**Original Research Article****DOI: 10.26479/2019.0503.32****STUDY OF BIODEGRADABLE PACKAGING MATERIAL
PRODUCED FROM SCOBY****Priyanka Aduri*, Kokolu Ankita Rao, Areeba Fatima, Priyanka Kaul, A. Shalini**Department of Biotechnology, Chaitanya Bharathi Institute of Technology,
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ABSTRACT: To overcome the various deleterious effects of plastic food packaging, the objective is to find out if in reality a material produced from biological sources could act as an alternative to plastic and could be put to use on a large scale. Micro-organisms have been found to be a better solution for production of high quality products with minimum complexity. Production of a food packaging material from a particular species of microorganisms might just be a solution to the problem of wide usage of plastic. SCOBY (symbiotic culture of bacteria and yeast) obtained on fermentation of Kombucha can serve as an edible packaging material. It is the gelatinous mat, a bacterial cellulose (BC) formed by Kombucha tea fermentation. Kombucha is a beverage that is produced by tea (Black tea/ Green tea) and sugar fermentation using SCOBY as starter culture. It is a conglomerate of yeasts and Acetic acid bacteria. Since SCOBY is a biologically consumable and a fully recyclable packaging option, it can be used to store food products with no waste thus giving a biodegradable, eco-friendly and a zero waste packaging if proved to be one. However, more research on the properties of SCOBY and its limitations if any are necessary for further conclusions.

KEYWORDS: Plastic, Food packaging, biodegradable, SCOBY, Bacterial cellulose, Kombucha fermentation.

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1. INTRODUCTION

Today, as food safety has become a global concern, scientists all over the world are working to find substitutes for “**Plastics**”. These have become an integral part of our daily lives and they continue to benefit the society because of which their ill effects are unheard or ignored. Plastic has been used since in various forms for different applications mainly due to it being inexpensive, corrosion-resistant, durable, strong and also its high thermal-electrical insulation properties. Due to these favorable properties, its production has increased over the last 60 years from around 0.5 million tonnes in 1950 to over 260 million tonnes today. Plastics are used in our day to day life in different ways, for example as carry bags, storage boxes, footwear, laboratory equipments, food packets, in tissue engineering, medicine etc[1].Packaging industry is now making 90% of their packaging material with plastic[2]. They prove deleterious to human health as well as environment. Research says they expose humans to toxic components like bisphenol A (BPA) and di-(2-ethylhexyl) phthalate (DEHP). Also major forms of plastics are non-biodegradable in nature which makes them potential sources of pollution, soil especially. Incineration of plastic results in the release of carbon dioxide, a greenhouse gas, and other air pollutants, including carcinogenic polycyclic aromatic hydrocarbons and dioxins [3].Due to reasons like these; it necessitates the need to find environment friendly alternatives to plastic packaging.In recent years, antimicrobial packaging has attracted much attention from the food industry because of the increase in consumer demand for minimally processed, preservative-free products. As a result of this demand, the preservative agents must be applied to packaging in such a way that only low levels of preservatives come into contact with the food [4].Viable edible films and coatings have been successfully produced from whey proteins having the ability to serve as carrier of antimicrobials, antioxidants, or other nutraceuticals, without compromising the desirable primary barrier and mechanical properties as packaging films which will add value to commercial applications in food industries [5].Several alternatives to plastic food packaging have been developed such as polymers extracted from biomass from plants, marine and domestic animals which include cellulose, chitin and starch, whey protein, casein, collagen, soy protein, myofibrillar proteins of animal muscle etc. Most common type of polymer produced by bacterial fermentation of sugar Polyhydroxy-butyrate (PHB) has been found useful in the areas of food packaging, cosmetic industry, pharmaceuticals and agriculture. Another biodegradable polymer includes Polyhydroxyalkanoate (PHA) produced from styrene monomer by bacterium *Pseudomonas putida* which is found to be water insoluble, biodegradable and compostable in nature. Currently, biodegradable polymer are molded into gels, films, bags, boxes with lids and trays and brought into the market for easy use and comfortable packaging[6].A recent report by a Polish design studentRozaJanuszsuggested that SCOBY(symbiotic

culture of bacteria and yeast) obtained on fermentation of Kombucha can serve as an edible packaging material. Kombucha is a beverage that is produced by tea (Black tea/ Green tea) and sugar fermentation using SCOBY. It is a slightly sweet, slightly acidic refreshing beverage consumed worldwide. Prolonged fermentation results in a vinegar like acidic flavor to the beverage It is a 6-10 days fermentation carried out under aerobic conditions at 20-30°C. Certain antimicrobial components are formed during Kombucha fermentation which inhibit the growth of *Shigellasonnei*, *Escherichia coli*, *Salmonella enteritidis* and *Salmonella typhimurium*. These components are substances other than organic acids, ethanol, proteins or tannins originally present in tea or their derivatives. They are mostly microbial metabolites produced by bacteria and yeasts during fermentation with tea and sugar as substrates [7]. Kombucha is a consortium of yeasts and Acetic acid bacteria [8]. The commercial brewing of Kombucha has increased in United States along with other parts of the world [9]. Medical beneficial of consuming Kombucha include prevention of few types of cancer, arthritis, heart disease, allergies, asthma, increase of T cell count etc [10]. The fermentation of bacteria and yeast will produce an organic membrane which can then be used to store a variety of lightweight foods like seeds, nuts, or even salads. The packaging material has long shelf life and stays edible for quite a long time due to its low ph. The food packaging is completely biodegradable and can be eaten after use thereby giving a zero-waste and eco-friendly alternative to plastic packaging. SCOBY is an abbreviation for Symbiotic Culture of Bacteria and Yeast. It is also known as a biofilm, pellicle, and zooglea (living skin), and yeast mat or near lichen. It is the gelatinous mat, a bacterial cellulose (BC) formed by Kombucha tea fermentation. The main bacterial strains that are present in the SCOBY are *Acetobacterxylinoides*, *Gluconacetobacterxylinus*, *Acetobacteraceti*, and *Acetobacterpasteurianus* etc. Yeasts include *Schizosaccharomyces pombe*, *Saccharomycode sludwigii*, *Saccharomyces cerevisia e*, *Torulaspora*, *Zygosaccharomyces bailii*, *Brettanomyces lambicus*, *Candida Pichia* species etc [11]. It can be referred to as a Biomaterial which can be defined as chemically unrelated product which is synthesized and catabolized by microorganisms which are subjected to different environmental conditions [12]. The best example of a biomaterial is Bacterial cellulose which is synthesized not only by plants but also by certain bacteria such as *Rhizobium* species, *Agrobacterium* species, *Acetobacter* species and *Alcaligenesspecies* [13]. Bacterial cellulose (BC) possesses unique properties, including high mechanical strength in the wet state, a high crystallinity, biocompatibility, non-toxicity, high porosity, an ultrafine fiber network and high water holding capacity [14]. Such features make BC an outstanding material suitable for technological applications in bioenergy, bioplastics, biomaterials, food chemistry and packaging [15]. The biochemistry behind the production of this BC includes 5 fundamental enzyme mediated steps: transformation of glucose to UDP glucose

via glucose-6-phosphate and glucose-1-phosphate and finally addition of UDP-glucose to the end of a growing polymer chain by cellulose synthase. Cellulose is formed between outer and the cytoplasm membrane. In the first step of cellulose formation glucan chain aggregates which has approximately 6-8 glucan chains which are elongated. These fibrils are assembled in the second step to form micro fibrils followed by their tight assembly to form ribbon as the third step. The matrix of interwoven ribbons constitutes the bacterial cellulose membrane [16]. Due to their favorable properties BC are known to have wide number of application in the industries such as food, paper, composite and cosmetic. The current biotechnology has enabled wide number of application in the field of medicine and has opened new opportunities in biomedical engineering.

1) Food industry

Offers health benefits because of which it is considered as “generally recognized as safe” (GRAS)[17]. BC is known to be a sweet dessert in the Southeast Asia this dessert is known as Nata de coco and Nata de pina whose flavors are controlled by coconut water based and pineapple water based culture mediums[18][19]. Other food product of fermentation using *Acetobacterspp* is Kombucha tea.

2) Nanocomposites, Silver particles

Preparation of BC membrane containing silver nanoparticles was done by Barud and his coworkers in 2008. BC can be used in as a template for hybrid nanocomposites. These hybrids are known to have application in filtration, toxic remediation and production of loudspeaker membranes [20].

3) Paper industry

BC can be used to improve the gloss on the paper, also reduce the gram mage of paper and its products. There has also been development of BC based carbon electronic papers. [21][22] Used BC based nanopaper which is known for ultra filtration technique.

4) Cosmetic industry

BC has been included in cosmetic formulation in order to produce oil in water emulsions because it provides high degree of hydration henceforth can be used in moisturizing cream, acts as an important component in finger polish and artificial nails, powders [23]. BC based gels facemasks have also been in the market for a while. [24] Introduction of nanoparticles in a BC based gel to encapsulate hydrophobic particles.

5) Biomedical industry

Production of hollow type spherical bacterial cellulose as a controlled release device which is known to have application as a drug delivery system. [25] Production of artificial blood vessels for microsurgery. Biomedical devices recently have gained a significant amount of attention because of an increased interest in tissue-engineered products for both wound care and the regeneration of

damaged or diseased organs. The nonwoven ribbons of microbial cellulose micro fibrils closely resemble the structure of native extracellular matrices, suggesting that it could function as a scaffold for the production of many tissue-engineered constructs. Microbial cellulose membranes, having a unique nanostructure, could have many other uses in wound healing and regenerative medicine, such as guided tissue regeneration (GTR), periodontal treatments, or as a replacement for Dura mater (a membrane that surrounds brain tissue). Though the potential use of SCOBY as a packaging film is a recent report, it has been used to produce a workable bio-textile, called "vegan leather" by Queensland University of Technology and the State Library of Queensland as reported in August 2016. SCOBY also has many topical uses. One of the terms for the SCOBY is zooglea, which translates as "living skin" and helps heal the skin from burns, wounds and other skin ailments. It can also be used in water filtration and soil remediation. It has also been reported to contain about 18 percent protein, 12 percent crude fiber, 4 percent phosphorus, and 6 percent calcium. It is nutrition packed culture and a great source of protein and is therefore, can be used for human and animal feed consumption. Since SCOBY being a biologically fully edible and a fully recyclable packaging solution, it can be grown by farmers to wrap their products and bring them to market with zero waste thus giving a biodegradable, eco-friendly and a zero waste packaging if proved to be one.

2. MATERIALS AND METHODS

Kombucha Fermentation

Kombucha tea fermentation is usually done in a glass vessel covered with clean cloth and incubated at room temperature (28–30°C) for 7–12 days [26]. After 2-3 weeks; a gelatinous layer was obtained as byproduct.

Drying of byproduct

The byproduct was washed under tap water and dried in the sun under the environmental conditions. This was done to obtain different shape and size of the material. The dried material obtained was our desired biodegradable packaging material.

Anti-bacterial analysis of the Kombucha tea and the obtained material

Kombucha tea and the prepared packaging material were tested against various bacterial strains and checked for anti-bacterial activity. Nutrient Agar media was prepared for 1000 ml and divided into small flasks of 100 ml and kept for autoclaving. 10 petri plates were autoclaved along with the media at 121°C for 15 minutes at 15 bars pressure. The petri plates and media were removed and cooled slightly. Under sterile conditions; 100 ml of Nutrient agar was distributed amongst the petri plates and left for solidification. Using spread plate technique, about 100 µl of bacterial strain (*Bacillus*, *Escherichia coli*, *Enterobacter*, *Staphylococcus*, and *Pseudomonas*) were spread onto 2 petri plates

each using a sterile spreader. For anti-bacterial analysis of the tea, a well was punctured at the center of 5 petri plate containing the solidified media. To this well, about 100 µl of the prepared Kombucha tea was added using a sterile micropipette. For antibacterial analysis of the material, a piece of the material was placed at the centre of the leftover solidified media, petri plates using sterile forceps. The petri plates were then incubated at 37⁰C and checked for growth inhibition after 12-15 hours.

Biodegradable packaging material checked for its packaging capacity

The packaging capacity was tested for 3 samples: Tomato (*Solanumlycopersicum*); Spinach (*Spinaciaoleracea*); Grapes (*Vitisvinifera*). The packaging material was stitched to make a bag to store fruits and vegetables samples in it. Plastic zip locks containing the same fruits and vegetables acted as standard. Three types of tests were done to see the food packaging capacity of the material:

(i) Physical Test

One set of the food samples were packed in the bag produced and the other set in plastic zip locks. Both set of the samples i.e. SET A in as well as the SET B was stored simultaneously in the refrigerator. After 8 days, samples were taken out and observed for physical changes i.e. change in appearance or any signs of spoilage. Apart from solid samples, a little amount of Milk was also taken and put into a small sachet prepared using the packaging material.

(ii) Nutritive analysis

Both set of the samples were also sent for nutritive analysis of the following parameters to check for any major deviations in the values. The parameters that were expected to show deviations, if any were Fats, Moisture content, Carotenoids, Vitamins and Minerals. As per convenience, in Carotenoids – Lutein, Lycopene and Beta-carotene were checked. For Vitamins – Vit-C, B5 and B6 were checked. For Minerals - Zn, Fe, Mn and Cu) were checked. The methods of the Association of Official Analytical Chemists were used for analysis of fat (2003.05) by solvent extraction method, using chloroform: methanol (2:1) (v/v) [27]. Elemental analysis was carried out after doing wet digestion according to AOAC (968.08) method. Briefly the powdered samples were digested using Suprapure HNO₃ (65%) and H₂O₂ in the ratio of 2:1(v/v), filtered and used for the analysis by flame atomic absorption spectroscopy (Varian- SpectrAA 220, USA). Carotenoids were determined as per the procedures described by Rodriguez-Amaya and Kimura [28]. They are extracted after saponifying the sample with petroleum ether in the presence of 12% (w/v) alcoholic potassium hydroxide. The total carotenoid was estimated by taking the optical density (OD) at 450 nm in Spectrophotometer (Analytikjena U-2800 SPECORD S.600).



Figure 1 : Samples of SET A packed in the bag produced from SCOBY



Fig 2: SET A -Before &After Incubation (Tomato, spinach and grapes to be packed in packaging bag)



Fig 3: SET B Before & After Incubation (Tomato, spinach and grapes that were packed in plastic bag)

(iii) Biodegradability Test

The following test was performed to check if the packaging material was biodegradable or not. A steel tray and a plastic container were taken and filled with moist soil covering 3/4th of the tray. 3 biodegradable packaging material samples were taken, which were previously produced in the project. First sample was not pre-treated it was used as it is. The second sample was pretreated with water

which makes it moist. The third sample was pre coated with beeswax. Plastic was taken as the standard and placed in a plastic container. All the 4 materials were placed on their respective containers of soil and covered with the soil till the rim. The tray and container were left to incubate for 10 days and results were viewed.



Figure 4: Samples of packaging material for biodegradability (Clockwise)- SCOBY sample (without treatment); pre-treated with water; Pre-treated with bees-wax.

Leak Test of the packaging material

The material was sprayed with oils to make it leak-proof. First, olive oil was sprayed onto the dried packaging material. In the second attempt, to obtain better results, beeswax was melted and added to olive oil in a bowl and sprayed onto the surface of the obtained packaging material. The material was made to cover a beaker head using rubber bands and few drops of water with dye was allowed to settle on the material and this setup was left for a day.

3. RESULTS AND DISCUSSION

Preparation of Kombucha and SCOBY material

Kombucha tea was prepared by black tea fermentation inoculated with SCOBY starter culture. Fermentation was carried out for about 2 weeks at 27-37°C after which a gelatinous mat was obtained. After washing the SCOBY, it was dried on a wooden plank.

Antibacterial activity

(i) For the Kombucha Tea: Antibacterial activity was observed for the following bacterial strains – *Bacillus*, *E-coli*, *Enterobacter*, *Staphylococcus* and *Pseudomonas*. The maximum zone of inhibition was observed in *Enterobacter* while the minimum zone of inhibition was seen in *Pseudomonas*.

(ii) For the packaging material: The SCOBY material inhibited growth of the following bacteria in its vicinity - *Bacillus*, *Enterobacter*, *Staphylococcus* and *Pseudomonas* but not for *E-coli*.

Biodegradable packaging material checked for its packaging capacity

Both SET A (Samples packed in packaging material) and SET B (samples packed in plastic zip locks) were observed after 8 days refrigeration and subjected to various tests.

(i)Physical Test: All the 6 samples (SET A and SET B included) were observed visually to check appearance and signs of freshness. All the samples remained fresh and showed no signs of spoilage even after 8 days of incubation. However, when Milk was packed in the scoby sachet, it leaked due to improper closure of the ends. It also made the scoby moist and soft. This shows that SCOBY still needed to be made non-porous in order to support liquid foods.

(ii)Nutritive Analysis: All the 8 samples (SET A and SET B included) were subjected to quantitative nutritive analysis for the following parameters: Fats, Moisture content, Carotenoids (Lutein, Lycopene Beta-carotene), Vitamins (Vit-C, B5 and B6), Minerals (Zn, Fe, Mn, Cu). Very minute changes in values were observed. This concludes, apart from keeping the samples fresh, this biodegradable packaging has no harmful effects on the nutritive quality of the foods on the whole.

(iii)Biodegradability Test: Over a period of 10 days, It was observed that the sample which was pre-treated with water degraded into the soil while the other 2 samples and the standard remained intact. It can be assumed that if the set-up was to be left for another 15-20 more days, the other two packaging material samples would have degraded as well.

Table 1: Nutritive analysis of Fats in SET A and SET B.

	Total Fat Content (g/100g)	
Type of sample	SET A	SET B
Tomato	0.43	0.46
Spinach	0.38	0.37
Grapes	0.56	0.58

Table 2: Analysis of Moisture content in SET A and SET B

	Total Moisture Content (g/100g)	
Type of sample	SET A	SET B
Tomato	93.13	93.32
Spinach	75.96	76.13
Grapes	90.17	90.04

Table 3: Nutritive analysis of Carotenoids in SET A and SET B.

	ug/100g			
Sample name	Total Carotenoids	Lutein	Lycopene	Beta Carotene
Tomato A	406	3.46	3.97	2.27
Tomato B	474	3.05	3.34	1.89
Grape A	100	0.57	0.00	0.00
Grape B	83	0.59	0.00	0.00
Spinach A	7816	171.55	0.00	46.98
Spinach B	8864	179.36	0.00	44.22

Table 4: Nutritive analysis of Vitamins in SET A and SET B.

	mg/100g		
Sample name	VIT C	VIT B5	VIT B6
Spinach A	2.02	5.16	0.089
Spinach B	2.04	4.15	0.093
Tomato A	20.93	6.75	0.071
Tomato B	20.68	6.74	0.064
Grapes A	8.04	0.68	0.054
Grapes B	8.11	0.65	0.052

Table 5: Nutritive analysis of Minerals in SET A and SET B.

	mg/100g			
Sample name	Zn	Fe	Mn	Cu
Spinach A	0.25	1.19	0.18	0.892
Spinach B	0.25	1.20	0.18	0.847
Tomato A	0.04	0.46	0.04	0.378
Tomato B	0.03	0.48	0.04	0.563
Grapes A	0.36	3.76	0.80	0.209
Grapes B	0.33	3.53	0.85	0.179

Leak Test

To make SCOBY leak proof, first olive oil and then secondly, a mixture of melted bees wax and olive oil was put together. It showed no water leakage but still absorbed the water showing it is still porous but not permeable. The main objective of this project was to find a better alternative to plastics which are known to be harmful and recalcitrant in nature. The results obtained can be said to be positive

because of the certain properties of the material (SCOBY) such as flexibility, recyclability, biodegradability and its antibacterial property which shows that it can inhibit the growth of food borne disease causing microorganisms thereby aiding in food preservation. However there were few shortcomings during the course of the project which needed to be addressed. During the production of SCOBY, it was observed that proper control of parameters were required for the Kombucha fermentation process to take place. The temperature at which the fermentation takes place i.e., 30-37°C had to be efficiently maintained. At temperatures lower than these, it resulted in slow growth of the SCOBY. A normal process for SCOBY to grow would take about 15 days but in colder climatic conditions it would take over a month for the growth which may or may not result in the proper growth of bacterial cellulose. Also, for a satisfactory thickness of the SCOBY, it is recommended to use a vessel of a proper height, as noticed in our project, it grew better in a large glass aquarium than a plastic tray. Another problem encountered was the proper covering of the container in which the fermentation was carried out. Since it is a combination of anaerobic and aerobic reaction, proper limitation of aeration by using a muslin cloth or cotton cloth is compulsory. Otherwise it results in contamination by fruit flies (*Drosophila melanogaster*) as they are attracted to acidic sweetened solutions [29]. Fungal spores can also result in the spoilage of the biofilm that is growing. In order to revive back the biofilm, addition of vinegar was done at regular interval for 3 days as referred from literature. However we could not revive back the SCOBY but it was observed that the large green spots were converted to small black spots for which the reason is unknown. It can be assumed that the acidic reaction from the vinegar resulted in the death of the microorganism which caused the contamination. Discard both Kombucha as well as SCOBY that shows signs of mold contamination and do not reuse them as inoculums. [30].

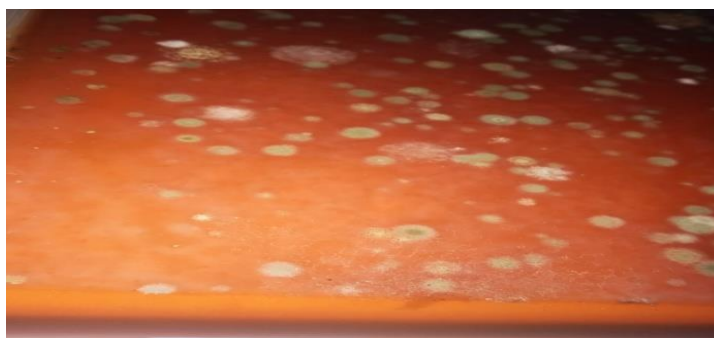


Figure 5: Fruit-fly contamination

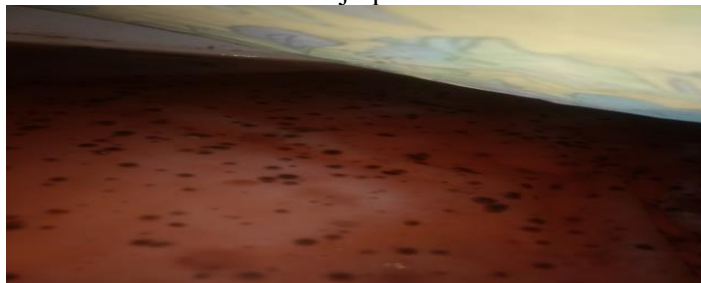


Figure 6: Black spots (Spots seen after addition of vinegar to SCOBY)

After the drying process was complete, one major problem was that the SCOBY dried out completely which resulted in it becoming extremely rigid- this could be because of the increased climatic temperatures. Hence this problem was solved partially by olive oil which was used to make the packaging material leak proof. However the problem still persisted after 4-5 days as the SCOBY absorbed the olive oil and it needed reapplication constantly. Beeswax was the solution to this problem besides making it waterproof. But beeswax alone could not be used since it could not solve the problem of drying completely. It was decided to use olive oil and beeswax as a combination. Logically both olive oil and beeswax help in providing a layer which prevents from leakage and beeswax helped SCOBY to retain its flexibility for a longer duration of time and prevented excessive drying. On conducting leak test, it was noticed that though SCOBY did not let water pass through it, it still absorbed the entire water that was put on it. This shows that it is porous but not permeable and hence, using it for storage of liquid foods, say milk or in fact water is not possible. The bag that was made of SCOBY turned soft after incubating the samples. This was mostly due to the absorption of water present in spinach and grapes. Therefore, efforts should be made to make SCOBY limit its moisture absorbing property to make it an efficient food packaging material.



Figure 7: Wet Bag (Bag becomes soft due to absorption of moisture)

The appearance of the material obtained could be made more acceptable by treating the material with edible dyes or food coloring agents. Likewise, odor from the SCOBY did not completely go even after drying. This could be possibly avoided by adding certain natural as well as edible fragrances such as rose water or aromatic extracts from edible plants. SCOBY production can be employed in large scale industries which will help in eliminating or reducing the extent of usage of plastics. An approximate economic comparison if done between production of plastic and of SCOBY will show that SCOBY production will cost lesser when compared to plastic. Since in the production of SCOBY, the only major requirement are the raw materials which are cheaply available, the only instrument required is a large sized covered container and the labor requirement is negligible as it can be done single handedly. In a plastic industry, setting up of a plant is a costly affair since it involves production of the polymer, molding, heat treatment etc. Since large scale production of SCOBY is not practically employed, we can only predict that its cost will be much lower than that of plastic because it cuts down expenses in equipment and the raw materials that will have a lower cost than LPDE grade grains. However, water can be the major expense in its production and a major risk of contamination is a problem to be looked upon when scaling up a SCOBY plant. On the bright side a SCOBY production unit can have a double gain by marketing the packaging material along with the prepared Kombucha tea which is a health drink gaining importance in the modern era. So no costs are incurred in effluent treatment as all the byproducts are used up. Also the Kombucha tea is known to be rich in various organic acids mainly acetic, gluconic, glucuronic, citric, L lactic, malic, tartaric, malonic, oxalic, succinic, and pyruvic. These acids can also be extracted from the tea for various other purposes. Therefore, SCOBY, bacterial cellulose produced in this project is proposed as a food packaging material but the applications it holds are limitless. It can be a successful alternative to plastic owing to its property of biodegradability and can be a boon to the modern society if most of its limitations are overcome.

4. CONCLUSION

Therefore, the microbial cellulose obtained from SCOBY can be a potential substitute to plastic polymers. As evaluated in the study, the SCOBY packaging neither has any detrimental effects on the physical appearance of packaged food nor on its nutritive value. In addition to this the major advantage is it can be easily degraded. So, usage of SCOBY as packaging material may overcome the environmental problems related to plastics. However, its two major limitations i.e. its ability to absorb the moisture and soften as well as the problem of excessive drying has to be overcome. By further studies and improved methods, SCOBY can be employed to use on a large scale.

CONFLICT OF INTEREST

Conflict of interest is none.

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