



## General Perspectives of Biopolymers-Review

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### ABSTRACT

The rising demand for sustainable alternatives to petroleum derived plastics has accelerated research into microbial biopolymers such as Polyhydroxyalkanoates (PHAs), Chitosan, Hyaluronic acid (HA) and Alginates. These polymers produced under optimized upstream bioprocess conditions, offer biodegradability and diverse biomedical and industrial applications. This review highlights microbial strains, media formulations and fermentation parameters that influence polymer yield and quality. PHAs are accumulated as intracellular carbon reserves in bacteria like *Cupriavidus necator*, *Pseudomonas* spp., and *Azotobacter* spp. They utilize substrates ranging from agricultural residues to kitchen waste. Chitosan which derived from fungal and bacterial chitin produced through fermentation of substrates such as rice straw, soybean residues and shrimp shells. pH, pretreatment and fermentation duration being critical factors for the production. HA synthesis, predominantly by *Streptococcus zooepidemicus* which enhanced through whey-based media and controlled glucose supplements. Collectively these findings emphasize the importance of substrate innovation, nutrient balancing, and process control in upstream bioprocessing to improve yield, reduce cost, and enable large-scale, ecofriendly biopolymer production. Future research should focus on integrated biorefinery approaches, genetic engineering of strains and valorisation of industrial and agricultural waste streams to establish a circular bioeconomy.

**Keywords:** Biopolymers, Polyhydroxyalkanoates (PHAs), Chitosan, Hyaluronic acid (HA), Sustainable, Bioeconomy, Ecofriendly, Microbes

### INTRODUCTION

The growing need of synthetic polymers is a problem in the global environment. Globally a large number of synthetic polymers are produced each year and their intricate degradation pose a significant problem in the environment that necessitates a trustworthy alternative (Saharan et al., 2023). Enzymes link with natural sources like sugars, amino acids, hydroxyl fatty acids and other building blocks to create polymeric materials are known as biopolymers. They can be chemically synthesized from biological material or produced by living organism (Akinsemolu & Onyeaka, 2023). Biopolymers can be either non-biodegradable (like bio polyethylene) or biodegradable (like polylactic acid). Alginate, cellulose, carrageenan, starch and plant glucomannans such as locust bean gum, mannan and guar gum are a few examples of commercially produced polysaccharides (Suput et al., 2015). The drawback of these biopolymers are their inconsistent quality and unreliable availability. Furthermore, it is inevitable that not all plants and seaweed polymers and their derivatives have rheological characteristics needed for applications at a reasonable cost. Biopolymers has various applications. History of pharmacology (Dr. S.Sreeremya, 2024a) and biotechnology had contributed in major advancements (Dr. S.Sreeremya, 2024b). Biopolymers can be synthesised by a wide range of microorganisms which serve diverse biological functions. Such biopolymers are differed from the conventional synthetic polymers, depending of their nature they serve specific properties. Bio functionality (stereoselectivity), biocompatibility, and biodegradability extremely helpful even in indispensable in various fields. Commodity chemicals (high volume low value products) and speciality chemicals (low volume high value products) can be produced by the use of biopolymers (Paul et al., 2020). Innovative biopolymers have potential uses in medicine (such as hyaluronate as a biomaterial), cosmetic products, food additives (such as xanthan and dextran), and packing relieved in recent developments in synthetic biology and bioengineering techniques. In microorganisms, enzymatic process creates biopolymers in the cytoplasm, organelles, cytoplasmic membrane, cell wall constituents and even extracellularly on the surface of cells. A biopolymer's synthesis may begin in one area of a cell and proceed in another as circumstance dictate (Swati Sharma et al., 2020). PHA are intracellular polyesters of microbes synthesized by various bacteria as intracellular carbon and energy storage reserves. Structurally diverse, they can be tailored for thermoplastic or elastomeric (B Dalton et al., 2022). Hyaluronic acid (HA) is an anionic, non-sulphated glycosaminoglycan composed of repeating disaccharide units (N-acetylglucosamine and glucuronic acid). HA serves as a lubricant, filter and scaffold in tissues with its remarkable properties of viscoelasticity, water-retaining ability and biocompatibility (Harth et al., 2024). Chitosan obtained by solid-state reaction has a heterogeneous acetyl groups distribution along the chain (Jayakumar et al., 2010b).

## METHODOLOGY

### UPSTREAM PROCESSING

Upstream bioprocess involves the preparation of the organism, media and bioreactor conditions to maximize polymer synthesis.

#### MEDIA PREPARATIONS AND PROCEDURES FOR SYNTHESIS OF PHA

For the synthesis of the microbial Polyhydroxyalkanoates which represent a group of biopolymers which are biodegradable in nature. It is considered similar to petroleum-based polymers. Numerous microorganisms under precisely defined working conditions in a particular substrate is referred to as PHA precursors. Many bacteria, such as *Cupriavidus* (C.) necator, different *Pseudomonas* species, strains belonging to *Azotobacter* (A.) species (*A. vinelandii*, *A. chroococcum*, *A. beijerinckii*), *Bacillus* (B.) spp, recombinant *Escherichia* (E.) coli, and *Burkholderia* (Bk.) spp, synthesize PHAs as intracellular carbon and energy storage, accumulating these polyesters of Hydroxyalkanoates as granules in the cytoplasm of cells. The cells can use the substrate as carbon source and integrate as PHA. Carbon dioxide, fossil resources like low-rank coal, renewable resources like starch, cellulose, and sucrose, waste products like molasses, whey, and glycerol, and chemicals like propionic acid are all relevant substrates for the synthesis of PHAs (Olimpia Pepe et al., 2017). Sesame oil samples from the sesame oil production facility were used to isolate the strain *S. epidermidis*. Agar plates were streaked with diluted sesame oil as a carbon source to produce the bacterial strains. Ten grams per litre of sesame oil, five grams per litre of  $\text{Na}_2\text{H}_2\text{PO}_4 \cdot 12\text{H}_2\text{O}$ , two grams per litre of  $(\text{NH}_4)_2\text{SO}_4$ , 0.4 grams per litre of  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , and 0.1% Tween-80. PHA accumulation cultures were cultivated in 500 mL Erlenmeyer flasks with media containing 1% (w/v) sesame oil as a carbon source for 48-96 hours at 35°C and 200 rpm (Wong et al., 2000). Molasses or sugar syrup can be converted into PHBpoly (b-hydroxybutyric acid) using strains of *A. latus*. After 93 hours of culture, a batch culture of *A. latus* ATCC 29713 was found to yield PHB up to 63% of dry cell mass. On sucrose, the average biomass yield coefficient was roughly 0.4 kg/kg. Only the first two of the four nitrogen sources of ammonium chloride, ammonium sulphate, ammonium nitrate, and urea were shown to sustain the growth of the culture and the synthesis of PHB (Grothet et al., 1999). Isolation of a halophile *Halomonas campaniensis* strain LS21 which could use kitchen wastes like mixed substrates as nutrients for production of bioplastic PHB. For 65 continuous days, the recombinant *H. campaniensis* LS21 with the PHB production genes *phbCAB* was cultured in artificial seawater with a variety of substrates that resembled kitchen garbage (soluble and insoluble cellulose, proteins, lipids, fatty acids, and starch). During the 65 days fermentation period, the recombinant produced almost 70% PHB at 37 °C with a pH of about 10 and 27 g/L NaCl. The benefits of this strain are demonstrated in this study with regard to inexpensive substrates (kitchen wastes like mixed substrates) (Yue et al., 2014). In order to provide highly concentrated carbon-rich feeding solutions with lower levels of inhibiting ingredients, optimization of upstream processing of feedstocks is important. Modest concentration of carbon substrate lactose of about 4-5 wt.-% contains in the sweet whey. An ultrafiltration step separates the proteinaceous whey retentate from the lactose-rich permeate fraction, about 21 wt.-% solubility limits of lactose be concentrate. To reduce the increase in volume of the cultivation broth, whey permeate can apply instead of native whey (WS Ahn et al., 2001).

#### MEDIA PREPARATIONS AND PROCEDURES FOR SYNTHESIS OF CHITOSAN

Chitosan, derived from the deacetylation of chitin which is the second most prevalent biopolymer, consisting of an extensive chain of N-acetylglucosamine. The study (Namboodiri et al., 2022) explains the production of chitosan from the rice straw substrate from the fungus *Penicillium citrinum*. Effect of pretreatment, particle size, moisture content and acetic acid addition were calculated and measured using Erlenmeyer flasks. Static and acidic pH (4.5) conditions were used for the fermentation process, and samples were taken every 24 hours for chitosan analysis. *Aspergillus niger* TISTR3245, *Rhizopus oryzae* TISTR3189, *Zygosaccharomyces rouxii* TISTR5058 and *C. albicans* TISTR5239 were used for the production of chitosan with the substrate media as soybean and mungbean residues. 30 g of sterilized substrate without moisture adjustment were injected with 1 ml of spore suspension (107 spores/ml) or 1 ml of yeast cell suspension (107 cells/ml) made from a 7-day-old slant in 500 ml Erlenmeyer flasks. For 16 days, the flasks were incubated at 30 °C. The moisture content, pH, fibre, fat, nitrogen content, carbohydrate, and ash of the substrates were all examined in (Suntornsuk et al., 2002). Fungal strain of *M. rouxii* DSM-1191 grown in potato Dextrose Broth (PDB; Merck, Darmstadt, Germany) under shake incubation condition at 28 °C for 75 hr. Centrifugation was used to harvest the mycelial growth, which was subsequently homogenized with 1M NaOH at 100°C for one hour after being twice cleaned with distilled water (Roberts, 2013). The alkali insoluble fraction was separated, washed and neutralized with 5% acetic acid (Tayel et al., 2010). Most of the publications using biological methods for chitin/chitosan mainly fermentation. Fermentation can be subdivided into lactic acid fermentation and non-lactic acid fermentation. The substrate of shrimp waste is used as the growth media *Lactobacillus* spp. strain B2. Fermentation parameters should be maintained in 30°C, for 4, 6 and 90 days, purification involves the demineralization HCl from 1.0 M to 0.2 M for 2 hr at 25°C. Then deproteinization by NaOH from 1.0M to 0.2M for 2 hr at 25°C (Cira et al., 2002). Demineralized prawn (*Nephrops norvegicus*) shell which contain carbon source as lactose, ideal media for the growth of *Streptococcus faecium* M74, *Lactobacillus plantarum*, and *Pediococcus acidilactici* for the fermentation. Pre fermentation process includes finely ground shell to 2 mm particle size in special reactor or fermentation at 25°C for 7 days (Healy et al., 1994). Screened and isolated *Streptomyces* species for chitinase production were reported and species like *S. viridificans*, *S. coelicolor*, *S. glaucescens*, *S. kanamycitis*, *S. lividans*, *S. parvies* and *S. venezuelae* observed their ability to produce chitinase. *S. viridificans* produce maximum levels of chitinase obtained at 1.5% colloidal chitin after six days of fermentation at 30 °C and 200 rev/min (P A Felse., 2000).

#### MEDIA PREPARATIONS AND PROCEDURES FOR SYNTHESIS OF HYALURONIC ACID

The generation of hyaluronic acid (HA) is mostly dependent on certain bacterial strains, especially *Streptococcus equi* subsp. *Zooepidemicus*. With 49 species and 8 subspecies, this gram-positive bacterium is incredibly varied (Liu & Catchmark, 2019). For the production of Hyaluronic acid (HA), optimization of cheese whey-formulated media of *Streptococcus zooepidemicus* were observed. N-acetyl glucosamine and glucuronic acid dimeric units combine to form the linear polymer known as hyaluronic acid (HA). This polymer is found in the cell walls of bacteria like *Streptococcus*

*zooepidemicus* and is a component of tissues like skin, cartilage, umbilical cord, avian crests, synovial fluid, and vitreous humour. The greatest amounts of HA were produced by culture media that contained either whey (W; 2.1 g/L) or whey hydrolysate (WH; 2.4 g/L) (Amado et al., 2015). *Streptococcus equi* subsp. *zooepidemicus* ATCC 39920 were used as organism as alyophilized culture kept in ampoules. In Brain Heart Infusion (BHI) broth with 10% glycerol and glass beads, the stock culture was kept frozen. The inoculum was made in two stages. First, five glass beads were put into each tube with five millilitres of the culture medium under study, and they were then incubated for twenty-four hours at 37 °C. After that, the tube's medium was moved to 125 mL Erlenmeyer flasks, which held 40 mL of the recently examined culture medium. The period needed to create an exponential phase inoculum, as previously established, was 10 hours, during which these flasks were incubated under reciprocal shaking at 150 rpm at 37 °C. The two stages of the inoculum preparation and the fermentations were conducted using the identical media in each instance (Pires et al., 2010). Hyaluronic acid with an average molecular weight of 1-4 MDa could be produced by *Streptococcus* fermentation. These bacteria only recover a small amount of energy during anaerobic fermentation. As a result, the bacterial fermentation process has produced a remarkably low yield of hyaluronic acid (0.1g/g glucose) (McLaughlin, 2005). A number of tactics were used to extend the synthase enzyme's lifespan, which increased *Streptococcus pyogenes* synthesis of hyaluronic acid and raised the cell's energy resource (glucose content) in the production media. The medium's pH should be optimized, and genes that negatively impact the hyaluronate synthase-producing gene should be mutated (P. Saranraj et al., 2013). *Streptococcus equi* subsp. *zooepidemicus* ATCC 39920 were cultured in the medium contained 60 g/L-1 yeast extract and salts (Swann et al., 1990). A pH of 7.5 was achieved before sterilization. To get IGCs between 0 and 90 g/L-1, a 20% (w/v) glucose solution was autoclaved separately and added to the medium. The stock culture was stored at -20°C in brain heart infusion (BHI) broth with glass beads and 10% glycerol. Glass beads were streaked onto BHI agar plates supplemented with 5% sheep blood (Biotério Boa Vista, São Paulo, Brazil) to create the pre-inoculum, which was then incubated for 24 hours at 37°C. After that, colonies were moved into the culture medium. Five millilitres of the inoculum were made at 37°C for twelve hours in order to cultivate it in shake flasks. For the cultivation in bioreactor, the inoculum was prepared with a series of transfers into the volumes of 25 and 250 mL of the same culture medium and both incubated at 37°C in a reciprocal shaker under 150 rpm during 12 and 6 hrs, respectively (A. M. B. Pires & Santana, 2010).

## CONCLUSION

Upstream bioprocessing plays a critical role in the efficient synthesis of microbial biopolymers such as PHA, chitosan and hyaluronic acid. The yield, quality, and cost-effectiveness of polymers are directly impacted by the selection of the microbial strain, substrate composition, and media optimization. By lowering reliance on fossil fuel-based feedstocks, renewable resources (such as starch, molasses, whey, oil wastes, and agricultural residues) and industrial byproducts have been shown to be sustainable substrates. Each polymer system requires careful management of certain growth conditions, including temperature, oxygen supply, pH, carbon-to-nitrogen ratio, and fermentation type. Research shows that using novel substrates (such as rice straw, shrimp shell, whey, and kitchen trash) in conjunction with ideal fermentation conditions not only increases output but also guarantees both environmental sustainability and economic viability. Thus, tailoring upstream bioprocess strategies to both microbial physiology and available feedstocks remains the foundation for large-scale, eco-friendly biopolymer production.

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