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ORIGINAL ARTICLE

A higher in value biopolymer product of polyhydroxyalkanoates (PHAs) synthesized by *Alcaligenes latus* in batch/repeated batch fermentation processes of sugar cane juice

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Abstract Biodegradable polymers of polyhydroxyalkanoates (PHAs) are produced and accumulated in various microorganisms, especially in bacteria. In this work, a cheap agricultural sugar cane juice (SCJ) was used as a carbon source by *Alcaligenes latus* during its growth coupled with the production of PHAs via batch/ repeated batch fermentation processes. In batch fermentation, the maximum PHAs and PHAs content of 2.20±0.14 g/L and 53.65 % were obtained within 24 h while in the case of four cycles of repeated batch fermentation, the PHAs productivity was reached at about 0.21 g/L·h that was 2.33 fold when compared to the first batch fermentation (0.09 g/L·h). Interestingly, the duration in each cycle or in batch operation during repeated batch fermentation was shorter than that found in the batch fermentation and PHAs productivity was also improved. After PHAs recovery, the physicochemical properties of nuclear magnetic resonance (NMR) and a fourier transform infrared (FTIR) spectrogram were characterized and revealed to be in full agreement with the standard polymer of polyhydroxybutyrate (PHB).

Keywords Polyhydroxyalkanoates · *Alcaligenes latus* · Sugar cane juice · Fermentation process

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Introduction

The polyhydroxyalkanoates (PHAs) is a group of biodegradable thermoplastics polymers produced by various microorganisms and accumulated as intracellular storage compounds of discrete granules in their cytoplasm for carbon and energy reserves (Tanamool et al. 2013) under conditions of limiting nutrients and an excess carbon source. Once the limiting nutrient is provided to the cell, these energy storage compounds are degraded and used (Kaewkannetra et al. 2008). Microbial polymers, unlike petrochemical polymers, are produced from various sugar-based renewable resources and contain the advantages of biodegradability and biocompatibility.

Nowadays, various microorganisms such as yeast, fungi, and especially more than 150 bacterial strains are used for the production of PHAs (Poirier et al. 1995). The PHAs bacterial producers can be divided into two main groups based on the culture conditions required for PHAs synthesis (Lee 1996a, b). The first group requires the limitation of essential nutrients such as nitrogen, phosphorous, magnesium, and sulphur for the synthesis of PHAs from excess carbon sources. The bacteria in this group are *Protomonas extorquens* (Suzuki et al. 1986), *Alcaligenes eutrophus* (reclassified and named as *Ralstonia eutropha* and later, *Cupriavidus necator*) (Kim et al. 1994; Kunasundari and Sudesh 2011), methylotrophs (Kim et al. 1996), etc. The second group, called growth-associated bacteria, which include *Azotobacter vinelandii* (Page and Cornish 1993), *Alcaligenes latus* (Hangii 1990; Braunegg and Bogensberger 1985; Hrabak 1992), and recombinant *Escherichia coli* (Lee et al. 1994; Lee and Chang 1995; Lee 1997) do not require nutrient limitation for PHAs synthesis and can accumulate polymer during their growth as well. However, the most popular bacterial strain used previously and currently is *A. latus* because it is a growth-associated PHAs producer and accumulates PHAs up to 80 % of dry cell weight (DCW) as a Gram-negative, non-sporulating, and obligate aerobe (Braunegg and Bogensberger

1985; Khanna and Srivastava 2005a; Suwannasing et al. 2011). Furthermore, it shows substantially different characteristics from *R.eutropha*, which accumulates PHAs during growth without any nutrient limitation. Moreover, it can utilize sucrose directly as a carbon source (Yamane et al. 1996), which is usually less expensive than glucose (Yamane 1993) and often found as a main sugar in various sugar-based agricultural materials. Previous studies successfully showed the production of PHAs by *A. latus* from several carbon sources such as when Yezza and Halasz (2007) used *A. latus* to produce PHAs from maple sap and found the maximum PHAs concentrations of 3.41 and 3.26 g/L obtained in a flask and a fermenter. Kumalaningsih et al. (2011) also studied production of PHAs from liquid bean curd waste by *A. latus*. The initial sucrose concentration of 25 g/L and incubation time of 60 h gave the maximum PHAs concentration (2.48 g/L).

Most reports on the PHAs production have been based on batch fermentation because it is an easy process. The process referred to is a partially closed system in which most of the materials required are loaded into the bioreactor, sterilized before the process starts, and then the product is withdrawn at the end of the process. A major drawback of batch fermentation is a long time for preparation of equipment and inoculums for the next batch. On the other hand, repeated batch fermentation could be an alternative process for PHAs production. It is similar to batch fermentation but the remaining broth at the end of the process needs to be used as a seed culture, to reduce the time and energy for preparation of the bioreactor in the next round and leading to reduction in the cost of production. Repeated batches for any number of culture cycles can be done until the batch is contaminated. Previous works such as El-Sayed et al. (2009) investigated production of polyhydroxybutyrate (PHB) from pure sucrose and glucose using a bioreactor with batch, two-stage batch, and fed batch cultures by *A. latus* and *R. eutropha*. In batch culture, *A. latus* produced PHB (56.59 %), which was more than the case of *R. eutropha* (8.46 %), while *R. eutropha* needed a two-stage batch culture for increasing the PHB content. Khanna and Srivastava (2005a) studied repeated batch fermentation of PHB by *R. eutropha*. The total PHB productivity obtained at the end of 67 h was 0.42 g/L·h. As compared to batch, two cycles of feeding led to a threefold increase in PHB productivity. Repeated batch culture has been successfully used to improve the productivity of many fermentation products such as acetic acid (Park et al. 1991), ethanol (Morimura et al. 1997), and L(−)-lactic acid (Yin et al. 1998) on a laboratory scale because it skips the turnaround time and the lag phase (Huang et al. 2008).

Since Thailand is one of the agro-industrial countries and abundantly available are not only microbial resources but also agricultural products such as cassava, rice, sugar cane, corn, potato, etc. Typically, these are used for human foods. In the age of the petroleum crisis, there was an argument about food crops versus energy crops. Many researchers have attempted to find energy crops such as soil bean, palm oil, sunflower, etc. to produce alternative energies. Finally, the comparisons for this issue had ended by attempts to find out second generation crops or non-food crops for use as raw materials for the production of alternative energies. Sugar cane is also one of the crops that are most widely planted in several areas of the country, especially in the northeastern area. Normally, its stems are squeezed for sugar cane juice (SCJ), and then they are mainly used to produce sugar. Other by-products from the process include molasses, bagasse, and filter cake that are primarily produced not only as an animal feed additive but also ethanol, enzymes, amino acids, organic acids, and compounds of pharmaceutical importance, etc. (Pandey et al. 2000). Presently, PHAs are becoming widely used for potentially several applications such as drug delivery, functional foods, coupling agents, and even non-food products such as membranes or nanofibrils for remediation of contaminated water, coated paper, etc. Therefore, the production of PHAs for use as a raw material to produce biodegradable plastic could help mitigate environmental problems, especially air pollution and waste management.

We are the first research group that has played attention to reduction of the cost for PHAs production covering upstream to downstream processes. Application of cheap agricultural crops such as sweet sorghum and sugar cane is also our target for reducing upstream process of fermentation. There was successful production in both flask and batch bioreactor from sweet sorghum juice (SSJ) and the SCJ using several isolated and pure cultures for several purposes (Kaewkannetra et al. 2008; Suwannasing et al. 2011; Tanamool et al. 2013). Presently, we are trying to improve the fermentation process from batch to other fermentation processes such as repeated batch, repeated fed batch, and even continuous modes.

Therefore, this present research aims to study some physicochemical properties of the SCJ and to evaluate its potential use as a carbon source for PHAs synthesized by a great many potential bacteria. Finally, batch and repeated batch fermentations processes of the SCJ for PHAs production were evaluated in terms of biomass yield and PHAs yield. In addition, some physicochemical properties of PHAs were also evaluated.

Materials and methods

Microbial strains

A strain of *Alcaligenes latus* (TISTR 1403) was purchased from the Thailand Institute of Scientific and Technological Research (TISTR), Bangkok, Thailand. The culture then was subcultured on nutrient agar, maintained at 4 °C, and

transferred monthly to fresh nutrient agar (3 g/L beef extract, 5 g/L peptone, and 15 g/L agar).

Sugar cane juice (SCJ)

A part of sugar cane stems, used throughout this study, were collected from the sugar cane plantation area of the Khon Kaen province of Thailand. Then, they were squeezed by conventional roller mills and the SCJ was obtained. The SCJ was preliminarily filtered by a cotton sheet and then kept at 20 °C for prevention of microbial contamination. It was left at room temperature and then sterilized prior to use.

Cultivation medium

Alcaligenes latus was cultivated in mineral medium 3 as described by Grothe et al. (1999). It consisted of (g/L): carbon source, 20.00; $(NH_4)_2SO_4$, 1.40; KH_2PO_4, 1.50; Na_2HPO_4, 1.80; $Mg_2SO_4{\cdot}7H_2O$, 0.20, and a trace element solution of 1 mL. The medium was adjusted to pH 6.5. The trace element solution contained (g/L): ammonium Fe (III) citrate, 6.00; $CaCl_2{\cdot}2H_2O$, 10.00; H_3BO_3, 0.30; $CoCl_2{\cdot}6H_2O$, 0.20; $ZnSO_4{\cdot}7H_2O$, 0.10; $MnCl_2{\cdot}4H_2O$, 0.03; $Na_2MoO_4{\cdot}2H_2O$, 0.03; $NiSO_4{\cdot}7H_2O$, 0.02, and $CuSO_4{\cdot}5H_2O$, 0.01.

Initial inoculum preparation

A loopful of *A. latus* was added into an Erlenmeyer flask (250 mL) containing 50 mL of nutrient broth (NB) medium (3 g/L beef extract and 5 g/L peptone). The inoculating flask was incubated on an incubator shaker (200 rpm) for 24 h under temperature control at 33 °C. The culture in this step was used as initial inoculums for capability tests by using different sugars contained in the SCJ.

Preparation of two-step inoculum in the bioreactor

The inoculums in the bioreactor were carried out by two-step cultivation in order to get a high biomass. First, initial inoculums described as above were prepared. In the next step, 10 % (*v/v*) of the initial inoculums was transferred into an Erlenmeyer flask (500 mL) containing 250 mL of NB plus 20 g/L total sugar in the SCJ. The culture flasks were incubated at 33 °C, 200 rpm for 12 h.

Capability test for using different sugars contained in the SCJ

Three main types of sugars usually found in the SCJ such as glucose, fructose, and sucrose were preliminarily separately tested by *A. latus* to evaluate its capability for utilizing each sugar as a carbon source not only for growth but also for PHAs synthesis. The batch experiment was carried out in 500 mL Erlenmeyer flasks containing 250 mL of cultivation medium (the initial sugar at 20 g/L). The culture flasks were inoculated with 10 % (*v/v*) of initial inoculums and incubated at 33 °C on an incubator shaker (200 rpm). Samples were collected for analyzing the DCW, PHAs, and sugar concentration.

Batch and repeated batch fermentation

Batch and repeated batch fermentation were carried out in parallel in a 2 L bioreactor with a working volume of 1.5 L of cultivation medium (the SCJ was used as a sole carbon source), and 10 % *v/v* inoculums of *A. latus* were inoculated into the culture medium. Temperature and dissolved oxygen (DO) were maintained at 33 °C and 30 %. The pH was adjusted to 6.5 and kept at a controlled constant throughout fermentation. Broth samples were withdrawn periodically during fermentation; DCW, total sugar, and PHAs concentration were analyzed following standard methods as described below. It should be noted that for repeated batch fermentation, the culture broth was withdrawn at the end of each cycle and the at least remaining 10 % of broth was used as the next seed culture and then added into a fresh production medium.

Cell growth and DCW measurement

Cell growth was monitored by measuring the optical density at 600 nm (OD_{600}) using a spectrophotometer (PG Instruments Limited, T60, UK). For DCW analysis, the culture broth (5 mL) was centrifuged (10,000 rpm, 10 min), and the supernatant was discarded. The pellets were washed twice with deionized water and dried in a hot air oven (80 °C) until gaining a constant weight.

Determinations of sugar contents and other minerals

The supernatant was used for sugar content analysis (sucrose, glucose, and fructose) using high performance liquid chromatography (HPLC) (Shimadzu, Japan) equipped with a refractive index detector (RID) by using a Vertical GES-NH_2 HPLC column (4.6×250 mm, 5 μm) and 75 % acetonitrile in deionized water as the mobile phase. The flow rate and the injection volume were constantly controlled at 1 mL/min and 20 μL. In the case of minerals such as Na, K, Mg, Ca, and Zn, they were estimated using a Perkin Elmer atomic absorption spectrophotometer (Model 2380, England) as described by the Association of Official Analytical Chemists: AOAC, 1998, while the organic nitrogen concentration was also measured by the standard method of total kjeldahl nitrogen (TKN).

Determination of intracellular PHAs and PHAs concentration

The presence of PHAs as an intracellular product was confirmed by the Sudan black B staining method. The culture was smeared on a glass slide and was heat fixed. This slide was

immersed by Sudan black B (0.3 % *w/v*) for 10 min and washed with xylene. Then, it was counter stained for 1 min with 0.5 % safranin and washed with deionized water. For PHAs concentration, it was determined by using the modified gravimetric method that was described by Grothe et al. (1999).

PHAs characterization

After recovery, the extracted PHAs film was characterized by its physicochemical properties using the two techniques of Nuclear Magnetic Resonance (NMR) and Fourier Transform Infrared Spectroscopy (FTIR). The structural film was recorded using purified PHAs in chloroform using 400 MHz of ^{13}C-NMR, while for the FITR it was scanned as a spectrogram between 4000 and 600 cm^{-1}. It should be noted that PHAs are mostly found in the form of polyhydroxybutyrate (PHB). Hereafter, the results obtained were compared to the polymer standard of PHB. (Sigma-Aldrich, USA).

Results and discussion

The composition of sugar cane juice (SCJ)

Some physicochemical properties of the SCJ were characterized and are shown in Table 1. Only three sugars (sucrose, glucose, and fructose) were found in the SCJ, but sucrose was mainly contained at a very high concentration (187.63 g/L). For total sugars they were also found in a high concentration at about 201 g/L. The main element of potassium (K) was found at about 5118 mg/L. On the other hand, other elements such as sodium (Na), calcium (Ca), magnesium (Mg), and zinc (Zn) were found in small amounts. The SCJ was rich in both sugars and elements. Thus, we have assumed that it may be suitable and could be used directly as a raw material for bacteria growth and the production of PHAs without requiring a pretreatment process such as hydrolysis. In addition, a high total sugar concentration is also one of the main factors affecting the efficient fermentation process. Therefore, some elements and nitrogen sources still needed to be added into the SCJ base medium because of a low concentration for them, which appeared when the SCJ was diluted to the desired initial concentration (20 g/L total sugar). It meant that the cost for the PHAs production could be reduced without a high sugar concentration of the SCJ.

Table 1 The composition of sugar cane juice

Component		Concentration
Sugar	Sucrose	187.63 g/L
	Glucose	8.69 g/L
	Fructose	5.32 g/L
	Total sugar	201.64 g/L
Elements	Potassium (K)	5118.00 mg/L
	Sodium (Na)	8.09 mg/L
	Calcium (Ca)	20.36 mg/L
	Magnesium (Mg)	140.00 mg/L
	Zinc (Zn)	0.29 mg/L
pH		5.50
Total soluble solids		20 °Brix
Nitrogen		1.20 % (w/v)

Effect of different sugars on growth and PHAs synthesis

Since, composition of the SCJ was mainly confined to three different sugars, the capability of *A. latus* in using each sugar for growth and PHAs synthesis was preliminarily tested. In Fig. 1 is shown DCW and PHAs concentrations in the three different carbon sources of glucose, fructose, and sucrose. Similar results were obtained among them, and this indicated that the bacterial strains could grow in many types of sugars. The DCW and PHAs were increased as incubation time increased and then reached the maximum value within 16 h of fermentation for all culture conditions. In addition, the maximum PHAs content (PHAs per DCW) was 73.33, 74.83, and 78.09 % on production medium containing glucose, fructose, and sucrose, respectively. The highest growth and PHAs production of *A. latus* were reached at 2.10±0.00 and 1.64±0.01 g/L on production medium containing sucrose. This is in agreement with previous studies; El-Sayed et al. (2009) and Santhanam and Sasidharan (2010) reported that *A. latus* produced the maximum PHAs concentration when sucrose was used as a carbon source. Braunegg and Bogensberger (1985) also reported that *A. latus* produced PHB up to 70 % during its growth when sucrose was used as a carbon source while Yezza and Halasz (2007) achieved production of PHAs content (74 %) from sucrose as well. In addition, Grothe et al. (1999) studied production of PHB by *A. latus* by using sucrose (20 g/L) as a carbon source. The maximum PHB was 60 % of dry cell mass after 93 h of incubation. Thus, the SCJ contained sucrose that mainly could be used as a sole carbon source for growth coupled with the PHAs synthesis.

Batch fermentation

The process was performed when the *A. latus* began to grow and produce PHAs after 9 h. The cells reached a stationary phase at 24 h (Fig. 2). The parameters in batch

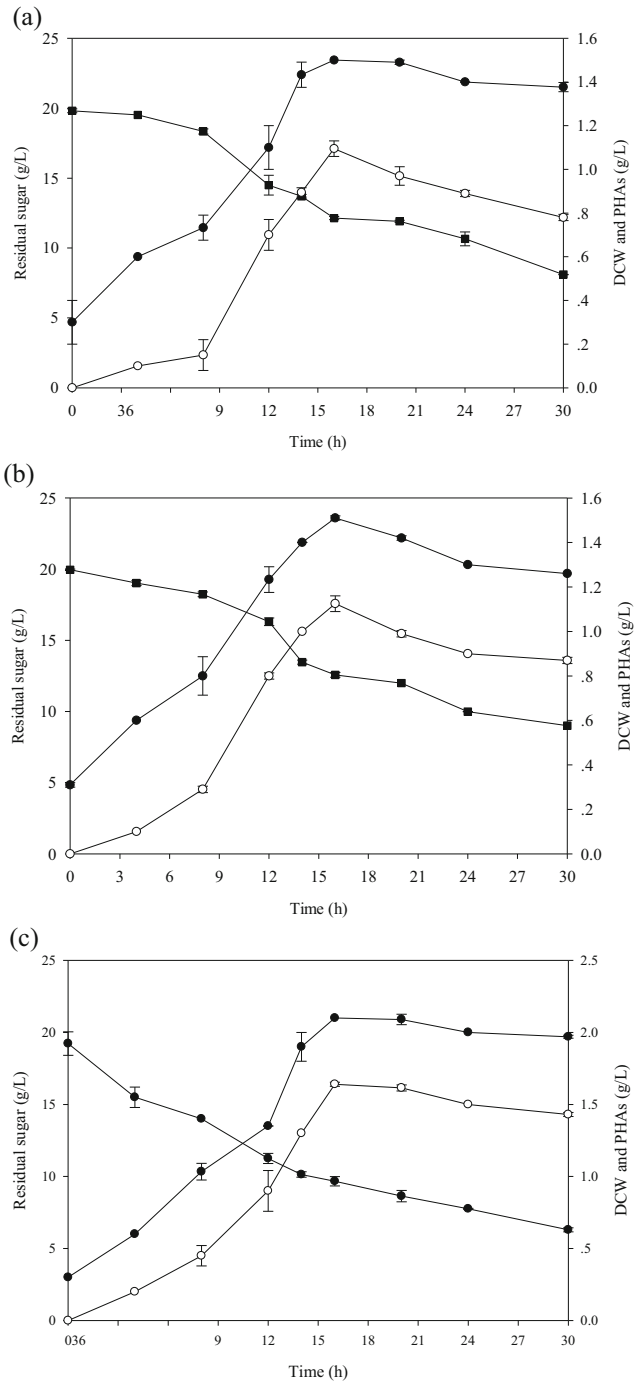

Fig. 1 Growth profile of DCW (*closed circles*), total sugar (*closed squares*) and PHAs production (*open circles*) by *A. latus* as function of time under batch fermentation in glucose (**a**), fructose (**b**), and sucrose (**c**)

fermentation are summarized in Table 2. PHAs production was initially detected after 9 h and reached 2.20±0.14 g/L within 24 h incubation while the highest DCW was obtained at 4.20±0.14 g/L during 30 h of incubation. Related to PHAs data in Table 2, it was indicated that PHAs produced by *A. latus* was increased during its growth. Specific growth rate (μ) and doubling time (t_d)

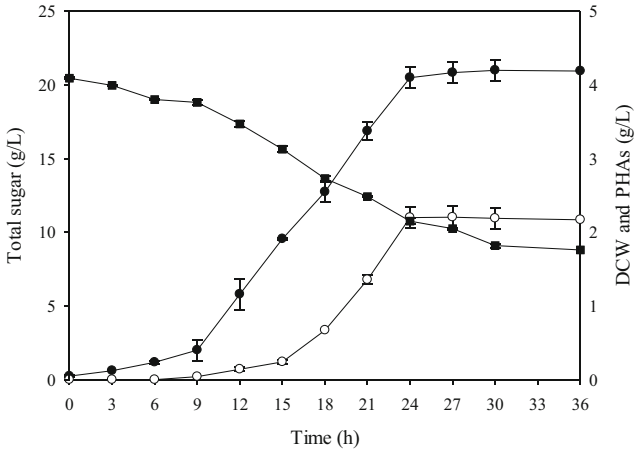

Fig. 2 Growth profile of *A. latus* during batch fermentation (DCW; *closed circles*, total sugar; *closed squares* and PHAs production; *open circles*)

were reached at 0.084 h^{-1} and 8.25 h. It was clearly found that *A. latus* could grow in the SCJ. Previously, Yezza and Halasz (2007) also reported that they used maple sap to produce PHB by *A. latus* because the sap was rich in sucrose that was similarly found in the SCJ. The profile of the SCJ fermentation by *A. latus* was used for further repeated batch fermentation. In addition, the intracellular PHAs product synthesized by the *A. latus* in this study was also preliminarily confirmed by the Sudan black B staining method. The photomicrograph of PHAs accumulated in cells is shown in Fig. 3.

Repeated batch fermentation

Repeated batch fermentation was undertaken in a 2 L bioreactor. The results obtained from repeated batch fermentation on the cell growth, PHAs production, and total sugar as functions of time are illustrated in Fig. 4.

In Fig. 4a is shown the fermentation kinetics of PHAs during repeated batch fermentation. The times for both cell growth and PHAs production were reduced gradually in each batch. The DCW and PHAs concentrations in the second, third, and fourth cycles were also increased as compared to the first batch, while in each batch of the repeated batch fermentation, the total sugar was decreased gradually during the process (Fig. 4b).

In Table 3, all parameters of the DCW, PHAs concentration, PHAs content, and their productivity were increased compared to the first batch fermentation. The PHAs productivity was obtained at about 0.21 g/L·h that was 2.33 fold when compared to the first batch fermentation (0.09 g/L·h). The duration time in each batch operated during the repeated batch fermentation was also shorter than that found in the batch fermentation. The results

Table 2 Growth and PHAs production by *A. latus* during batch fermentation

Time (h)	DCW (g/L)	PHAs (g/L)	Residual sugar (g/L)	Consumed sugar (g/L)	$Y_{X/S}$	$Y_{P/S}$	PHAs content (%)
0	0.05±0.01	0.00±0.00	20.47±0.05 [a]	0.00	0.00	0.00	0.00
3	0.12±0.00	0.00±0.00	19.99±0.03	0.48	0.25	0.00	0.00
6	0.24±0.02	0.00±0.00	19.02±0.03	1.45	0.17	0.00	0.00
9	0.40±0.14	0.04±0.00	18.83±0.18	1.64	0.24	0.02	10.00
12	1.11±0.21	0.14±0.03	17.37±0.19	3.10	0.36	0.05	12.61
15	1.91±0.01	0.24±0.03	15.65±0.22	4.82	0.40	0.05	12.57
18	2.55±0.14	0.67±0.00	13.66±0.21	6.81	0.37	0.10	26.27
21	3.37±0.12	1.36±0.06	12.43±0.03	8.04	0.42	0.17	40.35
24	4.10±0.14	2.20±0.14	10.76±0.21	9.71	0.42	0.23	53.65
27	4.17±0.14	2.20±0.15	10.25±0.07	10.22	0.41	0.22	52.75
30	4.20±0.14	2.19±0.14	9.10±0.14	11.37	0.37	0.19	52.14
36	4.19±0.00	2.17±0.00	8.80±0.00	11.67	0.36	0.19	51.79

[a] The initial sugar was 20.47± 0.05 g/L

$Y_{X/S}$ = Biomass yield (DCW per consumed sugar)

$Y_{P/S}$ = PHAs yield (PHAs produced per consumed sugar)

obtained from this study were similar to a previous study (Kim et al. 1996) that reported repeated batch fermentation could improve the volumetric hyaluronic acid productivity to be 2.5 fold of the batch fermentation. In addition, Khanna and Srivastava (2005b) also stated that, with two cycles of repeated batch fermentation of PHB by *R. eutropha*, they obtained a total PHB productivity at about threefold as compared to batch fermentation.

Characterization of PHAs film

The PHAs film was further characterized in its structure using 400 MHz of ^{13}C NMR techniques. The spectrum of ^{13}C NMR obtained is shown in Fig. 5a. Four groups of signal peaks at

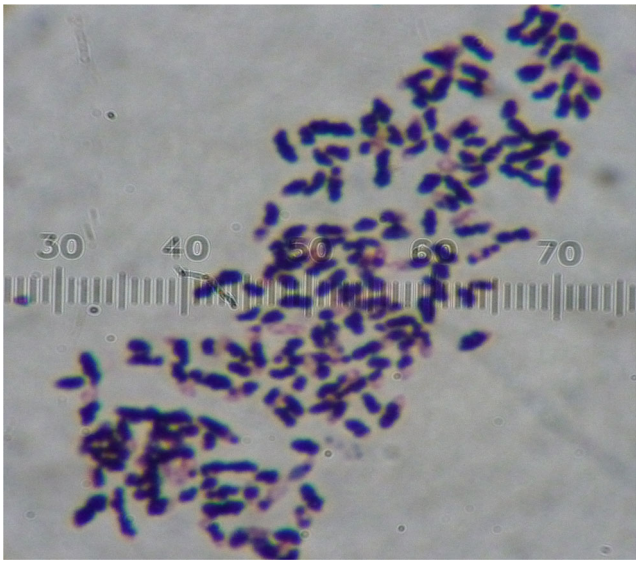

Fig. 3 Photomicrograph (100 x) of PHAs accumulated in cells during batch fermentation of the SCJ detected by the Sudan black B staining method after 24 h of incubation time

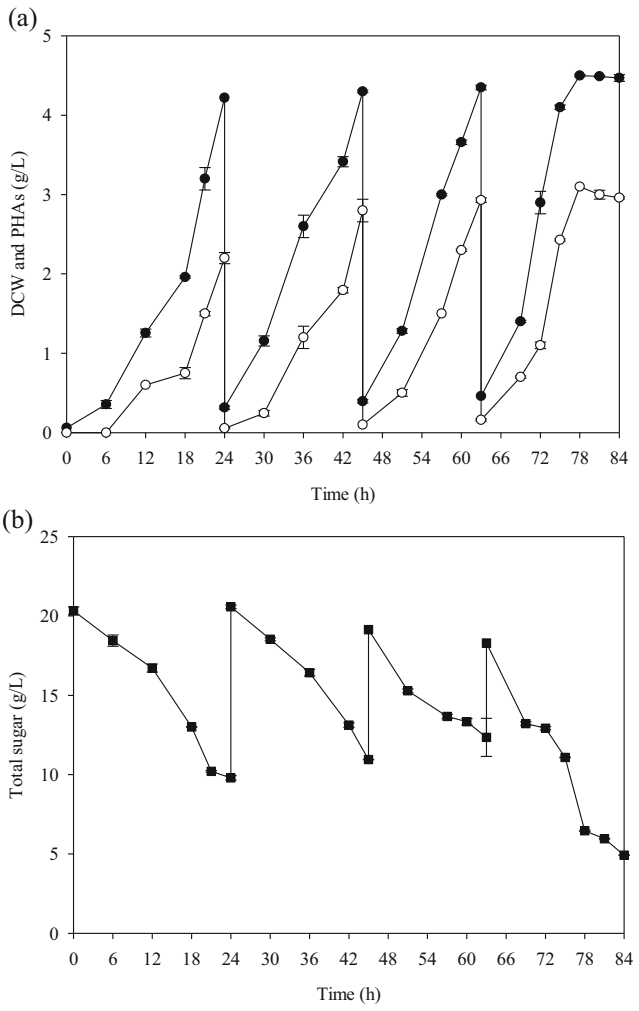

Fig. 4 Kinetics of PHAs production in repeated batch fermentation of the SCJ by *A. latus*; (**a**) DCW and PHAs concentration (**b**) total sugar (DCW; *closed circles*, total sugar; *closed squares* and PHAs production; *open circles*)

Table 3 Growth and PHAs production of *A. latus* during repeated batch fermentation processing

Batch number	Time (h)	DCW (g/L)	PHAs (g/L)	Residual sugar (g/L)	Consumed sugar (g/L)	$Y_{X/S}$	$Y_{P/S}$	PHAs content (%)	Productivity (g/L·h)
1	24	4.22±0.00	2.20±0.07	9.80±0.14	10.53	0.40	0.21	52.13	0.09
2	21	4.30±0.01	2.80±0.14	10.95±0.01	9.63	0.45	0.29	65.11	0.13
3	18	4.35±0.03	2.93±0.03	11.35±1.20	7.79	0.56	0.38	67.35	0.16
4	15	4.50±0.01	3.10±0.00	6.46±0.04	11.84	0.38	0.26	68.89	0.21

$Y_{X/S}$ = Biomass yield (DCW per consumed sugar)

$Y_{P/S}$ = PHAs yield (PHAs produced per consumed sugar)

19.717, 40.752, 67.568, and 169.089 ppm were found that corresponded to C-H_3, C-H_2, C-H, and C = O, respectively. The results obtained from fermentation of the SCJ were in full agreement with those obtained from a standard spectrogram of standard PHB (Fig. 5b) under the same conditions. Furthermore, they corresponded to the different carbon atoms

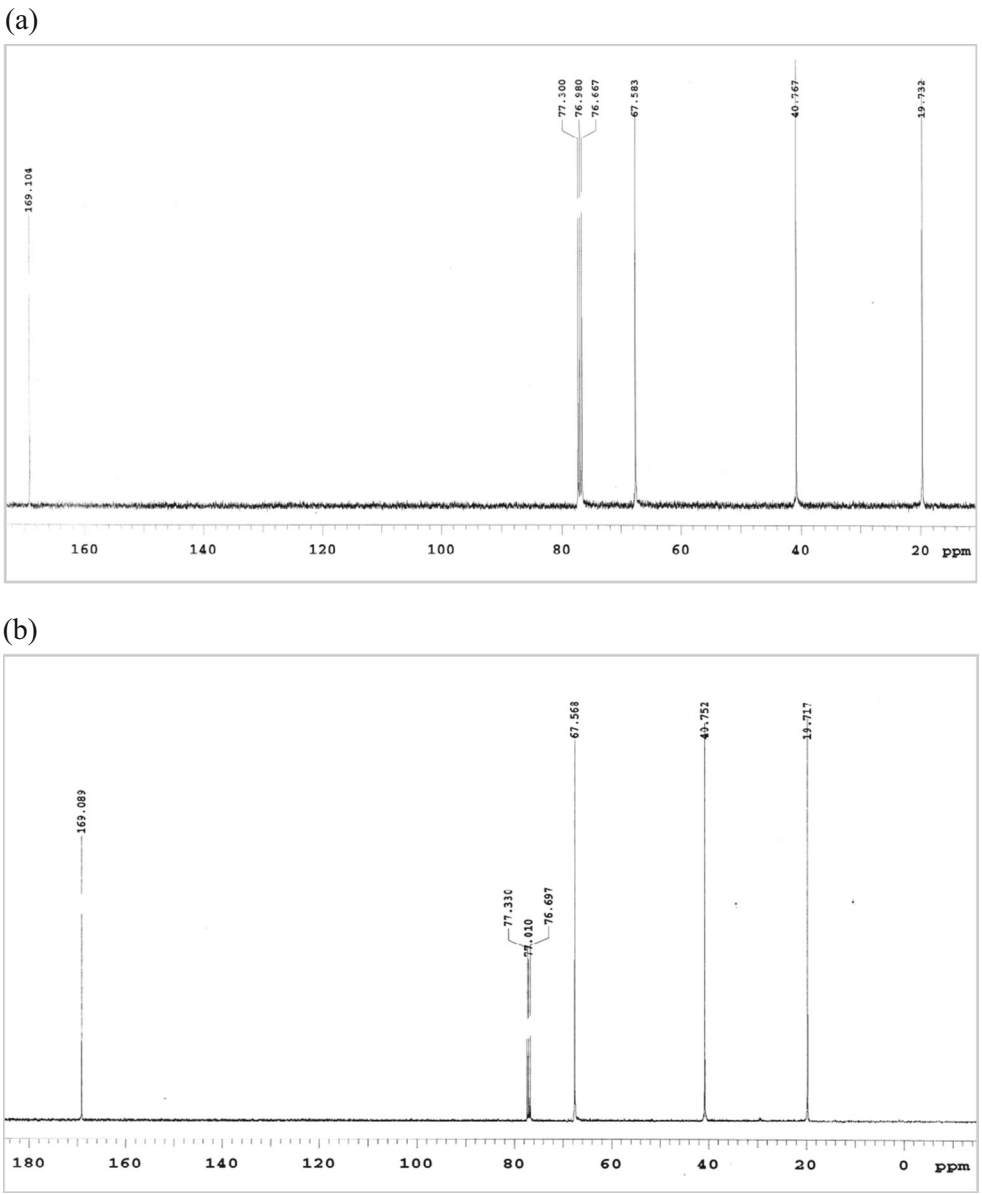

Fig. 5 ^{13}C NMR spectrograms obtained from (**a**) standard PHB and (**b**) synthesized PHAs

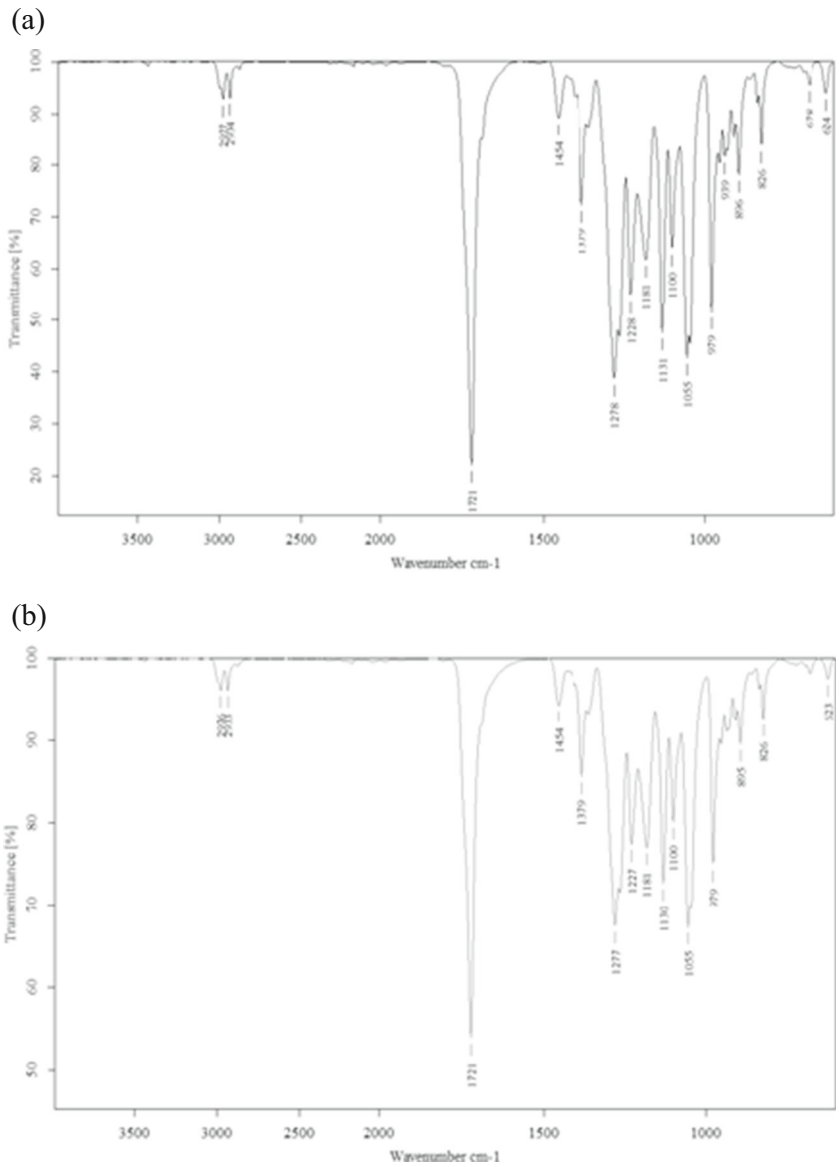

Fig. 6 FTIR spectrogram obtained (**a**) standard PHB and (**b**) synthesized PHAs

present in the PHB structure, $[-O\text{-}C\text{-}H\text{-}(C\text{-}H_3)\text{-}CH_2\text{-}, (C=O)\text{-}]_n$. There was no surprise in that PHAs produced from the SCJ was mainly the dominant form of PHB. A previous report stated that even in PHAs production from various sugar-based raw materials as carbon sources, the PHAs product obtained was dominated by the form of the homo-polymer of PHAs by various bacteria (Khanna and Srivastava 2005a).

The identified molecule scanned by the FTIR spectrophotometer (Fig. 6) was at a wavelength between 4000 and 600 cm^{-1}. The peak at 1721 and 1277 cm^{-1} corresponds to the C = O and the –CH group, respectively. The prediction of PHAs produced from the SCJ was a polymer type of the polyhydroxybutyrate (PHB). The FTIR spectrogram showed similar results obtained from a previous study reported by Kumalaningsih et al. (2011).

Conclusions

Inexpensive SCJ can be successfully used directly as a carbon source by *A. latus* for its growth coupled with the production of PHAs because of its abundance in the main sugars and others elements, vitamins, and aromatic compounds. Repeated batch fermentation, which has a non-productive downtime for cleaning, filling, and sterilization, can be eliminated, thereby increasing productivity in batch fermentation until the batch is contaminated. This leads to a reduction in the cost of PHAs production. The identity of PHAs was confirmed by ^{13}C NMR and FTIR techniques and illustrated that PHAs produced from the fermented SCJ was similar to the standard for PHB.

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