Production of Alginate by Different Isolates of *Azotobacter* species.

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Abstract: Ten *Azotobacter chroococum* isolates Were isolated from different soil sample collected from El-Khurma Governorate. KSA.The highest alginate yield on production media were obtained by *Azotobacter* Isolates n.1 and n.8. Biomass and alginate production by Azotobacter isolates were studied under shake flasks and fermentor as a batch culture. The optimum conditions to enhancement of biomass and alginate production by the two isolates were investigated. The highest biomass and alginate yield was obtained in about 5days incubation period on optimum medium at 28°C and 170 rpm shaking speed. The viscosity of the culture broth was 99 and 91 Cp. and the alginate concentration reached 3.4 g/l and 2.8 g/l by *Azotobacter* n.8 and n.1 respectively. The alginate production by *Azotobacter* n.1 and n.8 in batch culture under optimal conditions was studied using 3-L stirred tank bioreactor using *Azotobacter* n. 8.on producing media with 1.0% potato starch as a carbon source and 0.8 corn steep liquor as a nitrogen source. The maximal alginate productivity and yield % were 102.78 and 37.0% at 600 rpm after 42 hr incubation period at 28°C under controlled pH culture at 7.0.
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Key words: Alignate Production, *Azotobacter* sp., carbon sources, Nitrogen sources and Fermentation process.

1. Introduction:

Alginate is negatively charged biopolymer and presence in viscous solution form as solution (in absence of divalent cations) or in gel like structure (in the presence of divalent or trivalent cations) alginates are linear polysaccharides composed of (1– 4)-β-D-mannuronic acid and its C-5-epimer α-Lguluronic acid**. Auhim and Shatha, (2013).** With common molecular formula (C6H8O6)n with a molar mass varied between 10,000 and 600,000. **(Charles *et al.*, 2012).**

The importance of alginates is based on their wide industrial applications as thickening agents, stabilizers, gelling agents and emulsifiers in food, textile, paper making industries **(Brownlee *et al.*, 2005).** In addition, alginates are widely used as micron capsulating agent for probiotics to increase their viability during process and application as food additives **(Krasackoopt *et al.*,2006 and Kim *et al.*, 2008).** As alginates are known as biocompatible, biodegradable and safe biopolymers, thus they are widely used in many pharmaceutical and medical applications as anti-inflammatory agent, radioactive suppressive agent and wound healing **(Mirshafiey *et al.*, 2007 and 2009).** Moreover, based on chemical structure of alginate of abundance of free hydroxyl and carboxyl groups distributed along the polymer chain backbone, different modifications of these two active groups were conducted to improve their chemical and physical properties for more applications **(Yang *et al.*, 2011).** On the wide applications of alginates, the worldwide annual industrial production is estimated to be 30,000 metric tons in 2009 **(Donati *et al.*, 2009).**

Two genera of bacteria have been shown to secrete alginate, *Pseudomonas* and *Azotobacter*. Most of the research into the molecular mechanisms behind bacterial alginate biosynthesis has been conducted on the opportunistic human pathogen *Pseudomonas aeruginosa* or the soil dwelling *Azotobacter vinelandii*. Although these two genera utilize very similar molecular mechanisms to produce alginate, in nature, they secrete alginate for different purposes with different material properties: Some *P. aeruginosa* strains (known as mucoid strains) can secrete copious amounts of alginate to aid in the formation of thick highly structured biofilms **(Hay *et al.*, 2009 and 2013),** whereas *Azotobacter* produces a stiffer alginate (with typically a higher concentrations of G residues) which remains closely associated with the cell and allows the formation of desiccation resistant cysts **(Sabra and Ping Zeng., 2009).**

This study was undertaken to screening alignate production by different isolates of *Azotobacter chroococum* and to investigate the different nutritional factors in addition to study kinetics of cell growth and alginate production under optimized conditions using 3-L stirred tank bioreactor, cultivation process.

2-Material and Methods

2-1- Microorganisms

Azotobacter Strains were isolated from different soil sample collected from El- Khurma Governorate. KSA. And purified using the soil dilution plate technique described by **(Williams and Davies, 1965).**

All strains were identified according to **Bergye's Manual of Systematic bacteriology. (George *et al.*, 2001).**

2-2- Media used

2-2-1- Ashbys Mannitol Agar M706 **(Subba Rao., 1977).**

Ashbys Mannitol Agar is used for cultivation of *Azotobacter* species that can use mannitol and atmospheric nitrogen as source of carbon and nitrogen respectively. This medium has the following composition (g/L):

Mannitol, 20; Dipotassium phosphate, 0.20; Magnesium sulphate, 0.20; Sodium chloride, 0.20; Potassium sulphate, 0.10; Calcium carbonate, 5; Agar,15. Final pH (at 25°C) 7.4±0.2

Sterilization was carried out at 15 lbs pressure (121°C) for 15 minutes.

2-2-2- The standard production medium was that proposed by **(Clementi et al., 1995)**. It has the following ingredients (g/L): It has the following ingredients (g/L): Glucose, 20; $(NH4)_2$ SO4.,6; Na_2HPO_4. 2.0 MgSO4. $7H_2$. 3 yeast extract, 6.0 Final pH (at 25°C) 7.0

Sterilization was carried out at 15 lbs pressure (121°C) for 15 minutes.

2-3- Optimization of biomass and alginate production.

2-3-1-Growth conditions:

Fifty ml portion of the production medium were dispensed into 250 ml cotton plugged Elenmeyer flasks. Each flask was inoculated with 1 ml (standard inoculum contained $1x10x^7$ cells) and incubated at 28±2 ^oC on a rotary shaker incubator at 170 rpm for 5 days Shake flasks**.**

2-3-2- Factors affecting on biomass and Alginate production.

A) The shake flask:

The shake flask system was used to study the effect of different nutritional and environmental conditions on alginate production by Azotobacter isolates. Modifications of standard medium were carried out including the replacement of the original carbon source (on carbon- basis) with different carbon sources (mannose, glucose, galactose, fructose, sucrose, maltose, mannitol, glycerol, sorbitol, sodium pyruvate. Sodium succinate or sodium citrate). The effect of different concentrations of yeast extract (0.1, 0.2, 0.3, 0.4, 0.5, 0.6 and 0.7%), and Na_2HPO_4 (0.05, 0.1,0.15, 0.2, 0.25 and 0.3%) were also investigated. The carbon source was replaced by different carbonic raw materials namely rice starch, maize starch and potato starchy waste (0.2, 0.5 and 1% concentrations). Finally the nitrogen source was replaced by corn steep liquor as raw material in concentrations of 0.2, 0.4, 0.6, 0.8 and 1.0%.

B) Batch fermentor:

Three liters dished bottom fermentor (Cole-parmer Instrument) was used. The production medium with standard inoculum was added to the fermenting vessel to give a final working volume of 2 liters. Culture was 100% aeration and pH was adjusted by automatic pH controller at 7.0, temperature was kept at 28 ± 2^oC during batch cultivation. Two agitation speeds were compared with respect to alginate production i.e 300 or 600 rpm. The system was modified to evaluate alginate production using the best concentration of the selected raw material under such stabilized cultural conditions.

The fermentation process was used to study the effect of potato starchy waste 1 % at agitation 300 or 600 rpm. Finally, the nitrogen source was replaced by corn steep liquor (0.8%) at agitation speed of 600 rpm.

2-4-Analytical methods:

2-4-1- Biomass net weight:

An inoculums of 2.5%(v/v) of isolates was inoculated into 250 ml conical flasks, containing 100ml of **(Clementi *et al.*,1999)**. All flasks were incubated at 28 °C. After 72 hrs cells were harvested by centrifugation at 5000x for 5 min. The cells were dried at 70 °C for 48 hrs. Then, cells were weighted and calculated per 1 liter.

2-4-2- Determination of glucose:

Residual sugar (as glucose) was determined enzymatically using glucose oxidase kits according to the method of **(Trinder. 1969).**

2-4-3- Determination of Alginate viscosity:

It was determined viscometerically using Cole Parmer rotional viscometer with spindle No.5 and 60 rpm speed. The reading was expressed as centipoises (Cp.).

2-4-4- Alginate determination:

For quantitative determination of alginate samples were diluted four times with distilled water and then centrifugated at 11000 rpm at 4^oC for one hrs by using a refrigerated centrifuge. Precipitate which contain bacterial cells and capsular material was suspended in 5 mM ethylenediamine tetraacetic acid (EDTA) for 2 min to solubilize the cell associated alginate, and then centrifuged at 11000 rpm for 30 min. The supernatants were then recovered (containing both the exopolysaccharide and the solubilized capsular material) and separately treated three times with cold 95% vol/vol ethanol **(Page and Sadoff, 1976).** After centrifugation at 9500 rpm at 4^oC for 30 min. both precipitates were dried at 90^oC until reaching a constant weight and used to express the alginate concentration in grams per liter of culture broth.

3- Results And Discussion

Ten *Azotobacter chroococcum* were isolated from different soil samples collected from El- Khurma Governorate. KSA. and purified using the soil dilution plate technique described by **(Williams and Davies, 1965)**.

Selection of Azotobacter isolates for biomass and alginate Production.

Medium composition has great influence on the production of alginate and the growth of the microorganism. The components of the medium provide nutritional requirement for the optimal growth and polymer production by the bacteria **(Clementi *et al.*, 1995). (Alibutt *et al.*, 2011).**

Data in **fig: (1)**. showed that *A. chroococum* isolates n. 1 and n.8 exhibited the highest biomass and alginate production.

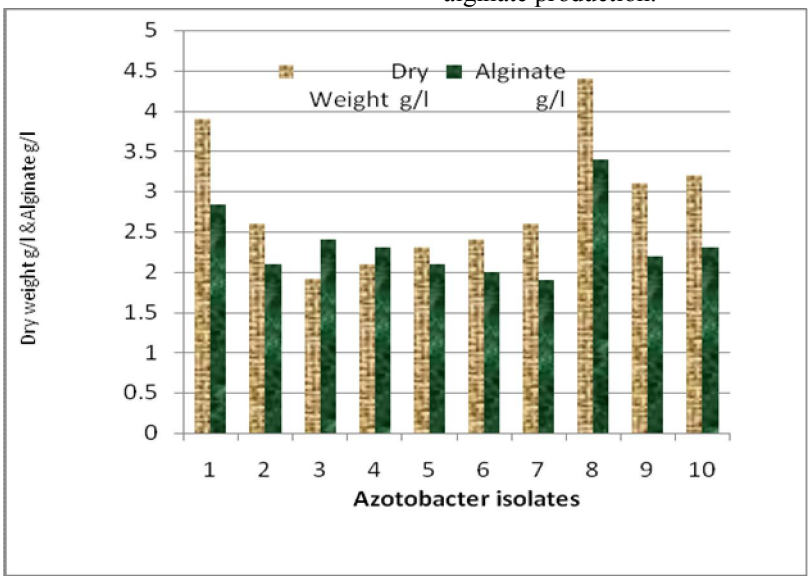

Fig.(1) Dry weight and alginate production by ten *Azotobacter chroococcum* on production medium.

Effect of different carbon sources on biomass and Alginate production.

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 culture to gain good production **(**Okabe et al., 1981., Clementi et al., 1999., Moreno et al., 1999 and Alibutt et al., 2011). In the present study**,** the Effect of different carbon sources (mannose, glucose, galactose, fructose, sucrose, maltose, mannitol, glycerol, sorbitol, sodium pyruvate. Sodium succinate or sodium citrate). were examined for alginate production.

Glucose proved to be the best carbon source and maximum alginate production (2.84 g/l and 3.4 g/l) and biomass g/l (3.9 g/l and 4.3 g/l) for *Az. chroococum* Isolates n.1 and n.8 respectively. **Figs: (2-A &2-B)**.

Emtiazi *et al.*, (2004) showed that *Azotobacter* AC2 produced maximum

alginate (7.5 mg/mL) in media with sucrose as the only carbon source while *Azotobacter chroococum* 1723 was able to produced exopolysaccharide greater than 5 mg/mL when used lactose as carbon source. **Saude and Junter, (2002)** revealed that polysaccharide was produced in after organism had entered the stationary phase. This confirms other results showing that alginate production by *A. vinelandii* is partially associated to growth.

The effects of carbon sources (1%) on alginate production from *Azotobacter* sp. showed that trehalose and sucrose gave similar alginate yield (156.67 and 206.67 g/mol, respectively) that had apparent110 and 295 cP, respectively. The corresponding cell viable counts were 1.25x108 and 9.1 x 107 cfu/mL in 72 h and at 30oC. A reduction of alginate production and the growth of *Azotobacter* sp. were decreased when sucrose concentration was greater than 1% (v/v). The alginate production and bacterial cell growth were increased with an increase of glucose concentration for 1% (w/v) **(Anyanee, 2008).** It might be due to the reason that sucrose is readily metabolized and easily employed by the bacteria **(Asami *et al.*, 2004).** Other carbon sources might be slowly metabolized by the bacteria.

Prasertan *et al.* (2008) reported that a high concentration of sucrose in the medium inhibits the growth of *Azotobacter* sp. and the production of alginate, and the inhibitory effect is attributed to high osmotic pressure.

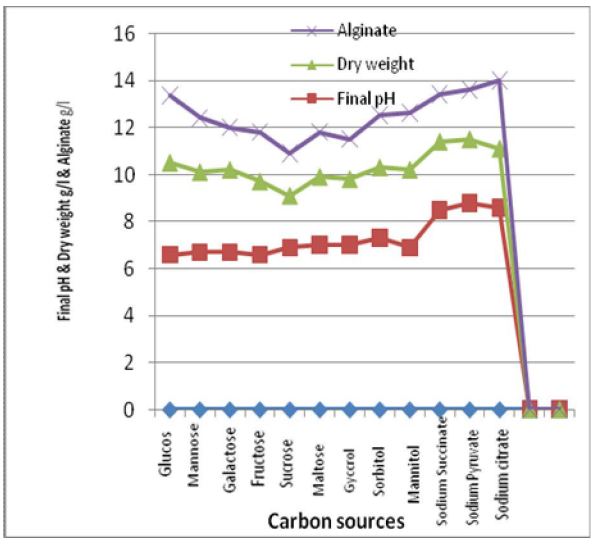

Fig: (2-A). Effect of different carbon sources on Dry weight g/l and Alginate production g/l by *A. chroococum* n.1. after 5 days incubation using shake flasks.

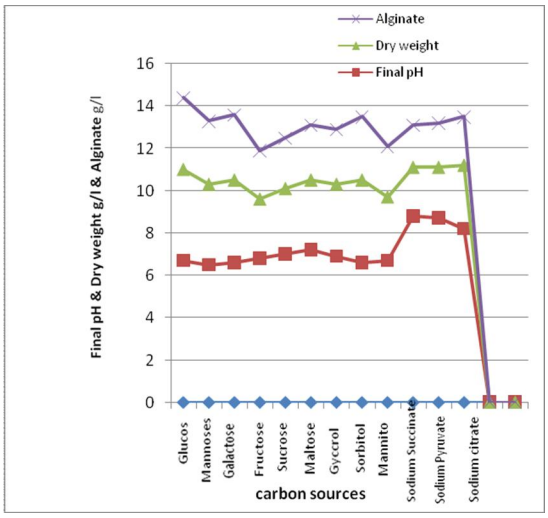

Fig: (2 - B). Effect of different carbon sources on Dry weight g/l and Alginate production g/l by *A. chroococum* n.8. after 5 days incubation using shake flasks.

Table (1): Effect of different concentration of Yeast extract on alginate production gl-1by Aztobacter isolates after 5 days incubation period using shake flasks as a batch culture.

Concentration of yeast extraet %	Az. Isolate (1)						Az. Isolate (8)					
	Cell dry weight (gl-1)	Alginate (g-1)	Y%	Y/c/x (mgg-1 dry cell)	Content%	P (mgl-1 h-1)	Cell dry weight (gl-1)	Alginate (g-1)	Y%	Y/c/x (mgg-1 dry cell)	Content%	P (mgl-1 h-1)
0	2.2	1.9	9.5	863.64	86.36	15.83	2.7	2.1	10.5	777.78	77.78	17.50
0.1	2.6	2.4	12.0	923.08	92.31	20.0	2.9	2.4	12.0	827.59	82.76	20.00
0.2	2.8	2.5	12.5	892.86	89.29	20.83	3.0	2.7	13.5	900.00	90.00	22.50
0.3	3.1	2.9	14.5	935.48	93.55	24.17	3.1	2.9	14.5	935.48	93.55	24.17
0.4	3.3	3.1	15.5	939.39	93.94	25.83	3.7	3.3	16.5	891.89	89.19	27.50
0.5	3.6	3.2	16.0	888.89	88.89	27.67	3.9	3.9	19.5	1000.0	100.00	32.5
0.6 (control)	4.2	3.8	19.0	904.76	90.48	31.67	4.9	4.5	22.5	918.37	91.84	37.50
0.7	3.9	3.5	17.5	897.44	89.74	29.17	4.4	4.1	20.5	931.82	93.18	34.17
0.8	3.5	3.1	15.5	885.71	88.57	15.83	4.0	3.8	19.0	950.0	95.00	31.67

Table (2): Effect of different concentration 0f Corn steep liquor waste on alginate production gl-1by Aztobacter isolates after 5 days incubation period using shake flasks as a batch culture.

Concentration of Corn steep liquor waste %	Az. Isolate (1)						Az. Isolate (8)					
	Cell dry weight (gl-1)	Alginate (g-1)	Y%	Y/c/x (mgg-1 dry cell)	Content%	P (mgl-1 h-1)	Cell dry weight (gl-1)	Alginate (g-1)	Y%	Y/c/x (mgg-1 dry cell)	Content%	P (mgl-1 h-1)
0.2	1.9	1.8	9.0	947.37	94.74	15.0	3.10	2.7	13.5	870.97	87.10	22.5
0.4	3.00	2.3	11.5	766.67	76.67	19.17	3.72	3.2	16.0	860.22	86.02	26.67
0.6	3.86	3.4	17.0	880.83	88.08	28.33	5.2	4.9	24.5	942.31	94.23	40.83
0.8	5.56	5.1	25.5	917.27	91.73	42.50	6.39	6.1	30.5	954.62	95.46	50.83
1.0	5.1	4.6	23.0	901.96	90.20	38.33	5.5	5.00	25.0	909.09	90.91	41.67

Effect of different concentration of Organic nitrogen source on biomass and Alginate production:

Different concentrations of organic nitrogen sources (Yeast extract and Corn steep liquor at concentrations of 0.0,0.2,0.4,.06,0.8and 1.0 %) were also evaluated for alginate production.

Tables (1&2) Represented that Yeast extract at 0.6 % and C.S.L. at 0.8% gave the better Alginate productivity (31.67$gl^{-1}h^{-1}$ & 37.5067$gl^{-1}h^{-1)}$ yield % of

Alginate (19.0 g% & 22.5 g%) &(42.50 $gl^{-1}h^{-1}$ & 50.83 $gl^{-1}h^{-1}$) yield % of Alginate (25.5 g%& 0.5 g%) by *A. chroococum* isolates n.1 and n.8 respectively. It might be due to the fact that organic nitrogen provides better available nitrogen for the bacterial growth and alginate production. This data sported by the results recorded by **(**Savalgi, 1992., Garcia et al., 2001., Khanafari and Sepahei, 2007and Alibutt et al.,2011)

Also **Anyanee, (2008).** notice that addition of inorganic nitrogen led to enhance growth of *Azotobacter* sp. Where the highest value was found in the presence of 0.5-2% (w/v) of DAP ($2.15x10^7$, $2.31x10^8$, $5.3x10^9$ and $9.6x10^9$ cfu/mL, respectively), and 0.5% (w/v) NH4H2PO4 ($8.15x10^9$) when compared LG medium (without addition of nitrogen). Adding NH_4Cl has effect on alginate production and viscosity. **Gayathri *et al.* (2012)** notice that increasing yeast extract and ammonium nitrate as organic and inorganic nitrogen sources showed production as high and least respectively.

But **Emtiazi *et al.* (2004)** showed that addition of vitamin, different nitrogen sources (Ammonium salts, yeast extract and peptone) did not effect exopolymer production in *Azotobacter* spp.

Effect of phosphate concentrations:

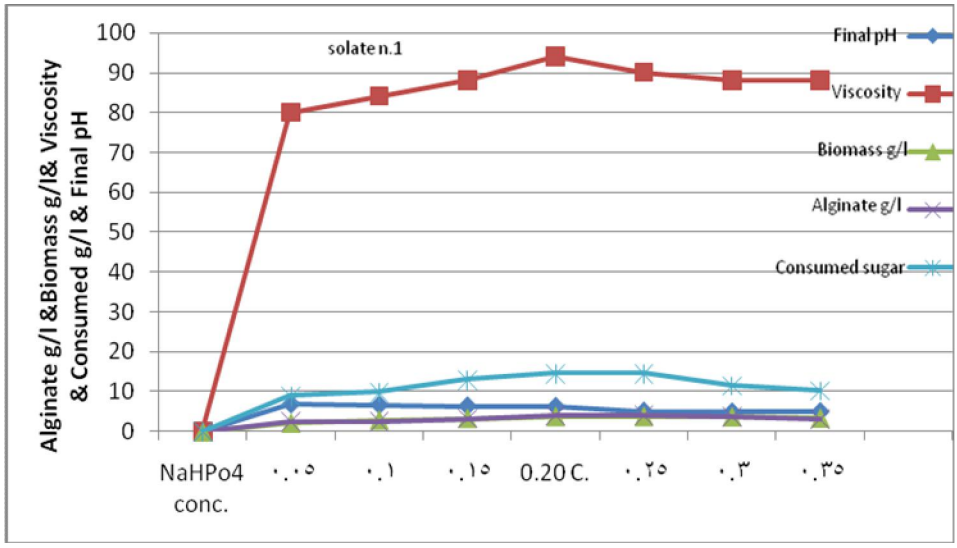

Fig: (3 - A). Effect of different concentrations of NaHPo4 on Biomass g/l and alginate production g/l after 5 days incubation using shake flasks.

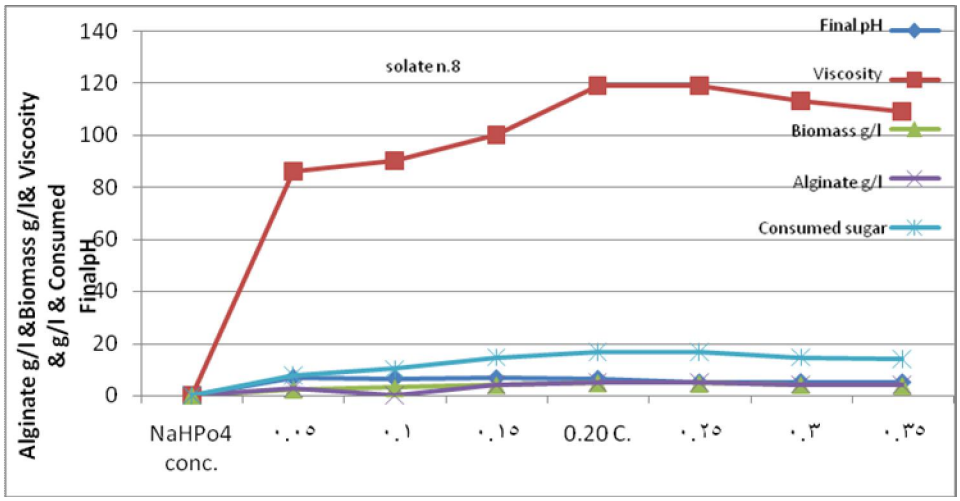

Fig: (3 - B). Effect of different concentrations of NaHPo4 on Biomass g/l and alginate production g/l after 5 days incubation using shake flask.

Data in **Figs: (3- A & 3- B)**. Show that in shake flasks experiments the maximum biomass and alginate production in shake flasks were obtained at0.20 % phosphate concentration (control) which resulted in (4.10 g/l & 5.10 g/ l) and (3.9 g/l & 4.8 g/l) by *A. chroococum* isolate n.1 and n.8 respectively. The final pH and Viscosity were also determined at the same phosphate concentration (20 %) the final pH was (6.4 & 6.5) then it decrees reached to minimum value 5.0 & 5.2 at 0.3 % phosphate concentration by *A. chroococum* isolate n.1 and n.8 Viscosity that indicate Alginate production increase gradually reaching the highest value (94 Cp.& 119 Cp.) at 20% phosphate. In general, the best alginate production was obtained at the highest phosphate concentration in shake flasks., these pronounced differences may be due to the heterogeneous pO2 profile during the fermentation run in flasks.

The obtained results is parallel with the results recorded by **(Sabra *et al.*, 1999).**

Effect of some Raw materials on Biomass and Alginate production:

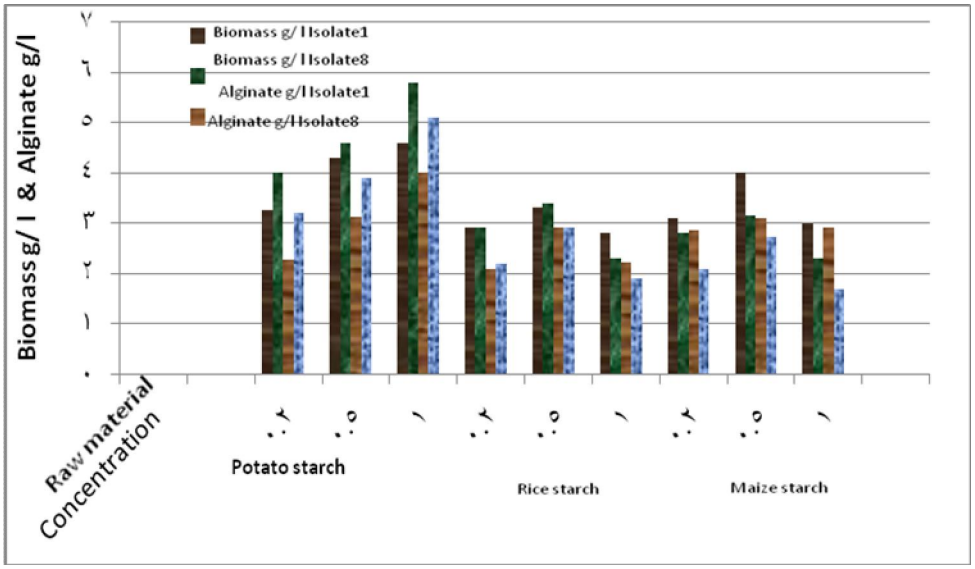

Fig: (4- A). Effect of different raw material concentrations on Biomass g/ l and Alginate /l by Azotobacter isolates after 5 days incubation period at 28°C. using shake flasks.

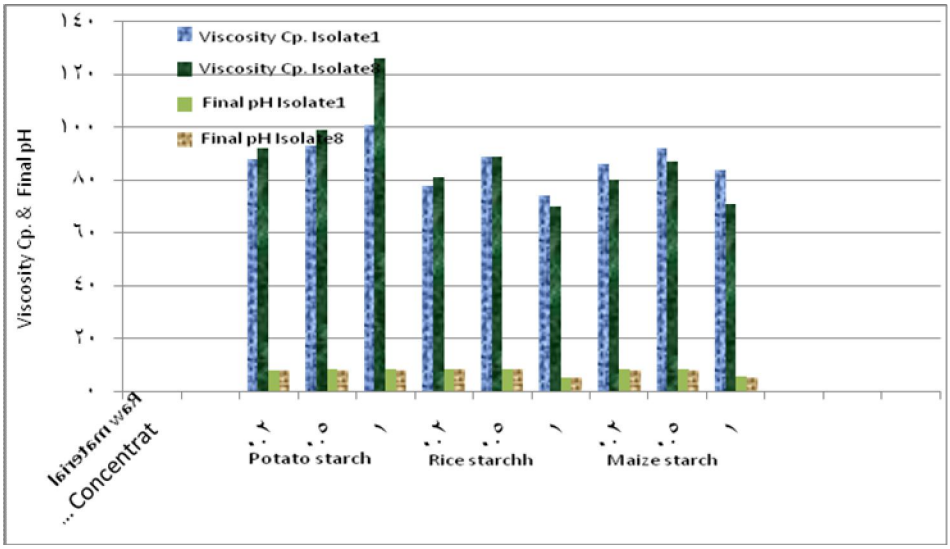

Fig: (4-B). Effect of different raw material concentrations on Viscosity Cp.and final pH by Azotobacter isolates after 5 days incubation period at 28°C.using shake flasks..

Table (3): Effect of different concentration 0f Potato starch waste on alginate production $gl^{-1}$by Aztobacter isolates after 5 days incubation period using shake flasks.

Concentration 0f Potato starch waste %	Az. Isolate (1)						Az. Isolate (8)					
	Cell dry weight (gl^{-1})	Alginate (g^{-1})	Y%	$Y_{c/x}$ (mgg^{-1} dry cell)	Content%	P (mgl^{-1} h^{-1})	Cell dry weight (gl^{-1})	Alginate (g^{-1})	Y%	$Y_{c/x}$ (mgg^{-1} dry cell)	Content%	P (mgl^{-1} h^{-1})
0.5	4.26	3.10	15.5	727.70	72.77	25.83	4.10	3.29	16.45	802.44	80.24	27.72
1.0	5.10	4.90	24.5	960.78	96.08	40.83	5.70	5.26	26.30	922.81	92.28	43.83
1.5	5.11	4.70	23.5	782.78	78.28	39.17	5.60	5.19	25.95	926.79	92.68	43.25
2.0	4.80	4.30	21.5	895.83	89.58	35.83	5.00	4.81	24.05	962.00	96.20	40.08
2.5	5.00	4.40	22.0	880.00	88.00	36.67	4.92	4.72	23.60	959.35	95.94	39.3
3.0	4.72	4.10	20.5	868.64	86.86	34.17	4.70	4.65	23.25	989.36	98.94	38.75

Effect of raw materials such (potato starch & rice starch & Maize starch at concentrations (0.2 & 0.5 & 1.0%) on biomass & alginate production & viscosity and final pH by *A. chroococum* isolates n.1 and n.8 are shown in **Figs. (4- A & 4-B).** In general the 1.0 % potato starch gave the highest biomass and alginate production being 2.0 folds than other raw materials. **Gayathri *et al.* (2012)** recorded that *Azotobacter chroococcum* is regarded as an efficient alginate biopolymer producer when subjected to different fermentation substrates of carbon sources like whey, butanol, molasses and glucose re. The biopolymer production was minimum in mannitol broth (38.74%), while maximum value was obtained when butanol (47.13%) and whey (45.15%) were used as substrates.

Viscosity increasing from 88 Cp. & 92 Cp. For n.1 and n.8 reaching the maximum value 101 Cp. & 128 Cp. at the same treatment, whoever pH decrease gradually from 8.0 to 5.1 for both isolates on rice starch and maize starch, respectively. The obtained results is parallel with the results recorded by **(Charles *et al.*, 2012).**The pH profile of uncontrolled culture clearly observed that the pH value was dropped gradually during cultivation from 7.1 (initial) to almost 3.0 after 20 hours. It is well known that Azotobacter can grow over a wide range of pH.

Also, different concentrations of potato starch on alginate production by Azotobacter isolates, showed that alginate productivity and yield % were 40.86 $mgl^{-1}h^{-1}$ &43.83$mgl^{-1}h^{-1}$ and 24.50 % & 26.30%. **Table (3).**

Effect of Agitation speed on Biomass and Alginate production:

Data illustrated in **Table (4)** clearly show that, the maximum alginate productivity & alginate yield % and alginate yield coefficient were obtained by *A. chroococum* isolate n.8 after 42 hrs incubation period at 1% potato starch as a carbon source with agitation speed 600 rpm and control pH at 7.0 being 102.87 $mgl^{-1}h^{-1}$ & 37.00% and 973.68 mg g^{-1}dry cell respectively. **Anyanee, (2008)** reported that alginate formation was partially growth associated due to both alginate concentration, and growth cell sharply increased as stirrer speed increased at 100-500 rpm. and slightly increased when up to 500 rpm within 24 h. Since the intensity of agitation influences 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." Both alginate and biomass concentrations increased with increasing the agitation speed till 600 rpm but beyond this value both alginate and biomasses decreased sharply till 1,000 rpm. By excess turbulence, it is most likely that the decreased biomass and alginate production may be due to damaging cell membranes and limited mass transfer in localized zones **(Toma *et al.*, 1991)**. The growth of *A. vindlanii* under diazotrophic conditions was always accompanied by the presence of alginate capsule around the cell. However, this layer was easily removed upon shaking and hence no mass transfer resistance in sample take from the agitated fermenter occurred **(Peters *et al.*, 1989and Lobas *et al.*,1992).** Additionally, at higher agitation intensity, the alginate capsular material was rich in gulronic acid forming harder gel resistant to dissolution. Moreover, this capsule layer was not decreased in thickness with the increase in agitation speed. Increased agitation or shaking speed was frequently used by many authors for optimizing the aeration rate for alginate production by *A. vindlanii* **(Jarman *et al.*, 1978; Jarman, 1979; Annison and Couperwhite, 1984; Brivonese and Sutherland, 1989; Clementi *et al.*, 1995; Pena *et al.*, 1997; Parente *et al.*, 1998 and 2000).**

Hydrogen ion concentration has a significant influence on industrial fermentation due to 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. *Azotobacter* species are known to growth over a wide range of pHs. The initial pH of 7.0 was found to be optimum for both growth and polysaccharide production by commercial polysaccharide-producing bacteria

(Prasertsan *et al.*, 2008) and decreased when pH dropped to 5.8 **(Vermani *et al.*, 1997).** pH also affects the permeability of the bacterial cell membrane thus affecting the biochemical activities of the cell required for biopolymer production **(Embuscado *et al.*, 1994).** This was within the optimum pH range (6.0-7.5) for synthesis of polysaccharides **(Lawson and Sutherland, 1978).** Beside of substance uptake is dependent on the external pH and adequate control of pH value is essential both in batch and in continuous culture for alginate synthesis **(Sutherland *et al.*, 1979).**

Table (4): Effect of agitation speed on Alginate production using Azotobacter isolate n. (8) using fermentor as a batch culture on producing media containing potato starch wast 1% as a carbon source.

Time (hrs)	Az. Isolate (8)											
	Cell dry weight (gl^{-1}		Alginate (g^{-1})		Y%		$Y_{c/x}$ (mgg^{-1}dry cell)		Content%		P ($mgl^{-1}h^{-1}$)	
	300	600	300	600	300	600	300	600	300	600	300	600
0	0.02	0.02	0.003	0.003	0.015	0.015	150.00	150.00	15.00	15.00	0.042	0.042
6	0.05	0.05	0.009	0.010	0.045	0.050	180.00	200.00	18.00	20.00	0.125	0.139
12	0.17	0.20	0.160	0.180	0.800	0.900	941.18	900.00	94.12	90.00	2.22	2.500
18	0.71	0.83	0.680	0.770	3.400	3.850	957.75	927.71	95.78	92.77	9.44	10.69
24	2.92	2.89	2.700	2.800	13.500	14.000	924.66	968.86	92.47	96.89	37.50	38.89
30	3.20	3.40	3.000	3.000	15.000	15.000	937.50	882.35	93.75	88.24	41.67	41.67
36	4.10	4.60	3.800	4.200	19.000	21.00	924.83	913.04	92.48	91.30	52.78	58.33
42	6.90	7.60	6.500	7.400	32.500	37.000	942.03	973.68	94.20	97.27	90.28	102.78
48	6.80	7.30	6.200	7.000	31.000	35.000	911.76	958.90	91.18	95.89	86.11	97.22
54	6.50	7.30	6.000	6.800	30.000	34.000	923.08	931.51	92.31	93.15	83.33	94.44
60	6.30	7.20	5.800	6.400	29.000	32.000	920.63	888.89	92.06	88.89	80.56	88.89
66	5.90	6.40	5.600	6.100	28.000	30.000	949.15	953.13	94.92	95.31	77.78	84.72
72	5.70	6.10	5.500	5.900	27.500	29.000	964.91	967.21	96.49	96.72	76.39	81.24

Table (5):show that the Alginate yield % & alginate productivity and alginate yield coefficient were obtained by *A. chroococum* isolate n.8 after 48 hrs incubation period at 1% potato starch as a carbon source and 0.8 % Corn steep liquor as a nitrogen source with agitation speed 600 rpm and control pH at 7.0 being 37.00% & 125.00 $mgl^{-1}h^{-1}$ and 119.40 mg g^{-1}dry cell, respectively.

Reyes et al., (2003)., Rocha-Valadez et al., (2007) and Charles et al., (2012) despected that pH control is one of the key cultivation parameters for alginate production. Cultivation under controlled pH condition is required to increase polysaccharide production yield **(Reyes *et al.*, 2003 and Rocha-Valadez *et al.*, 2007).**controlling pH value at 7.2 during cell cultivation showed positive effect on cell productivity. Maximal alginate production yield of 0.15 g alginate/g cells was obtained in controlled pH culture. This value is almost double of those obtained in pH uncontrolled culture after 30 h cultivation. This directly indicates that the high volumetric alginate production in controlled pH culture was mainly due to high cell mass. Meanwhile, the cell performance for alginate production was almost 50% less than in this culture compared to pH controlled one.

Table (5): Biomass and Alginate production from Azotobacter isolate n.8 using fermentor as a Batch culture on media containing Potato starch 1% as a carbon source and C.S.L. as a nitrogen source.

Time (hrs)	Az. Isolate (8)					
	Cell dry weight (gl^{-1})	Alginate (g^{-1})	Y%	$Y_{c/x}$ (mgg^{-1}dry cell)	Content%	P ($mgl^{-1}h^{-1}$)
0	0.028	0.004	0.02	142.86	14.29	0.07
6	0.070	0.010	0.05	142.86	14.29	0.17
12	0.21	0.200	1.00	952.38	95.24	3.33
18	0.82	0.79	3.95	963.42	96.34	13.17
24	3.6	3.46	17.30	961.11	96.11	57.67
30	4.6	4.38	21.90	952.17	95.22	73.00
36	5.4	5.4	27.00	1000.00	100.00	90.00
42	6.1	5.8	39.00	950.82	95.08	96.67
48	6.7	7.5	37.50	1119.40	111.40	125.00
54	6.2	6.0	30.00	967.74	96.77	100.00
60	5.1	4.9	24.50	960.78	96.08	81.67

Recommendations

1- Through this study we conclude that *Azotobacter vinelandii*

2- The best productivity Produced Large amount of poly sacchariedes called Alginate of alginate and Biomass production were obtained under Batch culture condition.

3- After screening of Azotobacter isolates for Alginate production we noticed that isolate n. 1 and n. 8 were the highest biomass and alginate production using shake flasks after 5 days incubation period at 28 °C at 170 rpm.

4- The highest biomass production and alginate yield were obtained using 2 %Glucose as a carbon source & 0.8 % Corn Steep Liquor as a nitrogen source and 0.2 % Na_2HPO4.

5- We obtained the largest amount of biomass and alginate yield % by using batch culture fermentation by Azotobacter isolate n.8 on potato starch 1.0% and C.S.L. 0.8 % with agitation speed 600 rpm under controlled p H at 7.0.

6- Therefore we recommended using potato starch and corn steep liquor (Raw materials) at pH 7.0 with 600 rpm by Azotobacter isolates for maximum alginate production.

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