ABSTRACT

The demand for cellulose is increasing owing to its desirable properties such as biodegradability, affordability, availability, manufacturing technologies, recyclability and eco-friendly. This demand will continue to scale up in the forthcoming years as cellulose has the potential to replace petroleum-based products for sustainability, and cost effectiveness. This process stimulates research and technological development, resulting in a set of innovativeness in value-added products. Several fundamental properties of cellulose make it very attractive in growing industries and suitable for various applications. Bacterial cellulose is gaining attention due to its purity and promising applications in many industries such as biomedical, food, cosmetics, tissue engineering, electronics, packaging industries, food additives, paper products, and biomedical devices because of its exclusivity of being a better bio-compatible coating agent, binding material, etc. It could be produced by certain species of bacteria and is also very conductive due to its unique properties, like good biocompatibility, excellent mechanical properties, a high degree of polymerization, and biodegradability.

In the present study, a unique bacterium, *Leifsonia soli* has been isolated from rotten fruit wastes. Identification studies of the isolated bacterium have been done with phylogenetic analysis by 16S rDNA gene sequencing. Morphological and biochemical characterization of the bacterium has also been done. This bacterium produced cellulose extracellularly when cultured in appropriate culture medium. Amongst all the parameters used for optimizing the bacterial cellulose production, maltose, soy whey, and calcium chloride had an intense effect in enhancing its synthesis from the bacteria. The isolated strain, *Leifsonia soli* yielded maximum of 5.97 g/L of cellulose at optimal growth conditions of pH 6.5, 30 °C under 7 days of the incubation period.

In addition, all selected parameters were optimized through ANOVA-based response surface methodology. Along with that, impacts of carbon/nitrogen ratios on the production of bacterial cellulose in static cultivation of *Leifsonia soli* were examined by using central composite design and response surface analysis. Amongst selected sources, soy-whey and maltose were found to be favorable for BC production. The C/N-ratio of 9:1 stimulated the propagation of bacteria. Additionally, bacterial cellulose characterization was

accomplished by using field emission scanning electron microscopy, X-ray diffraction, and Fourier-transform infrared spectroscopy studies to estimate its properties for appropriate applications. From the characterization studies, it has been found that obtained cellulose is highly porous and semi-crystalline with all absorption peaks. Keeping all these views in mind the present work has been formulated to go for bacterial cellulose production by utilizing a potential strain of *Leifsonia soli* in an eco-friendly and sustainable manner.

A novel study has been done which is based on the concepts of kinetics and morphology of produced cellulose by the *Leifsonia soli*. With the help of atomic force microscopy and field emission scanning electron microscopy, it has been observed that the cellulose layer formation in the culture flask was increasing up to the 7^{th} day of incubation. Further, the semi-crystalline nature of cellulose has been found through an x-ray diffraction technique and it revealed that the structure of cellulose was allomorph of Iα and Iβ. Additionally, the growth kinetics of bacteria was also co-related to cellulose production. Further, the purity of obtained cellulose was examined by elemental analysis where it was observed that the sample holds the value of carbon 44.1 ± 0.20 % and hydrogen 6.2 ± 0.3 %, respectively. In addition, the calculated water-holding capacity of the sample was found to be 73 %.

Two distinct applications have been done from the obtained cellulose, both of which are very eco-friendly and sustainable. It has been seen that obtained bacterial cellulose has a profound role in the production of bioethanol 10.83 % (v/v) concentration when the saccharification conditions were kept as 10 h and fermentation with *Saccharomyces cerevisiae* for 24 h. In addition, a BC-based film was prepared using a combination of sodium alginate, glycerol, and gallic acid, and characterization studies were performed on the obtained film. The results are quite promising for its greater applications in food packaging and biotechnological sector.

Keywords: 16S rDNA gene sequencing, soy-whey, C/N ratio, bioethanol, AFM, growth kinetic, BC-based film