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PULLULAN PRODUCTION AS AFFECTED BY *Aureobasidium pullulans* STRAINS AND CULTURING CONDITIONS

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ABSTRACT

Six pullulan producing strains of *Aureobasidium pullulans* were obtained and tested for pullulan production on three different media. All aureobasidium tested strains gave the maximum pullulan production on Reeselev & Jensen medium. Only one strain was selected as highly efficient pullulan producing fungus known as *Aureobasidium pullulans* ATCC 42023. The effect of some nutritional and environmental factors on pullulan production by this strain on Reeselev & Jensen medium were evaluated using shake flask as a batch culture. *A. pullulans* ATCC 42023 gave the highest pullulan production parameters after 5 days fermentation period at 28°C in modified Reeselev & Jensen medium containing 5 % sucrose and 0.05 % glutamic acid (as a carbon and nitrogen sources, respectively) and the ratio of flask size to working volume was 2.5 : 1. Using HCDI two-stage batch culture for pullulan production on modified Reeselev & Jensen medium (20 % sucrose) led to increase cell dry weight and pullulan productivity by 2.28 and 2.65 fold than that obtained in batch shake flasks technique.

Keywords: *A.pullulans*, Batch fermentation, Two-stage batch pullulan parameters.

INTRODUCTION

Pullulan is a microbial exopolysaccharide commercially produced by fermentation with the yeast-like fungus *Aureobasidium pullulans*. The pullulan polysaccharide is a linear homopolysaccharide composed of glucose units. Glucopyranose units are polymerized [linked by α (1→4) glucosidic bonds] into maltotriose units [which are joined by α (1→6) glucosidic bonds] (Szymanska and Galas, 1993). Due to its excellent properties, pullulan is used as a low-calorie ingredient in foods, gelling agent, coating and packaging material for food and drugs, binder for fertilizers and as an oxidation-prevention agent for tablets. Other applications include contact lenses manufacturing, biodegradable foil, plywood, water-solubility enhancer and for enhanced oil recovery (Schuster *et al*, 1993; Israilides *et al*, 1998 and Leathers, 2003). The effect of nutritional factors on the production of pullulan by *A. pullulans* in shake flasks as batch culture was studied by many investigators (Imshenetskii *et al*, 1981 and Silman *et al*, 1990). Imshenetskii *et al* (1981) studied the influence of some carbohydrates on pullulan accumulation by *A. pullulans* strains and found that pullulan was synthesized most actively in maltose containing medium. Gibson and Coughlin (2002) found that sucrose was superior to glucose as carbon source based on yield and pullulan produced. Pullulan titer was 26.2 g l^{-1}. Sucrose often has been described as the optimal substrate for pullulan production with concentrations

started from 20 to 60 g l^{-1} (Audet *et al*, 1998; Roukas & Serris, 1999 and Leathers, 2002).

Shabtai and Mukmenev (1995) used glutamic acid at a high concentration (12 g l^{-1}) in the first stage of fermentation process. In the second stage, when glutamic acid was exhausted (below 5 % of initial amount), production of pullulan was started and 35 g l^{-1} of the polysaccharide was achieved. Schuster *et al* (1993) found that the maximum rates of pullulan yield and glucose consumption strictly depended on the amount of available nitrogen. By surveying the most available publications for investigating the suitable temperature for pullulan production, it was found that 28°C was widely applied for pullulan production (Roukas & Biliaderis, 1995; Madi *et al*, 1997; Roukas & Serris, 1999 and Roukas, 1999). Using temperature at 30°C was less extent (West & Reed-Hamer, 1993a; Audet *et al*, 1998 and Lee *et al*, 1999). Roukas and Biliaderis (1995) found that pullulan concentration (11.0 g l^{-1}) and pullulan yield (47.2 %) was increased with increasing the initial pH up to 6.5.

A high oxygen transfer rate could reduce, enhance, or have no effect on the production of pullulan depending on the strain ploidy (Imshenetskii *et al*, 1981). Rho *et al* (1988) concluded that oxygen is necessary for the biosynthesis of pullulan by *A. pullulans* in both growth (nitrogen containing) and non-growth (nitrogen-free) media. Wecker and Onken (1991) reported that *A. pullulans* strain QM 3090 gave an optimal pullulan yield by decreasing the dissolved oxygen in fermentation medium. Gibbs and Seviour (1996) observed that high oxygen levels dramatically reduced the yield of pullulan. Pullulan production has been the subject of numerous studies conducted as batch culture (Roukas, 1999; Szymanska *et al*, 1999; Barnett *et al*, 1999 and Lazaridou *et al*, 2002).

The present investigation was constructed to study the effect of some nutritional and environmental factors on improving pullulan production. This was carried out using shake flasks as a batch and two-stage batch culture.

MATERIALS AND METHODS

1. Fungus strains used

Six *Aureobasidium pullulans* strains were obtained from American type culture collection namely *A. pullulans* ATCC 16628, 62921, 12535, 16629, 42023 and 62922. All strains were subcultured on malt agar slants at 30°C, maintained at 4°C, and transferred monthly.

2. Media used

- Malt agar medium (CAIM, 1987) was used for propagation and preservation of aureobasidium cultures.
- Shabtai & Mukmenev's medium (Shabtai and Mukmenev, 1995), Bulmer's medium (Bulmer *et al*, 1987) and Reeselev & Jensen's medium (Reeselev and Jensen, 1995) were used for pullulan production by all tested aureobasidium cultures.

3. Standard inoculum

Standard inoculum was prepared by transferring a loop of the tested culture into 250 ml conical flasks containing 50 ml of tested medium. The inoculated flask was incubated on a rotary shaker at 210 rpm for 48 h at 30°C. The content of this flask was used as a standard inoculum (1 ml contained 6.0–7.0 x 10^5 viable cells) for batch shake flasks experiments (5 %). In two stage batch experiments, the content of inoculum flask was used as a high cell density inoculation (HCDI) after centrifuged at 12000 x g for 15 min, and then cells were washed twice with sterile distilled water and harvested to inoculate production medium as the method described by Shabtai and Mukmenev (1995).

4. Fermentation process

Fermentation was carried out in 250 ml Erlenmeyer flasks, each containing 100 ml sterile tested medium and inoculated with 5 % standard inoculum. The inoculated flasks were then incubated for 5 - 6 days at 28°C using rotary shaker at 210 rpm. Samples (10 ml) were taken from the growing culture periodically (every day) till the end of fermentation period under aseptic condition. The biomass was separated by centrifugation at 4000 X g for 10 min and the sediment was washed twice with distilled water and then dried at 70°C for constant weight. The residual sugar, pullulan produced and pH were also determined in the supernatant.

4.1. Batch shake flasks

These experiments were carried out to evaluate the effects of some nutritional and environmental factors on pullulan production by *A. pullulans* strains as following:

4.1.1. Selection of suitable medium for pullulan production

Three media for pullulan production being Shabtai & Mukmenev's medium, Bulmer's medium and Reeselev & Jensen's medium were tested for selecting the most suitable medium for securing high pullulan production by six aureobasidium strains.

4.1.2. Various sugars as carbon sources

Five trials were done to replace glucose as a carbon source by five different sources of carbon named sucrose, lactose, fructose, cellobiose and starch in Reeselev and Jensen (1995) medium at 3 %.

4.1.3. Sucrose concentrations

Five sucrose concentrations, i.e. 3, 5, 10, 15 and 20 % were used. Correlation coefficient of pullulan concentration as a function of sugar concentrations was calculated.

4.1.4. Nitrogen sources

Nine sources of nitrogen were tested for highest pullulan production by *A. pullulans* ATCC 42023. These sources were peptone, glutamic acid, ammonium sulfate (control), ammonium chloride, ammonium nitrate, ammonium acetate, ammonium citrate, sodium nitrate and urea at different concentrations ranged from 0.5 to 3.0 g l^{-1}.

4.1.5. KH_2PO_4 concentration

Different KH_2PO_4 concentrations ranged from 0 to 7 g l^{-1} were tested. Correlation coefficient of pullulan concentration, pullulan yield and pH as a function of KH_2PO_4 concentrations, were calculated.

4.1.6. Incubation temperature

The experiments were performed to detect the optimum temperature for the growth and pullulan production during the stages of inoculum preparation and pullulan formation. Five different temperatures, 26, 28, 30, 32 and 34°C were investigated. The biomass formation, pullulan concentration, chlamydospores count and residual sugar were determined. Pullulan parameters and correlation coefficient were calculated.

4.1.7. Initial pH values

Cultures of *A. pullulans* ATCC 42023 were grown at different initial pH values of 2.0, 3.0, 4.0, 5.5 (control), 7.0, 8.0 and 9.0 on productive medium for pullulan production.

4.1.8. Effect of flask size to working volume ratio

This experiment was designed to study the influence of aeration on pullulan production by *A. pullulans* ATCC 42023. Different volumes of modified productive medium (25, 50, 100 control, 150 and 200 ml) were dispensed in 250 ml Erlenmeyer flasks to get different ratios, namely 10 : 1, 5: 1, 2.5 : 1, 1.67 : 1 and 1.25 : 1, size of flask / volume of fermented medium.

4.2. Two- stage batch culture

These experiments were also carried out in shake flasks for pullulan production as influenced by different inoculation techniques and sucrose concentrations during pullulan production stage as follows:

4.2.1. Influence of inoculation techniques on pullulan production

Three inoculation techniques were examined to select the optimum one for high pullulan production in two-stage batch culture. The first technique (A) was conducted by centrifuging all content of inoculum flask (50 ml) at 12000 x g for 15 min to harvest cells. Then cells were washed twice before inoculation. The second procedure (B) was obtained by the first without washing the cells. The third technique (C) was carried out by using the content of inoculum flask as crude cells to inoculate the second step flask. The control treatment was also carried out by using 5 % standard inoculum.

4.2.2. Influence of sucrose concentrations

HCDI two-stage cultures of *A. pullulans* ATCC 42023 were grown at different initial sucrose concentrations ranged from 5 to 250 g l^{-1} during production stage on modified Reeselev & Jensen's medium. Statistical analyses between sugar concentration and pullulan parameters were also calculated.

5. Pullulan determination

Pullulan was precipitated in the culture supernatant with 2 volumes of ethanol at 4°C for 1 h. The precipitate was centrifuged at 4000 x g for 10 min followed by drying at 80°C overnight and was then weighed (Göksungur *et al*, 2004).

6. Chemical determinations

Total residual sugars were determined in the fermented liquor according to the method described by Flood and Priestly (1973). Organic carbon of pullulan was determined according to the method suggested by Jackson (1973). Ash content was determined using the method of Herbert *et al* (1971). Total nitrogen of pullulan was determined using Kjeldahel method as described by Jackson (1973).

7. Calculations

The specific growth rate (μ) and doubling time (t_d) were calculated from the exponential phase according to Painter and Marr (1963). Correlation coefficient and regression analysis were carried out according to the method described by Steel and Torrie (1981).

RESULTS AND DISCUSSION

I – Using shake flask batch culture for pullulan production

1. Selection of suitable medium and fermentation period for pullulan production

Data presented in Table (1) show that the highest figures of growth were recorded on Shabtai & Mukmenev and followed on Bulmer *et al* media by *A. pullulans* ATCC 62922 being 11.64 and 5.96 g l^{-1}, respectively after five days fermentation period. Whereas, strain (ATCC 42023) gave the highest pullulan concentration on Reeselev & Jensen medium (7.92 g l^{-1}) followed on Shabtai & Mukmenev (6.2 g l^{-1}) and Bulmer *et al* (5.04 g l^{-1}) media, which resulting the highest pullulan yield of 26.4, 20.67 and 11.2 %, respectively.

Table (1): Pullulan production by different *Aureobasidium pullulans* strains on different media after 5 days of incubation period at 28°C using shake flasks as a batch culture.

Media used	*Aureobasidium pullulans* strains	Cell dry weight (g l^{-1})	Pullulan concentration (g l^{-1})	$Y_{p/x}$ (g g^{-1})	Pullulan yield (%)
Shabtai & Mukmenev (1995)	ATCC 16628	0.98	0.69	0.7	2.3
	ATCC 62921	2.1	2.06	0.98	6.8
	ATCC 12535	0.2	0.2	1	0.6
	ATCC 16629	0.34	0.68	2	2.2
	ATCC 42023	3.38	6.2	1.83	20.67
	ATCC 62922	11.64	4.14	0.35	13.8
Bulmer *et al* (1987)	ATCC 16628	1.04	0.46	0.131	1
	ATCC 62921	3.5	0.88	0.85	0.19
	ATCC 12535	2.48	2.3	0.93	5.11
	ATCC 16629	3.6	2.56	0.71	5.6
	ATCC 42023	4.48	5.04	1.125	11.2
	ATCC 62922	5.96	1.46	0.24	3.2
Reeselev & Jensen (1995)	ATCC 16628	2.2	4.7	2.1	15.6
	ATCC 62921	3.32	4.74	1.42	15.8
	ATCC 12535	2.0	6.94	3.47	23.1
	ATCC 16629	3.74	7.2	1.9	24
	ATCC 42023	3.3	7.92	2.4	26.4
	ATCC 62922	2.4	6.8	2.79	22.67

In medium of Reeselev and Jensen (1995), pullulan was without pigmentation.

So, Reeselev & Jensen medium (1995) will be used in the further studies as a productive medium for pullulan production by *A. pullulans* ATCC 42023. Many authors have exhibited attention to strain *A. pullulans* ATCC 42023, as a model pullulan producer (West & Reed-Hamer, 1995; Shabtai & Mukmenev, 1995; Guterman & Shabtai, 1996; West & Strohfus, 1997 and Lee *et al*, 1999).

2. Comparison between various sugars as carbon sources for pullulan production

Data plotted on Fig (1) show that *A. pullulans* ATCC 42023 grew exponentially during the first 48 h on all carbon sources, except cellobiose, where log phase increased to 72 h. The highest growth of *A. pullulans* ATCC 42023 was attained after 5 days in cellobiose medium, whereas the highest pullulan concentration was observed after five days of incubation on sucrose medium, being 8.11 g l^{-1}. Data in Fig (2) show that the highest growth parameters were achieved on medium containing sucrose than other sugars. Specific growth rate (μ), doubling time (t_d) and number of generations (N) of *A. pullulans* ATCC 42023 grown on sucrose medium, were 0.07 h^{-1} , 9.9 h and 4.8, respectively. The corresponding figures for pullulan yield coefficient relative to biomass ($Y_{p/x}$), conversion coefficient, pullulan yield and productivity were 3.3 g g^{-1}, 38.9, 27 % and 0.07 g $l^{-1}h^{-1}$ on sucrose medium, respectively. The pullulan productivity on sucrose medium increased 1.03 fold than glucose medium. So, sucrose was the obvious choice as appropriate sugar substrate for pullulan production by *A. pullulans* ATCC 42023. These results confirmed the results obtained by Silman *et al*, (1990), Schuster *et al* (1993) and Shabtai & Mukmenev (1995) who stated that the best results of pullulan production by *A. pullulans* were observed on media containing sucrose.

3. Effect of different sucrose concentrations

Data illustrated by Figs (3 & 4) reveal that *A. pullulans* ATCC 42023 grew exponentially during the first 24 h fermentation period on media containing sucrose concentrations ranged from 15 to 200 g l^{-1}, except medium containing 30 g l^{-1} sucrose, which prolonged the log phase to 48 h. Whereas, the highest figures of the specific growth rate (μ), multiplication rate (MR) and lowest doubling time were recorded in medium containing 50 g l^{-1} sucrose. Data also clearly show that increase of the sucrose concentration resulted in increasing the pullulan concentration till reaching the maximum figures (22.36 g l^{-1}) after 6 days fermentation period in the productive medium containing 150 g l^{-1} sucrose, whereas, the maximum pullulan yield and conversion coefficient values, being 30.6 and 37.4 % were recorded after 5 days incubation period at 50 g l^{-1} sucrose concentration. These parameters were decreased with the increase of sucrose concentration than 50 g l^{-1}. A positive correlation coefficient between sucrose and pullulan concentrations was also obtained being 0.72. Therefore, it could be stated that 50 g l^{-1} sucrose was the best carbon source for pullulan production by *A. pullulans* ATCC 42023 resulting 3.9 % increase in pullulan yield as compared to control (30 g l^{-1}). The aforementioned results are in agreement with those of Yamasaki *et al* (1993) and Shabtai & Mukmenev (1995), they stated that sucrose concentration had a marked effect on pullulan production.

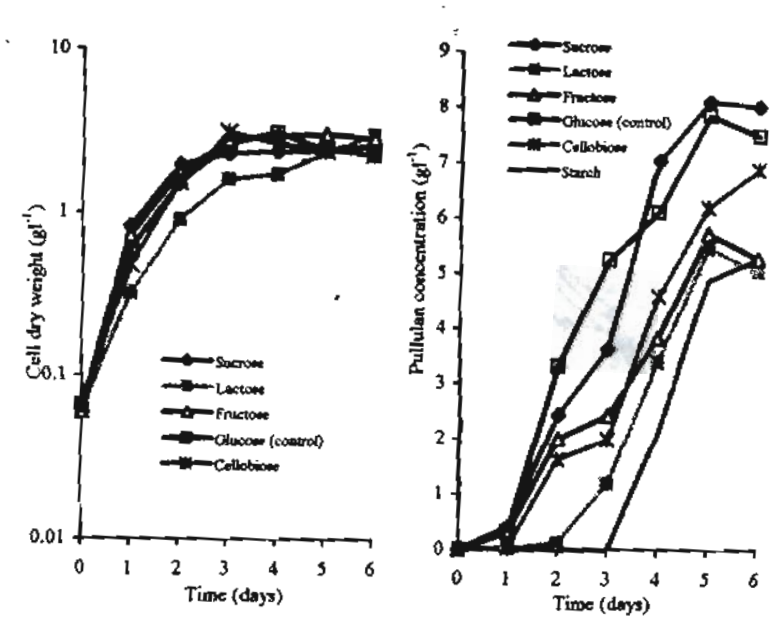

Fig.(1): Growth curves and pullulan production by *A. pullulans* ATCC 42023 as influenced by various carbon sources during 6 days of incubation period at 28°C using shake flasks as a batch culture.

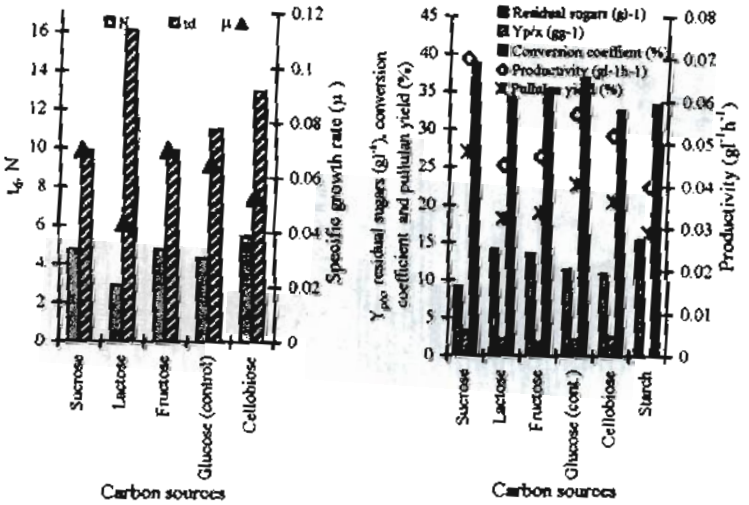

Fig.(2) Parameters of growth and pullulan production by *A. pullulans* ATCC 42023 as influenced by various carbon sources after 5 days incubation period at 28°C using shake flasks as a batch culture.

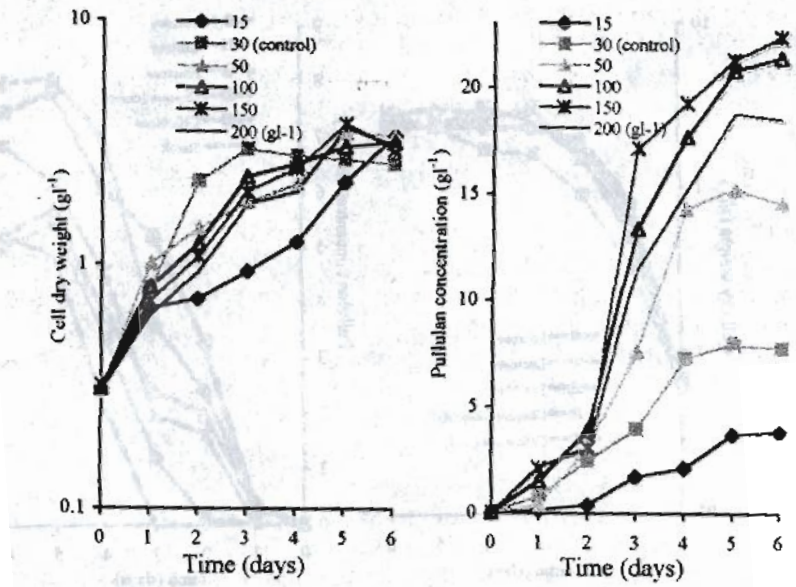

Fig.(3): Growth curves and pullulan production by *A. pullulans* ATCC 42023 as influenced by various sucrose concentrations during 6 days of incubation period at 28°C using shake flasks as a batch culture.

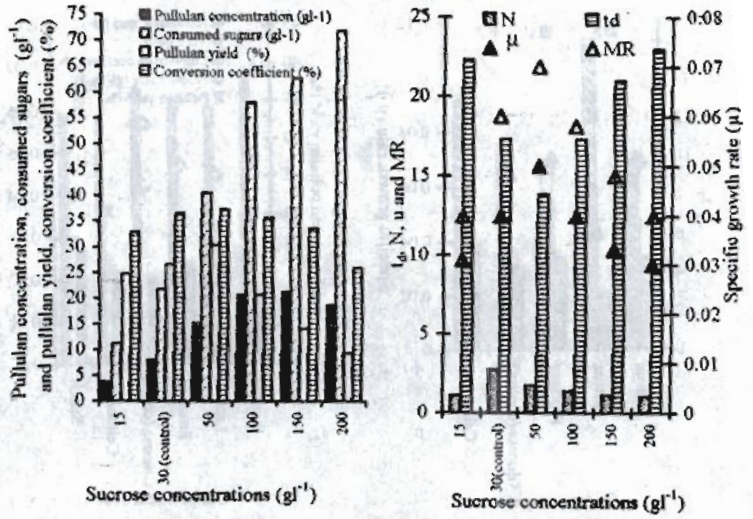

Fig.(4): Parameters of growth and pullulan production by *A. pullulans* ATCC 42023 as influenced by various sucrose concentrations during 6 days of incubation period at 28°C using shake flasks as a batch culture.

4. Effect of different Nitrogen sources

Data illustrated by Fig (5) show pullulan production by *A. pullulans* ATCC 42023 as influenced by different organic and inorganic nitrogen sources at various concentrations in medium containing 50 g l^{-1} sucrose. The highest figure of cell dry weight was attained in medium containing 2.5 g l^{-1} ammonium acetate followed by that containing 3 g l^{-1} peptone. The highest concentration of pullulan was obtained in medium supplemented with 0.5 g l^{-1} glutamic acid and followed in media containing 1.0 g l^{-1} glutamic acid and 1.0 g l^{-1} ammonium acetate. Also, it could be noticed that using 0.5 g l^{-1} glutamic acid increased the pullulan concentration 1.9 fold as compared to control (0.6 g l^{-1} ammonium sulphate). So, it could be recommended to use glutamic acid as nitrogen source for high pullulan production by *A. pullulans* ATCC 42023. These results are in agreement with those of Shabtai & Mukmenev, (1995). Desmond *et al* (1990) in a similar study reported that glutamate at a low concentration was equal to ammonium salts for pullulan production.

5. Effect of different KH_2PO_4 concentrations

The influence of increasing concentrations of potassium dihydrogen phosphate on growth, pullulan formation by *A. pullulans* ATCC 42023 grown on modified productive medium was illustrated by Fig (6). The highest pullulan concentration, pullulan yield coefficient relative to cell dry weight, pullulan yield and pullulan productivity were obtained on productive medium containing 2.98 g l^{-1} KH_2PO_4. Increasing KH_2PO_4 concentration more than 2.98 g l^{-1} led to a decrease in pullulan production.

The correlation coefficient between KH_2PO_4 concentration and each of cell dry weight and pullulan production were 0.05 and -0.5, respectively. These results are in agreement with those of McNeil & Kristiansen (1990) and Silman *et al* (1990), they used 5.0 and 1.0 KH_2PO_4 g l^{-1}, respectively for highest pullulan production by *A. pullulans*.

6. Effect of incubation temperature

As seen in Fig (7), final cell dry weight and pullulan concentration were increased as incubation temperature increased to reach the maximum being 6.4 and 2.89 g l^{-1} at 32°C on inoculation medium, respectively. The corresponding figures for cell yield, productivity and chlamydospores were 21.3 %, 0.13 g $l^{-1}h^{-1}$ and 6.1 %, respectively. Statistical analysis revealed a positive correlation coefficient (R) between temperature degrees, and cell dry weight, pullulan concentration, as well as chlamydospores being 0.47, 0 52 and 0 9 respectively. The later value indicated that the temperature has an obvious effect on the yeast-chlamydospores transition. In the modified productive medium (Fig, 8) incubated at 28°C for 5 days, *A. pullulans* ATCC 42023 consumed highest amount of sucrose (40.5 g l^{-1}) and produced highest pullulan concentration being 18.8 g l^{-1} and recorded the highest figures of pullulan parameters. Statistical analysis revealed positive and negative correlation coefficients (R) between temperature degrees, and both of cell dry weight and pullulan concentration, being 0.9 and -0.8, respectively. Data discussed was in line with that of McNeil & Kristiansen (1990) who found that the optimum temperature for highest growth and pullulan formation were 32 & 24°C, respectively. Also, West & Reed-Hamer (1993b) found that 26°C to be

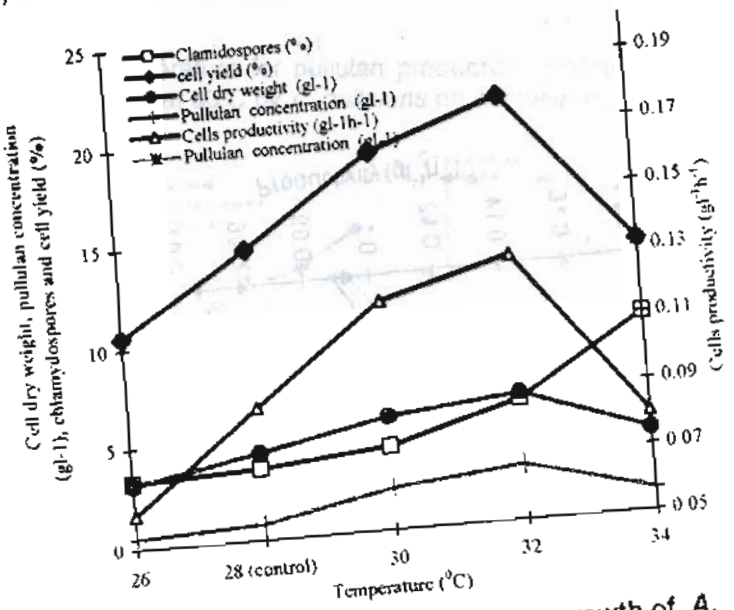

Fig.(7): Effect of incubation temperature on the growth of *A. pullulans* ATCC 42023 after 48 h of propagation on Inoculation med.5 using shake flasks as a batch culture.

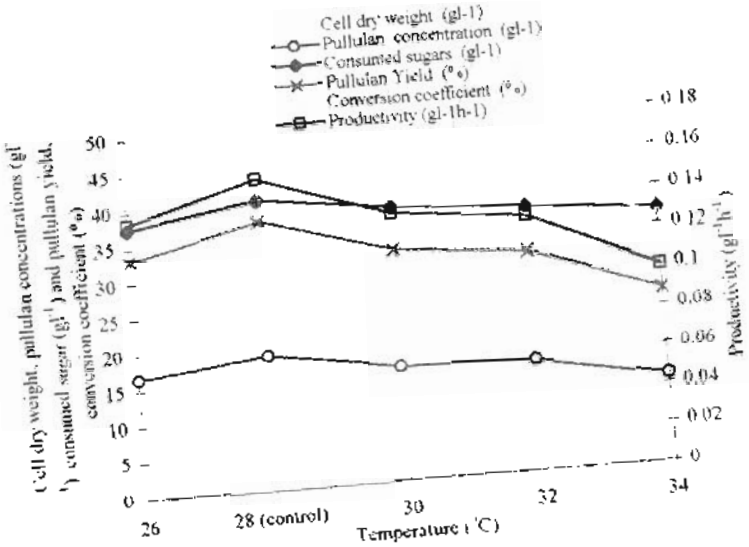

Fig.(8): Effect of incubation temperature on pullulan production by *A. pullulans* ATCC 42023 after 5 days incubation period on modified Reeslev & Jensen's medium using shake flasks as a batch culture.

7. Effect of initial pH values

Data illustrated by Fig (9) indicate that pH 7.0 was the most favorable value for pullulan production (20.2 g l^{-1}) by *A. pullulans* ATCC 42023. The corresponding figures for pullulan yield coefficient relative to biomass, pullulan yield and productivity were 7.77 g g^{-1}, 40.4 % and 0.168 g l^{-1} h^{-1}, respectively. In this respect, Roukas (1999) found that pullulan yield and concentration increased with increasing initial pH up to 6.5. It could be stated that biomass yield was favored at higher acidity than lowers. A highly positive correlation coefficient (R) between pH values (less than pH 8.0), and pullulan concentration was observed, being 0.99. Initial pH value of 7.0 was applied in further work, using modified Reeselev & Jensen medium.

8. Effect of the ratio of flask size to working volume.

Data in Fig (10) reveal that decreasing the ratio of flask size to volume of fermented medium resulted in increasing the cell dry weight and pullulan production as well as pullulan parameters until reaching the maximum at ratio 2.5: 1. Decreasing the ratio, less than 2.5: 1, showed a bad effect either on pullulan production or cell dry weight. These results are in line with those of Wecker and Onken (1991) who reported that optimal pullulan yields from strain QM 3090 was obtained at decreased dissolved oxygen condition. In this respect, Gibbs and Seviour (1996) observed that high oxygen levels dramatically reduce pullulan yields. Therefore, the ratio 2.5: 1 of flask size to volume of fermented medium will be used for further studies.

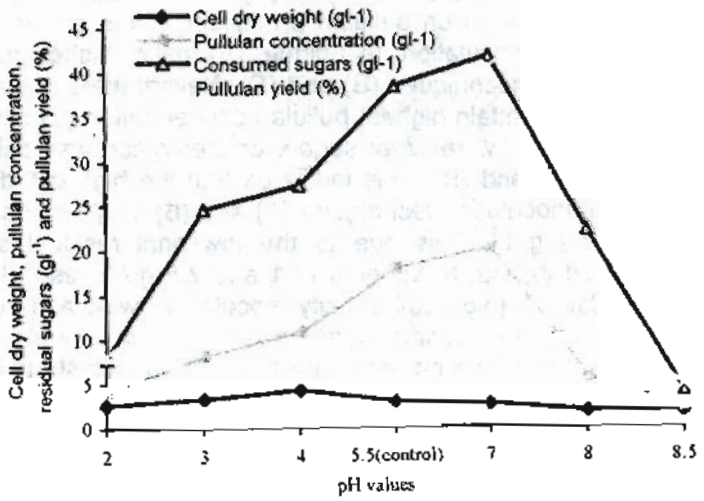

Fig.(9): Effect of different initial pH values in modified med. 5 on pullulan production by A. pullulans ATCC 42023 after 5 days of incubation at 28°C using shake flasks as a batch culture.

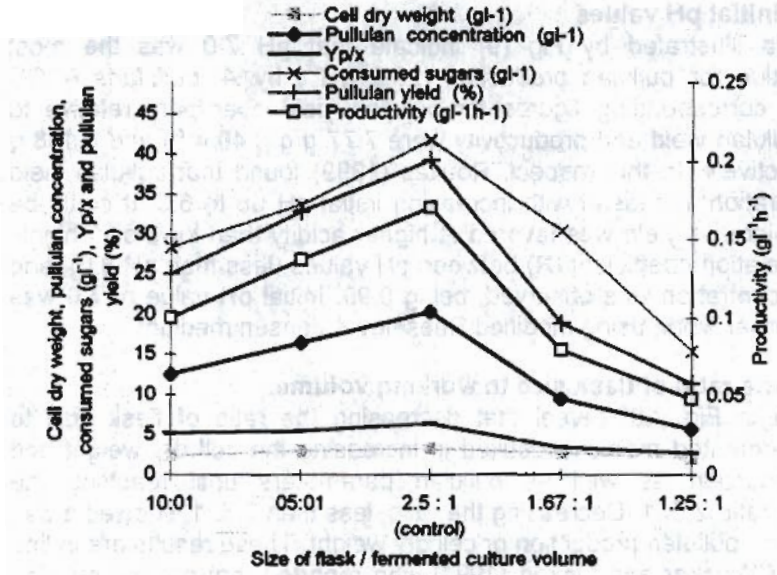

Fig.(10): Effect of different working volume of modified med. 5 on pullulan production by *A. pullulans* ATCC 42023 after 5 days of incubation at 28°C using shake flasks as a batch culture.

II – Using two-stage batch culture for pullulan production

1. Influence of different inoculation techniques

The influence of inoculation techniques, i.e. inoculation by washed cells (A), inoculation by cells without washing (B) and inoculation by crude cells (50 ml standard inoculum) on pullulan production (C) is shown in Fig (11). It was found that inoculation technique (A) gave higher pullulan production (17.2 g l^{-1}) than techniques (B) and (C). Nevertheless, it was still 5% inoculation (control), to attain highest pullulan concentration (19.81 g l^{-1}). On the other hand, a very low residual sugars concentration was detected using both techniques (A) and (B). This indicates that the high cell density inoculation in cases of inoculation techniques (A) and (B) may require more carbon source than 50 g l^{-1}. This due to the low final residual sugars concentrations obtained in both treatments (1.1 and 2.5 g l^{-1}, respectively). So, inoculation technique A (high cell density inoculation two-stage culture) was selected for subsequent study with higher initial sugar concentrations.

2. Influence of initial sucrose concentration on HCDI two-stage batch pullulan production

A direct relation between sucrose concentrations and cell dry weight was observed during production stage of two-stage batch cultivation of *A. pullulans* ATCC 42023 as seen in Fig (12). The highest figure of cell dry weight was obtained at 200 g l^{-1} sucrose, being 18.54 g l^{-1}. Increasing the sucrose concentration resulted in increasing the pullulan parameters and consumed sugar to reach the maximum at 250 g l^{-1} sucrose, whereas the maximum pullulan yield value being 22.67 % was recorded at 200 g l^{-1} sucrose.

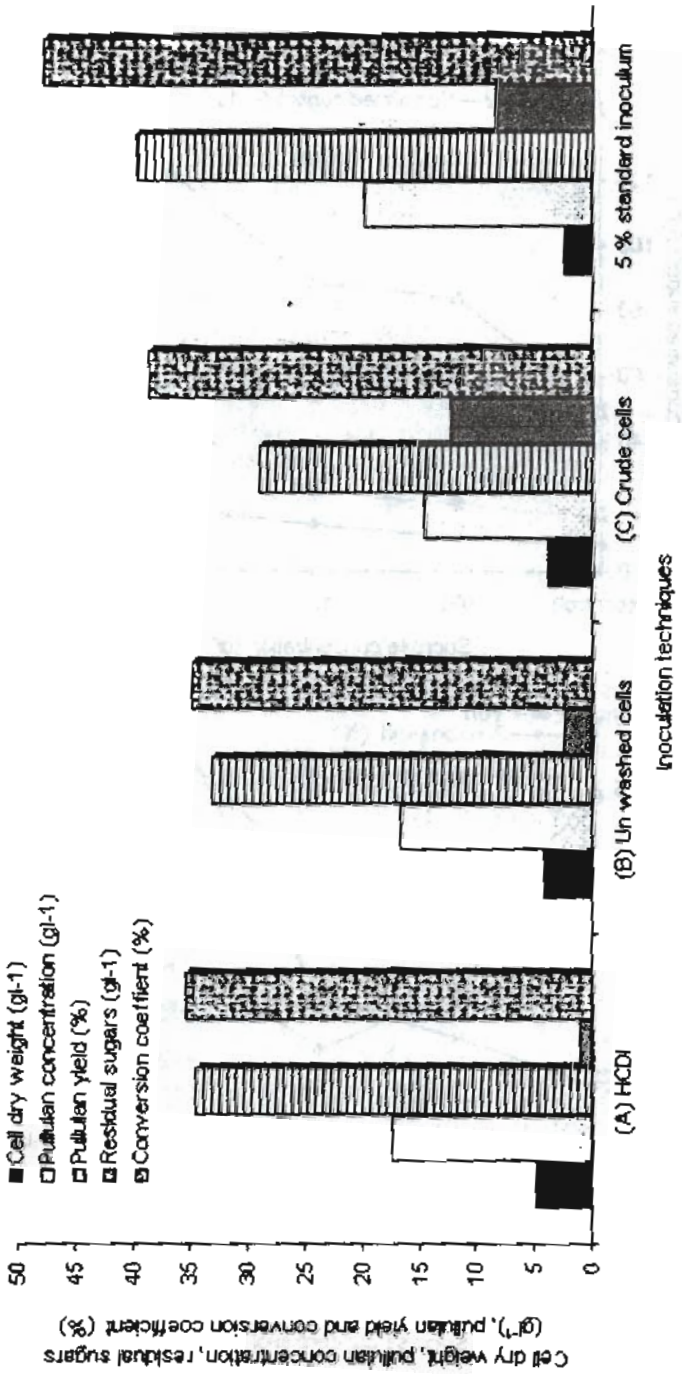

Fig.(11): Pullulan production by *A. pullulans* ATCC 42023 as influenced by different inoculation techniques on modified Reeslev & Jensen's medium incubated at 28°C for 5 days fermentation period using shake flasks as a two-stage batch culture.

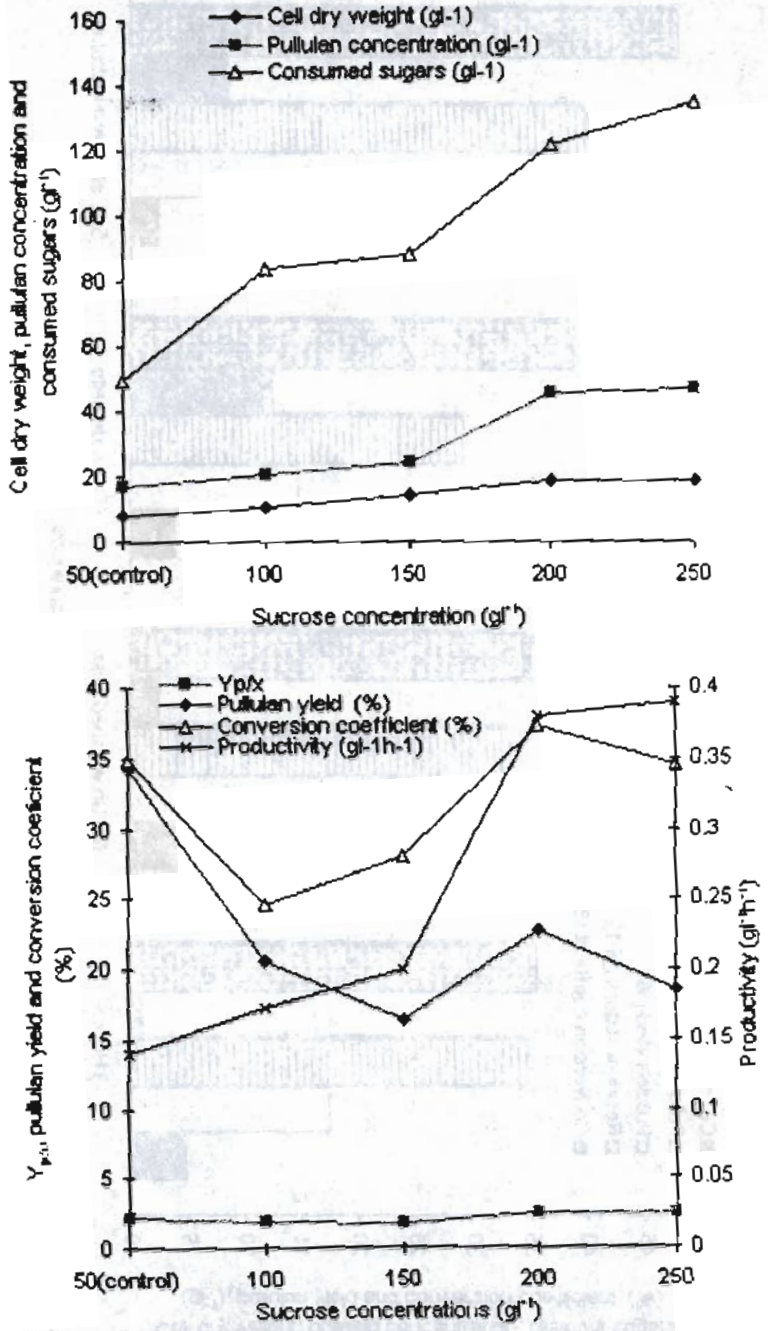

Fig.(12): Pullulan production by high cell density of *A. Pullulans* ATCC 42023 as influenced by different sucrose concentrations on modified Reeslev & Jensen's medium incubated at 28°C for 5 days fermentation period using shake flasks as a two-stage batch culture.

A high positive correlation coefficient (R) between sucrose concentrations, and each of cell dry weight, pullulan concentration and consumed sugar (g l^{-1}), being 0.97, 0.94 and 0.97, respectively. Therefore, it could be stated that 200 g l^{-1} sucrose was the most favorable concentration for pullulan production on modified Reeselev & Jensen's medium using shake flasks as a two-stage batch culture.

From the aforementioned data, it could be concluded that using modified Reeselev & Jensen's medium containing 200 g l^{-1} sucrose in two-stage batch culture for pullulan production increased the cell dry weight and pullulan concentration by 2.28 and 2.65 fold, respectively as compared to the production on 50 g l^{-1} sucrose for batch culture (control). Also, Gibbs and Seviour (1996) applied the high-cell-density-inoculum two-stage batch for pullulan production using a 30 g l^{-1} glucose medium. Whereas, Shabtai and Mukmenev (1995) used a 30 g l^{-1} sucrose medium in the second stage. Pullulan produced by *A. pullulans* ATCC 42023 gave close physical properties to standard pullulan. However, lower carbon content was recorded in produced pullulan being 38.6 %. Produced pullulan also contained higher amount of total nitrogen and ash being 0.17 % and 0.21 %, respectively (Table 2).

Table (2): A comparison between physicochemical properties of standard pullulan and pullulan produced by *A pullulans* ATCC 42023 in the present study.

Physicochemical properties	Standard pullulan specifications	Specifications of produced pullulan
Appearance	White powder	White powder
Solubility	Soluble in water	Soluble in water
Carbon content	42 %	38.6 %
Total nitrogen	0.09 %	0.17 %
Ash	0.1 %	0.21 %

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إنتاج البوليولان متأثراً بسلالات *Aureobasidium pullulans* و ظروف التنمية

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قسم الميكروبيولوجيا الزراعية – كلية الزراعة – جامعة عين شمس – ص ب٦٨حدلق شبرا ١١٢٤١ – القاهرة – مصر

تم في هذا البحث إختبار قدرة ٦ سلالات من ميكروب *Aureobasidium pullulans* على إنتاج البوليولان فى ثلاث بيئات مختلفة. و قد أعطت كل السلالات أعلى إنتاج للبوليولان على بيئة Reeselev & Jensen و قد تم إنتخاب السلالة *A. pullulans* ATCC 42023 ذات الكفاءة العالية لإنتاج البوليولان. و بدراسة تأثير بعض العوامل الغذائية و البيئية على إنتاج البوليولان بواسطة هذه السلالة لوحظ أن تعديل تركيب البيئة بإحتوائها على ٥٠ % سكروز ، ٠,٠٥ % حمض جلوتاميك (كمصدر كربون و نتروجين على التوالى) أعطى أعلى قياسات لإنتاج البوليولان و ذلك بعد فترة تخمير خمسة أيام و على درجة تحضين ٢٨°م بإستخدام تكنيك المزرعة المهتزة ذات الدفعة الواحدة. كما أن إستخدام تكنيك المزرعة المهتزة ذات المرحلتين لإنتاج البوليولان فى البيئة المعدلة و المحتوية على ٢٠ % سكروز أدى الى زيادة وزن الخلايا الجافة و إنتاجية البوليولان الى ٢,٢٨ ، ٢,٦٥ ضعف و ذلك مقارنة بالكميات المتحصل عليها عند إستخدام المزرعة ذات الدفعة الواحدة.