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ENHANCED PRODUCTION OF POLYHYDROXYBUTYRATE (PHB) FROM AGRO-INDUSTRIAL WASTES; FED-BATCH CULTIVATION AND STATISTICAL MEDIA OPTIMIZATION

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ABSTRACT

Bacillus megaterium SW1-2 showed enhanced growth and polyhydroxybutyrate (PHB) production during cultivation on date palm syrup (DEPS) or sugar cane molasses. FT-IR and NMR spectroscopic analyses of the polymer accumulated during growth on DEPS revealed specific absorption peaks characteristic for PHB. 1.65 g/L of PHB (56.9% CDW) was produced during growth on medium supplemented with 2 g/L of DEPS. Approximately, 36.1% CDW of PHB were recorded during growth on sugar cane molasses. Six runs of different fed-batch cultivation strategies were tested, the optimal run showed approximately 6.87-fold increase. Modified E2 medium was prefered recording 10.11 and 11.34 g/L of total PHB produced for runs 1 and 2, at the end of 96 h incubation period, respectively. Decrease in PHB was recorded during growth on complex medium (run 3 and run 4). In another independent optimization strategy, ten variables were concurrently examined for their significance on PHB production by Plackett-Burman statistical design for the first time. Among variables, DEPS-II and inoculum concentration followed by KH_2PO_4 and $(NH_4)_2SO_4$ were found to be the most significant variables encourage PHB production. Indeed, DEPS-II or Fresh syrup is more significant than commercial syrup DEPS-I (*p*-value= 0.05). RPM, incubation period have highly negative effect on PHB production. Role of ago-industrial wastes, especially DEPS, in enhancement of PHB production was closely discussed.

Keywords: Date syrup or DEPS, *Polyhydroxybutyrate* (PHB), *Bacillus megaterium*, Plackett-Burman design, fed-batch cultivation, optimization

INTRODUCTION

Among the most commonly known biopolymers, bacterial polyhydroxyalkanoates (PHAs) polyesters gain more interest. It is synthesized and intracellular accumulated as storage materials to be used as energy source during unbalanced growth **(Anderson and Dawes, 1990; Steinbuchel 2001; Du and Yu, 2002; Ojumu *et al.*, 2004)**. PHB production by microorganisms attracted attention of many scientists due to close resemblance to synthetic petroleum–based plastics such as polypropylene **(Mokhtari-Hosseini *et al.*, 2009)**, biodegradability and biocompatiblility **(Madison and Huisman, 1999; Ojumu *et al.*, 2004; Khanna and Srivastava, 2005)**. Several applications in medicine, veterinary practice, tissue engineering materials, food packaging and agriculture have been reported **(van der Walle *et al.*, 2001; Zinn *et al.*, 2001; Luengo *et al.*, 2003; Chen and Wu, 2005; Bucci *et al.*, 2005; Urtuvia *et al.*, 2014)**. Many bacilli have been reported to accumulate PHA and co-polymers of 3HB during growth on different substrates **(Belma et al., 2002; Tajima et al. 2003; Valappil *et al.*, 2007; Adwitiya *et al.*, 2009; Reddy *et al.*, 2009)**. In spite the privilege of microbial PHAs production compared with petroleum-derived plastics, costs of the feedstock are the main limiting factor for their mass production **(Ojumu *et al.*, 2004; Nikel *et al.*, 2005)**. Economically, the use of agro-industrial wastes, improved fermentation strategies and downstream processes have positive contribution on the overall biopolymer production costs **(Liu *et al.* 1998; Tamer *et al.* 1998; Kim, 2000; Halami, 2008)**.
Recently, scientists have been exploring different cultivation strategies involving inexpensive, renewable carbon substrates in order to reduce production cost and obtain high productivity **(Ojumu, 2004)**. Many carbon sources derived from wastes like whey, cane molasses, sugar beet molasses and date syrup can be used in production **(Beaulieu *et al.*, 1995; Lee *et al.*, 1997; Liu *et al.*, 1998; Omar *et al.*, 2001; Khanafari *et al.*, 2006; Albuquerque *et al.*, 2007; Hamieh *et al.*, 2013)**. Nowadays, PHB proved to be produced from relatively cheaper substrates such as methanol **(Kim et al., 2003; Mokhtari-Hosseini *et al.*, 2009)**, carbon dioxide **(Ishizaki *et al.*, 2001)**, and several agro-industrial by-product such as rice bran, pulp, paper and cardboard industry, whey, dairy wastes, sea water and municipal wastes **(Law *et al.*, 2001; Nikel *et al.*, 2005; Nath *et al.*, 2008; Santimano *et al.*, 2009; Pandian *et al.*, 2010; Bhuwal *et al.*, 2013; Hamieh *et al.*, 2013; Singh *et al.*, 2013; Watanabe *et al.*, 2014)**. Furthermore, fed-batch cultivation in chemically defined medium for optimization of PHB production has been applied **(Wang and Lee., 1997; Liu *et al.*, 1998)**. Investigations have also focused on reducing the total cost of PHB through optimizing fermentation processes. Recently, application of statistical methods has gained a lot of impetus for medium optimization and understanding the interactions among various physiochemical parameters involved in biopolymer production **(Khanna and Srivastava, 2005; Nikel *et al.*, 2005; Sharma *et al.*, 2007; Mokhtari-Hosseini *et al.*, 2009; Pandian *et al.*, 2010; Berekaa and Al Thawadi., 2012; Hamieh *et al.*, 2013)**.
The main objective of this study was to investigate the possible production of PHB from several agro-industrial wastes mainly; date palm syrup (DEPS) and sugar cane molasses. Produced polymer was identified by chemical characterization using FT-IR, C^{13}NMR and H^1NMR spectroscopy. Furthermore, optimization of PHB production by application of six different fed-batch cultivation strategies was closely investigated. Special emphasis was given to the application of statistical experimental design (Plackett-Burman) for optimization of PHB production from DEPS for the first time.

MATERIAL AND METHODS

Microorganism

Group of bacilli previously isolated, maintained and screened for PHB production **(Berekaa and Al Thawadi, 2012; Berekaa, 2012)**, were tested for PHB production from agro-industrial wastes namely; date syrup (DEPS) and sugar cane molasses. The potent PHB producing bacterial strain *Bacillus megaterium* SW1-2 used in this study was identified by 16S DNA gene analysis as previously reported **(Berekaa and Al Thawadi, 2012).**

Growth and production conditions

The bacterium was grown in 50mL aliquot of nutrient broth dispensed in 250 mL Erlenmeyer flask and incubated at 37°C for 24h or 48h at (150 rpm). 1.5% inoculums of the overnight culture was used to inoculate modified E2 medium of

the following composition (g/L): ammonium sulfate; 2.5, KH_2PO_4; 1.5, Na_2HPO_4; 3.5, $MgSO_4.7H_2O$; 0.2, traces of yeast extract and 1 mL of trace element solution ($FeSO_4.4H_2O$, $CaCl_2.2H_2O$, $MnSO_4.4H_2O$, $ZnCl_2$ 1 mM each) at 37°C. To test the possible production of PHB, date palm syrup (DEPS) or sugar cane molasses was used as a sole carbon source at different concentrations. Two types of date syrup (DEPS) were used in this study, the commercial (DEPS-I) composed mainly of (per 100 g); carbohydrates 55 g (mainly sucrose), vitamin B complex 0.57 mg, calcium 685 mg, phosphate 75 mg, iron 16 mg and magnesium 258 mg) and Freshly prepared date palm syrup (DEPS-II) as following; 500 g of date palm (Khalas from Kaseem province) were placed in 1.5 L dist. water and boiled for 90 min with mixing. At the end of incubation period the extract was filtered in cloth for 3-4 times. Subsequently, the extract kept in oven at 60°C till net volume of 200 mL. Finally, the fresh date syrup was kept in refrigerator till use. Sugar cane molasses used in this study were obtained from an industrial sugar manufacturing plant, Egypt. Sugar content of molasses was 76% (w/v), and composed mainly of sucrose (62%), and fructose (38%). At the end of incubation period, PHB was determined and the cell dry weight was estimated.

Fed-batch cultivation and PHB production strategies

For optimization of PHB production from date syrup (DEPS), fed-batch cultivation was applied. During fed-batch cultivation, two different feeding strategies were applied. The first pulsed feeding of DEPS-I was carried out after 24 and 60 h time intervals, while the second was performed after 48 and 84 h, respectively, with final concentration of 15 g/L. Furthermore, three basal media were tested in this study namely; modified E2 medium, nutrient broth medium and a mixture of nutrient broth and modified E2 medium (50% w/w each) with the sum of six different fed-batch cultivation strategies (run 1 to run 6). In each experiment, sample was taken during different time intervals, PHB was determined and the cell dry weight was estimated.

Extraction of PHB from the isolate

PHB was extracted from the cell masses by using modified hypochlorite method as previously described **(Rawte and Mavinkurve, 2002; Berekaa and Al Thawadi, 2012)**.

Analytical procedures

Cell dry weight

After centrifugation of the culture medium, supernatant was discarded and cell pellet was washed with distilled water. The washed pellet was resuspended in 1 mL distilled water, transferred to pre weighed boats and dried to constant weight at 60°C.

Chemical analysis of polymer

Characterization of PHB by FT-IR

The presence and characterization of PHB in dry cell matter was confirmed by Fourier Transform Infrared Spectroscopy (FT-IR) **(Hong *et al.*, 1999)**. Dry PHB polymer from *B. megaterium* SW1-2 was used to prepare KBr discs. Spectra between 400 and 4000 cm^{-1} were recorded using Nicolet 6700 FTIR spectrometer from the Nicolet Instrument Corporation, USA.

Characterization of PHB by C^{13} NMR and H^1 NMR analysis

Extracted PHB biopolymer from *B. megaterium* SW1-2 was characterized by spectroscopic analysis. H^1NMR spectrum was recorded on a JEOL JNM-LA 500 MHz spectrometer at 30°C in $CDCl_3$ as solvent. While, C^{13} NMR spectral experiments were performed at 125.65 MHz with the following acquisition parameters: 32 k data point, 0.967 s acquisition time, recycle delay 1 s and contact time 4.50 ms.

Fractional factorial design

Plackett-Burman experimental design

Screening design namely; Plackett-Burman experimental design was applied to investigate the significance of various medium factors on PHB production **(Plackett and Burman, 1946)**. 10 chemical and environmental independent variables were examined in two levels: –1 for low and +1 for high level based on Plackett-Burman design (Table 1). According to the matrix shown in Table 2, the independent variables were screened in 14 combinations. The main effect of each variable is the difference between average of measurements at high setting (+1) and average of measurements observed at low setting (-1) of that factor. Plackett-Burman experimental design was based on the first order model (Equation 1):

$$Y=\beta 0+\Sigma\ \beta iXi \quad (1)$$

Where, Y is the predicted response, β0 and βi are constant coefficients, and Xi is the coded independent variables estimates or factors. The quality of fit of the polynomial model equation was expressed by the coefficient of determination R^2.

Table 1 Variables and their settings employed in Plackett-Burman design for optimization of PHB production by *Bacillus megaterium* SW1-2 during growth on date syrup or DEPS.

Medium Composition	Levels	
	-1	**1**
DEPS-I (%)	2	4
DEPS-II (%)	2	4
$(NH_4)_2SO_4$ (g/L)	1.5	3
Yeast Extract (g/L)	0.5	1
Na_2HPO_4 (g/L)	2	4
KH_2PO_4 (g/L)	1	2
Inoculum conc. (%)	1	2
Temperature (°C)	28	37
RPM	60	120
Incubation Period	48	72

Table 2 Effect of different environmental conditions on PHB production from *Bacillus megaterium* SW1-2 during growth on date palm syrup or DEPS

Exp. No.	DEPS-I (%)	DEPS-II (%)	$(NH_4)_2SO_4$ (g/L)	Yeast Extract (g/L)	Na_2HPO_4 (g/L)	KH_2PO_4 (g/L)	Inoculum (%)	Temperature (°C)	RPM	Incubation Period (h)	PHB (% CDW)
1	4	4	3	0.5	4	2	1	37	60	48	**46**
2	4	2	3	1	2	2	1	28	60	72	**9.8**
3	1.5	1.5	2.5	0.5	3.5	1.5	1.5	37	120	48	**31**
4	4	2	3	0.5	2	1	2	37	120	48	**27.4**
5	4	4	1.5	1	2	1	1	37	120	72	**11.5**
6	2	2	1.5	1	4	2	1	37	120	48	**7.1**
7	2	2	3	1	4	1	2	37	60	72	**32.7**
8	2	4	3	1	2	2	2	28	120	48	**53.6**
9	2	4	3	0.5	4	1	1	28	120	72	**10.6**
10	4	2	1.5	0.5	4	2	2	28	120	72	**21.3**
11	4	4	1.5	1	4	1	2	28	60	48	**42.4**
12	2	2	1.5	0.5	2	1	1	28	60	48	**23.8**
13	1.5	1.5	2.5	0.5	3.5	1.5	1.5	37	120	48	**28.6**
14	2	4	1.5	0.5	2	2	2	28	60	72	**46.1**

Statistical analysis of the data

Data of the PHB production were subjected to multiple linear regressions using MICROSOFT EXCEL 97 to estimate t-value, P-value and confidence level. The significance level (*P*-value) was determined using the Student's t-test. Factors having highest t-value and confidence level over 95% were considered to be highly significant on PHB production. Data presented in this study measured in duplicate.

RESULTS AND DISCUSSION

Growth of *Bacillus megaterium* SW1-2 on date syrup (DEPS)

Among a group of polyhydroxybutyrate-producing bacilli explored for potential production of PHB biopolymer from an agro-industrial waste namely; date palm syrup (DEPS), *Bacillus megaterium* SW1-2 showed potent growth on DEPS or date syrup as a sole source of carbon. Elemental analysis revealed that 70% of the polymer accumulated during growth on date syrup or DEPS was carbon (data not shown).

PHB accumulation by *Bacillus megaterium* SW1-2 cultivated on DEPS-I

For economic visibility of PHB biopolymer production by *B. megaterium* SW1-2 date syrup or DEPS was used as a natural carbon source. Monitoring of PHB accumulation in presence of different DEPS concentrations was carried out. Data shown in Figure 1 indicated that the agro-industrial date palm syrup or DEPS can be used as a suitable renewable carbon source during PHB production. Maximum PHB production was recorded at the end of log phase and approximately 1.65 g/L of PHB was accumulated after 48 h during cultivation on modified E2 medium supplemented with 2 g/L of DEPS-I or date syrup. Furthermore, lower concentration of DEPS-I enhanced PHB accumulation (average of 42% CDW as well as 52.65% CDW of PHB accumulated after 24 and 48 h at concentration of 2 and 2.5 (w/v) of DEPS-I, respectively). **Omar *et al.* (2001)** successfully used date palm syrup or DEPS as carbon source during PHB production. **Page (1992)** reported that the polymer production greatly enhanced in presence of unrefined sugars as well as complex nitrogen sources. It is assumed that various sugars namely; sucrose, glucose and fructose in date syrup or DEPs can be used as a carbon source by the *B. megaterium* SW1-2 cells during PHB production and may contribute to enhanced production. As previously recorded, glucose or sucrose can be used as carbon source during biopolymer production by many bacteria **(Zhang *et al.*, 1994, Valappil *et al.*, 2007; Adwitiya *et al.*, 2009; Reddy *et al.*, 2009, Wang, 2011, Belal, 2013)**, supporting this conclusion.

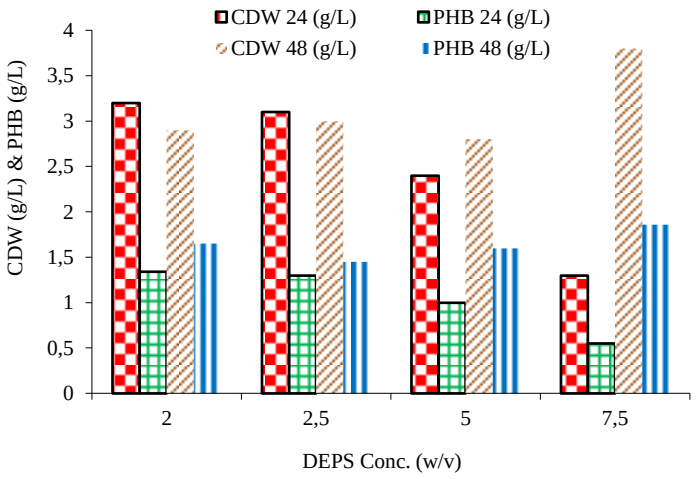


Figure 1 Effect of different date palm syrup or DEPS on growth and PHB production by *B. megaterium* SW1-2.

PHB accumulation by *Bacillus megaterium* SW1-2 cultivated on Molasses

In a trial to use other low-cost substrates for PHB production, *B. megaterium* SW1-2 allowed to grow on sugar cane molasses. Results revealed that *B. megaterium* SW1-2 showed clear growth and PHB accumulation during cultivation on modified E2 medium supplemented with sugar cane molasses as a sole carbon source (Figure 2). It revealed that 5 g/L of sugar cane molasses was the optimal concnetration for PHB production by *B. megaterium* cells. Approximately, 29.24% and 26.1% CDW of PHB accumulated after 24 and 48 h, respectively. Lower concentrations of molasses resulted in approximately 50% reduction in PHB, while clearly enhanced cell biomass. In concordance with our results, **Beaulieu *et al.* (1995)** used cane molasses together with ammonium salt for growth of *Alcaligenes eutrophaus* and PHB production. Recombinant *Escherichia coli* strain (HMS174/pTZ18u-PHB) capable of PHB production was reported by **Liu *et al.* (1998)**. **Belal (2013)** successfully used simple carbon sources as well as molasses as a source of carbon for PHB production. It is assumed that higher concentrations of sugars in cane molasses namely; sucrose and fructose contribute to enhanced production of PHB in *B. megaterium* SW1-2.

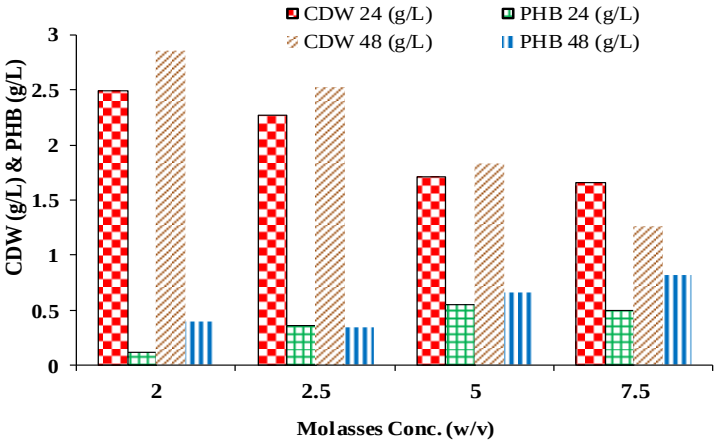


Figure 2 Effect of different sugar cane molasses on growth and PHB production by *B. megaterium* SW1-2.

FT-IR spectroscopy analysis

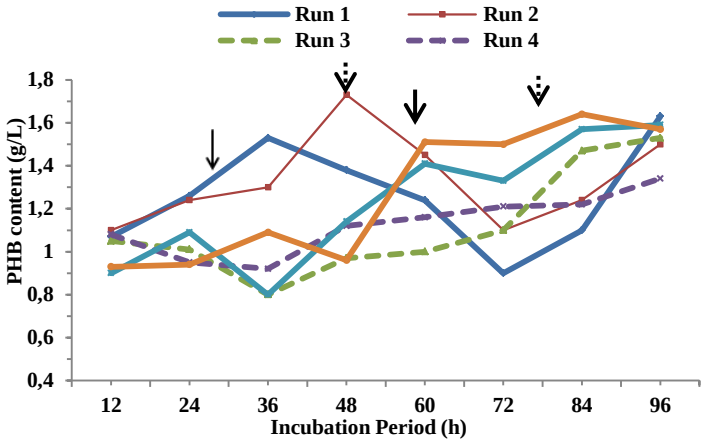
FT-IR Spectroscopic analysis of the polymer extracted from *B. megaterium* cells grown on modified E2 medium with date syrup or DEPS-I as a sole carbon source revealed a clear C–H and carbonyl stretching bands highly characteristic to PHB. Two absorption bands occurring at 2921.36 and 2859.92 cm^{-1} indicated the presence of aliphatic group $-CH_3$ and $-CH_2$. The two other absorption bands at 1718.26 and 1267 cm^{-1} in the PHB sample conforming the presence of C=O and C–O stretching groups and were identical to PHB from some bacilli **(Hong *et al.*, 1999, Pandian *et al.*, 2010; Valappil *et al.*, 2007)**.

H^1NMR and C^{13}NMR spectroscopy

Furthermore, analysis of extracted polymer by H^1NMR revealed three groups of distinctive signals of the PHB polymer. A doublet at 1.22 and 1.25 ppm represent methyl group ($-CH_3$) coupled to one proton and 2.28 ppm resulted from methylene group ($-CH_2$) adjacent to an asymmetric carbon atom. The third signal was at 5.2 ppm attributed to a methyne group (-CH). Furthermore, C^{13}NMR analysis was used to determine the structure of the isolated polymer from *B. megaterium* SW1-2 grown on the modified E2 medium. Four narrow lines appeared which were identical to the C^{13}NMR spectra of PHB **(Doi and Abe, 1990)**. The four peaks assigned for methyl (CH_3; 21.2 ppm), methylene (CH_2; 42.7 ppm), methine (CH; 68.5 ppm) and carbonyl (C=O; 169.7 ppm) carbon resonance of PHB **(Doi and Abe, 1990)**. Analysis collectively confirmed the molecular composition of the polymer to be PHB.

Fed-batch cultivation and application of different pulsed feeding strategies

Since PHB is a carbon-based biopolymer, it depends mainly on the nature and concentration of carbon source during synthesis and accumulation. Unfortunately, longer incubation period during batch cultivation leads to nutrient limitations especially for C-source, hence fed-batch cultivation may provide promising solution. In this study commercial date syrup or DEPS-I was added in two pulsed feeding experiment. Results presented in figure 3 indicated that fed-batch cultivation can be used as successful strategy to optimize PHB production by *Bacillus megaterium* SW1-2 especially when using 48 h (runs 6 and 4) and 84 h (runs 2 and 4) pulsed feeding process. Modified E2 medium was preferred for PHB accumulation, recording total amount of 10.11 and 11.34% CDW of PHB for run1 and 2 after 96 h cultivation, respectively. On the other hand, the lowest amount of PHB accumulated, 8.93% and 9% CDW of PHB during use of complex nutrient broth medium and applying the two different pulsed feeding processes run 3 and run 4, respectively. Surprisingly, there was general trend of increase in PHB production after feeding process and the total amount of PHB after fed-batch cultivation for 96 h recorded 6.86-fold increase in comparison to batch cultivation. **Albuquerque *et al.* (2007)** developed several strategies for polyhydroxyalkanoate (PHA) production from sugar cane molasses. They reported that beet molasses successfully replaced glucose as sole carbon source to produce poly-b-hydroxybutyrate by a recombinant *Escherichia coli* strain (HMS174/pTZ18u-PHB). Also, the positive impact of fed-batch cultivation on PHB production was reported by **Hameih *et al.*, (2013).**



Legend:

- Runs 1,3,5 = Feeding after 24 and 60 h & Runs 2,4,6 = Feeding after 48 and 84 h
- Runs 1,2 = MSM & Runs 3,4 = NB (conc.= 50%) & Runs 5,6 = MSM+NB (50% NB+50% MSM)
- Arrows, pulsed feeding: straight = 24 and 60 h & dashed = 48 and 84 h

Figure 3 Strategies for fed-batch cultivation and PHB production by *B. megaterium* SW1-2 using two pulsed feeding processes.

Evaluation of factors affecting PHB production
Optimization of PHB production by application of Plackett-Burman design

For optimization studies a series of 14 experiments, with two central points (3 and 13) having the same concentrations used in basal medium, were carried out to screen for the most significant variables affecting PHB production. As presented in Table 1, ten crucial variables; covering biochemical and cultivation parameters were prescribed into 2 levels, coded -1, and +1. The design of the optimization experiment is given in Table 2 together with the experimental results (% CDW). Regression analysis was performed to fit the response function (PHB production) with the experimental data. Analysis of variance indicated that PHB production can be well described by a polynomial model with a relatively high coefficient of determination ($R^2 = 0.93$).

$$Y_{\%\ yield} = 27.99 - 1.29X_1 + 7.34X_2 + 2.33X_3 - 1.51X_4 - 1.01X_5 + 2.96X_6 + 9.56X_7 + 0.77X_8 - 5.78X_9 - 5.69X_{10}$$

One of the advantages of the Plackett-Burman design is to rank the effect of different variables on the measured response independent on its nature (either nutritional or physical factor) or sign (contributes positively or negatively) as in (Figure 4A). Ranking of factor estimates in a Pareto chart (Figure 4B) benefits in displays the magnitude of each factor estimate and is a convenient way to view the results of Plackett-Burman design **(Strobel and Sullivan, 1999)**. It can be seen that, among those variables DEPS-II and inoculum concentration followed by KH_2PO_4 and $(NH_4)_2SO_4$ found to be the most significant variables that encourage PHB production. Interestingly, **Beaulieu *et al.* (1995)** reported that the best growth and PHB production were obtained with ammonium sulfate and sugar cane molasses as the growth activator. Indeed, DEPS-II or date syrup-II (Fresh syrup) is more significant than DEPS-I (commercial syrup) as indicated by the lower *p*-value (0.05) (Table 3).

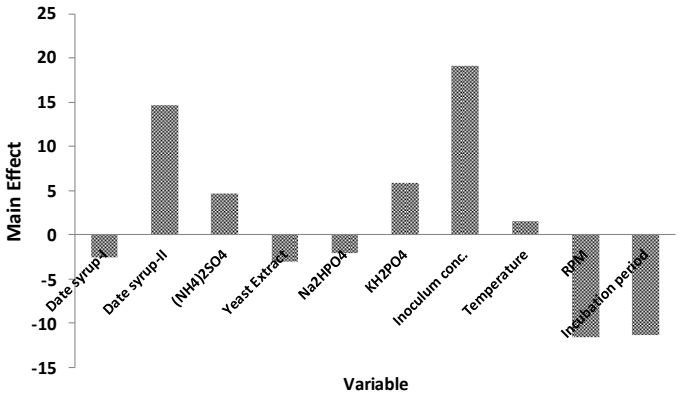


Figure 4A Effect of different environmental conditions on PHB production from *Bacillus megaterium* SW1-2.

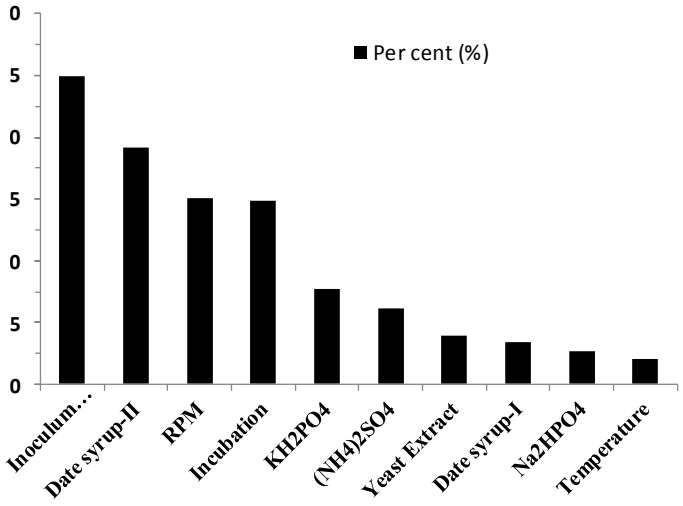


Figure 4B Pareto plot for Plackett-Burman parameter estimates of PHB production by *Bacillus megaterium* SW1-2.

Table 3 Statistical analysis of Plackett-Burman design showing coefficient values, t- and P-values for each variable.

Variable	Coefficient	*t Stat*	*P-value*
Date syrup-I	-1.29	-0.57	0.61
Date syrup-II	7.34	3.26	0.05
$(NH_4)_2SO_4$	2.33	1.03	0.38
Yeast Extract	-1.51	-0.67	0.55
Na_2HPO_4	-1.01	-0.45	0.68
KH_2PO_4	2.96	1.31	0.28
Inoculum conc.	9.56	4.24	0.02
Temperature	0.77	0.34	0.75
RPM	-5.78	-2.56	0.08
Incubation period	-5.69	-2.52	0.09

Interestingly, cane molasses contains many trace elements and vitamins such as thiamine, riboflavin, pyridoxine, and niacinamide **(Crueger and Crueger, 1984)** thus can be used as growth activators and help in enhancement of PHB production **(Beaulieu *et al.*, 1995)**. On the other hand, agitation (RPM) and incubation period have highly negative effect on PHB production. While, other factors namely; DEPS-I, yeast extract and Na_2HPO_4 still showed negative effect. Interestingly, the presence of growth activators in fresh DEPS-II as well as the absence of any preservative materials may explain its positive effect in comparison with the commercial DEPS-I. Furthermore, higher content of growth activators in fresh date syrup or DEPS-II makes it suitable substitutes for yeast extract that showed negative effect.

Results revealed that PHB production yield by *B. megaterium* SW1-2 was 53.6% CDW when cultivated on optimized medium developed by Plackett-Burman. Therefore, the statistical experimental design proved to be a powerful and useful tool for enhancing PHB production and confirm the necessity of the optimization process. In concordance with the obtained results in this work, enhanced PHB production by *Lactobacillus acidophilus* using statistical experimental design was reported by **Hamieh *et al.*, (2013)**. Interestingly, results reported in this study represent the first investigation on optimization of PHB production by application of statistical experimental design using date syrup or DEPS.

CONCLUSION

One of the most crucial variables affecting PHB production-economy is the nature of carbon source. *B. megaterium* SW1-2 stain exhibited nutritional versality in terms of varied growth and PHB accumulation during cultivatrion on different concentrations of the carbon-based agro-industrial wasts namely; date palm syrup (DEPS) or sugar cane molasses. PHB was successfully synthesized and intracellular accumulated in *B. megaterium* SW1-2 in presence of any of the used agro-industrial wastes as proven by FT-IR and NMR spectroscopy. Application of six different fed-batch cultivation strategies provides promising solution for nutritional limitation problems and clearly showed improved PHB production. Fed-batch cultivation recorded 6.87-fold increase in total amount of PHB produced after 96 h as compared with the amount produced under batch

cultivation. Experimental design namely; Plackett-Burman proved to be a powerful and useful tool for enhancing PHB production (53.6% CDW). The use of cheaper carbon sources such as date syrup (DEPS) or sugar cane molasses rather than glucose or sucrose greatly lower process economy and increase promises for biotechnological production of PHB biopolymer on industrial scale.

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