INT. J. BIOL. BIOTECH., 17 (4): 777-782, 2020.

POTENTIAL APPLICATIONS OF CATALYTIC EFFICIENCY OF ALGINATE LYASE IMMOBILIZED ON SILVER NANOPARTICLES: A SHORT REVIEW

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ABSTRACT

Alginate lyase catalyzes degradation of a linear polymer present widely in brown algae, called alginate. Degraded products of alginate have promising biotechnological applications in various fields. Applicability of enzyme in industry is hampered by its cost and low stability. Solution of this is immobilizing the enzyme on a support matrix most preferably nanomaterial due to their small size and large surface area. Attributed to their various unique features, silver nanoparticles are most suited. AOS is the prime product of enzyme that is important in food and pharmaceutical industry due to its vast health benefits and ecofriendly nature. Other applications of alginate lyase include production of bio fertilizer, biofuel, in food industry and in pharmaceutical industry. This review attempts to summarize the uses of immobilized alginate lyase on silver nanoparticles in different industrial sectors.

Keywords: Algino Oligosaccharides (AOS), Alginate, Ag-nanoparticles, Immobilization.

Abbreviations: UV-Vis (Ultra Violet- visible spectroscopy), TEM (transmission electron microscopy), XRD (X-ray Diffraction), HRTEM (High Resolution TEM), FTIR (Fourier-Transform Infrared (spectroscopy), ELISA (Enzyme Linked Immunosorbent Assay), SEM (Scanning Electron Microscopy), FRET (Forster resonance energy transfer), AOS (Algino Oligo Saccharide), TNF (tumor necrosis factor).

INTRODUCTION

Alginate lyase is a novel enzyme having ability to degrade a linear copolymer, alginate. The enzyme can be isolated from various sources, which are specific for their substrates, including algae, mollusks, fungi and some viruses (Chen *et al*., 2018). Although this enzyme has potential catalytic effect, its commercial use has some challenges such as lack of stability. But this problem can be solved by immobilizing the enzyme on the nanoparticles. Most frequently used and benign ones are silver nanoparticles which can be designed and synthesized by different methods. Immobilization of alginate lyase on silver nanoparticles can not only retain the enzyme activity and increase its stability but also can make the recovery of enzyme from the reaction medium much easier (Lee and Au-Duong, 2018).

Alginate lyase: Sources and reactivity

Alginate, abundantly found copolymer in brown alga, can be converted into alpha-L-guluronate and its C5 epimer β -D-mannuronate by a β-elimination reaction (Fig. 1). Enzyme can be isolated from various biological origins including *Laminaria hyperborean*, *Ectocarpus, Colpomenia, Laminaria, Fucales, Dictyota, and Sargassum* (Szekalska *et al*., 2016). It targets M-rich (Mannuronic acid) and G-rich (Glucuronic acid) alginates for their main cleavage action. They are classified to two main classes: endolytic-alginate lyases and exo-lytic alginate lyases.

Table 1. Some source organisms of novel enzyme alginate lyase.

Serial No.	Source Organism	Origin	Reference
1	*Isoptericola halotolerans*	Marine bacterium	Benwei *et al.*, 2018
2	*Nitratiruptor sp. SB155-2*	Deep sea thermal vents	Akira *et al.*, 2016
3	*Bacillus licheniformis*	Bacterial strain	Chen *et al.*, 2018
4	*Defluviitalea phaphyphila*	Marine Thermophile	Shiqi *et al*, 2019
5	*Bacillus sp. strain ATB-1015*	Soil bacteria	Nakagawa *et al.*, 1998
6	*Vibrio sp. SY08*	Marine bacterium	Shangyong *et al., 2016*
7	*Pyropia yezoensis*	Marine red alga	Akira *et al*., 2015

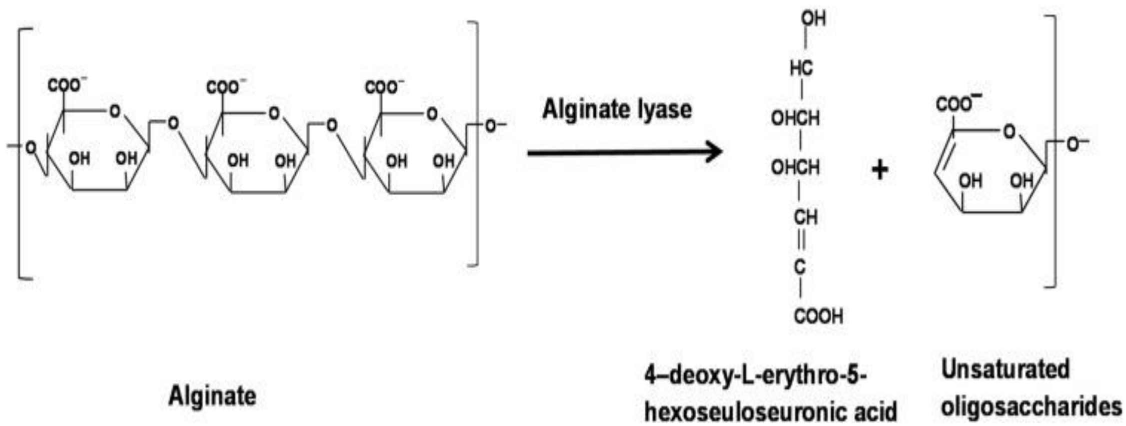

Fig 1. Action of alginate lyase on linear copolymer of alginate.

Advantages of immobilizing enzyme

Immobilized enzymes can be reused, thus reducing the cost of a process. Instability of enzyme structure can be removed when it is attached to a support matrix. It can be easily extracted at the end of the process. When enzyme gets attached to a support, its catalytic properties get coupled with optical, electrical, physical and chemical properties of nanoparticles (Ansari and Husain, 2010). Nanomaterials can be best served as support matrix and the most remarkable are nanoparticles. Among all the metals, silver holds prominent position.

Unique properties of silver nanoparticles

They have unique shape, size, crystallinity, composition, optical properties and excellent conductivity. Some biological activities are given in table 2.

Table 2. Different bioactivities shown by Ag nanoparticles.

Bioactivity	**Test organism / cell**	**Characterization**	**Reference**
Anti-bacterial	*Bacillus cereus*	Agar well diffusion method	Patil *et al.*, 2018
Anti-fungal	*Fusarium solani*	UV-VIS, TEM, XRD	Khatami *et al.*, 2018
Anti-cancer	Colon cancer cell lines	HRTEM, FTIR, XRD	Elella *et al.*, 2018
Anti-inflammatory	*Sambucus nigra*	FTIR, XRD	David *et al.*, 2014
Anti-viral	HIV1 retrovirus	ELISA	Zodrow *et al.,2009*
Cardio protection	*Millingtonia hortensis*	NA	Savitha *et al.*, 2016
Wound Dressing	*Acetobacter xylinum*	NA	Jacob *et al.*, 2018

UV-Vis (Ultraviolet- visible spectroscopy), TEM (transmission electron microscopy), XRD (X-ray Diffraction), HRTEM (High Resolution TEM), FTIR (Fourier-Transform Infrared (spectroscopy)), ELISA (Enzyme Linked Immunosorbent Assay).

Characterization of silver nanoparticles

Characterization is done to assess various features of nanoparticles such as size, shape, orientation, crystallinity, pore size and surface area. Each of the technique efficiently measures any aspect of nanoparticle (Table 3).
SEM (Scanning Electron Microscopy), FRET (Forster resonance energy transfer).

Methods of immobilization of enzyme on nanoparticles

Through covalent bonding

It is one the most commercial and conveniently applied approach due to its various attributes such as, high thermal stability of immobilized enzyme, reusability, easy handling, suitable economy, high pH stability and easy extraction of products (Ansari and Hussain, 2010). Enzyme is attached to the matrix whose surface is activated with the help of a chemical substance called as modifier/ linking agent.

Table 3. Some techniques used to assess features of synthesized Ag Nanoparticles.

Feature	Technique	Reference
Particle size	AFM/ TEM/ SEM	Choi *et al.,* 2007
3D- picture of particle	AFM	Hutter *et al.,* 2004
Distribution	DLS	Abul *et al.,* 2018
Crystallinity	XRD	Yoosaf *et al.,* 2007
Plasmon Resonance	UV-VIS spectrophotometry	El-Nour *et al.,* 2010
Surface charge	Doppler electrophoresis	Murdock *et al.,* 2008
Drug release kinetics	Dialysis	Anhalt *et al.,* 2012
Critical micelle concentration (CMC)	Light scattering, flourescent probes	Accardo *et al.,* 2004
Turbidity	Spectrophotometry	Isaacs *et al.,* 2006
Stability analysis	Gel permeation chromatography	Yokoyama *et al.,* 1993
Micelle stability	FRET	Chen *et al.,*2008
Critical aggregation concentration	Conductivity, Light Scattering	Accardo *et al.,* 2004

Through electrostatic adsorption

Forces involved can be Vander walls, hydrogen bonding or ionic bond for stabilizing these interactions. The enzyme and matrix must have a sort of affinity towards each other through some charged groups/moieties for attraction. However, some surface changes can be made according to the demand by using chemical modifiers (carriers)/ linking agents (Jesionowski *et al.,* 2014).

Applications of alginate lyase

Production of Algino Oligosaccharides (AOS) and its applications

Degraded products of alginate, by the action of alginate lyase, having 20-25 monomers are called as algino-oligosaccharides (AOS). The role of AOS as immuno-modulators in human beings has been reported. Alginate lyase produced from *Pseudoalteromonas* sp. has shown to enhance the secretion of TNF (tumor necrosis factor). Mollah *et al.* (2009) and Cao *et al.* (2007) have cited the production of poly G-preferred lyase from a *Streptomyces* sp. strain A5, and its role in production of AOS. He also explained the use of AOS in promotion of growth of certain plants including banana and red amaranth. AOS might be used as effective anti-oxidants, however, some more experimentation is required to prove its the effectiveness as anti-oxidant (Jun *et al.,* 2019). Enzymatically degraded products of alginate are proved to have prebiotic effects in the micro flora of intestine that has many health benefits (Ramnani *et al.,* 2012).

Production of Bio fertilizer and its Application

From many centuries, seaweeds are thought to be potential source of nutrients, good soil conditioners and fertilizers (Mingpeng *et al.,* 2016). Alginate is widely present in the seaweed being an important component of the cell wall of brown alga. Difficulty in the breakage of cell wall and extraction of all components hamper its applicability as a bio fertilizer. Alginatelyse is used for extracting nutrients from cell wall of seaweed. These nutrients are further polished to use as biofertilizer (Clarens *et al.,* 2011).

Medicinal Applications

Alginate lyase, added with antibiotics usually administered in case of lung infection as the potential enzyme is able to degrade exo-polysaccharide biofilm made by *Pseudomonas aeruginosa* (Cotton *et al.,* 2009). Increased inhibition efficiency of alginate lyase against bio film maker pathogen *Helicobacter pylori* in combination with a conventional antibiotic is analyzed by Bugli *et al.* (2016). Islan *et al.* (2013) has reported synergistic effect of alginate lyase and antibiotic against cystic fibrosis. He demonstrated the production of biopolymeric microspheres serving as delivery system of drug to the target against infection caused by *Pseudomonas aeruginosa*. Jang *et al.* (2016) has also supported the therapeutic potential of alginate lyase in cystic fibrosis by analyzing catalytic mechanism of enzyme. The enzyme is also an enhancer of revascularization of the tissues (Campbell *et al.,* 2018).

Hunt *et al*., (2017) reported treatment of retinal diseases by help of alginate scaffold used as encapsulators of microbial cultures.

Application in Bioremediation

The wastewater present in the treatment plants of the industries has a large number of populations of micro-organisms. They release polysaccharides causes fouling of membranes of plants. Alginate lyase can act as de-fouling agent (as it degrades alginate) and prevent the fouling of membrane (Dong and Michael, 2011).

Production of Biofuel

Biomass of brown seaweed is an excellent raw material for the production of biofuel such as bioethanol and biodiesel. Saccharification is the main step which requires synergistic effects of alginate lyases with endo- and exo-action modes. Alginate lyase serves as biocatalyst for the process of saccharification (Shin *et al.,* 2015). The novel alginate lyase produced from *Halomonas* sp. is shown to have some suitable properties for industrial production of biofuel. Properties include temperature tolerance, osmotic tolerance, pH tolerance (Yang and Wu, 2016).

Application in Food Industry

Alginate can act as emulsifier, additive and thickening agent. In some processed foods it is a component and can be consumed directly. It acts as good encapsulator of micronutrients and probiotics (Petzold *et al.,* 2019). *Sargassum horneri* is a common alga that is fermented to get extract which is proved to have effects in promotion of growth of roots and seedlings. Alginate lyase produced from. Xue *et al.* (2019) has reported the use of alginate as an additive to increase the nutrition level of product and to enhance its taste.

Concluding remarks and future prospective

The above review encompasses various source organisms to isolate novel alginate lyase. The list is going long day by day as researchers are exploiting more potential sources of this enzyme. This alginate degrader has specific catalytic effect, but it requires more experimentation to get a detailed insight of its mechanism of action and targeted utilization of the enzyme. Some shortcomings such as decreased enzyme activity and difficulty in separation and purification steps and pathogenicity of producing strains create hurdles in the way of commercialization of product at industrial scale. Up to now, there is no strategy reported in literature that claims high productivity and low cost enzyme yielding methods. Immobilization is done to modify enzyme and to innovate its applications. Silver nanoparticles results in a significant payoff for the field of immobilization. Presently it has many promising applications in industries, but further research and expertise are required for enzyme to revolutionize specialty chemicals market in the near future. From the collective information available presently on alginate and alginate lyases we can say that these are important enzymes in a broad spectrum of biological roles.

CONFLICT OF INTEREST

Authors have no conflict of interests.

ACKNOWLEDGEMENTS

We acknowledge Director of Industrial Institute of Biotechnology and Vice Chancellor of Government College University Lahore, Pakistan.

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(*Accepted for publication October 2020*)