Seaweed as natural raw material for industrial applications; extraction, physio-chemical characterization and antioxidant potential of alginate based biofilm

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Abstract: Brown seaweed *Jolyna laminarioides* was collected during low tide from coastal area of Karachi and was used as renewable source for production of sodium alginate. Starch was mixed with alginate as secondary polymer along with high (30%) and low (15%) concentration of bio-plasticizer (sorbitol and glycerol) to produce an eco-friendly biofilm by casting technique. Thickness, moisture content, solubility, bio-degradation test, density, scanning electron microscopy (SEM), Fourier transform infra-red spectroscopy (FTIR) and DPPH (2,2-diphenyl picrylhydrazyl) assay was performed for each blend of biofilm. The obtained results showed the affinity of sorbitol and glycerol with alginate-starch matrix with significant moisture content, thickness and biodegradation properties. The antioxidative potential of alginate based biofilms make it suitable for pharmaceutical and cosmeceutical applications like bio-packaging and manufacturing of eco-friendly production.

Keywords: Seaweeds, sodium alginate, casting technique, bio-plasticizer.

INTRODUCTION

Global advancement in technology and industrialization facilitate mankind in several ways, although some negative consequences still remain as conflict of environmental contamination. Among other toxic waste plastic is considerable pollutant, it is non-degradable and it may remain suspended in landfills and marine environment for several years (Tan *et al.*, 2022). Its small pieces may enter into the body of living organisms and can cause several diseases of lungs and guts. (Reddy *et al.*, 2014). Its recycling involves high cost, massive energy consumption and emits hazardous gases during incineration process (Vollmer *et al.*, 2020).

To combat these economic and environmental problems several pharmaceutical, cosmeceutical and food industries have been made an attempt towards the bio-plastic packaging (Wang and Rhim, 2015). Bio-plastic is a biopolymer based plastic and easily degraded by microorganisms and non-toxic to the environment. The industry of bio-plastic developed rapidly and tremendous increase in its production has been observed since (Jabeen *et al.*, 2015).

Marine macro algae (seaweeds) produce unique and valuable sulfated polysaccharides like agar, alginate and carrageenan (Aliya and Shameel, 2003; Kanagesan *et al.*, 2022). These polysaccharides are commercially important due to their nutritional and medicinal prospective (Sudhakar *et al.*, 2016). Sodium alginate is a long chain polymer consists of α-L glucuronic and β-D manuronic acid units, specifically extracted from members of phaeophycota (Rizvi, 2003). It is widely known as a thickener, stabilizer, retains moisture and capable to produce smooth films with other polymers, which makes it suitable for production of biodegradable and eco-friendly biofilms (Kadar *et al.*, 2021; Lim *et al.*, 2021). The alginate based bioplastic films are playing a vital role in drug delivery, skin tissue replacement, scaffolding of wound healing dressings, antioxidants and as a coating material for various pharmaceutical products (Sidek *et al.*, 2019).

Yadav and Satoskar, (1997) combined bio-plasticizer with sodium alginate to increase the softness and flexibility of polymer by reducing the intermolecular forces between long chain molecules without any alter in chemical composition. Thermal, mechanical, chemical and morphological properties of biofilm also depends upon biopolymers and plasticizer concentration (Pereira *et al.*, 2011; Lim *et al.*, 2021). Alginate is a linear polymer and capable to composite with other polymers to produce active biofilms with enhanced antioxidative and antimicrobial potential (Sidek *et al.*, 2019), active bioplastic film can extend the shelf life of product by maintaining the safety and quality of product (Hadi *et al.*, 2015; Li *et al.*, 2022). Few reports on formation of bioplastic film from brown seaweed are available therefore, the main objective of this study was to determine the potential of alginate based bioplastic film as raw material for industrial manufacturing. Additionally, study of its physio-chemical composition and antioxidative potential to evaluate its commercial application.

MATERIALS AND METHODS

Collection of seaweeds

Brown seaweed *Jolyna laminarioides* was collected during low tide from buleji coast Karachi. Washed
thoroughly with tap water to remove all exotic material, shade dried and kept in polythene bags at room temperature for further analysis. Seaweed was identified on the basis of morphology and anatomical features by Prof. Dr. Aliya Rehman, Department of Botany, University of Karachi.

**Extraction of sodium alginate**
Sodium alginate was extracted according to previously described method (Rasheed et al., 2018) with slight modifications; dried seaweeds grounded until fine powder obtained to increase the surface area. Twenty grams of seaweed powder was soaked with 4% Na₂CO₃ for 12 hrs. Gelatinous solution was filtered and precipitated with 2% dilute sulphuric acid to extract sodium alginate. Sodium alginate washed with distilled water then dried in oven at 60 °C followed by washing, drying and milling process. The extracted yield of sodium alginate was calculated by following formula (Viswanathan and Nallamuthu, 2014).

**Formation of bioplastic**
The bioplastic was prepared by the help of method described by Lim et al., 2021 with some alterations in formulations (table 1). Sodium alginate and starch was dissolved in distilled water then mixed with different types and concentrations of plasticizer (sorbitol and glycerol). The mixture was heated until transparency achieved and poured into casting plates for dryness of bioplastic films.

**Thickness**
Thickness of biofilm was measured by using digital micrometer screw gauge (INSIZE 0-25mm/0-1). Five readings were taken from different points of each formulated blend to evaluate the mean value of thickness.

**Density**
According to Oluwasina et al., (2021), the density (g/cm³) of bioplastic was calculated by measuring the mass and volume of bioplastic films.

**Moisture content**
By following the (Hadi et al., 2022) bio-plastic samples (3 cm x 3 cm) weighed initially (M₁) and dried in oven at 110°C. After 24 hours samples were weighed again (M₂) and moisture content determined by:

\[
\text{Moisture} (\%) = \left[ \frac{M_{1} - M_{2}}{M_{1}} \right] \times 100
\]

**Solubility**
The solubility of bioplastic film was determined by the method of Tapia-Blacido et al. (2011), samples (3cm x 3 cm) were placed in oven at 103°C for 90 minutes and weighed (S₁). The dried samples were then immersed in test tubes containing 50 ml distilled water. After 24 hours the remaining un-dissolved residues filtered, oven dried and weighed (S₂). Solubility rate calculated by:

\[
\text{Solubility} (\%) = \left[ \frac{S_{1} - S_{2}}{S_{1}} \right] \times 100
\]

**Biodegradation test**
According to Hira et al., (2018), to observe degradation rate, bioplastic samples were cut into 3 cm x 3 cm in size, initial weight (D₁) measured and buried in pots containing moist and fertile garden soil. Samples were again weighed at interval of 15 days (D₂) and buried afterwards to complete the incubation period of 30 days (no significant weight detected). Degradation rate was calculated by:

\[
\text{Degradation rate} (\%) = \left[ \frac{D_{1} - D_{2}}{D_{1}} \right] \times 100
\]

**Structural morphology**
All the samples of bioplastic with different compositions were observed at the magnification of 1000 x by scanning electron microscope (JSM-6380, Japan). Gold was incorporated to coat the samples up to 300°A.

**Chemical composition**
For analysis of chemical composition 2 gm of samples were mixed with potassium bromide to prepare a disc. By using spectrometer (Shimadzu FT-IR-8900) FT-IR spectra was measured between the ranges of 4000-500 cm⁻¹ in transmission mode with the resolution of 2 cm⁻¹.

**DPPH assay**
DPPH assay for alginate based biofilm samples was performed by following Li et al., (2022) with slight modification. 20 mg sample of biofilm was dissolved into water, then 0.1 ml sample solution and 3.9 ml DPPH ethanol solution (0.1mM) were mixed and incubated for 20 minutes. Later optical density was measured at 517 nm by using visible spectrophotometer and radical scavenging activity was calculated by using following formula:

\[
\text{DPPH radical scavenging activity (\%)} = \left[ \frac{A_{c} - A_{s}}{A_{c}} \right] \times 100
\]

Where Ac is absorbance of control and As is absorbance of sample to calculate the DPPH radical-scavenging activity (%).

**STATISTICAL ANALYSIS**
All the work done in triplicates and mean values evaluated statistically by one way analysis of variance (ANOVA). Duncan’s multiple range test was applied with 95% level of confidence among variable treatments to calculate significant difference (p<0.05) by using ‘Statistica’ software (Version 8).
Table 1: Formulation of bioplastic using sodium alginate and starch with high and low concentration of sorbitol and glycerol.

<table>
<thead>
<tr>
<th>S #.</th>
<th>Sample code</th>
<th>Formulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control film (CF)</td>
<td>Alg 3% + Starch 3%</td>
</tr>
<tr>
<td>2</td>
<td>Low concentration of sorbitol (LCS)</td>
<td>Alg 3% + Starch 3% + Sor 15%</td>
</tr>
<tr>
<td>3</td>
<td>High concentration of sorbitol (HCS)</td>
<td>Alg 3% + Starch 3% + Sor 30%</td>
</tr>
<tr>
<td>4</td>
<td>Low concentration of glycerol (LCG)</td>
<td>Alg 3% + Starch 3% + gly 15%</td>
</tr>
<tr>
<td>5</td>
<td>High concentration of glycerol (HCG)</td>
<td>Alg 3% + Starch 3% + gly 30%</td>
</tr>
</tbody>
</table>

Table 2: Effect of type and concentration of plasticizer on density of alginate-based bioplastic.

<table>
<thead>
<tr>
<th>S #.</th>
<th>Bioplastic samples</th>
<th>Mass (g)</th>
<th>Volume (cm³)</th>
<th>Density (g/cm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CF</td>
<td>0.117±0.773</td>
<td>0.021±0.005</td>
<td>5.793±1.267</td>
</tr>
<tr>
<td>2</td>
<td>LCS</td>
<td>0.137±0.002</td>
<td>0.102±0.010</td>
<td>1.358±0.161</td>
</tr>
<tr>
<td>3</td>
<td>HCS</td>
<td>0.141±0.001</td>
<td>0.243±0.009</td>
<td>0.576±0.020</td>
</tr>
<tr>
<td>4</td>
<td>LCG</td>
<td>0.148±0.002</td>
<td>0.207±0.009</td>
<td>0.717±0.030</td>
</tr>
<tr>
<td>5</td>
<td>HCG</td>
<td>0.153±0.001</td>
<td>0.609±0.022</td>
<td>0.251±0.008</td>
</tr>
</tbody>
</table>

Note: Above Results are expressed as Mean±Sd value by One-way ANOVA for mass (g), volume (cm³) and density (g/cm³).

Fig. 1: Physical appearance of alginate-based bioplastic films.

Fig. 2: Physio-chemical characteristic (a) thickness (mm), (b) moisture content (%), (c) biodegradation (%) and (d) water solubility (%) of bioplastic calculated by One-way ANOVA and expressed as Mean±Sd value.
RESULTS

**Yield of alginate**
In this study, the brown seaweed *Jolyna laminarioides* (20 gm) was dried and grounded into a fine powder to provide large surface area for alkali (4% Na₂CO₃) and acid (2% H₂SO₄) treatment. The obtained alginate yield was around 26.5% and seems light brown in color. The dried powder of alginate remained kept in polythene bag for further application.

**Physical appearance of bioplastic**
Alginate-starch based film (CF) was brittle, stiff and difficult to peel out from the casting plate. Natural plasticizer were added to improve the physical appearance of biofilm, addition of plasticizer (sorbitol and glycerol) at low (15%) concentration increased the flexibility, elasticity and smoothness of biofilm. Though, high (30%) concentration of sorbitol (HCS) increased the softness and thickness of biofilm, while glycerol (HCG) blend became slightly sticky.

**Physio-chemical characteristic**
In this study, the effect of plasticizer type and concentration on physio-chemical properties of biofilm was observed. Sorbitol and glycerol were combined with alginate-starch matrix at low (15%) and high (30%) concentration to determine the following characteristics of biofilm:

**i-Thickness**
The fig. 2 (a) represented the thickness of bioplastic film (0.023 mm to 0.676 mm) and glycerol (0.113 mm to 0.27 mm) at the concentration of 15% and 30% respectively. It is observed that sorbitol-based bioplastic has relatively greater thickness as compared to the glycerol-based bioplastic films.

**ii-Moisture content**
The moisture content of un-plasticized film (3.214%) greatly influenced by different type and concentration of plasticizers. It is found that the moisture content of biofilm increases with an increase in plasticizer concentration (15%-30%) regardless of plasticizer type (fig. 2b). In comparison, bioplastic film casted with glycerol significantly contains more moisture content as compared to the sorbitol.

**iii-Biodegradation test**
Fig. 2 (c), showed that within 15 days of soil incubation, bioplastic films with glycerol (LCG and HCG) exhibited slightly faster reduction in molecular weight of macromolecules than sorbitol (LCS and GCS). Moreover, after the period of 30 days, all type of bio-plastic blends (CF, LCG, LCS, HCG and HCS) were completely decomposed by the micro-organisms found in the soil.

**iv-Water solubility**
Fig. 2 (d), represented that alginate-based bioplastic film (CF) is moderately soluble in water, addition of sorbitol and glycerol as plasticizer increased the solubility of control film. Plasticizer concentration also effect the solubility, it is observed that blends formulated with sorbitol (LCS and HCS) slightly resistant as compared to blends formulated with glycerol (LCG and HCG).

**Density**
It was observed that all the plasticized bioplastic films exhibited lower density as compared to the control film (5.793 g/cm³). Table. 2 shows the effect of plasticizer type and concentration on density of alginate-based bioplastic. Raising the concentration of plasticizer (15% to 30%) lowers the density, regardless of plasticizer type. The density values of sorbitol plasticized film found slightly higher as compared to the glycerol.

**Antioxidant activities from biofilm**
In this study polysaccharide extracted from *Jolyna laminarioides* mixed with starch and natural plasticizer (sorbitol and glycerol) to assist the antioxidative potential of biofilm. In our findings (fig. 3), it is observed that all the formulated bioplastic blends (CF, LCS, LCG, HCS and HCG) exhibited antioxidant potential in DPPH radical-scavenging assay. Further rise in concentration of plasticizer (30%) put slightly down the antioxidant activity of alginate-based biofilms.

**Scanning electron microscopy**
The electron microscopic images were obtained at the magnification of 1000 x to illustrate the surface morphological features of synthesized bioplastic samples. In micrographs (fig. 4) the surface structure of plasticized biofilm (LCS, HCS, LCG and HCG) was compared with un-plasticized film (CF), to observe the effect of sorbitol and glycerol at different concentrations. The alginate-starch based biofilm exhibited irregular surface structure. The biofilms casted with 15% sorbitol (LCS) and glycerol (LCG) reduced the insolubility with homogenized surface structure. Further increased in the concentration (30%) of plasticizer presented more compact, smooth and dense surfaces of biofilms. However, in glycerol-based films (LCG and HCG) some insoluble particles are seemed as white spots.

**Chemical composition (FTIR spectroscopy)**
FTIR spectrum (fig. 5) specified the chemical interaction of bio-polymers (alginate, starch) with different concentration (15% and 30%) of sorbitol and glycerol. All the bioplastic blends CF, LCS, LCG, HCS and HCG showed broad spectrum absorption at 3000-3600 cm⁻¹ are attributed to O-H bond formation between polymers and plasticizers, followed by vibrations around 2900 cm⁻¹ are assigned to C-H aliphatic bonding. Whereas the vibrations around 1400-1600 cm⁻¹ are assigned to stretching of carboxylate group. Moreover, the vibrations at 990-1200
are linked to the presence of C-C, C-O bond stretching of C-O-C groups in glycosidic bond of alginate. The vibration between 770-950 cm$^{-1}$ is related to the C-O stretching attributed to uronic acid residues as fingerprints of alginate.

In this study, the brown seaweed *Jolyna laminarioides* was collected from bulleji coast of Karachi, for the extraction of sodium alginate. According to Vasuki et al., (2015), the extraction techniques known to influence the yield and physio-chemical properties of alginate. It is observed that sodium alginate showed great compatibility with starch and plasticizer (sorbitol and glycerol) to produce homogenous bioplastic films. In addition, plasticizer type and concentration are directly linked with the appearance and other morphological features of bioplastic film. Similar kind of trend was also reported from Malaysia (Lim et al., 2018; Kanagesan et al., 2022).

**DISCUSSION**

In current work physio-chemical properties are examined at 15% and 30% concentrations of sorbitol and glycerol. An increase in thickness of biofilm has been confirmed with the increase in concentration of plasticizer, further the high molecular weight of sorbitol as compared to glycerol ultimately subsidizing the thickening of LCS and HCS bioplastic samples. Similar kind of results reported earlier (Arham et al., 2016; Sanyang et al., 2016; Oluwasina et al., 2021).

Generally biopolymers are hydrophilic in nature and their moisture retaining capacity increases with addition of plasticizer (Femenia et al., 1999; Ghasemlou et al., 2011). Hydroxyl group of glycerol have more affinity to bind with water molecule than to sorbitol (Cerqueira et al., 2012; Sanyang et al., 2016), which may lead to high moisture content of LCG and HCG bioplastic blends.

According to Shravya et al., (2021), bio-plastic made from biopolymers and bio-plasticizers are easily degradable in natural environment and do not produce toxic substances. Alginate and starch are biopolymers which retain moisture and increases the mechanism of deterioration, plasticizer further increases the degradation rate. Our findings showed that formulation with glycerol decomposed faster as compare to with sorbitol which is also reported by Hadi et al., 2022 and Kanagesan et al., 2022.

Bioplastic with high insolubility in water is more suitable for food packaging (Perezgago and Krochta 2001; Yin et al., 2007), with an exception of some food materials which required soluble packaging (Aguirre et al., 2013). Plasticizer type and concentration greatly affect the solubility of polymers (Müller et al., 2008). Sorbitol makes a strong bonding with intermolecular chains of biopolymer hence the lesser chance of water molecules...
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penetration (Lim et al., 2021). In this study, LCG and HCG found with higher solubility as compare to LCS and HCS. Maran et al., 2013 and Sanyang et al., 2015 also stated similar kind of tendencies in water solubility of biofilm.

In another experimental response, higher the concentration of plasticizer decreases the density, regardless of plasticizer type. This might linked with the high molecular weight and density of plasticizer. Our results are in agreement with the findings of Razavi et al., (2015) and Sanyang et al., (2016), who also reported the sorbitol plasticized films with slightly higher density values.

In this study the DPPH assay is used to determine the antioxidant activity of biofilm, all the plasticized and un-plasticized blends possess antioxidant activity (fig. 3) regardless of plasticizer type ad concentration. However, HCS and HCG blends showed slightly decreased in activity as compare to LCS and LCG respectively. Similar kind of results also stated by Hadi et al., (2014), who linked the phenolic compounds with antioxidant potential of alginate-aloevera Vera composite films. Our results also in agreement with Doh et al., 2020 and Li et al., 2022, who reported antioxidant potential of biopolymer film developed from brown seaweeds, being antioxidative biofilms have capability to protect the packaging material from oxidation and also prevent the decay of microorganisms.

Micrographs (fig. 4) from scanning electron microscopy illustrate the morphological features of biofilm with high magnification. Plasticizer provide the flexibility among polymers chain by lowering the intermolecular forces. Fabrication of bioplastic films with sorbitol interpreted dense and compact surfaces without any phase separation which indicates its compatibility with polymers. Previous reports also suggested sorbitol to produce smooth and homogenized bioplastic as compared to glycerol (Tapia-Blacido et al., 2011; Sanyang et al., 2016; Marium et al., 2021).

FTIR spectrum (fig. 5) specified the chemical bonding of different functional groups present in polymers and plasticizers. Vibrations visible at 3000-3600 cm$^{-1}$ are ascribed to free-OH groups indicating the hydrogen bonds formation between polymers and plasticizer, similar results were reported by Safari et al., 2014 and Sanyang et al., 2016. The absorption band of C-H observed around the wave number 2900 cm$^{-1}$ whereas the region between 1400-1600 cm$^{-1}$ indicated the presence of carbonyl group in bioplastic film, similar kind of chemical bonding also observed by Badita et al., 2020 by using Laminaria japonica and Sargassum natans in formation of nanocomposite film. The vibrations between 990-1200 cm$^{-1}$ are corresponded to the C-C, C-O bond stretching, our results are in agreement with Doh et al., (2020) observed 996-1031 cm$^{-1}$ and Sanyang et al., (2016) stated 1004 cm$^{-1}$. The vibrations between750-950 cm$^{-1}$ is related to the C-O stretching attributed to glutaric and mannuronic acids of alginate. Our findings suggested that alginate-starch matrix is compatible with sorbitol and glycerol to produce bioplastics films as all the blends produced vibrational peaks with similar kind of chemical bonding (Sanyang et al., 2016).

CONCLUSION

This study highlights the commercial importance of sodium alginate extracted from brown seaweed (Jolyna laminariosa) inhabiting Karachi coastal area. Sodium alginate was casted with starch and plasticizer (glycerol and sorbitol) to produce bioplastic films. Our finding suggested that alginate can corporate with other polymers and plasticizers to produce homogenous and biodegradable bioplastic films. Biopolymers from natural resources like seaweeds are ecofriendly and also reduce the manufacturing cost of bioplastic. Moreover, as compare to synthetic plastic alginates-based bioplastic can be consider as suitable and profitable raw material for industries, as it can play an important role to achieve sustainable and pollution free environment.

REFERENCES


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