



OPTIMIZATION THE ALGINATE PRODUCTION CONDITIONS IN SWEET WHEY MEDIUM BY *AZOTOBACTER CHROCOCCUM*

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Walaa^{1*}, S. Hendawy, El Kanani Y.M.², El Batawy O.I.² and El mongy, T. M.¹

1. Microbiology Dept., National Center for Radiation Research & Technol., Atomic Energy Authority, P.O. Box 29, Nasr City, Cairo, Egypt
2. Food Sci. Dept., Fac. of Agric., Ain Shams Univ., P.O. Box 68 Hadyek Shoubra11241, Cairo, Egypt

*Corresponding author: walaahendawy78@gmail.com

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ABSTRACT

Sweet whey was used as medium for microbial alginate production by *Azotobacter chroococcum* NCBI MH249629. To optimize alginate production and achieve high yield of alginate, sweet whey medium was prepared using different lactose concentrations (20, 25, 30, 35, 40, 45, 50 g/L), added organic and inorganic nitrogen sources (yeast extract, Malt extract, ammonium sulphate and ammonium nitrate), kept at different temperatures (25, 30, 32, 35 & 37°C), different pH values (4, 5, 6, 7, 8, 9), different agitation speed (100, 120, 150, 170 & 200 rpm), different inoculum size (1,2,3,4,5,6,7,8,9,10%) and cultivated for different fermentation time (0, 12, 24, 36, 48, 60, 72, 84, 96, 108 & 120h). After fermentation time, produced microbial alginate was measured. The results showed that sweet whey could be used as a fermentation medium by *Azotobacter chroococcum* to produce microbial alginate. The highest yield of alginate (12.56%) as well as productivity of alginate (0.08 g/L/h) was obtained by fermentation of *Azotobacter chroococcum* in sweet whey medium optimized to 45 g/L lactose concentration as a carbon source and by providing the following conditions which yeast extract 1.5 g/L and ammonium nitrate 0.1 g/L as a nitrogen source and growth promoter, Na₂HPO₄ 1 g/L as mineral salts, Initial pH at 7, size of standard inoculums was 9 % of fermentation medium and the incubation period was 72 hrs.

Key words: Alginate production, Sweet whey, *Azotobacter* spp., Fermentation conditions.

INTRODUCTION

Alginates are a polysaccharides composed of variable ratios of β -D-mannuronate (M) and its C-5 epimer α -L-guluronate (G) linked by 1–4 glycosidic bonds. Alginates were first isolated from brown seaweeds in the 1880s, and its commercial production begun in the early 20th century. Alginate can be produced by various genera of brown seaweed and two genera of bacteria, *Pseudomonas* and *Azotobacter* spp. The ratio of M and G residues and thus the material properties vary depending on the source of the alginate. Its unique physical properties enable it to be used as a stabilizer, viscosifier and gelling agent in the food, beverage, paper, printing and pharmaceutical industries (Draget et al 2005 and Donati & Paoletti, 2009). The desirable material properties as well as its apparent biocompatibility has led to it being used increasingly in the medical, pharmaceutical and biotechnology industries for applications such as wound dressings (Thomas, 2000); the encapsulation or controlled release of drugs, enzymes or cells; or as matrices for tissue engineering (Andersen et al 2012 and Lee & Mooney, 2012).

Azotobacter are non-symbiotic diazotrophic bacteria generally found in neutral or slightly alkaline soils, including aerially transported dust and mostly abundant in plant rhizosphere and phyllosphere region. Members of the genus *Azotobacter*, are free living, gram negative, aerobic and heterotrophic in nature (Garg et al 2001). *Azotobacter* spp. is well known to produce large quantities of extracellular polysaccharides (EPS), commonly apparent as large mucoid colonies during isolation

from soil habitat (Vargas-Garcia et al 2002). EPS provide protection to cells against desiccation and predation by protozoans or phage attack (Looijesteijn et al 2001). This polysaccharide also protects the penetration of toxic metal ions inside the cell (Aleem et al 2003) and nitrogenase against high oxygen concentration environment (Sabra et al 2000 and Soto-Urzúa & Baca, 2001).

In the case of the dairy industry, one such 'waste' which is produced in enormous quantities is the whey resulting from cheese and casein manufacture. Whey is the serum part of milk remaining after separation of the curds during casein or cheese making (Zadow, 1986). It is rich in nutrients and contains at least half the solids found in whole milk (Horton, 1993). The other perspective, whey has a very strong polluting capacity, with a Biological Oxygen Demand (BOD) of 40,000 to 45,000 mg/L (Hacking, 2006 and Kemp & Quickenden, 1989). On a world wide scale, only 50 % (v/v) of total whey produced is utilized, and the remainder is disposed of disposed whey is either discharged into the sewage or ocean outfall or is sprayed on pastures (Zadow, 1987 & 1992). In recent years, value added products from whey have attracted much interest. One such area is the production of microbial biopolymer (Schwartz and Bodie, 1985, Fu and Tseng, 1990, Flatt, et al 1992 and Konicek et al 1993).

Therefore, the aim of this study was to optimize alginate production conditions by identified *Azotobacter chroococcum* NCBI MH249629 in shake flasks using sweet whey medium.

MATERIALS AND METHODS

Materials

Sweet whey (0.27% protein, 4.9% lactose, 0.5% ash and 5.8% total solids) from Gouda cheese manufacturing was obtained from Dina for Agricultural Investments Co., Alexandria Desert Road, It was characterized for its composition using the Milko Scan method (Microbalance 4503 MP6).

Azotobacter chroococcum was isolated from rhizospheric soil samples and identified according to Bergye's Manual of systematic bacteriology. (George et al 2001) Screening and selection of the strain have been made on the basis of their exopolysaccharides production (EPS) ability in nitrogen-free glucose Burk's medium (glucose,

20g; K₂HPO₄, 0.2 g; FeSO₄, 0.1 g; MgSO₄, 0.2 g; NaCl, 0.2 g; CaCO₃, 5 g; Na₂MoO₄ 0.01 g; water, 1000 ml). The pH was adjusted to 7 with a concentrated NaOH solution. To avoid precipitation during autoclaving, the solutions of FeSO₄ á 7H₂O and Na₂MoO₄.2H₂O were separated from the other medium components during sterilization (121 °C, 35 min). (Vela and Rosenthal, 1972). The selected strain was identified by 16S rDNA sequencing and designated as SSB81.

For 16S rDNA sequencing, total genomic DNA was extracted from the isolate following the method (Agrawal et al 2015). Amplification of 16S rDNA gene by PCR using universal primer, Forward (5'-AGAGTTTGATCCTGGCTCAG-3'), Reverse (5'- AAGGAGGTGATCCAGCCGCA-3'). All data reported in this study are from triplicate measurement.

Culture media and conditions

Sweet whey was autoclaved at 121°C for 5 min to precipitate the whey protein; the precipitate was removed by filtration through Whatman paper No.1 (Ricciardi and Clementi, 2002). The whey production medium for the highest alginate producing strain (*Azotobacter chroococcum* NCBI MH249629) contained: 0.1% (w/v) yeast extract, 0.05% (w/v) malt extract, 0.05% (w/v) Na₂ HPO₄, 0.01% (w/v) NH₄NO₃, 0.01 % (w/v) MgSO₄. Culture was performed in 250 ml Erlenmeyer flasks with a working volume of 50 ml then Each flask was inoculated with different inoculum size (1, 2, 3, 4, 5, 6, 7, 8, 9, 10%) which prepared with different lactose concentration (20, 25, 30, 35, 40, 45, 50 g/L), added organic and inorganic nitrogen sources (yeast extract, Malt extract, ammonium sulphate and ammonium nitrate 1g/L), kept at different temperatures (25, 30, 32, 35 & 37°C) and different agitation speed (100, 120, 150, 170 & 200 rpm/h), cultivated for different fermentation time (0, 12, 24, 36, 48, 60, 72, 84, 96, 108 & 120h), different pH (4.5, 6, 7, 8, 9) to optimize alginate production and achieve high yield of alginate (Butt et al 2011).

Growth curve

Samples were taken every 24 h to measure optical density (OD 400nm) by spectrophotometer assay using (model V-200-RS, visible light, LW scientific), according to the method described by Painter and Morr (1963).

Growth and biological activity parameter

The specific growth rate (μ) and doubling time (t_d) were calculated from the exponential phase according to **Painter and Morr (1963)** using the following equation: -

$$\mu = (\ln x - \ln x_0) / (t - t_0)$$

$$\mu = \ln 2 / t_d$$

where

- μ = Specific growth rate (h^{-1})
- x_0 = Amount of growth at t_0 time
- x = Amount of growth after t time
- t_d = Doubling time (h)
- t_0 = Time in hours at the beginning of log phase
- t = Time in hours at the end of log phase

Number of generations was calculated using the following equation according to **Stanier et al (1970)**:

$$N = t/t_d$$

Where

- N = Number of generation
- t = The period of exponential phase
- t_d = Doubling time (h)

Measurement of produced microbial alginate

10- ml of sample was centrifuged at 6000 rpm at 4°C for 30 min. The precipitate (biomass) was suspended in 10 Mm EDTA with NaCl for 2 min to solubilize the cell associated alginate then centrifuged at 8000 rpm at 4°C/30 min and washed twice

with distilled water, centrifuged and dried at 90°C till constant weight for 18 h. Alginate polysaccharides in cell-free supernatant was recovered by precipitation with three volumes of propanol (95% v/v), after centrifugation at 8000 rpm at 4°C/30 min and dried at 70°C till constant weight (**Pena et al 2011**).

Statistical analysis

The final, optimized experiments were run in triplicate and the data plotted are the mean value \pm SD, the calculations were performed using IBM SPSS Statistics V.22 software through One-Way ANOVA technique and multiple comparisons were made through Least Significant Difference test (**Steel et al 1997**).

RESULTS AND DISCUSSION

Effect of lactose concentration in sweet whey media on the production of microbial alginate

Carbon source had a dual effect as the building block and the energy requirement for the organism. Suitable and readily absorbable sugars can be the best choice for bacterial growth to gain good production (**Alibutt et al 2011**). In this study the effect of different lactose concentrations in sweet whey medium on the production of microbial alginate by *Azotobacter chroococcum* NCBI MH249629 is shown in **Table (1)**.

Table 1. Effect of different concentrations of lactose (milk sugar) in sweet whey medium on alginate production by *Azotobacter chroococcum*.

Why dilution (why/water) (V/V)	Why Lactose conc. (g/L)	CDW (g/L)	Alginate conc. (g/L)	Productivity (g/L/h)	Yield (%)	Final pH
4 : 6	20	0.658±0.002	0.645±0.023	0.0054±0.001	3.23±0.020	6.91
1 : 1	25	0.706±0.006	0.682±0.020	0.0057±0.001	2.73±0.026	6.85
6 : 4	30	0.732±0.002	0.716±0.012	0.0059±0.001	2.39±0.021	6.63
7 : 3	35	0.829±0.006	0.754±0.015	0.0063±0.004	2.15±0.021	6.50
8 : 2	40	0.976±0.002	0.923±0.026	0.0077±0.001	2.31±0.025	6.39
9 : 1	45	0.939±0.007	0.983±0.006	0.0082±0.002	2.18±0.010	6.20
1 : 0	50 (Control)	0.794±0.014	0.763±0.010	0.0064±0.003	1.53±0.032	6.43

*Initial pH=7

*The present values are the average of three replicates \pm SD.

*Whey medium; After 5 days of incubation period at 30 °C; 170 rpm.

*CDW: Cell dry weight

The results indicate that, variation of lactose concentration in sweet whey medium had a significant effect on the production of alginate produced by *Azotobacter chroococcum* NCBI MH249629. The data illustrate that the highest alginate concentration, productivity (0.983 g/L, 0.0082 g/L/h) respectively at 4.5 % lactose (v/v), (5%) inoculum size, 30°C/120h. While at 4 % lactose gave maximum biomass (0.976g/L) among all treatments. Increasing lactose concentration to 5% (control treatment) led to a significant decrease in alginate concentration compared with treatment containing 4.5% lactose. This is may be due to the effect of increasing the concentration of lactose in the medium inhibits the growth of *Azotobacter* spp. and decrease the production of alginate (Alibutt et al 2011). This inhibitory effect might be attributed to high osmotic pressure (Prasertan et al 2008). Lactose is the most abundant constituent of whey and it forms at least 78 % (w/w) of the whey's total solids, this indicates that for an organism to efficiently use whey as substrate, it must be able to metabolize the lactose as its carbon source. Such organisms should produce sufficient lactose hydrolyzing enzymes (β -galactosidase) to hydrolyze the lactose to its monomers (Belder, 2010 and Kang et al 2010). These findings are in harmony with the previous results obtained by (Emitazi et al 2004) who revealed that *Azotobacter chroococcum* 1723 was

able to produce alginate greater than 5 mg/L when used lactose as carbon source.

Generally, it could be concluded that 4.5% lactose (v/v) was the most favorable concentration as a carbon source for alginate production by *Azotobacter chroococcum* NCBI MH249629 in sweet whey medium.

Effect of different nitrogen sources in sweet whey media on the production of microbial alginate

As shown in Table (2) it could be stated that using different nitrogen sources either organic or inorganic in sweet whey medium have a significant effect on the values of (CDW) and alginate concentration produced by *Azotobacter chroococcum* NCBI MH249629. the control treatment (without any additives) was the lowest alginate concentration (0.719 g/L) among all treatments, whereas yeast extract gives the highest alginate concentration (0.984 g/L). This is means that, fortification the sweet whey medium with 1% different nitrogen sources (yeast extract, malt extract, peptone, ammonium sulphate, ammonium nitrate) caused significant increase in alginate concentration produced by *Azotobacter chroococcum* NCBI MH249629.

Table 2. Effect of different nitrogen sources in sweet whey medium on the production of microbial alginate.

Nitrogen sources (1 g/L)	CDW (g/L)	Alginate conc. (g/L)	Productivity (g/L/h)	Yield (%)	Final pH
0.0	0.815±0.035	0.719±0.004	0.006±0.002	1.59±0.051	7.75
Yeast extract	1.155±0.02	0.984±0.004	0.008±0.003	2.18±0.041	7.32
Malt extract	0.964±0.038	0.945±0.002	0.008±0.003	2.10±0.032	7.50
Peptone	0.897±0.022	0.899±0.026	0.008±0.006	1.99±0.04	7.38
Ammonium sulphate	0.685±0.025	0.667±0.007	0.006±0.006	1.48±0.032	7.64
Ammonium nitrate	0.831±0.048	0.755±0.005	0.006±0.004	1.67±0.051	7.70

* Legend as Table (1).

From the presented data, it could be stated that, using the inorganic nitrogen sources in sweet whey medium such as (ammonium nitrate) give significant more alginate concentration produced (0.755 g/L) by *Azotobacter chroococcum* NCBI MH249629 than that using ammonium sulphate (0.667g/L). Our results are agreeing with (Anyanze 2008) who notice that addition of inorganic nitrogen led to enhance growth of *Azotobacter* spp.,

Fig. (1) represent the highest values of cell dry weight, alginate concentration, productivity and yield were attained in sweet whey medium supplemented with (1.5g/L) yeast extract being (1.155g/L, 0.984g/L, 0.008g/L/h and 2.18%) respectively. **Fig. (2)** represent the highest values of cell dry weight, alginate concentration, productivity and yield were attained in sweet whey medium supplemented with (0.5g/L) ammonium nitrate as sole nitrogen source being (1.323g/L, 1.260g/L, 0.01g/L/h, 2.8%). Addition of (1.5g/L) yeast extract

and (0.5 g/L) ammonium nitrate in sweet whey medium gave higher values of (CDW) and alginate concentration (g/L) compared with other organic or in-organic nitrogen sources.

Increasing yeast extract concentration in the mixture nitrogen source (organic and inorganic nitrogen sources) of productive medium led to increase the cell dry weight, alginate concentration, productivity and yield to reach the maximum values being (1.155g/L, 1.17 g/L, 0.0097 g/L/h & 2.6%)

respectively at 1.5g/L yeast extract after 5 days at 30°C **Fig. (1)**. It might be due to the fact that organic nitrogen provides better available nitrogen for bacterial growth and alginate production. This data sported by the results recorded by (**Alibutt et al 2011**). Also, **Gayathri et al 2012** notice that increasing yeast extract and ammonium nitrate as organic and inorganic nitrogen sources showed highest production of alginate.

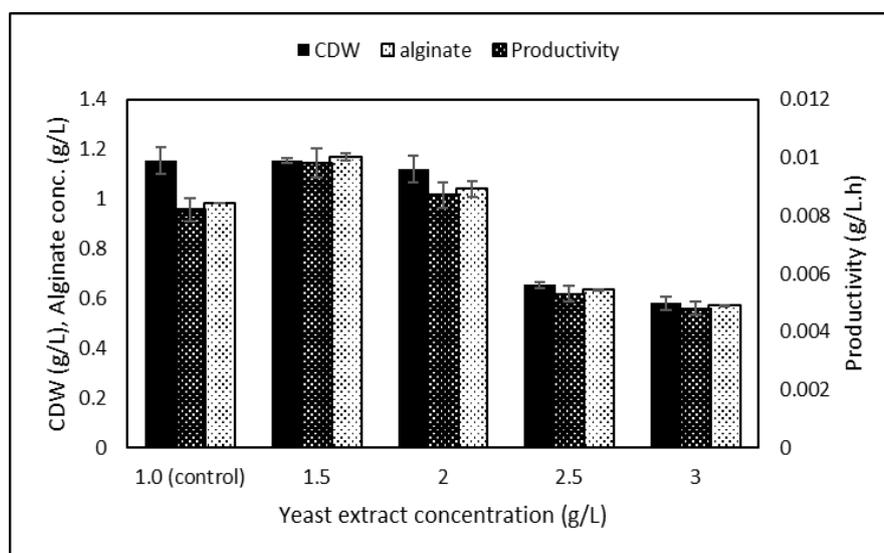


Fig. 1. Effect of different yeast extract concentrations in sweet whey medium on the production of microbial alginate.

* Legend as Table (1).

Effect of different disodium hydrogen phosphate concentrations in sweet whey medium on alginate production by *Azotobacter chroococcum*

Phosphate might simply act to buffer the medium which will tend to become more acidic as alginate is secreted and use a phosphate rich medium (**Brivonese and Sutherland 1989**). The data illustrated by **Fig. (2)** revealed the stimulatory effect of Na₂HPO₄ in the sweet whey medium used for produce microbial alginate by *Azotobacter chroococcum*. The highest cell dry weight, alginate concentration, productivity and yield obtained from sweet whey medium containing Na₂HPO₄ at 1 g/L (2.493g/L, 2.48g/L, 0.021g/L/h & 5.51%) respectively. While increasing the concentration of Na₂HPO₄ up 1% led to significant decrease in cell dry weight, alginate concentration, productivity and yield obtained by *Azotobacter chroococcum*. These

results are in line of those obtained by **Deavin (1976)** and **Deavin et al (1977)**, they stated that the alginate production by a mutant *A. vinelandii* in continuous culture was depended on inorganic phosphate concentration. Also, **Sabra et al (2000)** observed the drop in alginate yield in medium containing excess of phosphate concentration and for their continuous culture studied a phosphate limitation gave maximum alginate production.

Effect of initial pH of sweet whey medium on alginate production by *Azotobacter chroococcum*

Hydrogen ion concentration has a significant influence on industrial fermentation due to much its importance in controlling bacterial growth, fermentation rates and product formation. The variation in growth rate related to the pH presents an optimum value and extreme limits.

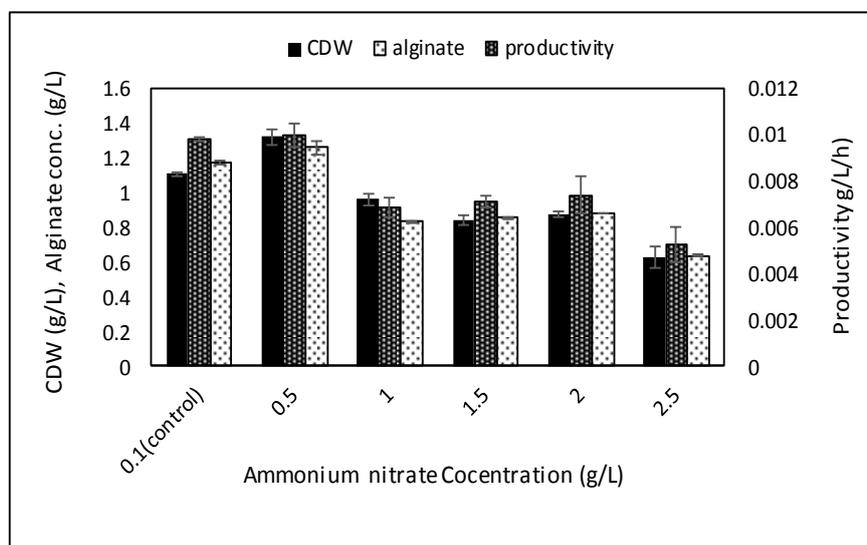


Fig. 2 Effect of different Ammonium nitrate concentrations in sweet whey medium on the production of microbial alginate.

* Legend as Table (1).

The specific pH affected on the molecular weight of the polysaccharide produced (Jeanes, 1977), and affected directly on synthesis of those enzymes responsible for polysaccharide production and biochemical activities of the cell this was within the optimum pH range (6.0 - 7.5) for synthesis of polysaccharides (Lawson and Sutherland, 1978). In this experiment, all nutritional and environmental conditions were adjusted to the best findings in previous experiment, except that the only variable factor was the pH.

Results of alginate production as influenced by initial pH values were illustrated in Fig. (3). Increasing or decreasing the initial pH value of sweet whey medium than 7.0 led to significant decrease in values of cell dry weight and alginate concentration produced by *Azotobacter chroococcum*. These results indicate that control sweet medium at pH 7.0 was the most favorable medium could be used for produce cell dry weight and alginate concentration, productivity and yield (2.489g/L, 2.48g/L, 0.021g/L/h and 5.51%) respectively by *Azotobacter chroococcum*. These results are in agreement with those obtained by Alibutt et al (2011) they noticed that the maximum alginate production by *Azotobacter vinelandii* was attained at pH=7 in shake flasks fermentation. The initial PH of 7.0 was found to be optimum for both growth and polysaccharide production by commercial polysaccharide-producing bacteria and decreased when pH dropped to 5.8, also pH affects the permeability of

the bacterial cell membrane, thus affecting the biochemical activities of the cell required for biopolymer production (Prasertsan et al 2008).

Effect of agitation of sweet whey medium on alginate production by *Azotobacter chroococcum* after 5 days at 30°C

As shown in Fig. (4), it could be found that the maximum CDW, alginate conc., alginate productivity and yield (2.580g/L, 2.774g/L, 0.03g/L/h and 6.2%) respectively were at 200 rpm after 5 days of incubation period on whey production medium fermented by *Azotobacter chroococcum*. Alginate concentration in fermented sweet whey medium by *Azotobacter chroococcum* was increased by increasing the ratio of agitation. This increase may be due to the intensity of agitation influences on the transport of nutrients into cells, increased agitation may increase microbial productivity, due to better mixing and the elimination of the so-called "dead zone". These results agreement with Moral and Sanin (2011) found that the growth rate of *A. vinelandii* ATCC9046 increased from 0.165 to 0.239 h⁻¹ by the increase of mixing speed from 200 to 400 rpm. Whereas, alginate production was found to be the most efficient at 400 rpm with the highest value of 4.51 g/l achieved at the end of fermentation. The viscosity of culture broth showed similar trends to alginate production.

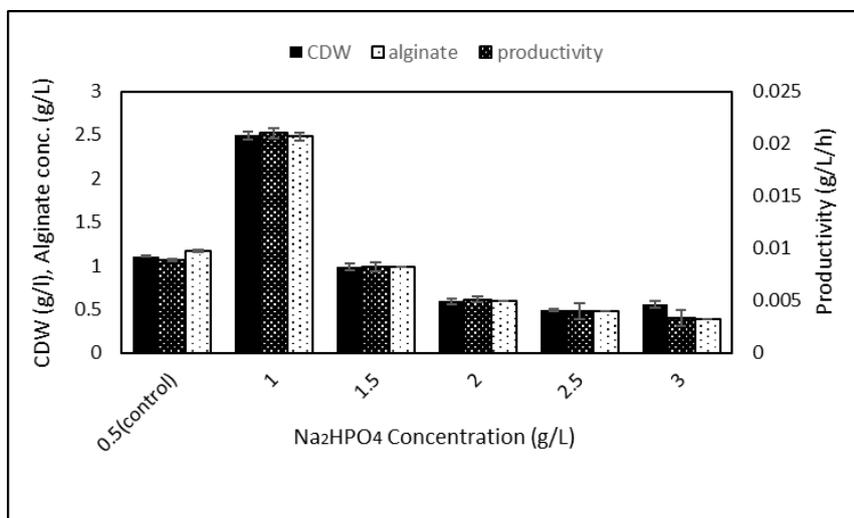


Fig. 3. Effect of different disodium hydrogen phosphate concentrations on alginate production by *Azotobacter chroococcum*

* Legend as Table (1).

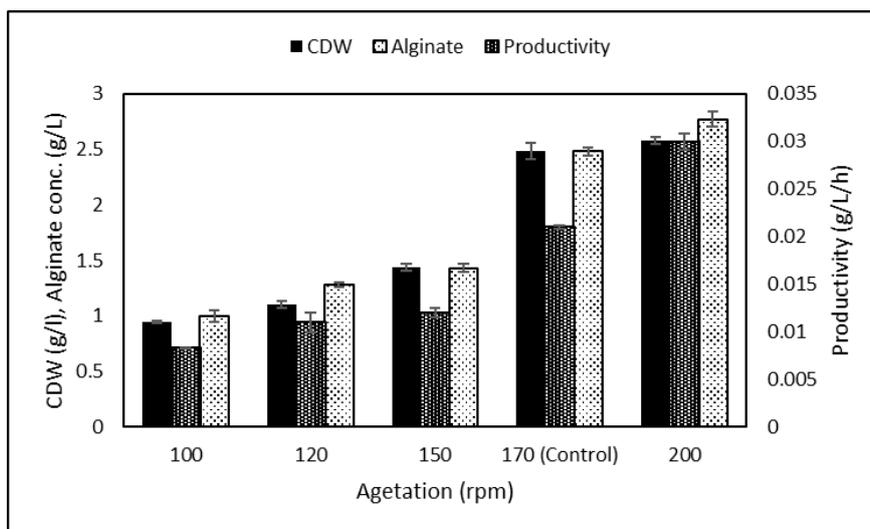


Fig. 4. Effect of agitation of sweet whey medium on alginate production by *Azotobacter chroococcum* after 5 days at 30°C.

Effect of inoculums size in sweet whey medium on alginate production by *Azotobacter chroococcum* after 5 days 30°C

Data in **Table (3)** shows that inoculation with 9 % of standard inoculums *Azotobacter chroococcum* in modified sweet whey medium at 30°C for 5 days on rotary shaker (200 rpm) gave the highest amount of alginate, cell dry weight and productivity of alginate being (3.522 g/L, 3.470 g/L & 0.029 g/L/h) respectively. The inoculums size used by

Alibutt et al (2011) was 2% of *A. vinelandii* NRRL-14641 for maximum alginate production (6.17 gL⁻¹) in optimized medium after 110 h at 30°C.

The size and the physiological conditions of the inoculum have a profound effect on alginate synthesis by *Pseudomonas* and *Azotobacter*. For alginate production in a shaking flask 1-2 vol- % inoculum is transfer to a shake flask (500 ml, baffled) with 100 ml PIA-medium or PIA-medium with reduced salt.

Table 3. Effect of inoculum size on alginate production by *Azotobacter chroococcum* after 5 days 30°C.

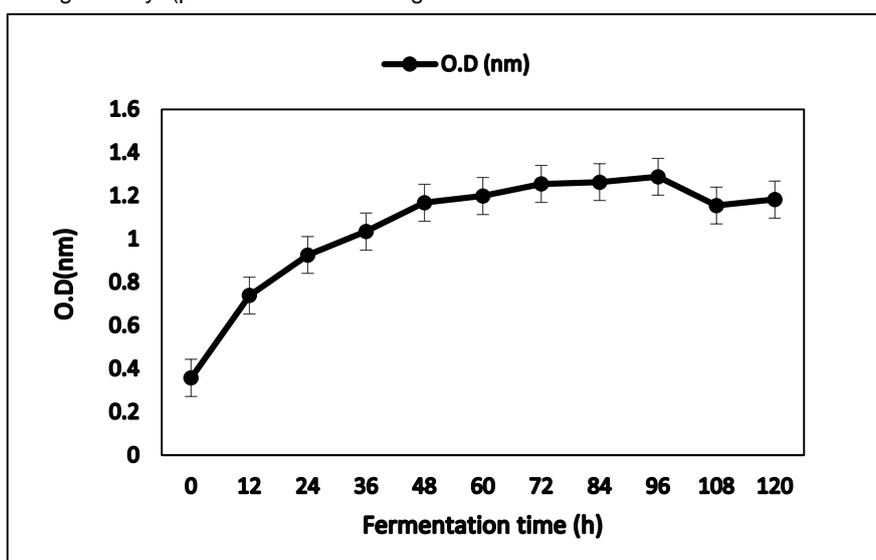
Inoculums size (%)	CDW (g/L)	Alginate con. (g/L)	Productivity (g/L/h)	Yield (%)	Final pH
1	0.927±0.004	0.839±0.006	0.0069±0.0006	1.68±0.09	7.5
2	0.935±0.004	0.946±0.005	0.0079±0.0008	2.10±0.32	7.2
3	0.988±0.005	0.985±0.008	0.0082±0.0006	2.18±0.40	6.9
4	1.039±0.070	1.173±0.030	0.0098±0.0029	2.60±0.30	6.5
5 (Control)	2.486±0.021	2.481±0.053	0.021±0.0053	4.96±0.50	6
6	2.645±0.022	2.530±0.035	0.021±0.0071	5.06±0.10	6.2
7	2.781±0.350	2.690±0.061	0.023±0.009	5.38±0.20	6.7
8	2.843±0.009	2.721±0.044	0.022±0.008	5.44±0.60	6.72
9	3.522±0.090	3.470±0.068	0.029±0.006	6.94±0.60	6.76
10	2.985±0.053	2.991±0.035	0.025±0.0.012	5.98±0.50	6.74

But for alginate production in fermentor 2-3 vol-% inoculum from shake flask is transferred to a 3 liter fermentor (Applicon), with 1.4 liter PM5-medium. The fermentations are performed at 25 °C and pH from start is adjusted to 7.0-7.2 pH. High concentrations of alginate, approximately 20 g/L was obtained (Gimmestad et al 2009).

Effect of fermentation time of sweet whey media on Alginate production by *Azotobacter chroococcum* on whey production medium.

The production of microbial exopolysaccharides has been identified as a potential growth area for the fermentation industry (Sutherland, 2001). Data illustrated by Fig. (5) shows that *Azotobacter chroococcum* MH249629 grow exponentially during the first 6 hrs of incubation, thereafter the growth rate decreased gradually (phase of decelerating

growth during 6-18 hrs) to more constant (stationary phase) during the period of 18 to 72 hrs, then slightly increase during 72 to 120 hrs of incubation. Data illustrated by Fig. (6) shows that cell dry weight was obtained after 72 hrs being 5.654 gL⁻¹ with growth parameters of 9.3 h⁻¹, 13.4 h & 2.7 for specific growth rate (μ), doubling time (t_d) and number of generations (N), calculated during exponential phase respectively. Gradual increase in alginate production was observed during the first 18 hrs fermentation, and then slight increase was attained during stationary phase of growth to record the highest alginate concentration being 5.654 g/L after 72 hrs. Thereafter, alginate production decrease with increasing the fermentation time for 120 hrs. After 72 hrs fermentation period, the alginate yield and productivity were 12.56% and 0.08 g/L/h, respectively.

**Fig. 5.** Effect of growth densities on Alginate production by *Azotobacter chroococcum* on modified whey sweet production medium.

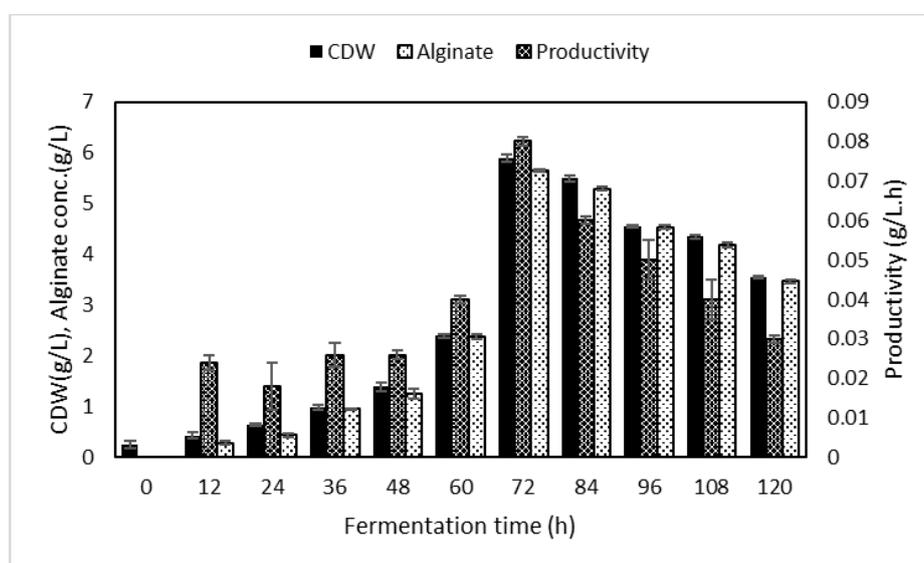


Fig. 6. Effect of fermentation time on Alginate production by *Azotobacter chroococcum* on modified sweet whey production medium.

In similar studies, **Clementi et al (1995)** noticed that the stationary values of *Azotobacter* cell and alginate concentrations were reached after 24 and 36 hrs, respectively. Also, **Peña et al (2011)** stated that pH 7 was the optimum value for alginate production. Also, it could be noticed that the final pH value was increased from 7.0 to 9.74 during the fermentation period.

REFERENCECS

- Agrawal, P.K., Agrawal, S. and Shrivastava, R., 2015.** Modern molecular lar approaches for analyzing microbial diversity from mushroom compost ecosystem. **3 Biotech. 5, 853–866.**
- Aleem, A., Isar, J. and Malik, A., 2003.** Impact of long-term application of industrial wastewater on the emergence of resistance traits in *Azotobacter chroococcum* isolated from rhizospheric soil. **Bioresour. Technol. 86, 7–13.**
- Alibutt Z., Ikram-ul-haq and Abdulqader, M., 2011.** Alginate production by a mutant strain of *Azotobacter vinelandii* using shake flasks fermentation. **Pak. J. Bot., 43 (2), 1053-1067.**
- Andersen, T., Strand, B.L., Formo, K., Alsberg, E., and Christensen, B.E. , 2012.** Alginates as biomaterials in tissue engineering. In "Carbohydrate Chemistry". **Chemical and Biological Approaches. Vol. 37. Rauter, A.P. (ed.). pp. 227–258.** Cambridge, UK: The Royal Society of Chemistry.
- Anyance Prompaphagorn, 2008.** **Algnate production by Azotobacter spp. And its Application in enzyme immobilization.** Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master Science in Biotechnology Suranaree University of Technology Academic.
- Belder, A.N., 2010.** **Dextran, Polysaccharides and their derivatives, 3rd Ed. pp. 417-499,** Academic Press, London, U.K.
- Brown, M.E., Burlingham, S.K. and Jackson, R.M., 1962.** Studies on *Azotobacter* species in soil I. Comparison of media and techniques for counting *Azotobacter* in soil. **Plant Soil 17, 309–319.**
- Butt, Z.A., Ikram-ul-haq and Qadeer, M.A. 2011.** Alginate production by a mutant strain of *Azotobacter vinelandii* using shake flask fermentation. **Pakistan J. Bot. 43(2), 1053-1067.**
- Donati, I., and Paoletti, S., 2009** **Material properties of alginates.** In **Alginates: Biology and Applications. Rehm, B.H.A. (ed.), pp. 1–53.** Berlin, Heidelberg, Germany: Springer Berlin Heidelberg,
- Draget, K., Smidsrød, O. and Skjåk-Bræk, G., 2005.** Alginates from algae. **Biopolymers Online. doi: 10.1002/ 3527600035.bpol6008.**
- Emtiazi, G., Ethemdifara Z. and Habibib M.H., 2004.** Production of extra-cellular polymer in *Azotobacter* and biosorption of metal by exo-

- polymer. *African Journal of Biotechnology*. **3**, 330-333.
- Fu, J.F. and Tseng, Y.H., 1990. Construction of lactose utilizing *Xanthomonas campestris* and production of xanthan gum from whey. *Appl. Environ. Microbiol.*, **56**, 919-923.
- Gauri, S.S., Mandal, K.C., Dey, S. and Pati, B. R., 2009. Enhanced production and partial characterization of an extracellular polysaccharide from newly isolated *Azotobacter* sp. SSB81. *Bioresource Technology*. **100**, 4240-4243.
- Gayathri Pandurangan., Jyothi S., Karunakaran T., and David K.J., 2012. Small Scale Production and Characterization of Alginate from *Azotobacter chroococcum* using different substrates under various stress conditions. *International Journal of Applied Biology and Pharmaceutical Technology*. **3** (1), 40-45.
- George, M.G., Winters M. and Searles D.B. 2001. *Bergey's Manual Systematic Bacteriology 2nd Ed.*, 320 p. Taxonomic outlines of the Prokaryotic Genera, New York, USA,
- Hacking, A.J., Taylor I.W.F., Jarman, T.R. and Govan, J.R.W., 2006. Alginate biosynthesis *Pseudomonas mendocina*. *J. Bacteriol.*, **188**(15), 5551-5560.
- Horton, B.S., 1993. Whey processing and utilization. *Bull. Int. Dairy Fed.*, **279**, 46-49.
- Konicek, J., Lasik, J. and Safar, H., 1993. Production of exopolysaccharide by a new mutant strain of *Achromobacter delicatulus* using whey fermentation. *Folia Microbio.*, **38**, 68-70.
- Lee, K.Y. and Mooney, D.J. 2012. Alginate: properties and biomedical applications. *Prog Polym Sci.*, **37**, 106-126.
- Looijesteijn, P.J., Trapet, L., de Vries, Abee E., , T. and Hugenholtz, J., 2001. Physiological function of exopolysaccharides produced by *Lactococcus lactis*. *Int. J. Food Microbiol.*, **64**, 71-80.
- Painter, P.R. and Marr, A.G., 1963. Mathematics of microbial populations. *Annual Rev. Microbiol.*, **22**, 219 - 221.
- Pena, C., Galindo, E. and Buchs, J., 2011. The viscosifying power, degree of acetylation and molecular mass of the alginate produced by *Azotobacter vinelandii* in shake flasks are determined by the oxygen transfer rate. *Process Biochem.* **46**, 290-297.
- Prasertsan, P., Wichienchot S., Doelle H., and Kennedy J. F., 2008. Optimization for biopolymer production by *Enterobacter cloacae* WD7. *Journal of Carbohydrate Polymer.*, **71**, 468-475.
- Ricciardi, A. and Clementi, F., 2002. Exopolysaccharides from lactic acid bacteria: Structure, production and technological applications. *Italian Journal of Food Science*, **1**, 23-45.
- Sabra, A., Zeng, P., Lonsdorf, H. and Deckwer, W.D., 2000. Effect of oxygen on formation and structure of *Azotobacter vinelandii* alginate and its role in protecting nitrogenase. *Appl. Environ. Microbiol.* **66**, 4037-4044.
- Schwartz, R.D. and Bodie, E.A., 1985. Production of high viscosity whey broths by lactose utilizing *Xanthomonas campestris* strain. *Appl. Environ. Microbiol.*, **50**, 1483-1485.
- Soto-Urzuá, L. and Baca, B.E., 2001. Mechanisms for protecting nitrogenase from inactivation by oxygen. *Rev. Latinoam Microbiol.* **43**, 37-49.
- Stanier, R. Y.; M. Doudoroff and E. A. Adelberg (1970). *General Microbiology* (3rd Ed.), pp. 302-306. Macmillan & Co. Ltd. London.
- Steel, R.G. D., Torrie J.H. and Dickey D.A., 1997. *Principles and Procedure of Statistics. A Biometrical Approach 3rd Ed.* McGraw Hill Book Co. Inc., New York. pp. 352-358.
- Thomas, S., 2000. Alginate dressings in surgery and wound management-Part 1. *J. Wound Care* **9**, 56-60.
- Vargas-García, M.C., López, M.J., Elorrieta, M.A., Suárez, F. and Moreno, J., 2002. Physiology of exopolysaccharide production by *Azotobacter vinelandii* from 4- hydroxybenzoic acid. *J. Ind. Microbiol & Biotechnol.* **29**, 129-133.
- Vela, G.R., Rosenthal, S., 1972. Effect of peptone on *Azotobacter* morphology. *J. Bacteriol.* **111**, 260-266.
- Williams, S.T. and Davies F.L., 1965. Use of antibiotics for selective isolation and enumeration of actinomycetes in soil. *J. Gen. Microbiol.*, **38**, 251-262.
- Zadow, J.G., 1987. Whey production and utilization in Oceania. *Bull. Int. Dairy Fed.*, **212**, 12-16.
- Zadow, J.G., 1986. Utilisation of milk components: Whey. In: Robinson, R.K. (Ed), *Modern dairy technology, Advances in milk processing.* *Appl. Sci.*, **1**, 273-317.
- Zadow, J.G., 1992. Review and report on whey utilization. A report for the dairy research and development. *Bull. Int. Dairy Fed.*, **314**, 21-23.



ضبط الظروف المثلى لإنتاجية الألبينات في بيئة الشرش المنتجة بواسطة ميكروب *Azotobacter chroococcum*

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- ولاء سعيد هنداوي^{*1} - يوسف مرسى الكنانى² - أسامة إبراهيم البطاوي² - طارق محمود المنجى¹
1. قسم الميكروبيولوجي - المركز القومي لبحوث وتكنولوجيا الإشعاع - هيئة الطاقة الذرية - ص.ب 29 - مدينة نصر -
القاهرة - مصر
2. قسم علوم الأغذية - كلية الزراعة - جامعة عين شمس - ص.ب 68 - حدائق شبرا 11241 - القاهرة - مصر

*Corresponding author: walaahendawy78@gmail.com

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الموجز

ومعدلات تلقيح مختلفة (1، 2، 3، 4، 5، 6، 7، 8، 9 و 10%) حيث تم قياس تركيز الألبينات والمحصول الناتج في نهاية فترة التخمر وكانت النتائج المتحصل عليها كالآتي: حيث سجلت أعلى إنتاجية للألبينات (8،، جم/لتر/ساعة) وأعلى محصول (12,56%) وذلك باستخدام تركيز 45 جم/لتر من اللاكتوز كمصدر للكربون وإضافة (1,5 جم/لتر) من مستخلص الخميرة (1،، جم/لتر) نترات الأمونيوم وإضافة داي صوديوم هيدروجين فوسفات بتركيز (1 جم/لتر) كمصدر للأملاح المعدنية وذلك على pH 7 ونسبة التلقيح 9% والتحصين على 30°م/72 ساعة.

الكلمات الدالة: إنتاج الألبينات، الشرش الحلو، أزوتوباكتر كروكوكوم، ظروف التخمر

تم استخدام الشرش الحلو لإنتاج الألبينات الميكروبية بواسطة ميكروب *Azotobacter chroococcum* NCBI MH249629 وذلك للحصول على أعلى إنتاج بعد ضبط ظروف الإنتاج وذلك باستخدام بيئة الشرش المحتوية على تركيزات مختلفة من اللاكتوز (20،25، 30، 35، 40، 45، 50 جم/ لتر) وإضافة مصادر نيتروجينية عضويه وغير عضويه (مستخلص الخميرة، مستخلص المولت، نترات الأمونيوم، كبريتات الأمونيوم)، وذلك على درجات حرارة مختلفة (25، 30، 32، 35 و 37°م) ودرجات مختلفة من الأس الهيدروجيني (4، 5، 6، 7، 8 و 9) وسرعات تقليب مختلفة (100، 120، 150، 170 و 200rpm)