD in SBR

Cl_2 dosage	Influent COD in SBR (mg/L)
0 mg/L Cl_2	8,666.66
2 mg/L Cl_2	7,933.33
4 mg/L Cl_2	5,166.67
6 mg/L Cl_2	3,483.33
8 mg/L Cl_2	1,041.67

Table 5
Specific growth rate models used [40,41]

Model	Equation
Growth limiting substrate	
Monod	$\mu = \frac{\mu_{\max S}}{K_s + S}$
Growth rate considering cell inhibition	
Contois	$\mu = \frac{\mu_{\max S}}{K_s\ S + S}$
Growth rate considering substrate inhibition	
Haldane	$\mu = \mu_{\max} \frac{S}{S + K_s + \frac{S^2}{K_i}}$
Haldane at higher substrate concentration ($K_s \leq S$)	$\mu = \mu_{\max} \frac{S}{S + \frac{S^2}{Ki}}$

The ability of prechlorination to enhance the COD removal rate might be explained that volatile fatty acids (VFA) production decreases as the COD loading rate in the SBR system decreases [35]. VFA accumulation should be avoided since it may cause the inhibition of organic substrates biodegradation [36]. Many researchers have also found similar results. They stated that the COD removal rate and its efficiency increased with decreasing COD loading rate and influent COD concentration in the SBR system [32,37,38].

Table 3 also shows the k-value for all reaction orders decreased as the Cl_2 dosage increased from 6 to 8 mg/L. This phenomenon was probably caused insufficient influent COD load which the organic degrading microorganisms require for their growth [35].

3.1.2. Effect of Cl_2 dosage adjustment on the maximum specific growth rate

Four specific growth rate models were evaluated to determine the value of the maximum specific growth rate (μ_{max}) as presented in Table 5. Eight variations in SBR operation were conducted to study the effect of Cl_2 dosage. The sum of the squared errors (SSE) was used as the basis of model validation since this parameter is widely used to measure the closeness of the observed and predicted values. A well-fitting regression model indicated by minimum SSE value was chosen for all SBR treatment [39].

Table 6 shows the estimated μ_{max} values using many specific growth rate models for all Cl_2 dosages. The μ_{max} values from the best fit model for each variation of SBR operation are marked by bold font. The growth rate generally increased when the applied Cl_2 dosage increased. A decrease in the influent COD due to Cl_2 dosage addition seemed to be an important reason for the growth rate improvement. It has been known that the concentration of organic substrate has a significant influence on the growth of microorganisms [41].

The highest μ_{max} of 2.681 h^{-1} was obtained when alkaline pre-chlorination using a Cl_2 dosage of 6 mg/L. The higher μ_{max} in SBR will produce lower effluent COD concentration that means this condition is becoming more effective. The μ_{max} obtained in this study is also higher much higher compared to the activated sludge process in municipal wastewater treatment which only ranged between 0.23 and 0.34 h^{-1} [42]. Nevertheless, μ_{max} value was found to decrease when the COD influent was decreased with an increase in Cl_2 dosage from 6 to 8 mg/L. A similar result was achieved in the research of Kumar and Subramanian [43]. They reported that the SBR process for the treatment of medium strength paper and pulp wastewater gave higher μ_{max} as compared to the treatment of high and low strength paper and pulp wastewater.

Table 6 also confirms that the Monod model is the best-suited model to describe the microorganism's growth rate, especially at a Cl_2 dosage of 8 mg/L. This result may be attributed to the effect of Cl_2 dosage addition on the reduction of the organic substrate. It is well known that the microorganism's growth rate can be inhibited at high substrate concentrations. Tazdaït et al. [44] reported that there is no inhibition in aerobic batch biodegradation for low organic substrate concentrations.

On the contrary, the SBR processes in this study using Cl_2 dosages below 8 mg/L are more suitable to the specific growth rate models considering inhibition effect than the Monod model. Furthermore, substrate inhibition kinetics by the Haldane model was predominant at 0 mg/L ≤ Cl_2

Table 6
The maximum specific growth rate (μ_{max}) values determined by four specific growth rate models in varied Cl_2 dosages

Model	μ_{max} (h^{-1})									
	Control		2 mg/L Cl_2		4 mg/L Cl_2		6 mg/L Cl_2		8 mg/L Cl_2	
	Value	SSE	Value	SSE	Value	SSE	Value	SSE	Value	SSE
No inhibition										
Monod	0.143	21.920	0.159	1,467.846	0.093	16.188	0.285	1.040	**0.002**	**0.0025**
Cell inhibition										
Contois	0.123	22.071	0.074	247.432	0.084	1,414.843	**2.681**	**0.057**	3.448	0.077
Substrate inhibition										
Haldane	**0.010**	**19.033**	0.028	362.751	**0.013**	**3.739**	0.718	4.097	0.328	6.002
Haldane (without considering K_s)	0.100	21.726	**0.020**	**184.461**	0.054	17.187	0.227	0.531	0.156	0.815

dosage £ 4 mg/L. This phenomenon seems to be associated with the enhancement of the organic loading rate with decreasing Cl_2 dosage in the prechlorination. Previous studies have shown that an increase in the organic loading rate can increase VFA production [45]. It has also been reported by Olafadehan and Alabi [46] that VFA accumulation led to substrate inhibition.

In this study, at a Cl_2 dosage of 6 mg/L, the specific growth rate is more suitable with the Contois model than the Haldane. This result was probably caused by the inhibitory effect of high microorganism's concentration. Fig. 1 shows that MLSS reaches its highest level at SBR operation using 6 mg/L Cl_2 as tapioca wastewater pretreatment. It is well known that the Contois equation is the most suitable kinetic model to describe the specific growth rate of the fermentation process with a high concentration of microorganisms [47]. The microorganism's existence is considered to inhibit its growth [48].

3.1.3. Effect of Cl_2 dosage adjustment on the substrate utilization rate

The cell yield coefficient ($Y_{X/S}$) values were obtained from the slope of each substrate utilization model in Table 7. The estimated $Y_{X/S}$ values from all models are shown in Table 8. The best fit $Y_{X/S}$ values at five different applied Cl_2 dosages are marked by bold font.

Unfortunately, the $Y_{X/S}$ value generally increases with increasing Cl_2 dosage. This fact does not seem to be beneficial since the higher value of $Y_{X/S}$ corresponds to a higher sludge production in a bioreactor [49]. Similarly, our previous study also reported that the alkaline pre-chlorination process for tapioca wastewater treatment can lead to an increase of $Y_{X/S}$ in anaerobic baffled reactor system [50]. An increase in $Y_{X/S}$ value is primarily caused by the effect of Cl_2 dosage adjustment on the change of specific growth rate (μ) in the SBR system. From Fig. 2, it can be seen that the slope of a plot of ln MLSS vs aeration time, as known as μ, was found to increase when the Cl_2 dosage to tapioca wastewater prior to the SBR process was increased. This finding was consistent with the study of Lele and Watve [51], which presented a positive linear correlation between μ of Aerobacter aerogenes in glycerol-NH_3 salt medium and its $Y_{X/S}$.

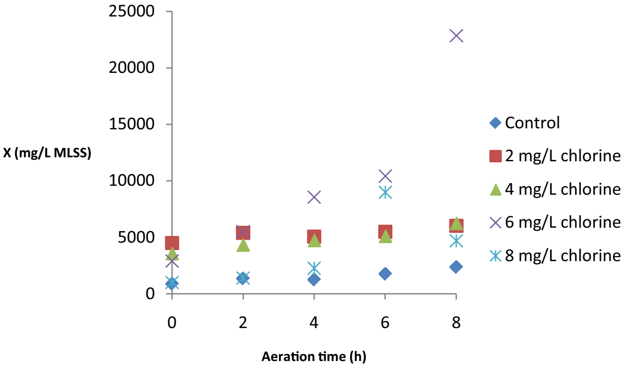

Fig. 1. MLSS profile in tapioca wastewater treatment using SBR with alkaline pre-chlorination as pretreatment.

Table 7
Models used to describe the substrate utilization [29,42]

Model	Equation
Monod	$X = X_0 + Y_{X/S}(S_0 - S)$
Monod considering the decay effect	$\frac{X_0 - X}{X_t} = Y_{X/S}\frac{S_0 - S}{X_t} - K_d$

3.2. Cycle time effects on the kinetics of PHB formation

The optimum result of Cl_2 dosage (6 mg/L) can be recommended as a promising technology for pre-treatment of tapioca wastewater. The application of alkaline pre-chlorination using the optimum Cl_2 dosage allows tapioca wastewater treatment using SBR to achieve the highest values of k and μ_{max}. Moreover, the study of cycle time effects was carried out by evaluating kinetics of PHB production from tapioca wastewater using a combination of alkaline prechlorination with Cl_2 dosage of 6 mg/L and SBR

Table 8
$Y_{X/S}$ values determination using Monod models

mg/L Cl_2	Monod		Monod considering decay rate	
	$Y_{X/S}$ (mg MLSS/mg COD)	R^2	$Y_{X/S}$ (mg MLSS/mg COD)	R^2
0	**0.299**	**0.851**	0.14	0.799
2	0.266	0.630	**0.793**	**0.951**
4	**0.705**	**0.875**	0.634	0.692
6	4.511	0.622	**1.384**	**0.936**
8	10.12	0.082	**0.508**	**0.166**

operation for 8 h of aeration time followed by 8 h of settling time. The recommended cycle time was determined based on its effects on the kinetics of PHB production.

3.2.1. Effect of cycle time on microbial conversion of COD into PHB

To study the effect of cycle time on microbial conversion of COD into PHB, equations in Table 7 were used by substituting $Y_{X/S}$ and X to yield coefficient of COD to PHB ($Y_{P/S}$) and PHB concentration (P), respectively. The estimated $Y_{P/S}$ values from all equations are shown in Table 9. According to R^2, the Monod equation with considers the decay rate effect appeared to be the most suitable mathematical model describing the microbial conversion of COD into PHB. Moreover, based on this model, the $Y_{P/S}$ value was stable until the aeration time of 6 h was achieved. $Y_{P/S}$ was also found to decrease with excessive aeration time and

Table 9
$Y_{P/S}$ values determination using Monod models

Cycle time	Monod		Monod considering decay rate	
	$Y_{P/S}$ (mg PHB/mg COD)	R^2	$Y_{P/S}$ (mg PHB/mg COD)	R^2
Aeration				
4 h	0.01	0.772	0.01	1
6 h	0.016	0.764	0.01	0.911
8 h	0.023	0.731	0.009	0.852
Settling				
2 h	0.017	0.417	0.002	0.844
4 h	0.013	0.233	0.002	0.811
6 h	0.01	0.151	0.001	0.563
8 h	0.008	0.098	0.001	0.582

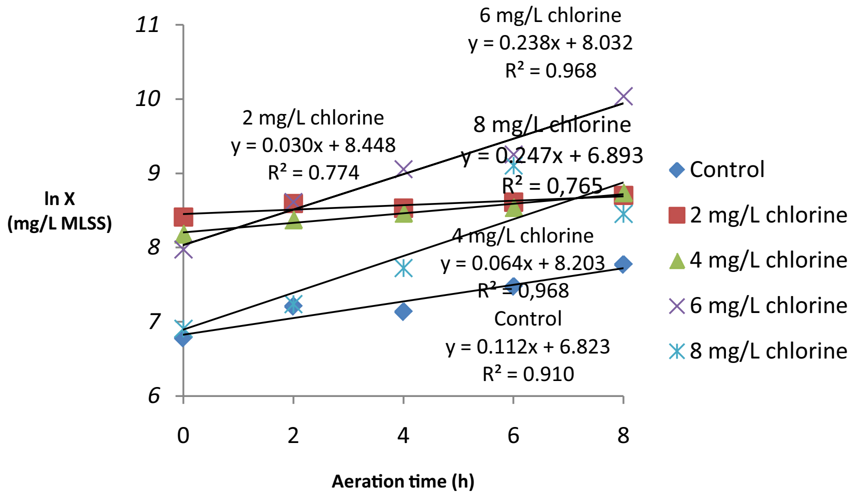

Fig. 2. Specific growth rate (μ) profile in tapioca wastewater treatment using a combination of alkaline pre-chlorination and SBR.

Table 10
Models expressing PHB production rate used [40]

Model classification	Equation
Growth associated product formation	$\frac{dP}{dt} = Y_{P/X} \frac{dX}{dt}$
Non-growth associated product formation	$\frac{dP}{dt} = \beta X$

settling time. It might be due to PHB utilization as a nutrient source by bacteria [52] under low COD concentration conditions [53]. Third et al. [54] have shown that the level of COD conversion to PHA decreased under excess oxygen.

3.2.2. Effect of cycle time on PHB production rate

Two models expressing the PHB production rate as described in Table 10 were validated. Generally, those models describe the relationship between PHB production rate (dP/dt), growth rate (dX/dt), and MLSS concentration (X) with growth associate PHB yield coefficient ($Y_{P/X}$) and non-growth associated PHB yield coefficient (β) as constants.

From R^2 value, it was observed that the production of PHB from tapioca wastewater effluent of prechlorination with a Cl_2 dosage of 6 mg/L at pH 8 in this study is followed non-growth associated product formation. It means that product formation is a function of cell concentration. Also, from Tables 10 and 11, there is a positive linearship between the microbial conversion of COD into PHB and PHB production rate per cell mass formed. A similar trend was also reported by Panda et al. [55]. They reported that substrate concentration has a direct impact on the biomass production and PHB production rate.

4. Conclusions

Alkaline pre-chlorination is proven its effectiveness in improving the performance of tapioca wastewater treatment using SBR. The SBR combined with alkaline pre-chlorination at a Cl_2 dosage of 6 mg/L as pre-treatment shows the best performance based on its highest values of COD removal rate constant (k) and maximum specific growth rate (μ_{max}). Four models, that is, first-order kinetic rate, Contois Model, substrate utilization rate based on Monod equation considering decay effect, and non-growth associated product formation were the appropriate model for organic removal kinetic, specific growth rate model, kinetics of COD conversion to cell mass and PHB, and PHB formation rate, respectively. Excessive aeration time and settling time application was found to decrease the level of COD conversion to PHB and PHB production rate per cell mass formed. The maximum yield coefficient of COD to PHB ($Y_{P/S}$) of 0.01 mg PHB/mg COD and non-growth associated PHB yield coefficient (β) of 9.07×10^{-4} mg PHB/mg MLSS/h were achieved at 6 h of aeration time. For optimization, SBR should be operated for treatment of effluent of tapioca wastewater prechlorination using Cl_2 dosage of 6 mg/L at pH 8 with cycle consisted of 1 h of filling, 6 h of aeration, and 0.5 h of settling.

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Table 11
Model validation of PHB production rate in SBR for treatment of tapioca wastewater effluent of prechlorination with Cl_2 dosage of 6 mg/L at pH 8

Cycle time	Growth associated product formation		Non-growth associated product formation	
	$Y_{P/X}$ (mg PHB/mg MLSS)	R^2	β (mg PHB/mg MLSS/h)	R^2
Aeration				
4 h	0.004	0.789	7.63×10^{-4}	0.901
6 h	0.007	0.859	9.07×10^{-4}	0.973
8 h	0.004	0.906	4.59×10^{-4}	0.906
Settling				
2 h	0.002	0.33	4.68×10^{-4}	0.868
4 h	0.001	0.115	5.52×10^{-4}	0.633
6 h	0.003	0.048	3.41×10^{-4}	0.365
8 h	0.002	0.016	3.19×10^{-4}	0.136

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