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Research Article

Production and characterization of alginate from *Azotobacter vinellandii* A3

Husam Sabah Auhim* and Shatha Salman Hassan

Department of Biology, College of Science, Baghdad University, Baghdad, Iraq.

ABSTRACT

Azotobacter vinellandii A3 was isolated from corn field at College of Agriculture in Abu-Ghraib, alginate of *A. vinellandii* A3 characterization was performed, alginate color was cream to light brown, gummy texture and soluble in water but insoluble in ethanol, methanol and isopropanol, alginate had high viscosity at a concentration of 1% solution at 35°C which was 146.94 centipoises, the viscosity decreased with increasing temperature ,and increase with lowering pH and reached the maximum at pH 5 , alginate had high affinity and binding capacity for copper and zinc ions and form water insoluble salt (gel formation)with them, the molecular weight of alginate was 223.23 KD.

Keywords: Alginate, *Azotobacter venillandii*, Polysaccharides, viscosity.

INTRODUCTION

Alginates are linear polysaccharides composed of (1–4)-β-D-mannuronic acid and its C-5-epimer α-L-guluronic acid. Alginates occur both as structural components in marine brown algae (Phaeophyceae) as well as capsular polysaccharides in some bacteria[1]. Due to properties of alginate, it has various industrial applications e.g. viscosifiers , stabilizing, thickening, emulsifying, alginates are also used in textile printing, paper industries, manufacturing of ceramics and production of welding rods[2], alginates have found wide ranging applications in food industry and in biomedical field[3], further more it is an important source for detoxification of metals and wastewaters [4].

Alginate has been used for a number of pharmaceutical applications, specifically in drug delivery[5]. The properties of alginates are particularly determined by their molecular weight, monomer composition and sequence pattern[3], the possibility of manipulating the molecular weight of alginate would allow the production of polymers for specific biotechnological / biomedical applications[6]. In this study alginate produce by *A.vinellandii* was extracted and characterized.

MATERIALS AND METHODS

Microorganism

The microorganisms were isolated by using routine microbiological techniques from the soil of corn field at College of Agriculture in Abu- Ghraib – Baghdad, the isolated organisms were maintained on slant agar medium at 4 °C. The isolated organism was identified as strain of *A. vinellandii* (coded as A3) depending on morphological, biochemical test and microscopic examination[7].

Alginate production

A. vinellandii A3 was inoculated into a 250-ml flask containing 100 ml of Enrichment medium[8],contained (per liter) Sucrose, 20 gm; K_2HPO_4, 0. 3 gm; KH_2PO_4, 0. 7 gm; $MgSO_4.7H_2O$, 0. 2 gm; $CaCl_2.2H_2O$, 0. 1 gm; $FeSO_4.9H_2O$, 0.05 gm; $Na_2MoO_4.2H_2O$, 0.005 gm; Yeast extract,5gm. pH 7, and cultivated at 28 °C for 18 h . 5% of the culture was then transferred into another 250-flask containing 100 ml of fermentation medium[9] contained (per liter) Sucrose, 20 gm; K_2HPO_4, 3.2 gm; KH_2PO_4, 0. 8 gm; $MgSO_4.7H_2O$, 0. 4 gm; NaCl,0.2 gm; $FeSO_4.9H_2O$, 0.02 gm; $Na_2MoO_4.2H_2O$, 0.03gm; $CaCO_3$, 0.05 gm.pH 7.2 ,the alginate produced by shaking the flask at 28°C for 5 days.

Extraction and partially purified of alginate

Capsular alginate was solubilized by adding 1 ml of 5.0M NaCl and 2ml 0.05 M disodium EDTA to 50 ml of the culture ,pH was adjusted to 7.0, and shacked for 5 min then centrifuged at 18000 rpm at 15°C for 30 min to precipitate the cells. Alginate in the culture supernatant fluid was precipitated by addition of 3-volumes of ice cold isopropanol, the precipitated alginate was collected on a Whatman filter paper No.1 and dissolved in water and precipitated again by addition of 3-volumes of ice cold isopropanol[10], collected and dissolved in water at room temperature prior to assay by carbazole assay method[11].

Determination of some physical properties of alginate

The following physical properties of the alginate were observed and recorded: Color, feature of alginate and the solubility in different solvents (water, methanol, ethanol and isoprpanol).

Measurement of alginate viscosity

The viscosity was measured by an Cannon-Fenske viscometer, the diameter of capillary tube of 1.27 mm and bulb volume of 3.1 ml, a 10 ml of 1% alginate solution was prepared and measurement was conducted at 35°C and pH 7, the density of alginate solution was measured by pycnometer (density flask) at 35°C. Distilled water was used as solvent its density and viscosity was measured at 35°C and pH 7[12]. Flow time of alginate solution was measured at least three times and alginate viscosity was calculated according to the following equation[13]:

$\eta = t\ d$

Where η = the alginate solution absolute viscosity, t = flow time of alginate solution, d = density of alginate solution, The unit of absolute viscosity is poise (g.cm-1 .s-1) or centipoise. One poise = 100 centipoises

Effect of temperature on alginate viscosity

To study the effect of temperature on alginate solution, 1% of alginate solution was incubated in water bath at different temperature (40, 60, 80 and 100) for 15 min, the viscosity of alginate solution was measured as described above after cooling and the change in viscosity % was calculated.

Effect of pH value on alginate viscosity

To study the effect of pH value on alginate solution, 1% of alginate solution was prepared at different pH values (3,4, 5, 6,7,8,9 and 10),and incubated at 30 °C for 30 min. the viscosity of alginate solution was measured .

Determination of molecular weight

Molecular weight of alginate was estimated by viscometer[14] alginate dilutions were prepared (0.2, 0.3, 0.4, 0.5,0.6,0.7,0.8 and 0.9 % wt/vol) in water, The molecular weight is calculated by Mark-Houwink equation[15]

$$[\eta] = K\ Mr\ \alpha$$

Where $[\eta]$ is the intrinsic viscosity, K and α the constants for the given polymer–solvent system (K= 1.228 ×10-4, α= 0.9) and Mr is the molecular weight[16]. To get the intrinsic viscosity $[\eta]$, four viscosities must be calculated:

- Absolute viscosity η was calculated for the solvent (water) and alginate solutions at 20 °C
- Relative viscosity $\eta_r = \eta / \eta_0$, where η = viscosity of alginate solution and η_0 = viscosity of the solvent.
- Specific viscosity $\eta_{sp} = (\eta / \eta_0) - 1$.
- Reduced viscosity $\eta_{red} = \eta_{sp} / c$.

Where c is the concentration of alginate dilutions .The relationship between the concentrations of alginate and the reduced viscosity was plotted. The intercept point of Y axis represents the intrinsic viscosity.

Gel formation by alginate with copper and zinc ions solutions.

Ten ml of alginate solution (1%) was added to 10 ml of each $ZnSO_4.7H_2O$ solution containing (1000 µg / ml) of Zn^{+2} and $CuSO_4$ solution containing (1000 µg / ml) of Cu^{+2} which was prepared by dissolving 4.398 gm of $ZnSO_4.7H_2O$ in 1000 ml D.W. and 2.5 gm of $CuSO_4$ in 1000 ml D.W.

RESULTS AND DISCUSSION

Determination of some physical properties and the nature of alginate.

The features and some characters of alginate were observed, alginate has a creamy to light brown color, gummy texture and soluble in water but insoluble in ethanol, methanol and isopropanol.

Alginate viscosity

The biological activity of biopolymer solution is generally determined under the conditions of the polymer molecule in solution. One of the most important parameters for the characterization of high molecular weight polysaccharide spatial structure is the solution viscosity, which is mainly affected by

concentration, solvent nature, kind and concentration of salts, pH and temperature[17-18].
It was found that alginate of *A. vinellandii* A3 has high viscosity at a concentration of 1% solution at 35°C which was 146.94 cps compared with commercial alginate 56.73 cps at the same conditions.
Suzuki et al.[19] mentioned that viscosity of alginate rich in gluronic and mannuronic acids at a concentration of 1% solution was 250 cps. low viscosity of commercial sigma alginate is 250 cps at 2% solution[20].

Effect of temperature on alginate
Many polysaccharides partially or fully degrade at high temperature, so the practical use of alginate required knowledge of its thermostability.Viscosity was measured for 1% alginate solution production by *A. vinellandii* A3 at different temperature (40, 60, 80 and 100°C), alginate viscosity decreased gradually and slightly (not sharp) when the temperature increased (figure 1).

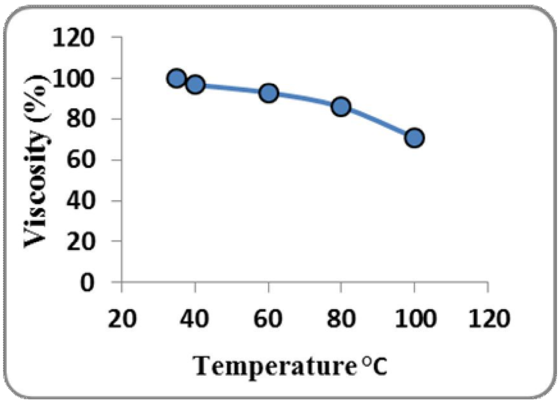

Fig. 1: Effect of temperature on alginate viscosity after incubation for 15 min

Viscosity at 40°C decreased slightly (3.09 %), while at 100°C decreased by 29.2 %, this indicates the thermostability of alginate at wide range of temperature, which is useful in many application using high temperature.

Effect of pH on alginate
The stability of the macromolecular structure of polysaccharides in solution in relation to the pH can be assessed by a change in viscosity, allowing optimum pH values and the pH range to be determined.
The viscosity of 1% alginate solution produced by *A. vinellandii* A3 was measured at pH values of 3-10. At neutral pH, viscosity of alginate solution was 146.94cps , at pH 6 ,5and 4, viscosity increased to 157, 162 and 160 cps respectively ,while at pH 3 the viscosity decreased to 121 cps , at pH 8,9 and 10 the viscosity decreased to 145.8,140 and 137.5 respectively (Figure 2).

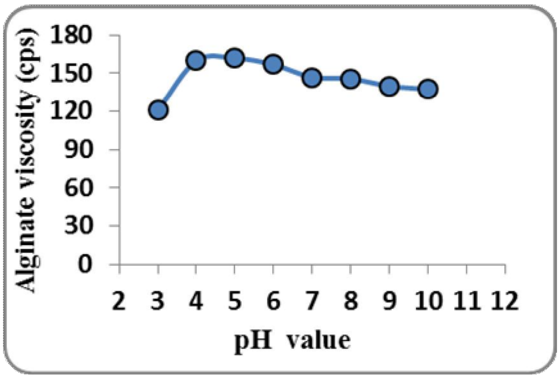

Fig. 2: Effect of pH value on alginate viscosity after incubation for 30 min

These results indicate that alginate solution has higher viscosity at acidic pH (4-6) than the neutral and alkaline pH, it can be concluded that *A. vinellandii* A3 alginate is stable at wide range of pH and can be used at acidic and alkaline conditions.
Alginates have COO- and COOH groups along the chain conferring different charge densities depending on the pH , the hydrophilic and hydrophobic groups along a molecule chain can be altered by the protonation and deprotonation of carboxyl groups in the backbone chain.
The initial pH value of the neutral alginate solution at 1% concentration was 6.6[21], which mean that the carboxylic acid groups are slightly dissociated. Two types of interactions play an important role in aqueous alginate solution, namely the charge repulsion between dissociated carboxylic groups and the hydrogen bonding formed between carboxylic acid and ionized carboxylate groups[22], with the decrease of pH ,the number of dissociated carboxylic groups in alginate chains were also decrease , which makes alginate lose its hydrophilicity to some extent, when some dissociated carboxylic groups in alginate chains are gradually protonated , the hydrophobic segments appear in alginate chains , as the pH value decrease from 6 to 4, the hydrophobic segments in the alginate chains increase and the hydrophilic segments decrease, the weakening of the mutual repulsion of ionized carboxyl groups promotes the development of intermolecular hydrogen bonds and possible entanglements[21].

Molecular weight

All polymers increase the viscosity of the solvent in which they are dissolved. This increasing allows for a convenient method of determining the molecular weight of polymers. Since the viscosity method is not based on rigorous physical laws, it must be calibrated by standards of known molecular weight with narrow molecular weight distributions.

The molecular weight of alginate produced by *A. vinellandii* A3 was calculated by Mark-Houwink equation:

$$\eta \text{ int} / = K\, M r\alpha$$

The constants K and α for the given polymer–solvent system (K= 1.228 ×10-4, α= 0.9)[16]. In order to calculate the intrinsic viscosity η int of alginate solution, four viscosities for diluted alginate solution was calculated as following in Table 1:

Table 1: Different viscosities for diluted alginate solution

Alginate conc.%	Absolute viscosity (cps)	Relative viscosity	Specific viscosity	Reduced viscosity gm/ml
0.2	11.97	2.67	1.67	8.39
0.3	23.11	5.17	4.17	13.9
0.4	29.77	6.66	5.66	14.15
0.5	39.82	8.9	7.9	15.81
0.6	49.7	11.11	10.11	16.86
0.7	63.14	14.12	13.12	18.75
0.8	79.02	17.67	16.67	20.84
0.9	109.58	24.5	23.5	26.12

The relationship between the concentrations of alginate and the reduced viscosity is plotted. The intercept point of Y axis represents the intrinsic viscosity (figure 3).

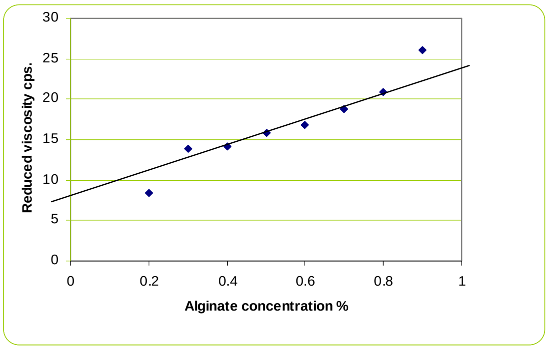

Fig. 3: The relationship between the concentrations of alginate and their reduced viscosity

The intrinsic viscosity η int for alginate produced by *A. vinellandii* A3 was 8 (dl/gm), and the molecular weight of alginate was 223.23 KD, results obtained from Zeki[23] showed that molecular weight of alginate produced by *pseudomonas aeruginosa* was 262 KD, while AL-Janabi[24] reported lower molecular weight, which was about 141KD. Variation in results may be due to the fact that alginate is an exo-polysaccharide polymer, so molecular weight as well as other properties of such molecules is differ among the producing strains and culture conditions[25].

Gel formation by alginate with copper and zinc ions solutions

Ion binding characteristics are the basis for the gelling properties of alginate, its affinity for multivalent cations depends on its composition, when the alginate produced by *A. vinellandii* was added to two salt solutions, gel was formed in solution contain Cu^{+2} ions and Zn^{+2} ions, (figure 4).

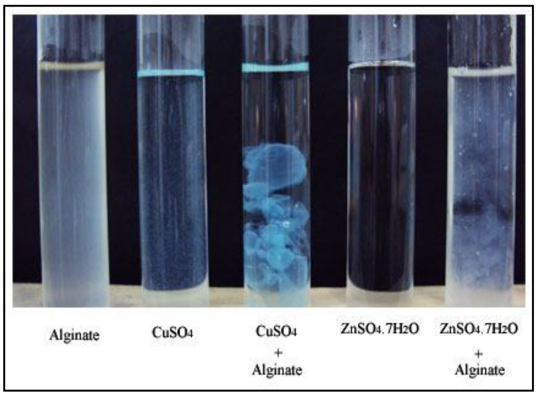

Fig. 4: Gel formation by alginate with copper and zinc ions solutions

The binding affinity of alginate produced by *A. vinellandii* increases markedly with content increasing of α -L-guluronate residue in the chain[26].

CONCLUSION

Bacterial alginate has cream to light brown color, gummy texture and soluble in water but insoluble in ethanol, methanol and isopropanol so it can be precipitate by this solvent, algiante viscosity depend on pH and temperature, algiante viscosity increase with lowering pH and decreased with increasing temperature, reached the maximum at pH 5, so bacterial alginate can be use in different application according to their properties.

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