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FULL LENGTH ARTICLE

Review on production of Bacterial Cellulose from wastewater and its applications

G.Gayathri, G.Srinikethan
Department of Chemical Engineering, National Institute of Technology Surathkal, Karnataka, India
Email: gaya30105@gmail.com

ABSTRACT
Bacterial cellulose (BC) a biopolymer, synthesized by aerobic bacteria is gaining importance due to its unique physiochemical properties that are beneficial for various applications. Researches mainly focus onbiosynthetic process using low cost materials which can act as microbial substratesand produce BC with higher yield and purity. This review discusses usage of the waste water/ raw effluent which contains huge amount of organic rich in Carbon and Nitrogen as the model effluent to verify whether that can be used as a crude substrate for growth of suitable microorganism and production of BC including its applications.
Keywords*—Bacterial/Microbial Cellulose, wastewater,Acetobacter xylinum*

INTRODUCTION

The wastewater generated from industries and other sources in huge quantities is a major concern worldwide. These industrial effluents which contains large amount of organic (carbon and nitrogen), phosphates contributing to high BOD, hence have to be subjected for various treatments and meet the regulatory standards before they are discharged into the environment. Currently worldwide technologies are developed on recycle, reuse and reduce concepts. Industries such as distilleries, paper and pulp, textiles generate large amount of wastewater containing organic and inorganic substances substances ([1]–[4]). In biological treatment through biochemical operations end products are carbon dioxide, water and biomass, whichare not used later. Instead the organic content and nutrients present in wastewatercan be utilized as substrate for microorganisms with twin objectives of reducing BOD and growth of useful microorganisms which can release biopolymer or other essential materials that can reduce overall cost. Therefore sustainable development process has to be developed to utilize the organic carbon and other nutrients which can be helpful for the growth of specific microorganisms so that useful biopolymer such as Cellulose can be recovered. One of the most prominent drawbacks of plant cellulose is that as they are made up of 2 more polymers other than cellulose i.e. hemicellulose and lignin, hence exhibit limited properties. The pulping and bleaching processes which are used to obtain the cellulose fibers release toxic effluents and chemicals which in turn cause environmental pollution. In this regard, microbial cellulose seems to better choice over the plant ([5], [6]). Moreover, the cellulose from micro-organisms could be obtained in a purer form and quicker as compared to cellulose from plants. Also, since this is an eco-friendly method for obtaining cellulose no harsh treatments are required for purification of bacterial cellulose because it is free from hemicelluloses and lignin, the adverse impact on the environment will be minimized.

BACTERIAL CELLULOSE

Bacterial cellulose (BC) was initially identified in *A. xylinum* as a growth of unbranched pellicle with chemically equivalent structure as plant cellulose [7]. This type of cellulose possess number of properties such as higher chemical purity (free from lignin and hemicelluloses), higher degree of polymerization, greater mechanical strength, high crystallinity index , higher water retaining capacity, in-situ moldability, larger surface area, biodegradability and biocompatibility ([8]-[13]).

A. Structure of BC and its metabolism

Bacterial Cellulose is made up glucose monomers that are linked by β-1→4 glycosidic linkages with molecular formula $(C_6H_{10}O_5)_n$. The glucan chains are held together by inter and intramolecular hydrogen bonding [14]. The synthesis of BC is carried by multi enzyme complex by a sequential steps: (a) glucose is phosphorylated to glucose-6-phosphate by the enzyme glucokinase; (b) glucose-6-phosphate is isomerized to glucose-1-phosphate by phosphoglucomutase; (c) glucose-1-phosphate is converted to uridinediphosphate glucose (UDP–glucose) by enzyme UDP-Glucose phosphorylase; and (d) UDP-glucose is converted to cellulose by cellulose synthase which involves the formation ofβ-1→4 glucan chains with the polymerization of glucose units followed by assembly and crystallization of cellulose chains ([15],[5]).

B. Cellulose producing microorganisms

Cellulose is produced by various microorganisms such as algae, cyanobacteria and bacteria. ([16], [17]).Predominantly bacterial strains of different genera such as*Acetobacter (Gluconacetobacter), Aerobacter, Achromobacter, Agrobacterium, Alcaligenes, Azotobacter, Pseudomonas, Rhizobium, Sarcina, Salmonella, Escherichia*, etc. have shown their ability to produce cellulose in the form of a mat or diffuse pellicle at the air–liquid interphase ([18], [5]). *Acetobacterxylinum*[19] is the the most efficient BC producer due to its ability to grow on various carbon and nitrogen sources ([15], [20], [21]). High cost and low yield production has limited the industrial production of BC and its commercial application. Therefore, it is important to look for a new cost-effective carbon source with shorter fermentation process for high yield BC production.

PRODUCTION OF BACTERIAL CELLULOSE

C. Fermentation conditions

BC production is produced in either by static or agitation/stirring conditions. Thick pellicle was reported under static condition while under agitated condition produced irregular shape spherical cellulose particle [22]. Under static condition aeration and carbon source forms the important parameters to enhance yield [27]. Pellicle grows downward indicating maximum BC production. At industrial scale so as to increase the yield and productivity Semi-continuous process is preferred [30].

Agitated condition results in formation irregular forms of cellulose either as spheres/pellet debris or irregular masses ([31], [32]). When compared to the pellicle in Static condition, the polymerization and mechanical strength of cellulose formed via agitation process is lesser. Studies have shown disruption of hydrogen bonds by aeration is responsible for this [33].

D. Media and optimization

The fermentation medium contains carbon, nitrogen and other macro- and micronutrients required for the growth of the organism. Exopolysaccharides is usually most noticeable when the bacteria are supplied with an abundant carbon source and minimal nitrogen sources to the culture medium.

1) Carbon sources

Generally, glucose and sucrose are used as carbon sources, although other carbohydrates such as fructose, maltose, xylose, starch, and glycerol have also used [23]. Initial glucose concentration plays an important role in thecellulose production as there is the formation of gluconic acid which decreases the pH of the culture and as a result Cellulose production decreases. The addition of polyphenol compounds or antioxidants tends to inhibit gluconic acid production [24]. Cellulose yields at initial glucose concentrations of 48 g/L were studied, and the consumption of glucose was found to be 28 % of the initial concentration [25]. Ethanol is used as additional carbon source and also to inactivate the growth of cellulose non-producing bacterial cells which tend to grow under submerged culture conditions [26].

So as to reduce the cost of synthetic culture for the production of BC, many studies have been carried out using agriculture waste and industrial by-product as a potential carbon source ([28], [29]). Some of them used as carbon source for BC production are waste beer yeast [34], thin stillage [35], maple syrup [36] and sugarcane molasses [37]. As a result, they reduce environmental pollution by suitable reuse of waste.

2) Nitrogen sources

Nitrogen is the main component of essential for the synthesis of nucleic acids and proteins, comprises 8–14 % of the dry cell mass of bacteria. Various nitrogen sources are studied such as the yeast extract, casein hydrolysate and peptone ([12], [38]-[43]). Excess of nitrogen favors biomass production but cellulose production drops [44].

3) Temperature

Temperature is an environmental parameter that influences both growth and cellulose production. Studies have shown the cellulose production was observed between 28 and 32 °C. Optimum temperature where maximum production was at 30 °C ([5], [38], [45], [43], [46],[47]).

4) pH

The optimum pH at which bacterial cellulose production is in the range of 4.0 to 7.0, the yield of cellulose decreasing below pH4 ([25], [38], [48], [43],[49], [5]). The pH decreases as fermentations occur because of the accumulation of gluconic, acetic or lactic acids in the culture broth [50]. Therefore, it is important to control the pH within the optimal range by monitoring at frequent intervals.

5) Dissolved oxygen

The dissolved oxygen in the culture medium is yet another factor affecting cellulose production. In static fermentation, it depends on diffusion; the oxygen availability might become the limiting factor for cell metabolism and could have a negative effect on cellulose production and quality of the cellulose [51]. Also too high dissolved oxygen in the medium increased the gluconic acid content and thus affected the cellulose production, whereas too low dissolved oxygen in the medium also affected the production as the microbial growth was limited. ([52], [56], [53]).

SCALE UP STUDIES

Attempts have been made by some researchers to scale up cellulose production under agitated culture using conventional fermenters which provided significant results ([54],[48], [52], [46]).

BC DETECTION AND ANALYSIS

Dyes are used for identifying both amorphous and crystalline form of cellulose. All these dyes are fluorescent brightening agents and their dye-cellulose complexes are stabilized by weak bonding forces such as Vander Waals forces and hydrogen bonding. Calcofluor white is one most commonly used [55].

Different methods/techniques are employed for analyzing various properties of bacterial cellulose such degree of polymerization is determined by viscometry ([57],[40]). To determine the structural features of bacterial cellulose, they are generally examined by X-ray diffraction, Raman, Infrared, and NMR spectroscopy. Conventional methods were used to determine other physicochemical parameters such as Young's modulus, Thermal stability, Water holding capacity and Mechanical strength after the bacterial cellulosic sheets were dried thoroughly ([58],[59], [10]).

PURIFICATION OF BACTERIAL CELLULOSE

Bacterial cellulose obtained after fermentation contains bacterial cells entrapped beneath with other impurities. De-ionized water was be used to remove organic and inorganic impurities (metal ions) in the cellulose mat which might be present after washing using tap water.

Cellulose mats produced in the medium was treated with 1.0 L solution of NaOH/KOH of different normality in the range of 0.1 N – 3.0 N at 80 ºC for 15 min. Decolouration of cellulose mat and was examined. After treatment with acid, cellulose mats were washed with 1.0 L de-ionized water for 10 min to remove excess acid ([5], [26], [48],[50], [5],[21]). Cellulose mats obtained after alkali and acid treatment were dipped in 0.5% solution of bleaching agents like sodium hypochlorite (NaOCl) and Hydrogen peroxide (H_2O_2) at 90 ºC for 8 h. The efficiency of the bleaching process was analyzed by observing the colour of the mat. Bacterial cellulose so obtained is dried either by freeze drying, air drying, drying in a vacuum or a simple conventional oven. In air drying method, the purified cellulose mats and was on placed/ spread on a Teflon / wooden slab and dried at room temperature for 48 h.Most of the workers have dried microbial cellulose in a vacuum oven ([54], [48], [41], [43]). In some reports, the purified bacterial cellulose was dried to a constant weight at 80 or 105 °C in a conventional oven [38]. The dry mass of bacterial cellulose without any microbial cells was measured after drying ([25], [50]).

APPLICATIONS

Due to its versatile properties such ashigh purity, hydrophilicity, high degree of polymerization and biocompatibility, they have been efficiently used in various areas including textile industry, paper, food, pharmaceutical, cosmetics, tissue engineering, and dentistry and predominantly in medical field ([61], [5])

Food applications: Chemically pure cellulose is used in processing foods as thickening and stabilizing agent. The first use of microbial cellulose in the food industries was in form of gel *Nata De Coco* which was synthesized by *A.xylinum* bacteria [62].

Paper industry:Ajinomoto Co. along with Mitsubishi Paper Mills in Japan is currently active in developing microbial cellulose for paper products.Bacterial cellulose has been found to be effective as binders in paper as they add on the strength and durability [63].

Pharmaceutical and medical applications: BC has been applied in multiple fields such as wound dressing ([65]-[67], [5]) blood vessel regeneration [64].BC's predominant application is a topical covering for severe wounds. Others include artificial skin, coverings for nerve surgery and arterial stent coating [65]. Bioprocess®, XCell®, and Biofill® products already are commercially available which serve in wound healing ([67], [68]).Tissue engineering as tissue replacement or gap substitute material to maintain the integrity of tissue, in scaffold development ([69], [75]).

Cosmetics: BC facial masks are used to treat dry skin due to its biocompatibility, low toxicity and ability to hydrate the skin [70]. BC is also playing a role in fabrication of contact lenses due to the properties such as transparency, light transmittance, and permeability to liquid and gases. BC-based contact lens was prepared by means high viscosity BC solution which remained intact in its shape and transparency for about 42 days [71].

Drug delivery: Recent studies have shown the release of the antibiotic amoxicillin (AMX) from BC at nearly neutral (7.4) pH conditions. The concentration of AMX significantly influenced the drug release. This system might provide a suitable way for antibiotic delivery to the wound [72] .
Immobilization material: Various studies have used Bacterial Cellulose as immobilized carriers for cells and enzymes ([73],[75], [32]).

CONCLUSION AND FUTURE PROSPECTS

To conclude, the versatile properties of bacterial cellulose makes it suitable for a wide variety of applications. But in order to meet the demand at commercial scale, a robust and feasible industrial production process and supply is crucial. As this form of cellulose is not associated with lignin or hemicellulose, purification steps are comparatively easy, therefore higher yield and purity BC can be obtained.

Newer approaches

Low cost waste materials contains organic and inorganic substances that can be used to meet the twin objectives of reducing cost of treatment i.e., reduction of BOD and as a Carbon source for the production of BC. Continuous production on large scale can make it an economical process by reducing the overall cost.

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