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Full Length Research Paper

Polyhydroxyalkanoates production via *Bacillus* plastic composite support (PCS) biofilm and date palm syrup

Mohammad A. Khiyami[1]*, Sulaiman M. Al-Fadual[1] and Ali H. Bahklia[2]

[1]King Abdulaziz City for Science and Technology, Kingdom of Saudi Arabia.
[2]King Saud University, P. O. Box 6086 Riyadh 11442, Riyadh, Kingdom of Saudi Arabia.

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Polyhydroxyalkanoates (PHAs) are a group of biopolymers resembling plastic due to their physical characteristics. PHAs are known to have great structural variety and biodegradability which add to their importance in replacing synthetic plastics and making them future green materials. Such biopolymers gained worldwide attention. Most bacteria produce biopolymers as an intracellular carbon and energy compounds. Several bacterial strains were isolated from desert soil and tested for the accumulation of PHAs. Off all isolates, only one was shown to have the ability to accumulate significant amounts of PHA. *Bacillus* spp. which named later as *Bacillus* SA was isolated and characterized by biochemical and molecular studies using the 16S rRNA. Several concentrations 5.0, 10.0, 15.0, 20.0 and 25.0% (v/v) of date syrup and plastic composite support tubes (PCS) were employed to develop PCS biofilm and motivate PHA accumulation. The stimulation of biofilm formation and production of high PHA accumulation was optimized, and 0.5 g/l urea, 120 rpm agitation and 15% (v/v) date syrup. The cellular dry matter (CDM) was 8.3 g/l with almost 70.5% PHA content. The average molecular weight range was between 3.7 to 4.1x10^{5}g/mol. This study demonstrated that a low cost raw material consisting of date syrup can be evaluated as a complex media supplement to meet the nutritional requirements for biopolymer production by suitable isolated bacterial strains. Also, the PCS tube stimulated the biofilm formation and increased the biopolymer accumulation. Consequently, biotechnology production lines of PHA may benefit from this approach to produce low cost PHA using low cost raw material and PCS tubes.

Key word: Date syrup, polyhydroxyalkanoates, plastic composite support (PCS) tube, biofilm, PCS biofilm, suspended cell.

INTRODUCTION

Polyhydroxyalkanoates (PHAs) are a group of biopolymers that have plastics' physical characteristics. PHAs exhibit a crystallinity ranging from 30 to 70% and a melting temperature of 50 to 180°C, these thermoplastic material properties make PHAs commercially relevant as renewable and biodegradable alternatives to oil-based plastics. Research has been devoted to reducing the cost and to quantitatively optimizing biopolymers production. The biopolymers production cost is a crucial parameter compared with that of chemical synthetic non-biodegradable plastics. The high costs of biopolymer associated with production methods and composition of media limit a more widespread use of PHA (Bengtsson et al., 2010). Hitherto, the low cost raw materials are a significant parameter in production process.

The biopolymers can be produced from a wide variety of substrates such as low cost renewable resources (for example, sucrose, starch, cellulose, triacylglycerols), fossil resources (methane, mineral oil, lignite, hard coal), by products (molasses, whey, glycerol), chemicals

*Corresponding author. E-mail: mkhiyami@kacst.edu.sa.

(propionic acid, 4-hydroxybutyric acid) and carbon dioxide (Kim, 2000). Innovative processes have been investigated for producing PHAs from the organic matters in wastewater (Ertesvag et al., 1994), industrial waste (Yu et al., 2002) and in municipal waste (Sheu et al., 2000). Dates are the main agricultural product in Saudi Arabia. They are a comprehensive nutritional substance. The Kingdom of Saudi Arabia is considered as one of the pioneer countries in date palm cultivation and dates production. According to the latest reports on palm dates, there are currently 32 million trees producing one million tons of dates (Khyami et al., 2011). The sugars in dates are sucrose, glucose, and fructose, which are easily assimilable to most microorganisms. The protein content of dates is in the range of 1 to 3%. This low amount of nitrogen is suitable for PHAs production; furthermore, the presence of vitamins, such as B1 (0.75 mg/100 g), B2 (0.2 mg/100 g) and nicotinic acid (0.33 to 2.2 ml/100 g), are very important for microorganisms growth. Also, dates contain important minerals for microbial growth, thus there is 650 to 750 mg potassium and 288 to 290 mg chloride per 100 g dates (Al-Eid, 2006).

Most bacteria produce biopolymers as intracellular carbon and energy storage compounds (Arcos et al., 2010). Biopolymers accumulate in the bacterial cells under unfavorable growth conditions in the presence of excess carbon source and limited nitrogen source (Anderson and Dawes, 1990; Steinbuchel, 1991; Byrom, 1994; Page, 1995; Lee, 1996). Under normal growth conditions, PHB content in the cells is usually not very high. Depending on bacterial strains, it is 2 to 10% weight of the dry cell mass. However, PHB content can reach up to 80% of the dry cell mass if growth is limited by the depletion of essential nutritional compounds (Du et al., 2001). The biopolymers content is related to biomass. Increasing biomass can be achieved by different methods such as biofilm which is considered to be a natural form of immobilized cells. Moreover, the plastic composite supports (PCS) have been shown to stimulate biofilm formation and enhance the productivity during the fermentation process (Demirci et al., 1989; Cotton et al., 2001; Velazquez, 2001). In current endeavour, a low cost raw material consisting of date syrup was evaluated as complex media supplements to meet the nutritional requirements for biopolymer production by suitable isolated bacterial strains. Also, the PCS biofilm was employed to increase the accumulating biopolymer via increasing biomass production.

MATERIALS AND METHODS

Isolation and screening

The screening procedure for PHA producers from the environment was studied according to Sheu et al. (2000). Serial dilutions of different soils samples were prepared in mineral salt media (MSM). Aliquots of the dilution series were spread onto MSM plates containing 1% (w/v) sucrose (Sigma), 1.5% (w/v) sodium gluconate (Wako), 1% (w/v) glycerol (Sigma) or 0.1% (w/v) sodium octanoate (Wako) as carbon sources and were cultured at 30°C overnight. The grown colonies were individually streaked onto MSM plates to obtain well-separated single colonies.

For PHA producer candidates analysis, 0.002 volume of a solution of 0.25 mg Nile red or Nile blue per ml dimethylsulfoxide (DMSO) was added to the MSM sterilized media to give a final concentration of 0.5 µg dye (ml media^{-1}). The agar plates were exposed to ultraviolet light (312 nm) after appropriate cultivation periods to detect PHA-positive strains.

Classification and identification

The PHA-positive strains were classified depending on their morphological and biochemical characterizations. Molecular procedures were carried out to identify the PHA-positive strains. Genomic DNA from strains was isolated (QIAamp DNA Mini kit, Valencia, CA), and 16S rDNA gene was amplified by PCR and sequenced by DNA sequencer (Macrogen Inc). Each sequencing data was identified using the basic local alignment search tool (BLAST) and ribosomal databases of Michigan State University. Sequencing data were aligned and the evolutionary relationship of the sequencing information was studied by phylogenetic analyses.

PCS biofilm formation

A computer controlled New Brunswick Bioflo 10 (Edison, NJ) benchtop fermentor equipped with pH, temperature, agitation and oxygen dissolved controls was employed with batch cultures. The 1.2 L vessel was equipped with filtered sterilized air in and out, alkali, acid, medium addition, and broth removal ports. To control pH, acid and alkali were added from graduated burettes, which were refilled aseptically from reservoirs with 1 N HCl and 1 N NaOH. The dissolved oxygen (DO_2) and pH controls were recorded automatically. The broth removal port was connected with two branched lines to withdraw a sample and to draw off all culture media from the vessel. The reactor was sterilized with water in the autoclave for 1.25 h at 121°C. After sterilization, media was used to dilute out water at a dilution rate of 0.6 h^{-1}.

Biofilm was developed on PCS tubes which bound to the agitator shaft in a grid fashion as described by Cotton et al. (2001). The fermentor was supplied with MSM media and inoculated with 5 ml (A620 of 0.5) of an 18 h PHA – positive strain (*Bacillus* SA culture). The fermentor was incubated as batch culture for six days with constant agitation (100 rpm), and 30°C. The development of the *Bacillus* SA biofilm was monitored on the PCS visually.

Preparation of date syrup

The date cultivar (variety) Khalas, a type of dates' purchased from the local market in Riyadh was used in this study, because of its economic affordability and its availability in large quantities in Saudi Arabia. The extraction of date syrup was performed as described by Al-Eid (2006) with some modification. The dates were sliced to pieces. Fifty grams of stone-free dates were soaked in 225 ml distilled water for 10 min.

The soaked dates were then aseptically blended in a sterile waring blender for 5 min at low speed. The homogenized mixture was transferred to a 500 ml Erlenmeyer conical flask and placed on magnetic stirrer for 30 min at 80°C. The slurry was filtered through a cloth using hand press. The collected raw date juice was then centrifuged at 7,000 x g for 30 min. The produced date syrup was autoclaved and then packed in sealed glass bottles and stored at room temperature. In each experiment batch, date syrup was mixed at 1:1 (volume) with filter sterilized mineral solution media (MSM),

adapted from Serafim et al. (2004), containing per liter: 720 mg $MgSO_4 \cdot 7H_2O$, 84 mg $CaCl_2 \cdot 2H_2O$, 687.6 mg NH_4Cl, 24 mg EDTA, 20 mg N-allylthiourea (to inhibit nitrification) and 1.2 ml of a trace elements solution (containing, per liter: 1500 mg $FeCl_3 \cdot 6H_2O$, 150 mg H_3BO_3, 30 mg $CuSO_4 \cdot 5H_2O$, 30 mg KI, 120 mg $MnCl_2 \cdot 4H_2O$, 60 mg $Na_2MoO_4 \cdot 2H_2O$, 120 mg $ZnSO_4 \cdot 7H_2O$ and 150 mg $CoCl_2 \cdot 6H_2O$).

A phosphate solution (containing 165.6 mg K_2HPO_4 and 81 mg KH_2PO_4 per liter of final mineral solution) was prepared separately to avoid precipitation during sterilization.

Batches protocols

Effect of different concentrations of date syrup

The PHA production was evaluated in eighteen repeated batch fermentations at 30°C with 1 L working volume, and 30 h incubations per batch. The repeated batch fermentations of MSM media were investigated with different concentrations of the date syrup including 5.0, 10.0, 15.0, 20.0, and 25.0% (v/v), and 10 gm/l glucose was used as control. The nitrogen source was 0.5 g/l of NH_4Cl. After each batch end the bioreactor was drained and biofilm was removed off PCS tubes by changing the agitation rate. The removed biofilm was analyzed for PHA quantity. Fresh MSM medium with a new date syrup concentration was then added and inoculated with 5 ml (A620 of 0.5) of fresh *Bacillus* SA culture. The new batch was treated as described previously.

Effect of agitation

The accumulation of PHA in cells at 120 and 300 rpm agitation and continuous oxygen addition were investigated. Thirty six repeated batch fermentations at 30°C with 1 L working volume, and 30 h incubations per batch were ran. Date syrup concentrations were 5.0, 10.0, 15.0, 20.0, 25.0% (v/v), and 10 gm/l glucose as control. The nitrogen source was 0.5 g/l of NH_4Cl.

Effect different nitrogen source

The accumulation of the PHA in cells with different nitrogen sources and different concentrations of date syrup was investigated. A hundred and twenty-six repeated batch fermentations at 30°C with 1 L working volume, and 30 h incubations per batch were run. The nitrogen sources (0.5 g/l) included ammonium oxalate, NH_4NO_3, $NaNO_3$, ammonium acetate, $(NH_4)_2SO_4$, NH_4Cl and urea. The date syrup concentrations were 5.0, 10.0, 15.0, 20.0, and 25.0% (v/v), and 10 gm/l glucose as control.

Comparison between suspended cells and PCS biofilm

The accumulation of the PHA in suspended cells and PCS biofilm with NH4Cl and urea as nitrogen sources and 15% (v/v) date syrup was investigated. Twelve repeated batches were run for 30 h at 30°C.

Time course of PHA production

The time course of developing PCS biofilm and accumulation and PHA was investigated. The repeated batches were run with 15% (v/v) date syrup, 0.5 g/l urea, for 30 h at 30°C. The PCS biofilm and PHA production were monitored in eighteen repeated batches at 5, 10, 15, 20, 25, and 30 h. In each interval time the PCS biofilm, PHA and sucrose consumption was determined.

EXPERIMENTAL ANALYSIS

Physiological of factors analysis

In each batch, biofilm formation was monitored by oxygen consumption, CO_2 production, and change in pH via probes in the bioreactor connected to a computer. Also, the biofilm formation was estimated via consumption of carbon sources. The concentration of sucrose was calculated from the amounts of fructose and glucose that were detected in cell free supernatants after inversion of sucrose in the presence of 94 mM sulfuric acid at 70°C for 3 h. Fructose and glucose were analyzed by HPLC using an ion exclusion HPX-87H (7.8 × 300 mm) column at 70°C (Biorad, Richmond, USA) and a RI-71 detector. The column was equilibrated with 6.5 mM sulfuric acid at 0.5 ml $min^{-}1$.

Extracellular protein was determined as concentrations according to the Lowery et al. (1951) reagent modified method on 0 and end of each batch culture. Protein concentrations were determined spectrophotometrically at 750 nm by using a Spectrasonic 20 (Milton Roy, Rochester, NY). Bovine serum albumin was used to establish a standard curve for extracellular protein concentration (50 to 400 g/ml*)* 0.999).

Urea was determined in cell free supernatants employing Urea Assay Kit (DIUR-500), (GENTAUR). The assay was run as recommended by the manufacturing company.

Ammonium was estimated in cell free supernatants employing ammonium test bars (Merck AG, Darmstadt, Germany) or a gas-sensitive type 152303000 ammonium electrode (Mettler Toledo GmbH, Greifensee, Switzerland).

The concentration of sucrose was calculated from the amounts of fructose and glucose that were detected in cell free supernatants after inversion of sucrose in the presence of 94 mM sulfuric acid at 70°C for 3 h. Fructose and glucose were analyzed by HPLC using an ion exclusion HPX-87H (7.8 × 300 mm) column at 70°C (Biorad, Richmond, USA) and a RI-71 detector. The column was equilibrated with 6.5 mM sulfuric acid at 0.5 ml min–1.

Isolation of PHA

The biopolymer was determined in removed biofilm after each batch. The biopolymer extraction procedure was performed according to Findlay and White (1983). The biofilm was lyophilized (Freeze dry systems, Labconco). The lyophilized bifoilm was placed in a Soxhlet extractor lined with glass wool. Enough chloroform was added to cover the sample, and the sample was sonicated for 10 min. The sample was extracted overnight in a total of 125 ml of chloroform. The extraction thimble of the Soxhlet extractor was heated so that the chloroform present boiled, maintaining solubility of the polymers. The chloroform was recovered and removed in a rotary evaporator in vacuum. Subsequently, the polymer was redissolved in hot chloroform and biopolymer was recovered from the chloroform by nonsolvent precipitation and filtration. Methanol was used as the nonsolvent (4 to 6 volumes).

Analysis of accumulated PHA

The extracted PHA was prepared for GC analysis. 10 ml samples were prepared as proposed by Oehmen et al. (2005) and Arcos-Hernandez et al. (2010). 10 ml samples were taken with 10 drops of 30 to 40% formaldehyde added to stop all activity. The samples were centrifuged at 5000 rpm for 5 min. The supernatant was removed and the pellet dried in an oven at 110°C until dry. Next, 2 ml of acidified methanol with benzoic acid as internal standard (3% w/w of H_2SO_4) and 2 ml of chloroform were added followed by digestion of the sample for 20 h at 100°C. After cooling to room temperature, 1 ml of MilliQ water was added to allow phase

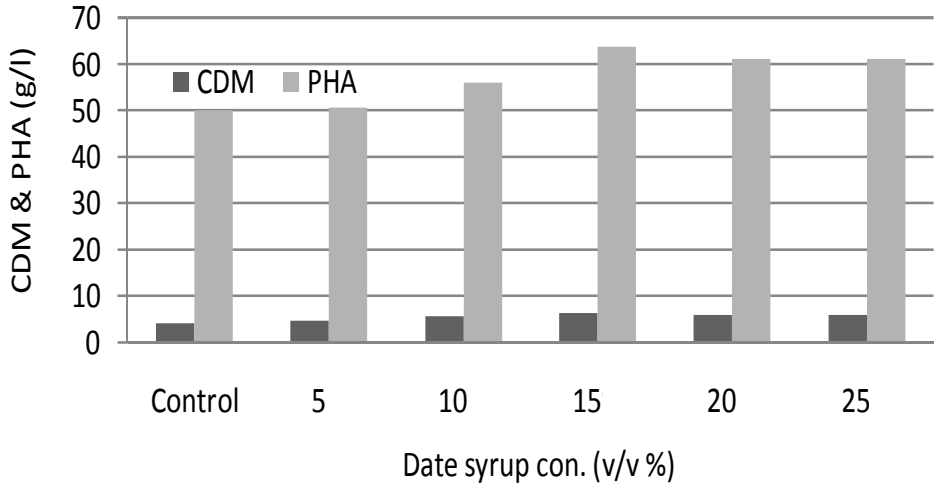

Figure 1. Effect different concentration of date syrup on developing PCS biofilm as cellular dry mater (CDM) and accumulation PHA.

separation. After 1 h settling, the organic phase was transferred to a vial for GC analysis.

Average molecular weight

The measurements were performed with polymer solution in chloroform (1% w/v) after filtration through 0.45 µm Sartorious membranes. The average molecular weights were determined using a Lachrom Merck–Hitachi gel permeation chromatography system with refractive index detector and PS4000, PS400, PS40 and PS4 Licrogel columns placed in series with exclusion limits 106, 105, 104, and 103 Dalton. Chloroform was used as eluent at a flow rate of 1.0 ml/min and injection volumes of 20 µl were used. Polystyrene Merck standards with narrow polydispersity were used for the calibration curve.

RESULTS AND DISCUSSION

A major limitation to the commercial production of readily biodegradable PHA is the lack of inexpensive feedstock that can compete with conventional thermal plastics made from oil. Bacteria efficiently convert different carbon sources into a diverse range of polymers with average chemical and material properties (Anderson and Dawes, 1990). Biopolymers accumulate in the bacterial cells under unfavorable growth conditions in limited nitrogen source and excess of carbon source (Steinbuchel, 1991; Byrom, 1994; Page, 1995; Lee, 1996). The carbohydrates in date syrup illustrated a great potential in this connection and our research found suitable microorganisms *Bacillus* spp. (*Bacillus* SA) could convert date syrup to PHA (Figure 1).

In this study, one date variety (Khalas) has been selected due to its availability in large quantities, nutrition compound and its low cost. Dates are a high energy food containing most of the basic dietary elements such as sugars, proteins, fats, and minerals and are also important raw materials for many food products (Mikki, 1998). However, it is important to use the best techniques for date syrup extraction to maximize fermentable sugars extraction. Three extraction techniques can be used for date syrup preparation. These techniques are classical extraction, fluidized bed, and super critical fluid extraction. The classical technique is the simplest and cheapest; however, different factors such as temperature and water rate can influence the extraction technique (Ramadan, 1998).

The fleshy part of dates contains carbohydrates, vitamins, salts and minerals, protein and small amounts of fats, oils, and acids. Dates are composed of large amounts of reducing sugars including sucrose, glucose, and fructose, (Al-Eid, 2006) which can be converted to by products. Presently, more than 100 different monomers have been identified as constituents of PHA produced by bacteria (Steinbuchel and Valentin, 1995). *Pseudomonas putida* IPT 046 accumulated higher amounts of PHA from glucose and fructose compared to *P. putida* KT2440, reaching 60% and 50% of the cell dry weight, respectively. *P. putida* KT2440 was used as a reference since it and other related strains have been used in studies aiming at the establishment of industrial processes (Ruben et al., 2003). The presence of monomers differing by two carbon atoms as well as the presence of constituents with more than 10 carbon atoms containing unsaturation clearly reflects the use of the fatty acid biosynthesis pathway as a supplier of intermediates for PHA biosynthesis.

Effect of different date syrup concentrations

Different concentrations of date syrup were used to

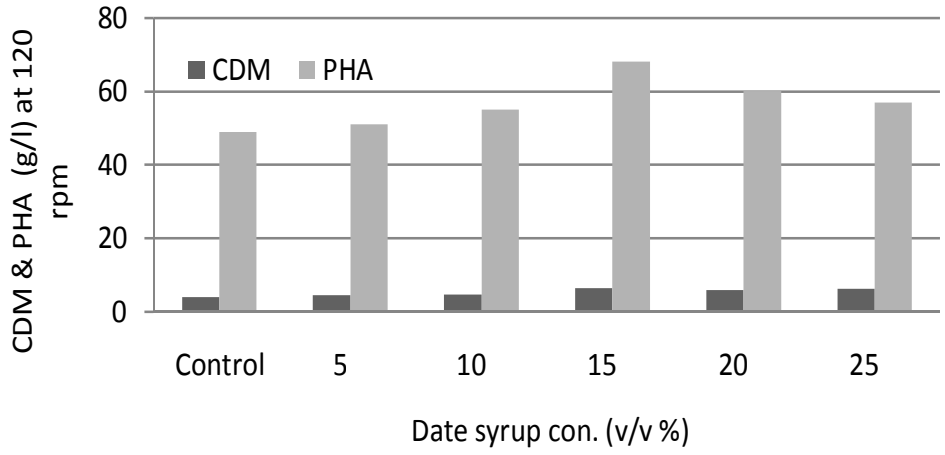

Figure 2. Effect of agitation 120 rpm on developing PCS biofilm as cellular dry mater (CDM) and accumulation PHA.

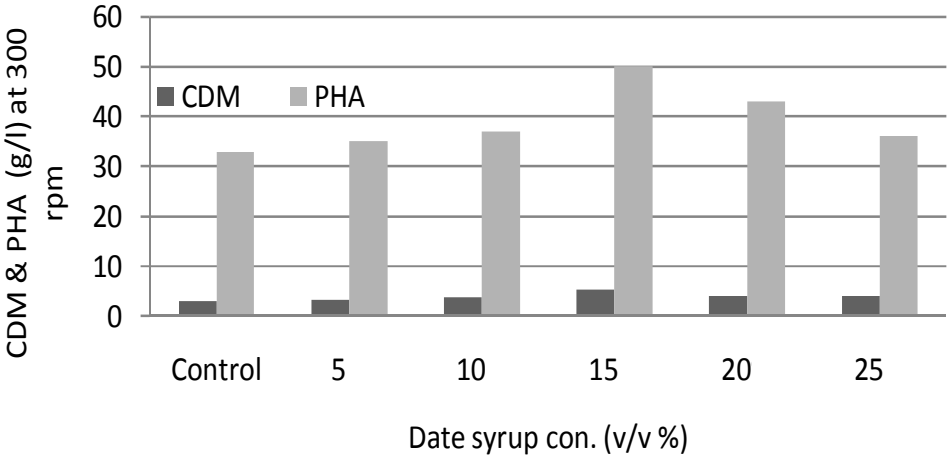

Figure 3. Effect of agitation 300 rpm on developing PCS biofilm as cellular dry mater (CDM) and accumulation PHA.

PCS bioflim in repeated batch fermentations. All date syrup concentrations stimulated biofilm formation. However, the low and high concentrations were not stable for PHA production (Figure 2). In general, PHA accumulation was significantly higher with date syrup at 15% (v/v). The biomass was 6.4 g/l and PHA was 63.6 g/l. The PHA was calculated based on the cellular dry matter which was 6.4 g/l with almost 63.6% PHA content. The low concentration might have a low sugar content which was not a sufficient amount for PHA production. While, the high concentration might have contained some inhibitors or undesirable compounds such as acetic acid, propionic acid, butyric acid and formic acid (Al-Eid et al., 2006) which might have affected PHA production. These inhibitors can affect the microorganism's fermenting ability both by producing ethanol and by stopping growth. The inhibition of growth and developing PHA might be noticeable with suspended cell when employing 5 and 10% of date syrup. The PCS biofilm developed more CDM and PHA with 15% date syrup and that is because the biofilm can resist toxic compounds (Khiyami et al., 2005).

Effect of agitation

The repeated batches were run at 120 and 300 rpm. PHA accumulation was significantly developed with 120 rpm and date syrup at 15% (v/v) compared with other date syrup concentrations (Figure 3). The biomass was 6.4 g/l and PHA was 68 g/l (Figure 3). Low and high concentration of date syrup with agitation of 300 rpm

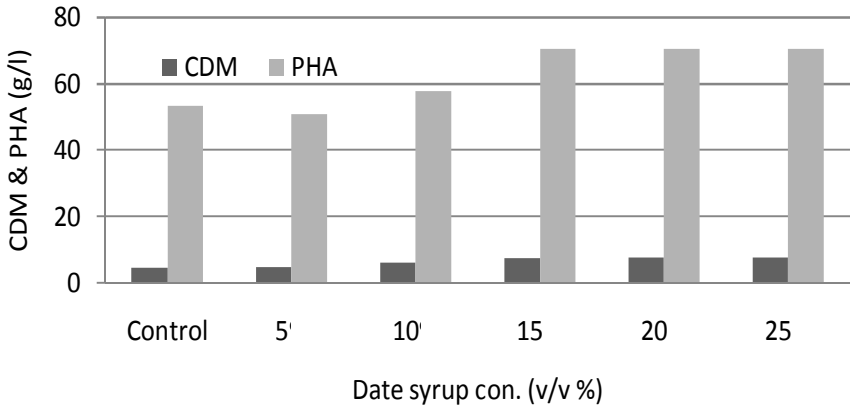

Figure 4. Effect of urea on developing PCS biofilm as cellular dry mater (CDM) and accumulation PHA.

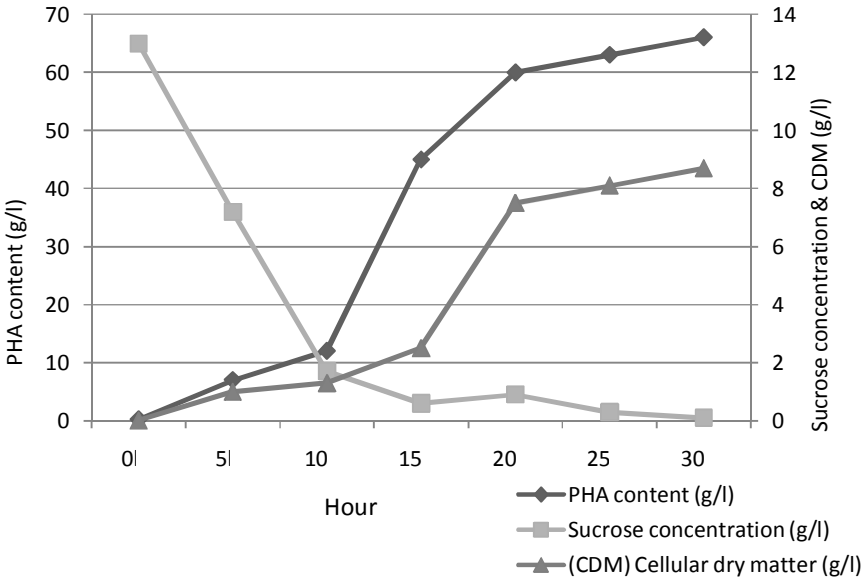

Figure 5. Time course of batch fermentation with PCS biofilm of *Bacillus* SA in mineral salts medium with 15% date syrup as sole carbon source and 0.5 (g/l) NH_4Cl. Cultivation conditions: temperature, 30°C; pH, 7; agitation 120 rpm.

reduce biofilm formation and PHA accumulation better than agitation 300 rpm (Figure 4). In most microorganisms, intracellular PHA is accumulated as a response to nutrient(s) limitation and unbalanced growth conditions (Kessler and Witholt, 2000).

Effect of different nitrogen sources

Since the nitrogen source is an important factor for the accumulation of PHA, different salts of ammonium oxalate, NH_4NO_3, $NaNO_3$, ammonium acetate, $(NH_4)_2SO_4$, NH_4Cl and urea were tested and provided at amounts of 0.5 g^{-l}. The nitrogen sources have no significant influence on PHA accumulation (Annuar et al., 2008), except for urea which was effective and increased the accumulation of PHA. It was hypothesized that the monomer compositions of the PHA produced would remained relatively constant irrespective of the nitrogen sources used. Yet, different types of carbon sources would influence the PHA accumulation. (Brandl et al., 1988; Gross et al., 1989; Preusting et al., 1990; Annuar et al., 2008). Moreover, this experiment was run with different concentration of date syrup. The 15% (v/v) was the most effective concentration with all salts except with NH_4NO_3 (Figure 5). However, growth and PHA

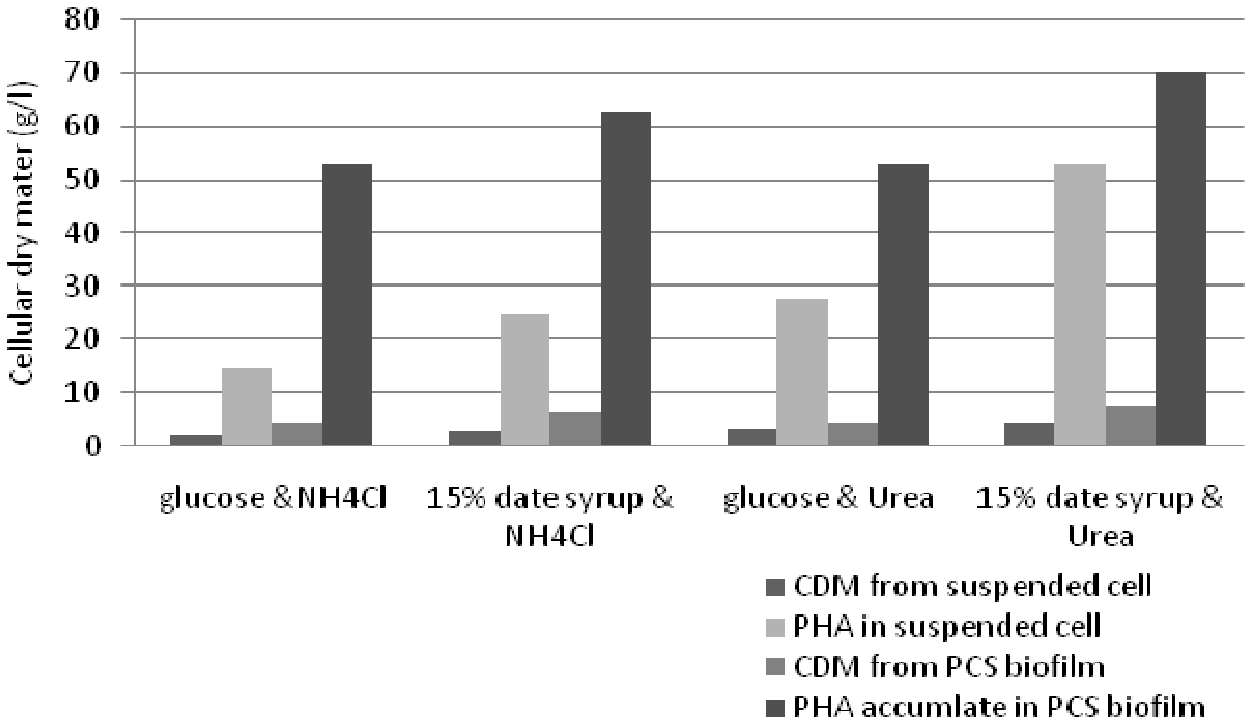

Figure 6. Comparison between accumulation PHA in suspended cell and PCS biofilms.

accumulation were high, when urea was used as a nitrogen source followed by NH_4Cl, $(NH_4)_2SO_4$, $NaNO_3$, ammonium acetate, ammonium oxalate, and NH_4NO_3 (Figure 5). Accumulation of PHA in the cells reached its maximum with urea as 70.5 g/l.

Accumulation of PHA in suspended cells vs biofilms

The comparison between accumulated PHA in suspended cells and PCS biofilm was determined (Figure 6). The ingredient of PCS and media composition stimulated the biofilm formation (Ho et al., 1997; Urbance et al., 2003). The PHA production was higher in PCS biofilm and that was due to the size of biomass production. Figure 6 also shows effect of NH_4Cl and urea as nitrogen source on developing biofilm and accumulation the PHA. Using urea as nitrogen with 15% (v/v) date syrup estimated developing biofilm and produce high amount of PHA.

Time course of PHA production

The effect of time on PHA accumulation was investigated (Figures 5 and 6). The biofilm could develop during 5 h of incubation. However, the biomass reached a maximum at 30 h with 8.3 g/l. The PHA accumulation was combined to developing biofilm and it reached a maximum at 70.5 g/l. After 30 h the biofilm and PHA development slowed and that was combined to the consumption the sucrose (Figure 7). Optimal growth and PHB accumulation in *Bacillus megaterium* was studied with 5% (w/v) date syrup. The production of PHB reached maximum amount at around 12 h. Cell density was 3 g/l with a PHB content of 50% (w/w) (Omar et al., 2001).

Average molecular weight of PHA

The medium-chain-length polyhydroxyalkanoates produced by *Bacillus* SA were cartegorized with respect to average molecular weight, monomer composition, representative structure and crystalline properties. Analysis by gas chromatography revealed that PHA is composed from essentially hydroxydecanoate (50 to 65%) and hydroxyoctanoate (25 to 30%) sequence units with a non-terminal double bond in about 6% of the side chains. The weight average molecular weight range between $3.7x10^5$ g/mol and $4.1x10^5$ g/mol. On the other hand, the molecular weights of polymers produced with fermented molasses had molecular weights between 3.5×105 g/mol and 4.3×105 g/mol (Bengtsson et al., 2010a). The molecular weights of polymers obtained from synthetic substrates (acetate and propionate) were 1.1 to 5.6×105 g/mol (Dai et al., 2007, 2008; Simon et al., 2010; Premurkumar et al., 2011). The average molecular weight might be changed depending on the media

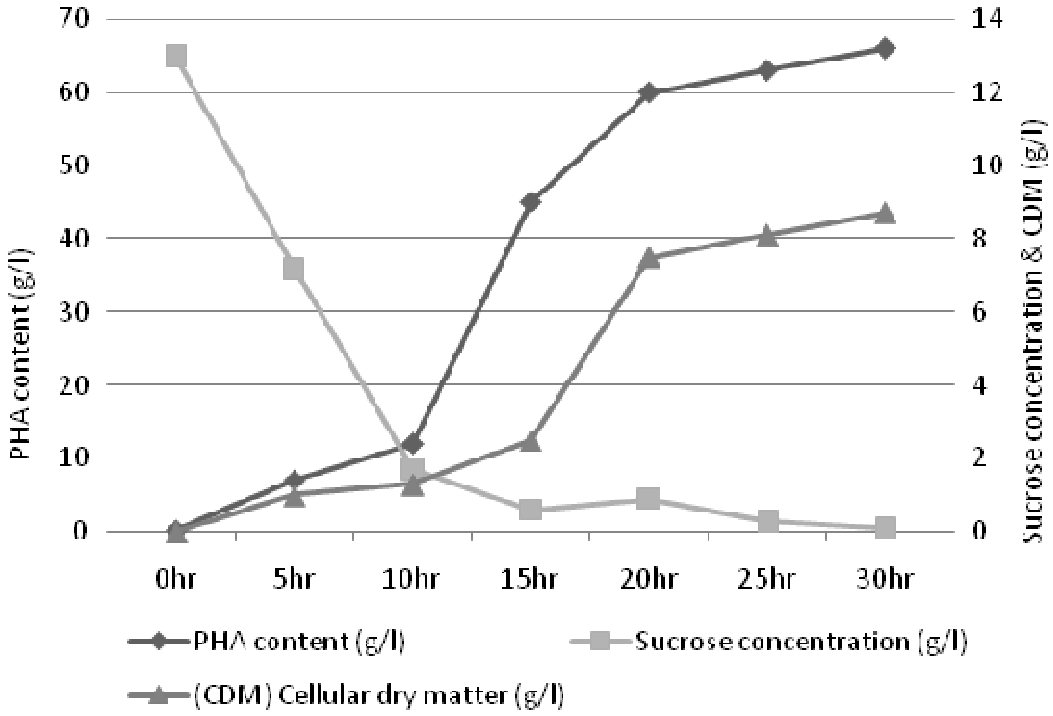

Figure 7. Time course of batch fermentation with PCS biofilm of *Bacillus SA* in mineral salts medium with 15% date syrup as sole carbon source and 0.5 (g/l) NH_4Cl. Cultivation conditions: temperature, 30oC; pH, 7; agitation 120 rpm.

compositions and microorganisms. Oil fatty acids media lead to produced unsaturated medium-chain-length poly(3-hydroxyalkanoates) which relatively have small molar mass and contain a high concentration of unsaturated side-chains (Van der et al., 1999; Ashby et al., 2000).

Conclusion

Biodegradable biopolymer medium-chain-length polyhydroxyalkanoates (PHA) was obtained by strains *Bacillus* spp. isolated from Saudi desert soil. 15% (v/v) of date syrup was the optimum concentration as carbon source to produce 7.3 g/l of PCS biofilm biomass with almost 70.5% of PHA content. The quality of the PHA was not affected by changing the culture conditions but the PCS stimulated the development of biofilm and changed the amount of PHA due the increase in biomass. The improvement of date syrup preparation will encourage fermenting high concentration of date syrup, which leads to develop more biofilm and accumulate higher amounts of PHA.

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REFERENCES

Al ESM (2006). Chromatographic separation of fructose from date syrup. Int. J. Food Sci. Nutri., 57: 83-96.

Anderson AJ, Dawes EA (1990). Occurrence, metabolism, metabolicrole and industrial uses of bacteria polyhydroxyalkanoates. Microbiol. Rev., 54: 450-472.

Annuar MSM, Irene KP, Ramachandran KB (2008). Evaluation of nitrogen sources for growth and production of medium-chain-length poly-(3 hydroxyalkanoates) from palm kernel oil by *Pseudomonas putida* PGA1. Asia Pacific J. Mol. Biol. Biotechnol., 16(1): 11-15

Arcos HMV, Gurieff N, Pratt S, Magnussonc P, Werkerc A, Vargas A, Lant P (2010). Rapid quantification of intracellular PHA using infrared spectroscopy: An application in mixed cultures. J. Biotechnol., 150: 372-379

Ashby RD, Foglia TA, Solaiman DKY, Liu CK, Nunes A, Eggink GV (2000). Properties of linseed oilbased medium chain length poly(hydroxyalkanoate) films: effects of epoxidation and curing. Int. J. Biol., 27: 355-361.

Bengtsson S, Pisco AR, Johansson P, Lemos PC, Reis MAM (2010a). Molecular weight and thermal properties of polyhydroxyalkanoates produced from fermented sugar molasses by open mixed cultures. J. Biotechnol., 147: 172-179.

Bengtsson S, Pisco AR, Reis MAM, Lemos PC (2010). Production of polyhydroxyalkanoates from fermented sugar cane molasses by a mixed culture enriched in glycogen accumulating organisms. J.

Biotechnol., 145: 253-263.
Brandl H, Gross RA, Lenz RW, Fuller RC (1988). *Pseudomonas oleovorans* as a source of poly(β-hydroxyalkanoates) for potential applications as biodegradable polyesters. Appl. Environ. Microbiol., 54: 1977-1982.
Byrom D (1994). Polyhydroxyalkanoates. In: Mobley, D.P. (Ed.), Plastics from Microbes: Microbial Synthesis of Polymers and Polymer Precursors. Hanser, Munich, Pp. 5-33.
Cotton JC, Pometto AL, Jeremic JG (2001). Continuous lactic acid fermentation using a plastic composite support biofilm reactor. Appl. Microbiol. Biotechnol., 57: 626-630.
Dai Y, Lambert L, Yuan Z, Keller J (2008). Characterisation of polyhydroxyalkanoate copolymers with controllable four-monomer composition. J. Biotechnol., 134: 137-145.
Dai Y, Yuan ZG, Jack K, Keller J (2007). Production of targeted poly(3-hydroxyalkanoates) copolymers by glycogen accumulating organisms using acetate as sole carbon source. J. Biotechnol., 129: 489-497.
Demirci A, Pometto AL, Lee B, Hinz P (1998). Media evaluation of lactic acid repeated-batch fermentation with *Lactobacillus plantarum* and *Lactobacillus casei* subsp. *rhamnosus*. J. Agric. Food Chem., 46: 4771-4774.
Du G, Chen J, Yu J, Lun S (2001). Continuous production of poly-3 hydroxybutyrate by *Ralstonia eutropha* in a two-stage culture system. J. Biotechnol., 88: 59-65.
Ertesvag H, Doseth B, Larsen B, Skjakbraek G, Valla S (1994). Cloning and expression of an *azotobacter vinelandii* mannuronan c-5-epimerase gene. J. Bacteriol., 2846-2853.
Findlay RH, White DC (1983). Polymeric betahydroxyalkanoates from environmental samples and *Bacillus megaterium*., Appl. Environ. Microbiol., 45: 71-78.
Gross RA, DeMello C, Lenz RW, Brandl H, Fuller RC (1989). Biosynthesis and characterization of poly (β-hydroxyalkanoates) produced by *Pseudomonas oleovorans*. Macromol., 22: 1106-1115.
Ho K, Pometto AL, Hinz P, Dickson N, Demirci A (1997). Ingredient selection for plastic composite supports for L-(+)- lactic acid biofilm fermentation by *Lactobacillus casei* subsp. *rhamnosus*. Appl. Environ. Microbiol., 63: 2516-2523.
Kessler B, Witholt B (2001). Factors involved in the regulatory network of polyhydroxyalkanoate metabolism. J. Biotechnol., 86(2): 97-104.
Khiyami M, Pometto AL, Brown R (2005). Detoxification of Corn Stover and Corn Starch Pyrolysis Liquors by *Pseudomonas putida* and *Streptomyces setonii* Suspended Cells and Plastic Compost Support Biofilms. J. Agric. Food Chem., 53: 2978-2987.
Khiyami M, Noura AF, Basel B, Sher H (2011). Food borne pathogen contamination in minimally processed vegetable salads in Riyadh, Saudi Arabia. J. Med. Plants Res., 5(3): 444-451.
Kim BS (2000). Production of poly (3-hydroxybutyrate) from inexpensive substrates. Enzyme Microb. Technol., 27: 774-777.
Lee SY (1996). Bacterial polyhydroxyalkanoates. Biotechnol. Bioeng., 49: 1-14.
Mikki MS (1998). Present status and future prospects of date and date palm industries in Saudi Arabia. Proceedings of the first international conference on date palms. Al-Ain, United Arab Emirates. Pp. 469-507.
Oehmen A, Keller KB, Zeng RJ, Yuan Z, Keller J (2005). Optimisation of poly-B-hydroxyalkanoate analysis using gas chromatography for enhancedbiological phosphorus removal systems. J. Chromatogr., 1070: 131-136.
Omar S, Rayes A, Eqaab A, VoB I, Steinbüchel A (2001). Optimization of cell growth and poly (3-hydroxybutyrate) accumulation on date syrup by a *Bacillus megaterium* strain. Biotechnol. Lett., 23: 1119-1123.
Page J (1995). Bacterial polyhydroxyalkanoates, natural biodegradable plastics with a great future. Can. J. Microbiol., 41: 1-3.
Premurkumar G, Sankaranarayanan R, Jeeva S, Rajarathinam K (2011). Cytokinin induce shoot regeneration and flowering of *Scoparia dulics* L (*Scrophulariaceae*)- an ethnomedicinal herb. APJTB, pp. 169-172
Preusting H, Nijenhuis A, Witholt B (1990). Physical characteristics of poly(3 hydroxyalkanoates) and poly(3-hydroxyalkenoates) produced by *Pseudomonas oleovorans* grown on aliphatic hydrocarbons. Macromol., 23: 4220-4224.
Ramadan BR (1998). preparation and evaluation of Egyptian date syrup. The first International Conference on Date palm. Al-Ain, United Arab Emerates, pp. 86-99.
Rubén J, Sánchez JS, Luziana F, Marilda K, Taciro JGC, Pradella J, Gregório CG (2003). Medium-chain-length polyhydroxyalkanoic acids (PHA_{mcl}) produced by *Pseudomonas putida* IPT 046 from renewable sources. Eur. Polym. J., 39(7): 1385-1394.
Serafim LS, Lemos PC, Oliveira R, Reis MAM (2004). Optimization of polyhydroxybutyrate production by mixed cultures submitted to aerobic dynamic feeding conditions. Biotechnol. Bioeng., 87: 145-160.
Sheu D, Wang Y, Lee C (2000). Rapid detection of polyhydroxyalkanoate accumulating bacteria isolated from the environment by colony PCR. Microbiol., 146: 2019-2025.
Simon B, Ana RP, Peter J, Paulo CL, Maria AMR (2010). Molecular weight and thermal properties of polyhydroxyalkanoates produced from fermented sugar molasses by open mixed cultures, J. Biotechnol., 147: 172-179.
Steinbüchel A, Valentin HE (1995). Diversity of bacterial polyhydroxyalkanoic acids. FEMS Microbiol. Lett., 128: 219-228.
Steinbuchel A (1991). Polyhydroxyalkanoic acids. In: Byrom, D. (Ed.), Biomaterials: Novel Materials from Biological Sources. Stockton Press, New York, NY, pp. 124-213.
Urbance S, Pometto AL, Di SA, Demirci A (2003). Medium evaluation and plastic composite support ingredient selection for biofilm formation and succinic acid production by *Actinobacillus succinogenes*. Food Biotechnol., 17: 53-65.
Vander WGAM, Buisman GJH, Weusthuis RA, Eggink G (1999). Development of environmentally friendly coatings and paints using medium-chain length poly(3-hydroxyalkanoates) as the polymer binder. Int. J. Biol., 25: 123-128.
Velazquez A, Pometto AL, Ho K, Demirci A (2001). Evaluation of plastic composite supports in repeated fed-batch biofilm lactic acid fermentation by *Lactobacillus casei*. Appl. Microbiol. Biotechnol., 55: 434-441.
Yu J, Si Y, Keung W, Wong R (2002). Kinetics modeling of inhibition and utilization of mixed volatile fatty acids in the formation of polyhydroxyalkanoates by *Ralstonia eutropha.* Process Biochem., 37: 731-738.