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Production of PHAs from Waste Frying Oil by *Pseudomonas fluorescens* S48 Using Different Bioreactor Feeding Strategies

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BIODEGRADABLE polyesters, polyhydroxyalkanoate (PHAs) are promising candidates for the development of environment-friendly and totally biodegradable plastics. The production of PHAs by *Pseudomonas fluorescens* S48 was examined using bioreactor as one-stage batch, two-stage batch, and fed-batch (pulsed, continuous and high cell density) cultures. Corn oil , soybean oil (extracted from their meal) and two types of waste frying oils (WFO) were used in productive medium as a carbon source. The highest values of polymer content in the one-stage bioreactor fermentation (52 % and 76.53 %) were obtained after 60 h on media supplemented with extracted corn oil and soybean oil, respectively. Whereas, the highest figure obtained on WFO type 2 was 30 % under the same circumstances. Using bioreactor as a two-stage batch culture increased the polymer content with soybean, corn oil and WFO type 2 by 2.2 %, 32.11 % and 58 %, respectively as compared with that obtained in one-stage batch culture. High-cell-density (0.64 gl^{-1}) at continuous feeding rate 0.55$ml l^{-1}h^{-1}$ of WFO type 2 recorded the highest polymer content, yield and conversion coefficient after 48 h. Moreover, this application increased the polymer content by 65.7 % than that obtained in one-stage batch culture as well as reduced the fermentation period 12 hr.

Keywords: *Pseudomonas fluorescens* S48, PHAs, Batch, Two-stage batch, High-cell-density, Bioreactor.

Growth in the human population has led to the accumulation of huge amounts of non-degradable waste materials across our planet. The increasing effect of non-degradable plastic wastes is a growing concern. Biodegradable polymers have extremely attracted much public and industrial interest as a consequence of extensive discussions looking for better waste-management strategies (Steinbüchel, 1995). Their physical properties make them very convenient in utilization. The use of biodegradable polymers allows composting as an additional way for waste disposal. Furthermore, the use of these polymers opens several new applications in medicine, agriculture and industry. Research into the production of PHAs as petrochemical alternatives for the future has been

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explored using bacterial and plant systems. Accumulation of intracellular storage polymers has been considered a strategy used by bacteria to increase survival in a changing environment. The ability to store PHB is an example of this characteristic and usually reflects a transient abundance of carbon sources with respect to other nutrients such as nitrogen and phosphorus (Jacquel *et al.*, 2008). PHA has been industrially produced by pure cultures including *Alcaligenes latus*, *Azotobacter vinelandii*, *Pseudomonas oleovorans*, recombinant *Alcaligenes eutrophus* and recombinant *Escherichia coli* (Lee & Choi, 1998; Grothe *et al.*, 1999 and Grothe & Chisti, 2000). From the literature, one of the limiting factors in the commercial success of PHB and other PHAs production schemes is the cost of the substrate used for PHA formation. Vegetable oils have been found to be possible substrates in the production of PHAs (Alias &Tan, 2005). Waste frying oils which are even cheaper than purified oils can be used too (Fernández *et al*., 2005). Since deep frying is popular, there is a potential to turn this waste resource into a useful biomaterial. Akiyama *et al.* (2003) reported a yield of 0.8 g PHA per g plant oil and 0.3 g PHA per 1 g glucose. In the past few years, a number of mechanistic models for the production of PHAs have been constructed. Models for fermentation with simple batch culture, two-stage fed-batch process and single fed-batch fermentations are frequently described (Patwardhan & Srivastava, 2004; Yan *et al*., 2005 and Lee & Gilmore, 2006). In a laboratory scale bioreactor, With canola oil as carbon source, the polymer content of the cell dry matter of *Wautersia eutropha* was 90% by fed-batch process (Lopez-Cuellar *et al*., 2011). Verlinden *et al*. (2011) stated that, when *Cupriavidus necator* grown on oils the biopolymer produced was found to be chemically pure PHB and the molecular weights of polymers from waste frying were similar to those from other oils and glucose.

This study was conducted to examine the feasibility of using extracted oil from soybean meal, corn meal as well as two types of waste frying oils (WFO) to produce PHAs using bioreactor as a batch culture, two-stage, fed-batch with pulsed and continuous feeding in addition to high cell density fed-batch technique. Fermentations were carried out to maximize the production of the polymer (PHAs) by choosing the accurate and reliable fermentation technique.

Materials and Methods

Bacteria used

Pseudomonas fluorescens S48 was used throughout this investigation as an intracellular bioplastic polymer producer which was previously isolated , identified and confirmed as PHAs accumulation by Gamal *et al*. (2011). The bacterial culture was maintained by transferring at regular intervals on nutrient agar slants. Slants were kept at 4^{o}C.

Media used

Nutrient agar medium (Jacobs & Gerstein, 1960) was used for bacterial culture preservation, while the medium without agar was used for inoculum preparation. Modified Kim medium (Gamal *et al.*, 2011) which contained (gl^{-1})

glucose 10, $(NH_4)_2SO_4$ 1.0, KH_2PO_4 1.5, $Na_2HPO_4.12H_2O$ 9.0, $MgSO_4.7H_2O$ 0.2 PH 6.8 and 1 ml of trace elements solution ($FeSO_4.7H_2O$ 10, $ZnSO_4.7H_2O$ 2.25, $CuSO_4.5H_2O$ 1.0, $MnSO_4.4H_2O$ 0.5, $CaCl_2.2H_2O$ 2.0, $Na_2B_4O_7.10H_2O$ 0.23, $(NH_4)_6Mo_7O_{24}$ 0.1 and 35 % HCl 10 ml). The productive medium contained modified Kim medium with the substitution of glucose with 2 % corn oil, 1% soybean oil or1 % waste vegetable oil (either deep-fries chips or eggplant which represented type 1 or type 2,respectively).

Preparation of bacterial inoculum

A conical flask (250 ml) containing 50 ml of nutrient broth medium was inoculated with a loop of *Ps. fluorescens* S48 and incubated at 28 – 30°C with shaking (300 rpm) for 24 h prior to inoculation .

Bioreactor experiments

A 2L dished bottom bioreactor Z6110/Coob (Cole-Parmer Instrument) was used, which consisted of 3 liter vessel equipped with lipseal stirrer assembly, automatic pH controller, automatic dissolved O_2 controller, CO_2 controller, automatic temperature controller, foam controller and multi-channel peristaltic pump (for feeding). The PHAs producing bacteria were grown in the bioreactor as batch, two-stage batch and fed-batch (pulsed, continuous and high cell density) cultivation. During all fermentations, temperature, dissolved O_2 and agitation speed were kept at 30°C, 20% of saturation and 500 rpm, respectively. Initial pH was adjusted to 7±0.1 which was not controlled during the fermentation period. Samples (10 ml) were aseptically withdrawn from the fermentation vessel periodically. The samples were centrifuged at 15000 x *g* for 4 min at 4°C. The sediment (biomass) was washed twice with distilled water, and then dried at 70°C to constant weight. Polymer in bacterial cells was determined using the chloroform-sodium hypochlorite method (Hahn *et al.*, 1994). Residual carbon was determined in supernatant according to Walinga *et al.* (1992). PHAs and copolymer parameters were calculated. The parameters of polymer production; yield (%), conversion coefficient (%) and carbon utilization efficiency were calculated according to Ramadan *et al.* (1985). Polymer content (%) and productivity were calculated according to Lee & Choi (1998) and Lee (1996), respectively.

Bioreactor as a batch culture (one-stage)

In this experiment, the fermentation vessel containing 1950 ml productive medium was autoclaved at 121°C for 20 min. The bioreactor was inoculated with 1% standard inoculum (5 x 10^8 cfu/ml) of *Ps. fluorescens* S48. The final working volume was 2 liter.

Bioreactor as two-stage culture

The production of PHAs was carried out by two-stage cultivation. In the first stage, 1 liter of nutrient broth inoculum culture of *Ps. fluorescens* S48 was centrifuged at 15000 x *g* for 4 min at 4°C and the bacterial cells were collected and suspended in additional sterile productive medium to inoculate the fermentation vessel to give a final working volume of 1 liter.

Bioreactor as fed-batch culture

Two fermentations were applied to produce PHAs by *Ps. fluorescens* S48 using pulsed and continuous feeding of oil. In the pulsed oil feeding, the amount of WFO type 2 (10 ml^{-1}) was added to fermentation vessel. Two, three and four additions of this amount of WFO were added during the first 12, 36 and 48 h of cultivation period (fed-batch by pulsed feeding). In the second fermentation, the WFO type 2 was fed continuously during the first 12, 18 and 24 h of cultivation period at rates of 0.83, 0.55 and 0.42 $ml^{-1}h^{-1}$, respectively (fed-batch by continuous feeding). The final working volume in bioreactors was 1 liter at the end of feeding period.

High-cell-density fed-batch culture

Three experiments of continuous fed-batch cultures were constructed to study the effect of washed high-cell-densities of *Ps. fluorescens* S48 (0.36, 0.64 and 0.74 gl^{-1}) on PHAs production. WFO type 2 was fed continuously at 0.55 $mll^{-1}h^{-1}$ during the first 18 h of cultivation.

Statistical analysis

The collected data were statistically analysed using IBM® SPSS® Statistics software (2011).

Results and Discussion

The research aimed to develop an advanced process for production of biodegradable PHAs by using renewable resource as the feedstock. It is known that the cost of raw materials for the production of bioplastic is one of the limiting economic factors in the scaling up of such process. Therefore**,** this study was constructed to examine the feasibility of using oil extracted from soybean meal, corn meal as well as two types of WFO to produce PHAs using bioreactor as one-stage batch, two-stage and fed-batch cultures techniques.

Batch culture (one-stage)

In one-stage batch culture, where any of the oils were used as the sole carbon source, increasing the fermentation period led to a gradual increase in cell dry weight of *Ps. fluorescens* S48 (Fig. 1). On the other hand, PHAs production was started during the exponential phase of growth (after 12 h) then sharply increased to achieve the highest figures of yield and content (26.09, 52 % and 36.71, 76.53%) during stationary phase after 60 h fermentation period on media supplemented with extracted corn oil and extracted soybean oil, respectively. The corresponding figures of productivity were 0.03 and 0.044 $gl^{-1}h^{-1}$, respectively.

Using any of the two types of WFO as the sole carbon source for PHAs production by *Ps. fluorescens* S48, recorded the lowest values of cell dry weight, PHAs concentration, content, yield and productivity than that obtained on media containing meal oils. It is also interesting to notice that PHAs production was delayed to start after 30 hr of fermentation period in medium contained WFO

type 1. WFO type 2 was chosen for further studies since it enhanced the production of the polymer to reach the maximum (30 % content and 0.69 gl^{-1}) after 60 h comparing with those obtained using WFO type 1 . Regarding to polymer parameters using WFO type 2, the highest values of PHAs yield, and productivity were 9.14 % and 0.012 $gl^{-1}h^{-1}$, respectively. In the same regard Fern´andez *et al*. (2005) reported that PHA accumulation ranged between 66.1%, 29.4% and 16.8% when waste-free fatty acids (from soybean oil) (WFFA), waste frying oil (WFO) and glucose were used as carbon substrate by *Pseudomonas aeruginosa* 42A2, respectively.

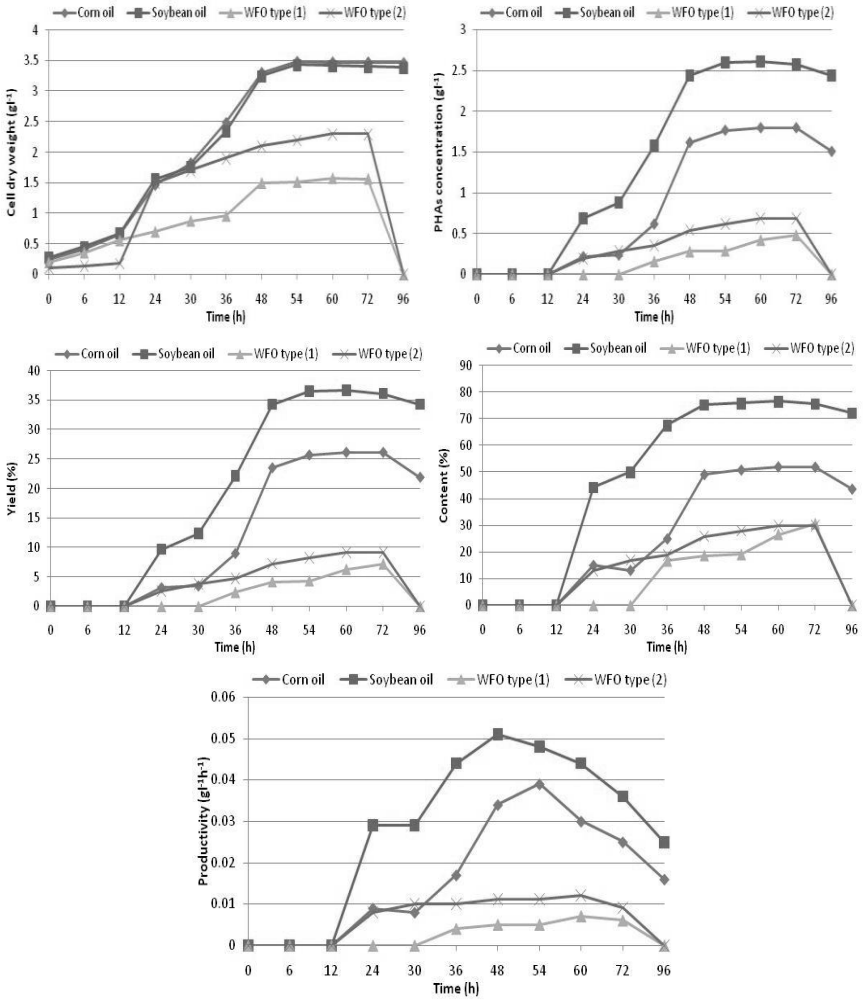

Fig. 1. Growth of *Ps. fluorescens* S48 and PHAs production in productive medium containing any of 2% corn oil, 1% soybean oil or 1% waste frying oil (WFO) (type 1 and 2) as carbon sources during 96hr at 30ºC using bioreactor as a batch culture.

Two-stage batch culture

In the two stage batch culture, cell dry weight of *Ps. fluorescens* S48 and PHAs concentration were increased gradually during the second stage of cultivation (production stage) to reach the maximum values after 60 hr in productive medium containing either of 2 % corn oil or 1 % soybean oil (4.35, 2.96 gl^{-1} and 4.53, 3.44 gl^{-1}), respectively (Fig. 2). The highest values of PHAs content was attained after 48 h (68.7 %) using corn oil and 78.2 % using soybean oil, respectively. The corresponding figures of yield, productivity and conversion coefficient were 39.9 %, 0.057 $gl^{-1}h^{-1}$, 74.9 % and 45.7 %, 0.066 $gl^{-1}h^{-1}$ & 67 % for corn oil and soybean oil, respectively.

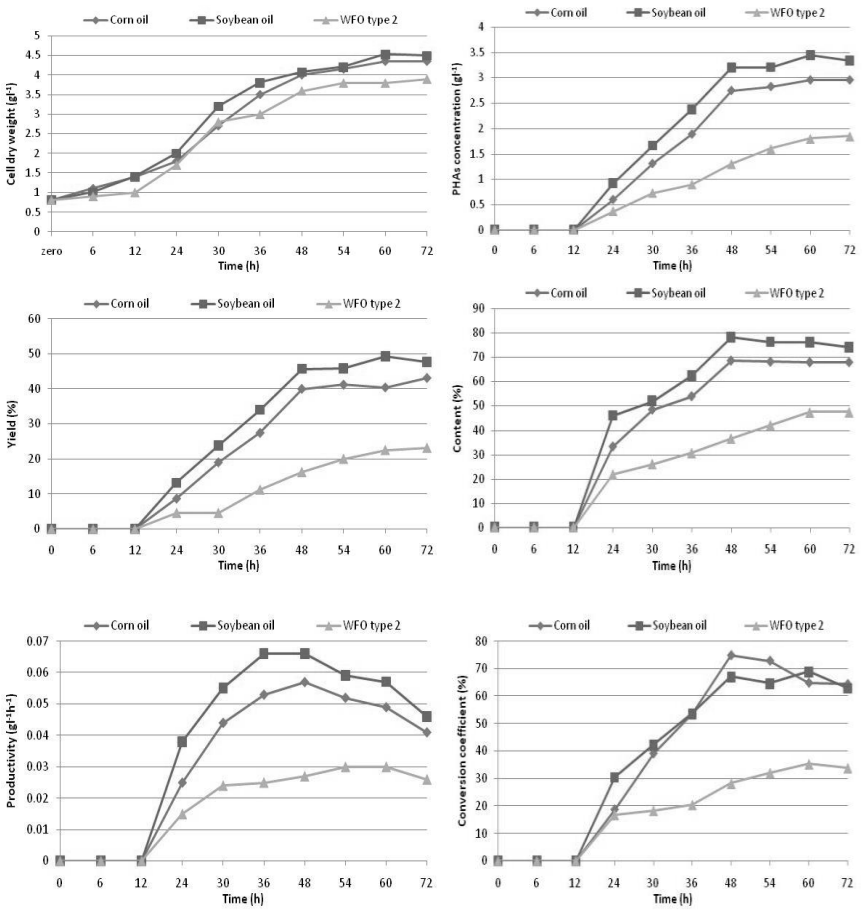

Fig. 2. Growth of *Ps. fluorescens* S48 and PHAs production in productive medium containing any of 2 % corn oil, 1% soybean oil or WFO type 2 as carbon sources during 72 h at 30°C using bioreactor as a two-stage culture.

With respect to potential of *Ps. fluorescens* S48 to produce PHAs on WFO type 2 as the sole carbon source, results indicated that the yield of cell dry weight was 3.8 gl^{-1} after 54 h of the second cultivation stage, whereas the maximum PHAs

concentration, content and yield were obtained at the end of fermentation period (72h) (1.85 gl^{-1}, 47.43 % and 23.13, respectively). The corresponding figures of productivity and conversion coefficient were 0.026 $gl^{-1}h^{-1}$ and 33.82%, respectively. From the above mentioned results, it could be concluded that using bioreactor as a two stage batch culture increased the polymer content on soybean oil, corn oil and WFO type 2 when used singly as sole carbon sources by 2.2 %, 32.11% and 58%, respectively as compared with that obtained by one-stage batch culture.

Fed-batch culture

Pulsed and continuous oil feeding

Pulsed addition of WFO type 2 was carried out every 12 or 24 h during the first 48 h of fermentation period. Two, four and three pulsed addition was applied during the first 12, 36 and 48 h of fermentation period with specific addition rate of 0.058 $mll^{-1}h^{-1}$, 0.039 $mll^{-1}h^{-1}$ and 0.023 $mll^{-1}h^{-1}$, respectively (Table 1). Generally, it could be noticed that after 60 h of cultivation, the highest figure of cell dry weight of *Ps. fluorescens* S48, PHAs concentration, yield, productivity and conversion coefficient were recorded at the specific addition rate of 0.058 $mll^{-1}h^{-1}$ (1.7 gl^{-1}, 0.35 gl^{-1}, 4.29 %, 0.006 $gl^{-1}h^{-1}$ and 6.13 %, respectively). Whereas, the highest values of polymer content and utilized carbon were 26.82 % and 6.82 %, respectively at the specific addition rate of 0.023 $mll^{-1}h^{-1}$. Data also revealed that the polymer productivity were constant at the specific addition rates of 0.039 and 0.023 $mll^{-1}h^{-1}$ after 48 h till the end of cultivation.

The effect of different continuous feeding of WFO type 2 on the production of PHAs during 96 h fermentation period revealed that cell dry weight and PHAs concentration increased gradually during fermentation period to reach the maximum after 72 hr. Whereas, the maximum values of carbon utilized and carbon utilization efficiency were recorded at the end of fermentation period (96 h) (Table 2). After 72 h, a slight increase in polymer concentration was noticed with decreasing the continuous oil feeding from 0.83 to 0.55 $mll^{-1}h^{-1}$ with a slight increase in polymer content from 41.2 % to 41.88 %. On the other hand, increasing the cell dry weight and polymer concentration at the feeding rate of 0.42 $mll^{-1}h^{-1}$, led to decrease the polymer content to 36.14 % with increasing of the yield and conversion coefficient to 7.5 % and 12.3 %, respectively. The highest polymer productivity (0.014 $gl^{-1}h^{-1}$) was recorded after 24 h at the feeding rate of 0.55 $mll^{-1}h^{-1}$.

The previous results indicated that the pulsed and continuous feeding of waste frying oil type 2 led to decrease the polymer content about 43.4 % and 11.7 % than that obtained by two-stage batch bioreactor technique. Whereas, continuous oil feeding at 0.55 $mll^{-1}h^{-1}$ increased the polymer content about 39.6% than that obtained by the one-stage batch technique. So, it could be stated that the fed-batch culture with continuous feeding have positive impact on the PHAs production by *Ps. fluorescens* S48 on productive medium containing WFO type 2 as carbon source. These results were in agreement with those obtained by Shahhosseini (2004), Valappil *et al.* (2007) and Sun *et al.* (2007), they used fed-batch culture technique for PHAs production by *Ralstonia eutropha*, *B. cereus* and *Ps. putida* KT2400, respectively.

High-cell-density fed-batch culture

This experiment was performed to study the behavior of *Ps. fluorescens* S48 to assimilate WFO type 2 for the production of PHAs using different concentrations of high-cell-density in bioreactor as a fed-batch culture at continuous WFO feeding of 0.55 $mll^{-1}h^{-1}$. Data recorded in Table 3 show that the cell dry weight of *Ps. fluorescence* S48 was increased gradually with increasing the fermentation period to reach the maximum values (1.75 and 3.92 gl^{-1}) after 48 and 54 h at the fed batch cultures started with cell densities of 0.36 gl^{-1} and 0.64 gl^{-1}, respectively. On the contrary, the cell dry weight decreased sharply during fermentation period at the highest cell density treatment. The highest figures of PHAs concentration, content, yield and conversion coefficient were attained after 48 h at 0.64 gl^{-1} cell density (1.72 gl^{-1}, 49.71 %, 21.5 % and 29 %, respectively). This treatment enhanced the productivity to three fold and minimized the fermentation period by 12 h comparing with the lowest inoculum density treatment (0.36 gl^{-1}) at 60 h. The highest utilized carbon and carbon utilization efficiency were recorded after 72 h at the cell density of 0.64 gl^{-1} (6.95 gl^{-1} and 86.88 %, respectively). Also, it could be stated that starting the fed-batch culture with feeding of WFO type 2 at 0.55 $mll^{-1}h^{-1}$ with 0.74 gl^{-1} cell density had negative impact on the PHAs production by *Ps. fluorescens* S48 on productive medium.

The potentiality of *Ps. fluorescens* to accumulate PHAs using oil in productive media were varied according to the feeding strategies, type of oil and fermentation technique which can be recorded in Table 4 and summarized in the following items:

- Productive medium containing soybean 1% using two-stage bioreactor reduced the time of maximum PHAs figure by 12 h comparing with batch bioreactor and gave approximately similar content of polymer (78.2 %). Whereas, PHAs content was increased to about 1.3 fold on productive medium containing 2 % corn oil being 68.7 % under the previous circumstances.

- High- cell- density (0.64 gl^{-1}) at continuous WFO type 2 feeding rate of 0.55$mll^{-1}h^{-1}$ increased the polymer content by 65.7% and reduced the fermentation period by 12 h comparing with one-stage batch culture.

From the holistic economic vision, using waste frying oil was the first choice, as the amount of disposed frying oils was several millions of tons annually in Egypt, since 40% of the expenditure of PHAs production is the cost of the carbon source. Here in the amount of soybean oil and corn oil which were extracted from their meals were 3.4% and 2.2%, respectively. The latter oils can be successfully used in the commercial scale if there are reliable and cheap methods for their extraction in order to minimize the expenditures of bioplastic production.

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TABLE 1. Growth of *Ps. fluorescens* S48 and PHAs production on productive medium using bioreactor as fed-batch culture by pulsed WFO type 2 feeding at specific addition rates of 0.058, 0.039 and 0.023 $mll^{-1}h^{-1}$.

Specific addition rate ($mll^{-1}h^{-1}$)	Time (h)	Cell dry weight (gl^{-1})	PHAs				Added oil/liter (mll^{-1})	Added carbon (gl^{-1})	Residual carbon (gl^{-1})	Utilized carbon (gl^{-1})	CC* (%)
			Concentration (gl^{-1})	Content (%)	Yield (%)	Productivity ($gl^{-1}h^{-1}$)					
	0	0.20	-	-	-	-	5.00	4.08	4.08	0.00	-
	3	0.21	-	-	-	-	0.00	0.00	3.00	1.08	-
	6	0.24	-	-	-	-	0.00	0.00	2.35	1.73	-
	12	0.27	-	-	-	-	5.00	4.08	5.83	2.33	-
0.058	24	0.46	-	-	-	-	0.00	0.00	4.38	3.83	-
	30	0.74	-	-	-	-	0.00	0.00	3.78	4.38	-
	36	0.93	0.13	14.19	1.62	0.004	0.00	0.00	2.78	5.38	2.45
	48	1.40	0.26	18.57	3.19	0.005	0.00	0.00	2.73	5.43	4.79
	54	1.60	0.32	20.00	3.92	0.006	0.00	0.00	2.60	5.56	5.76
	60	1.70	0.35	20.58	4.29	0.006	0.00	0.00	2.45	5.71	6.13
	72	1.30	0.27	20.76	3.31	0.004	0.00	0.00	2.00	6.16	4.38
Mean		0.82^{b}	0.27^{a}	18.8^{b}	3.27^{a}	0.005^{ab}	0.909^{a}	0.75^{a}	3.27^{a}	3.78^{a}	4.70^{a}
	0	0.20	-	-	-	-	2.50	2.04	2.04	0.00	-
	3	0.22	-	-	-	-	0.00	0.00	1.09	0.95	-
	6	0.28	-	-	-	-	0.00	0.00	0.99	1.05	-
	12	0.30	-	-	-	-	2.50	2.04	2.47	0.56	-
0.039	24	0.58	-	-	-	-	2.50	2.04	1.56	2.95	-
	30	0.64	-	-	-	-	0.00	0.00	0.95	3.56	-
	36	0.90	0.10	11.11	1.23	0.004	2.50	2.04	2.51	4.04	2.48
	48	0.94	0.15	15.95	1.84	0.003	0.00	0.00	2.00	4.55	3.30
	54	0.99	0.17	18.08	2.08	0.003	0.00	0.00	1.90	4.65	3.66
	60	1.03	0.20	19.41	2.50	0.003	0.00	0.00	1.71	4.84	4.13
	72	1.06	0.24	22.64	2.94	0.003	0.00	0.00	1.12	5.43	4.42
Mean		3.35^{a}	0.17^{c}	17.4^{c}	2.12^{c}	0.003^{c}	0.909^{a}	0.73^{c}	1.67^{c}	2.96^{c}	4.00^{b}
	0	0.20	-	-	-	-	3.33	2.72	2.72	0.00	-
	3	0.24	-	-	-	-	0.00	0.00	2.00	0.72	-
	6	0.38	-	-	-	-	0.00	0.00	1.55	1.17	-
	12	0.46	-	-	-	-	0.00	0.00	0.88	1.84	-
0.023	24	0.56	-	-	-	-	3.33	2.72	2.35	3.09	-
	30	0.70	-	-	-	-	0.00	0.00	1.69	3.75	-
	36	0.82	0.13	15.82	1.59	0.004	0.00	0.00	0.66	4.78	2.72
	48	0.99	0.22	22.22	2.70	0.005	3.33	2.72	2.45	5.71	3.85
	54	1.15	0.27	23.47	3.31	0.005	0.00	0.00	1.52	6.64	4.10
	60	1.19	0.30	25.21	3.68	0.005	0.00	0.00	1.47	6.69	4.48
	72	1.23	0.33	26.82	4.10	0.005	0.00	0.00	1.34	6.82	4.84
Mean		0.72^{c}	0.25^{b}	22.7^{a}	3.07^{b}	0.005^{ab}	0.900^{b}	0.74^{b}	1.69^{b}	3.75^{b}	3.60^{c}

*CC (%) = Conversion coefficient: Polymer concentration (gl^{-1}) / utilized carbon (gl^{-1}) X 100 (Ramadan *et al.*, 1985).
Yield (%) = PHAs (gl^{-1}) / initial carbon (gl^{-1}) x 100 (Ramadan *et al.*, 1985).
Productivity ($gl^{-1}h^{-1}$) = Polymer concentration (gl^{-1}) / fermentation time (h) (Lee, 1996).
Content (%) = Polymer concentration (gl^{-1}) /cell dry weight (gl^{-1}) x100.
Values in the same column followed by the same letter do not significantly differ from each other, according to Duncan's at 5 % level.

TABLE 2. Growth of *Ps. fluorescens* S48 and PHAs production on productive medium using bioreactor as fed-batch culture with continuous WFO type 2 feeding at the rates of 0.83, 0.55 and 0.42 $mll^{-1}h^{-1}$.

Addition rate ($mll^{-1}h^{-1}$)	Time (h)	Cell dry Weight (gl^{-1})	PHAs				Added oil (mll^{-1})	Residual carbon (gl^{-1})	Utilized carbon (gl^{-1})	CUE** (%)	CC* (%)
			Concentration (gl^{-1})	Content (%)	Yield (%)	Productivity ($gl^{-1}h^{-1}$)					
0.83	0	0.26	-	-	-	-	0.00	0.00	0.00	0.00	-
	3	0.28	-	-	-	-	1.25	0.20	1.80	22.50	-
	6	0.61	-	-	-	-	5.00	1.74	2.26	28.25	-
	12	0.74	-	-	-	-	10.00	4.83	3.17	39.63	-
	18	0.80	0.10	12.50	1.25	0.005	0.00	4.2	3.8	47.50	2.63
	24	0.84	0.20	23.80	2.50	0.008	0.00	3.58	4.42	55.25	4.52
	30	1.10	0.28	25.45	3.50	0.007	0.00	2.85	5.15	63.38	5.44
	48	1.16	0.46	39.65	5.75	0.010	0.00	1.94	6.06	75.75	7.59
	72	1.17	0.48	41.20	6.00	0.006	0.00	1.24	6.76	84.50	7.11
	96	1.09	0.36	33.11	4.51	0.004	0.00	1.19	6.81	85.13	5.31
Mean		0.81^{c}	0.36^{c}	32.66^{b}	4.45^{c}	0.007^{b}	1.81^{c}	1.95^{c}	4.05^{a}	50.49^{a}	5.99^{c}
0.55	0	0.21	-	-	-	-	0.00	0.00	0.00	0.00	-
	3	0.27	-	-	-	-	1.67	1.00	0.14	1.75	-
	6	0.64	-	-	-	-	3.33	1.66	1.00	12.50	-
	12	0.76	-	-	-	-	6.67	2.25	3.09	38.63	-
	18	0.87	0.21	24.13	2.63	0.012	10.00	3.37	4.63	57.88	10.15
	24	1.01	0.34	33.66	4.25	0.014	0.00	3.13	4.87	60.88	6.98
	30	1.09	0.40	36.69	5.00	0.013	0	3.00	5.00	62.50	8.00
	48	1.15	0.47	40.86	5.88	0.010	0.00	2.89	5.12	64.00	9.18
	72	1.71	0.49	41.88	6.13	0.007	0.00	2.40	5.60	70.00	8.75
	96	1.00	0.35	34.31	4.38	0.004	0.00	1.77	6.23	77.88	5.62
Mean		0.85^{b}	0.37^{b}	34.97^{a}	4.65^{b}	0.009^{a}	2.41^{a}	2.07^{b}	3.41^{b}	42.61^{b}	8.13^{b}
0.42	0	0.23	-	-	-	-	0.00	0.00	0.00	0.00	-
	3	0.82	-	-	-	-	1.25	0.20	0.80	10.00	-
	6	0.84	-	-	-	-	2.50	0.40	1.60	20.00	-
	12	0.92	-	-	-	-	5.00	1.57	2.43	30.38	-
	18	0.99	0.15	15.15	1.87	0.008	7.56	2.88	5.12	64.00	2.93
	24	1.18	0.26	22.03	3.25	0.011	10.00	4.41	3.59	44.88	7.24
	30	1.22	0.29	23.77	3.63	0.010	0.00	3.90	4.10	51.25	7.07
	48	1.62	0.51	31.48	6.38	0.011	0.00	3.41	4.59	57.38	11.11
	72	1.66	0.60	36.14	7.50	0.008	0.00	3.12	4.88	61.00	12.30
	96	1.62	0.50	30.86	6.25	0.005	0.00	2.11	5.89	73.63	8.49
Mean		1.12^{a}	0.43^{a}	28.86^{c}	5.40^{a}	0.009^{a}	2.09^{b}	2.12^{a}	3.10^{c}	38.72^{c}	9.24^{a}

Initial carbon concentration = 8 gl^{-1}.

*CC (%) = Conversion coefficient: Polymer concentration (gl^{-1}) / utilized carbon (gl^{-1}) X 100 (Ramadan *et al.*, 1985).

**CUE (%) = Carbon utilization efficiency: Utilized carbon (gl^{-1}) / initial carbon (gl^{-1}) X 100 (Ramadan *et al.*, 1985).

Yield= PHAs (gl^{-1}) / initial carbon (gl^{-1}) x 100 (Ramadan *et al.*, 1985).

Productivity ($gl^{-1}h^{-1}$) = Polymer concentration (gl^{-1})/ fermentation time (h) (Lee, 1996).

Content (%) = Polymer concentration (gl^{-1}) /cell dry weight (gl^{-1}) x100.

Values in the same column followed by the same letter do not significantly differ from each other, according to Duncan's at 5 % level.

TABLE 3. Growth of *Ps. fluorescens* S48 and PHAs production in productive medium using bioreactor as high-cell-density fed-batch culture with 0.36, 0.64 and 0.74 gl^{-1} cell density and continuous WFO type 2 feeding at 0.55 $mll^{-1}h^{-1}$.

Cell density (gl^{-1})	Time (h)	Cell dry weight (gl^{-1})	PHAs				Residual carbon (gl^{-1})	Utilized carbon (gl^{-1})	CC* (%)	CUE** (%)
			Concentration (gl^{-1})	Content (%)	Yield (%)	Productivity ($gl^{-1}h^{-1}$)				
0.36	0	0.36	-	-	-	-	0.00	-	-	-
	6	0.46	-	-	-	-	3.12	-	-	-
	12	0.50	-	-	-	-	5.88	-	-	-
	24	0.97	0.21	21.64	2.63	0.009	5.26	2.74	7.66	34.25
	30	1.10	0.29	26.36	3.63	0.010	3.90	4.10	7.07	51.25
	36	1.30	0.40	30.76	5.00	0.011	3.28	4.72	8.47	59.00
	48	1.75	0.66	37.71	8.25	0.014	2.80	5.10	12.94	63.75
	54	1.64	0.69	42.07	8.63	0.013	1.84	6.16	11.20	77.00
	60	1.60	0.74	46.25	9.25	0.012	1.17	6.83	10.83	85.38
	72	1.46	0.67	45.89	8.38	0.009	1.07	6.93	9.67	86.63
Mean		1.12[b]	0.52[b]	35.8[b]	6.54[b]	0.11[b]	2.83[c]	5.23[c]	9.69[b]	65.32[a]
0.64	0	0.64	-	-	-	-	0.00	-	-	-
	6	0.90	-	-	-	-	3.86	-	-	-
	12	1.10	-	-	-	-	6.86	-	-	-
	24	1.38	0.47	34.05	5.88	0.020	5.26	2.74	17.15	34.25
	30	1.40	0.50	35.71	6.25	0.017	4.86	3.40	14.71	42.50
	36	1.68	0.70	41.67	8.75	0.02	2.77	5.23	13.38	65.38
	48	3.46	1.72	49.71	21.50	0.036	2.07	5.93	29.00	74.13
	54	3.92	1.66	42.34	20.75	0.031	1.95	6.05	27.44	75.63
	60	3.10	1.15	37.09	14.38	0.019	1.44	6.56	17.53	82.00
	72	2.94	1.10	37.41	13.75	0.015	1.05	6.95	15.83	86.88
Mean		2.05[a]	1.05[a]	39.7[a]	13.04[a]	0.23[a]	3.01[a]	5.27[b]	19.29[a]	65.82[b]
0.74	0	0.74	-	-	-	-	0.00	-	-	-
	6	0.90	-	-	-	-	4.42	-	-	-
	12	0.28	-	-	-	-	7.58	-	-	-
	24	0.19	0.015	7.89	1.87	0.0006	5.46	2.54	0.59	31.75
	30	0.12	0.023	19.17	2.88	0.0007	3.20	4.80	0.48	60.00
	36	0.102	0.025	24.51	3.12	0.0006	2.91	5.09	0.49	63.63
	48	0.138	0.027	19.56	3.37	0.0005	1.60	6.40	0.42	80.00
	54	0.140	0.031	22.14	3.87	0.0005	1.41	6.55	0.47	81.88
	60	0.154	0.039	25.32	4.87	0.0006	1.25	6.75	0.58	84.38
	72	0.204	0.065	31.86	8.12	0.0009	1.12	6.88	0.94	86.00
Mean		0.30[c]	0.32[c]	21.5[c]	4.01[c]	0.001[c]	2.90[b]	5.57[a]	0.57[c]	69.66[a]

Initial carbon concentration in oil = 8 gl^{-1}.

*CC (%) = Conversion coefficient: Polymer concentration (gl^{-1}) / utilized carbon (gl^{-1}) X 100 (Ramadan *et al.*, 1985).

**CUE (%) = Carbon utilization efficiency: Utilized carbon (gl^{-1}) / initial carbon (gl^{-1}) X 100 (Ramadan *et al.*, 1985).

Yield = PHAs (gl^{-1}) / initial carbon (gl^{-1}) x 100 (Ramadan *et al.*, 1985).

Productivity ($gl^{-1}h^{-1}$) = Polymer concentration (gl^{-1}) / fermentation time (h) (Lee, 1996).

Content (%) = Polymer concentration (gl^{-1}) /cell dry weight (gl^{-1}) x100.

Values in the same column followed by the same letter do not significantly differ from each other, according to Duncan's at 5 % level.

TABLE 4. Comparative data on PHAs production by *Ps. fluorescens* using different oils, feeding strategies and fermentation techniques.

Fermentation technique	Media used	Incubation period (h)	Content (%)
One-stage bioreactor	Productive medium containing corn oil (2 %) extracted from meal	60	52.00[b]
	Productive medium containing soybean (1 %) extracted from meal	60	76.80[a]
	Productive medium containing WFO type 2 (1%)	60	30.00[c]
Two-stage bioreactor	Productive medium containing corn oil (2 %) extracted from meal	48	68.70[b]
	Productive medium containing soy bean (1 %) extracted from meal	48	78.20[a]
	Productive medium containing WFO type 2 (1 %)	60	47.37[c]
Fed-batch bioreactor, fed with WFO type 2	Pulsed at specific addition rate of 0.023 mll^{-1} h^{-1}	72	26.82[c]
	Continuous rate at 0.55 mll^{-1} h^{-1}	72	41.88[b]
	High cell density (0.64 gl^{-1}) at continuous rate of 0.55 $mll^{-1}h^{-1}$	48	49.71[a]

Values in the same column followed by the same letter do not significantly differ from each other, according to Duncan's at 5 % level.

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إنتاج البولى هيدروكسى الكونيت (PHAs) من مخلفات زيت القلى(WFO) بواسطة *Pseudomonas fluorescens* باستخدام المخمر بطرق تغذية مختلفة

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تعتبر مركبات البولى هيدروكسى الكونيت (PHAs) وسيلة لمواد بلاستيكية صديقة للبيئة و قابلة للتحلل تماماً. تم انتاج البولى هيدروكسى الكونيت (PHAs) من *Pseudomonas fluorescens* باستخدام المخمر بطريقة المزرعة ذات الدفعة الواحدة ، ذات الدفعتين وكذلك الدفعة الواحدة ذات التغذية المتزايدة. وقد استخدم زيت الذرة ، زيت فول الصويا ونوعان من مخلفات زيوت القلى كمصادر للكربون فى بيئة الانتاج، وقد أعطت المزرعة ذات الدفعة الواحدة أعلى قيمة لمحتوى الخلايا من البوليمر بعد ٦٠ ساعة من فترة التحضين على بيئة تحتوى على زيت الذرة (٥٢%) و زيت فول الصويا (٧٦,٥٣%) بينما كانت أعلى قيمة لمحتوى الخلايا من البوليمر مع الزيت رقم ٢ من مخلفات زيت القلى (٣٠%) . ووجد أن باستخدام المخمر بطريقة المزرعة ذات الدفعتين أعطت زيادة فى محتوى الخلايا من البوليمر مقدارها ٢,٢% ، ٣٢,١١% ، ٥٨% مع كلاً من زيت فول الصويا ، زيت الذرة و زيت القلى رقم ٢، على التوالى مقارنة مع ما تحصل عليه من طريقة المزرعة ذات الدفعة الواحدة . وباستخدام طريقة High cell density (٠،٦٤ جم / لتر) بطريقة التغذية المستمرة بزيت القلى رقم ٢ بمعدل ٠،٥٥ مللى / لتر / ساعة أعطت أعلى قيم لكلاً من polymer content ، conversion coefficient و yield بعد ٤٨ ساعة. علاوة على أن هذه الطريقة أعطت زيادة فى محتوى البوليمر تقدر بـ ٦٥،٧% مقارنة بطريقة المزرعة ذات الدفعة الواحدة مع تقليل وقت التخمير بمقدار ١٢ ساعة.