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Mini review on Alginate: Scope and Future perspectives

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Abstract

Alginate is an industrially needed linear copolymer composed of β-1,4-linked D-mannuronic acid. This industrial biopolymer has been widely obtained from seaweeds, but production of alginate from sea weeds farm is not possible for all climates. Hence, finding an alternate easily available bio resource to produce such polymers under minimal process with long time is essential. Some bacteria are capable of producing alginates, especially native and mutant strain of *Azotobacter vinellandi*. But mutant strains have the ability to produce higher amount of polysaccharides compared to native strains with value added by product through fermentation processes. Although seaweeds are the largest source for alginate production, they are not ideal resources for industrial production of alginates to meet commercial demand thus high lighting the significance of microbial alginate production. This review is an attempt to describe possible differences in the production of alginates from bacteria and seaweed and industrial application of both bacterial and seaweed alginate.

Introduction

Seaweeds are the largest source for the production of value-added polysaccharides and industrial byproducts all over the world. Alginates are one of the major products extracted from seaweeds especially brown algae. The second most common source for major exoployssacharde producers is bacteria. In seaweeds, polysaccharides are primary components of both the cell walls and the intercellular matrix. Biochemically Alginate comprises sequences of two blocks namely M block and G block. B-D-mannuronate (M) and its C-5 epimer α-Lguluronate (G) linked by 1–4 glycosidic bonds. The ratio of M and G are depends on the source of alginates but their physical properties are almost same being they extracted from different resources. They are used as a stabilizer, viscosity agent and gelling agent in food materials with annual industrial needs of alginates reaching ~ 30,000 metric tons. 40% of the alginates are obtained from seaweeds [Draget *et al*., 2005]. In recent years, this polysaccharide has been used for wide range of pharmaceutical applications like wound dressings [Thomas, 2000] and many more biological applications. Before 1975, commercial alginate production depended on seaweeds, where they were treated with alkali solution [Clark 1936] followed by filtration. The alginates are precipitated with Sodium / Calcium chloride finally the alginate salts can be transformed to alginic acid with dilute HCl. Finally, alginic acid is purified to produce water soluble sodium alginates [Rinaudo M.2008]. Later1980'stwo genera of bacteria are identified as major producers of alginate and alginic acids namely non pathogenic pseudomonas and Azotobacter. In nature they produce alginates for different reasons with various material properties. *P.auerogenisa* produces alginates for thick highly structured bio film [Nivens *et al.,* 2001and Hay *et al*., 2009]. But Azotobacter produces rigid alginate which is essential for the formation of desiccation resistant cysts [Sabra and Ping Zeng, *et al* 2009] against stress.

Biosynthesis of Alginates

Alginate biosynthesis begins with oxidation of a carbon source to acetyl-CoA, which enters the TCA cycle to be converted to fructose-6-phosphate through gluconeogenesis. Fructose-6-phosphate undergoes a series of biosynthetic transformations to be further converted to GDP-mannuronic acid, which acts as a precursor to alginate

synthesis [Gacesa, P *et al*. 1998] (Fig.1). In general, the biosynthetic operation can be divided into four stages (1) GDP-mannuronic acid precursor bio synthesis, (2) Cytoplasm membrane transfer and polymerization, (3) Periplasmic transfer and modification and (4) Export through the outer membrane. Post-polymerization modification of alginates occurs at stage (3), where polymannuronic acid is acetylated at the O-2 and/or O-3 positions by several transacetylases. Epimerization is then performed by a family of epimerase enzymes to convert some non-acetylated M residues to G residues (Franklin) finally; alginate is released from the cell through trans membrane porins. The regulatory mechanisms for the production of alginate are identical in both bacteria and algae with minimal slight difference. Even though alginate production are synthesized from almost similar biosynthetic pathway the characteristic properties of bacteria and seaweeds are different (Table.1)

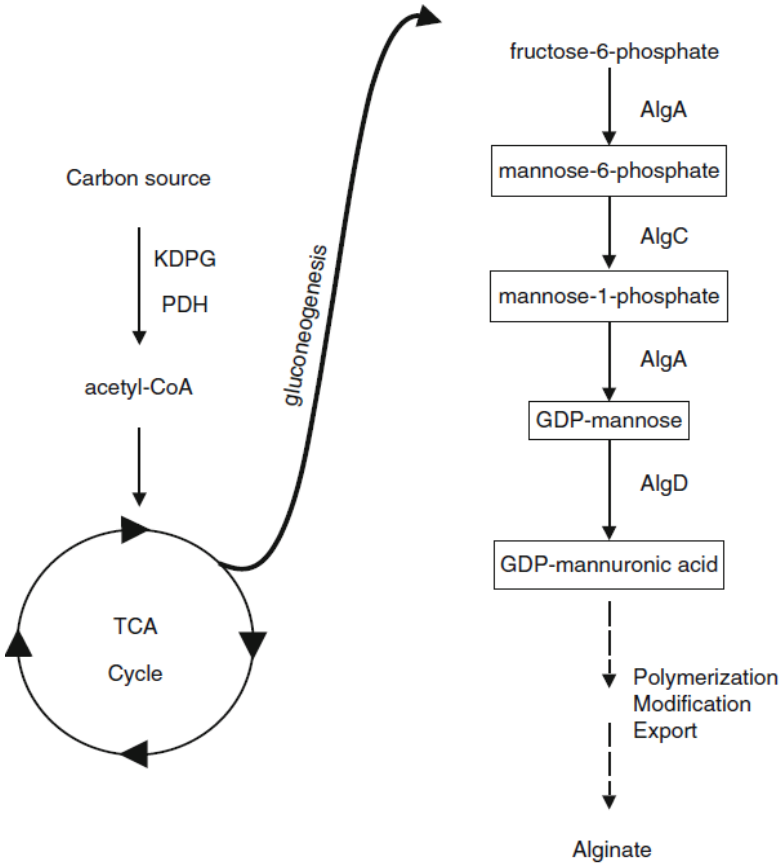

Fig.1. Alginate biosynthetic pathway in Seaweed and bacterial

[A].Biosynthesis route of alginate in seaweed. KDPG ketodeoxyphosphogluconatepathway (Entner–Doudoroff pathway), PDHpyruvatedehydrogenase, AlgAphosphomannoseisomerase–GDP-mannose pyrophosphorylase, AlgC phosphor-mannomutase, AlgD GDP-mannose dehydrogenase. Boxed intermediates are precursors of alginate

Dashed arrows indicate unknown biosynthesis steps

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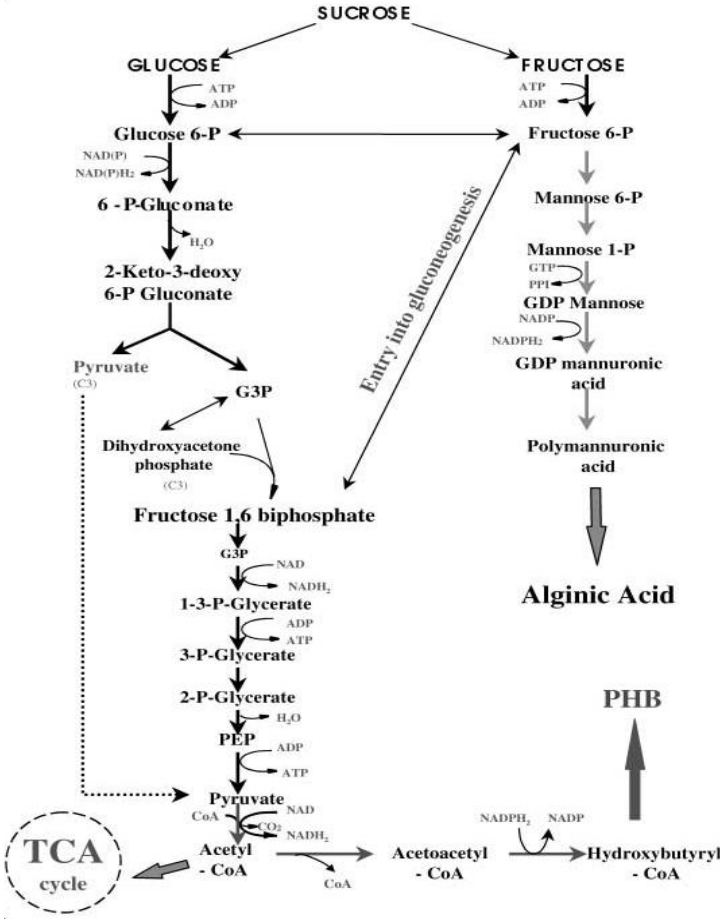


Fig.2 Biosynthesis route of alginate in bacteria.

KDPG ketodeoxyphosphogluconate pathway (Entner–Doudoroff pathway), PDH pyruvatedehydrogenase, AlgAphosphomannoseisomerase–GDP-mannose pyrophosphorylase, AlgC phosphor-mannomutase, AlgD GDP-mannose dehydrogenase. Boxed intermediates are precursors of alginate, Also byproduct of PHB production.

Table: 1 Difference between bacterial (Azotobacter) and seaweed alginate

S.No	Properties	Bacterial alginates	Seaweed alginates	Reference
1.	Nature of organism	Native	Mutant also possible	W. Sabra*et al*., 2001
2.	Molecular weight	154600-730000 gm/Mol	48000-186000gm/Mol	Clementi*et al*., 1998 Donnan& Rose *et al*., 1950
3.	Polyglucuronate blocks	Absent in Pseudomonas ps.,	Present	Sherbrock-Cox*et al*., 1984
4.	Nature of gel formation	Flexible	Rigid	Sherbrock-Cox*et al*., 1984
5.	*O*-acetylation	Essential for outer cyst wall formation	*O*-acetylation not occurs	Sherbrock-Cox*et al*., 1984
6.	Selective ion binding	Higher against Ca ion	Higher against Na ion	
7.	Production induced by change of Membrane potential	Possible	Not possible	Yuzoharada*et al.,* 1964
8.	No of genes reported	30 genes		Iain D. Hay, Zahid 2010
9.	Manufacturing process	Conversion of alginate to sodium alginate is necessary	No conversion of alginate to Sodium alginate	Chermapandi Parthiban, Kaliyamurthy Parameswari *et al.,* 2012
10.	Factor affecting production of alginate	Oxygen supply under diazortrophic condition (Dissolved O_2 is the limiting factor)	Not reported	
11.	Other function role beyond alginate production	Protective barrier in heavy metal toxicity and Nitrogen fixation role in the soil		W. Sabra *et al.,* 2001
12.	Fermentation process for production alginate / alginic acid	Possible for industrially modified alginate with mutant strains	Depends on the nature of sea weeds.	
13.	Biosynthetic pathway	Synthase Dependent	Synthase dependent	Rehmand valla *et al.,*1997
14.	By product formation	PHB		

Seaweed Alginate production and Limitations

Alginates a form of hydrocolloids substances derived from seaweed that interacts with water to form colloid systems either in the form of a gel or solubilized particles. These Hydrocolloid polysaccharides have much importance, both technologically and economically. Alginates are extracted in different ways depending on the application, but the most generally employed procedure described [Calumpong *et al.* 1999], involves extracting the alginate in the form of sodium alginate. This method is based on converting the insoluble calcium- and magnesium-alginates present within the brown seaweed cell walls to soluble sodium alginates that are subsequently recovered as alginic acid or calcium alginate. This conversion is carried out by sequential addition of acid, alcohol, and sodium carbonate. The extraction techniques available for alginate extraction face some difficulties in, e.g., relation to separation of the seaweed residuals that do not dissolve. As the alginate dissolves as sodium alginate, the thickness of the solution makes the process difficult one, hence filtration and the solution has to be done with large quantities of water. As the seaweed residuals are very fine and can clog the filter, filter aids must be provided making the process cost effective. In

addition, the chemicals used for extraction are believed to influence the physico-chemical properties of alginates (Vauchel, P *et al.*, 2008). To avoid the difficulties encountered in the traditional extraction techniques and the destructive effects they have on the functional properties there is a need for alternative extraction and processing techniques. To our knowledge, no attempts on enzymatic extraction of alginate from seaweed have been reported. The main issues alginate producers must face are: (1) Changing locations for alginate production - rising non-alginate uses for the same types of seaweed (2) Increasing government controls on the harvesting of natural seaweeds (3) All easily accessible large natural seaweed resources already being harvested. (i) Seaweeds produce alginate mainly in the form of Sodium alginate, the sodium salt of alginic acid, extracted from the (Biomass) seaweeds. Natural seaweed excretes alginate alone but is not stable in the marine environment later turned to sodium salt of alginates. (ii) Potassium alginate also obtained extract of from cells of marine seaweed (iii) calcium alginate is obtained from sodium alginate, where sodium is replaced with calcium.

Current alginate production by bacteria:

Screening strategies for Alginate producing strains

CPC method

1. Enrichment culture technique is the readily available method to screen the microorganisms capable of producing alginate lyase enzyme carried out by investigating their abilities to grow on alginate-containing solid media plates and occurrence of a clearance zone after flooding the plates with agents such as 10% (w/v) Cetylpyridinium chloride (CPC), which can form complexes with alginate.

Plate assay method

2. In this method, alginate-containing agar plates are flooded with Gram's iodine instead of CPC. Gram's iodine forms a bluish black complex with alginate but not with hydrolyzed alginate, giving sharp, distinct zones around the alginate lyase producing microbial colonies within 2-3 min. Gram's iodine method was found to be more effective than the CPC method in terms of visualization and measurement of zone size. The alginate-lyase-activity area indicated using the Gram's iodine method was found to be larger than that indicated by the CPC method.

Alginate production by Pseudomonas

Under *invivo* conditions non pathogenic, Pseudomonas produces alginate which will act as a protective layer against heavy metal toxicity; thus the secretion of alginate may be enhanced by Nacl and Ethanol in fluorescent pseudomonas, suggesting that Osmoloarity and dehydration may be plays a significant role in the production of Polysaccharide [Kidambi*et al.* 1995].

Alginate production by Azotobactersps.,

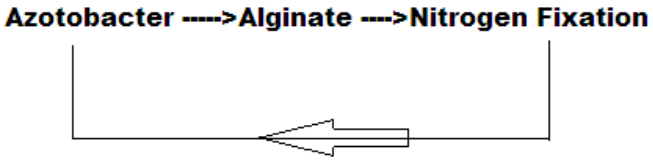
Azotobacter vinelandii is a major stable producer of acetylated alginate under various in vitro and in vivo conditions. Alginate not only behaves as an overflow metabolite, but also serves as a protective barrier against heavy metal toxicity and provides protection against attack and adverse environmental conditions [Fyfe *et al*., 1983]. *A. vinelandii* growing diazotrophically with various oxygen concentrations shows alginate capsules are formed, even under high shear stress in the fermenter [Sabra *et al*. 2000]. Moreover, the alginate capsule forming capacity of *A. vinelandii* is much compact and density also higher in the presence of higher dissolved oxygen concentration (pO_2), the postulated result that, the formation of alginate layer around the cell also act as a diffusion barrier of oxygen, which controls the transfer of oxygen sensitive enzyme namely Nitrogenase.

Influence of Medium Components

Effect of Nutrients on Bacterial alginate production:

Many attempts have been made to report the medium for formulation of Azotobacter alginate production in fermentation process. Both beneficial and harmful effect has investigated thoroughly along with fixed nitrogen level in the culture medium. The accumulated Nitrogen may act as a limiting factor for the production of Alginate for

Azotobactersps., [Brivonese and Sutherland 1989; Sabra 1998] (Fig:2). More over peptone used in the medium will alter the alginate production up to 30 %, suggesting more specific role for Nitrogenous nutrients.



Effect of Phosphate on Bacterial alginate production

The effect of phosphate on alginate production by *A.vinelandii* was also reported controversially (Table 1). [Brivonese and Sutherland *et al*.,1989] reported phosphate simply acting as buffer agent in the medium in the case of phosphate-rich medium (7.5 g K_2HPO_4).On the other hand medium with excess phosphate leads to maximum production of alginate. Moreover, correlation between phosphate limitation and respiratory requirement of cells in terms of RQ value (Respiratory quotient) and alginate production in diazotrophically grown A.vinelandii. The calculated RQ value is around 0.8 in the level of 2-5 % of Phosphate level.

Table: 2 Effect of Phosphate on Bacterial alginate production

Strain	Azotobacters ps.,	Dissolved PO_2	Medium	Condition	Alginate	yield	Productivity ($g\ l^{-1}h^{-1}$)	Reference
M	NCIB 9068	Uncontrolled	NFM	PO_4 rich	6.2	0.31	0.06	Chen *et al*., 1985
M	NCIB 9068-A	Uncontrolled	NFM	PO_4 rich	5.5	0.27	0.07	Chen *et al*., 1983
M	SM 52B of NCIB 9068	Uncontrolled	NFM	PO_4 Limited	5.0	0.25	0.05	Horan *et al.,* 1981
M	DSMZ 93-541b	Controlled 2%	NFM	PO_4 Limited	4.9	0.12	0.2	Sabara *etal.,* 1999
N	NCIB 9068	Uncontrolled	NFM	PO_4 Limited	3.0	0.075	0.04	Jarman *et al*., 1978
N	A.Vinelandii Native	Uncontrolled	NRM	PO_4 Limited	3.15	0.066	0.045	Deavin *et al*., 1977
N	NCIB 9068	Uncontrolled	NRM	PO_4 rich	6.0	0.15	0.04	Brivonese and Sutherland 1989
M	DSM 576	Uncontrolled	NRM	PO_4 rich	5.8	0.145	0.053	Savalgi and Savalgi 1992
M	DSM 576	Uncontrolled	NRM	PO_4 Limited	5.0	0.25	0.07	Clementi *etal*. 1999
M	ATTC – 9046	Uncontrolled	NRM	PO_4 rich	4.9		0.2	Clementi *et al*. 1995
M	DSM 576	Controlled at 2%	NRM	PO_4 rich	4.5		0.0625	Pena *et al*. 1997

M= mutant, N=native

Effect of dissolved oxygen on bacterial alginate production

Dissolved Oxygen plays a significant role in the alginate production in Nitrogen and phosphate rich medium by Azotobacter were studied in the fermenter at pH 7 and 35^{o} C, where batch fermentation was carried out without control of dissolved oxygen at 1, 2, 5 and 10 % DO where the bacterial growth was higher but maximum production of alginate was lower. No alginates at 10% DO but higher growth rate of bacteria was achieved between 5 and 2% DO but alginate was less were higher production of alginate was obtained without Dissolved Oxygen control.

Table: 3 Effect of dissolved O_2 in the medium

Azotobacter sps.,	Dissolved O_2 in %	Medium	Alginate production	Reference
Azotobacter DSM 579	1-10	Glucose	Biomass higher but alginate production is less < 5%, the identified condition for maximum DO for alginate production is (3 -5 %)	E Parente*, et al.,*
Azotobacter armeniacus	1- 5	Glucose	Alginate production is high at 3% DO	Carlos Pena, Mauricio *et al.,*
Azotobacter sp. AR	3-5	Glucose	Alginate production is high at 3% DO	M. A. Trujillo-Rold *et al.,*
Azotobacter beijerinckii	4	Glucose	Supports the maximum biomass but less alginate production	Haraguchi K, Kodama T *et al.,*1996
Azotobacter Vinelandi mutant	5-6	Glucose	Biomass production but comparatively low alginate production	Zahid Ali Butt *et al.,* 2011
Azotobacter sp.	10	Glucose	No effect in the alginate production	E. Soto Escuela *et al.,*2010
Azotobacter mutant AT268	1-5	Glucose	Alginate production is 25% less production compare to wild type between the dissolved Oxygen range	C Pena1, *et al*., 2002

The kinetics of growth and alginate production from glucose in a nitrogen and phosphate-rich medium by *Azotobacter vinelandi* were studied in a laboratory fermenter at pH 7 and 35°C. Batch fermentations were carried out both without control of dissolved oxygen concentration (DO) and at 1, 2, 5 and 10% DO. Although growth was faster at higher DO, maximum biomass concentration was lower. No alginate was produced at 10% DO. Alginate production was faster at 5 and 2% DO but higher alginate concentrations and yields were obtained without DO control. Alginate production was growth-associated at 5% DO, but significant amounts of alginate were produced after growth had stopped at lower DO values. In fermentations without DO control the molecular weight of the polymer reached a maximum (11–17.6) when specific growth rate was between 0.02 and 0.04 $h^{-1}$and residual concentration of ammonia nitrogen was between 0.01 and 0.02 g L^{-1} and then sharply decreased. The DO level between an ideal range for the identification of alginate production by Azotobacter is 3-5 %; where decreasing the activity of Nitrogenase helps the organism to produce more amount of alginate.

Effect of agitation in the medium for the production of Alginate

The effect of alginate production by Azotobactersps., was significantly affected by the various rpm range in the fermenter studies under controlled pH condition.[E Parente *et al.,* 2000] The reported maximum level of alginate production was 2 gm/l at 500-600 rpm. [Wen-Pin Chen *et al*, 1985]Mutant strain of *Azotobacter vinelandii* NCIB 9068 produces 6.22 g/L alginate at 170 rpm without pH control [Cigdem Moral *et al*., 2012]. The growth rate of bacteria increased from 0.165 to 0.239 h^{-1} by the increase of agitation from 200 to 400 rpm. On the other hand, 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. Increased agitation or shaking speed was frequently used by many authors for optimizing the production of alginate along with other factor like aeration and Phosphate concentration. However, no data are available on the independent effect of agitation speed and constant supply of oxygen. Moreover, no systematic study has been reported regarding the influence of increased agitation on cell morphology and capsule formation in *A. vinelandii* grown diazotrophically under particular phosphate limited conditions.

Applications of alginate (Bacterial and Seaweed alginate) in different industry

S.No	Industry	Application	Reference
1	Textile	Thickeners for the paste containing the dye	Hilton *et al* 1969
2	Paper	Improve crumpling and resistance of paper.	Arthur Johnson *et al* 1952
3	Pharmacy	adhesion agent of tablets	Miyazaki S, Nakayama A, *et al.,* 1994
	Facial plastic surgery	inject able fillers	Andre P, Moulongu *et al* I. 2014
		Drug delivery and encapsulation of drug	Wang A1, Tao C *et al*., 2008
4	Medicine	Treatment for hear burn and acid reflux	B. P. Daggy, D. A. Brodie *et al.,* 2000
		Alginate beads for the treatment of breast cancer	B Arıcaa, S Çalışa, *et al.,* 2002
5	Food industry	Gelling agent	Dipjyoti Saha & Suvendu Bhattacharya 2010
		Prevent moisture loss of meat during storage	Mastromatteo M. *et al.,* 2011
		Beverage additive for stabilization	G. Jackson, *et al.,* 1980
6	Dairy	Prevention of milk protein from agglutination	A. Syrbe, W. J. Bauer 1998
		Prevent milk protein and other solid particles from coagulation and fat from floating.	A. Syrbe, W. J. Bauer 1998
		Anti melting capacity	A. Syrbe, W. J. Bauer 1998
7	Cosmetics	Thickener and moisture retainer	M. A. Lesser.
		Retaining the color of lipstick on lip surface by forming gel-network.	M. A. Lesser.
8	Welding industry	Production of welding rod, as a binder of flux.	Protan 1984
9	Dental	Dental alginate impression	R.G. Craig 1988

Future perspectives

Biopolymer demand continues to grow every year because of wild application in different fields, but only 3–5% production was achieved between 1980s and 2000.Emerging markets in China, Eastern Europe, Brazil, etc has largely driven this growth. Sales of agar, alginates and carrageenan in the US and Europe are holding up reasonably well in spite of the recession. However, price increases to offset costs in 2008 and 2010 have begun to have a dampening effect on sales, especially in markets where substitution or extension with less expensive ingredients is possible. These higher prices have been driven by higher energy, chemicals and seaweed costs. The higher seaweed and process costs reflect the production of alginic acid in large scale. The Philippines and Indonesia are the dominant producers of the farmed *L. digitata*, *L. hyperborea* and *L. saccharina* because of their oceanic behavior supports the growth of alginate producing seaweed in whole year. Comparatively in India, the alginate producing seaweed availability is less and the viscosity of the alginate is not suitable for textile industry, hence there is a huge challenge to find bacterial resources for alginate production under minimal capital cost with higher viscosity nature. Production of alginate depends on the needs of various industries and the viscosity of the alginate production can be improved using recombinant strains of Azotobacter by genetic manipulation technique.

Conclusion

Although commercial production of alginate depends on cheap algal sources, the ability to engineer bacterial alginates will make bacterial fermentative production increasingly attractive. Better understanding of the biosynthesis of alginate and PHAs *in A. vinelandii* and development of cell culture systems for biopolymers production will lead to the emergence of new fermentation strategies to obtain alginate and PHAs with specific chemical characteristics and more defined material properties that could be used in specific applications in pharmaceutical and biomedical fields. Increased understanding of alginate production will also help to overcome the production bottleneck currently seen in alginate production for the purpose of important biopolymer for medical and biotechnological applications. Moreover, an increased understanding of alginate composition and material properties will help meet medical and pharmaceutical specifications thus providing enormous opportunity for the use of engineered bacteria for the production of alginates.

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