



*Original Article*

## Physicochemical properties of cassava starch and starch-keratin prepared biofilm

Oluwasina Olugbenga Oladayo<sup>1,3\*</sup>, Umunna Queendaline C.<sup>1</sup>, Olusegun Sunday Joseph,<sup>2</sup>  
and Wahab Oluwasegun<sup>1</sup>

<sup>1</sup> *Department of Chemistry,*

<sup>2</sup> *Department of Metallurgical and Material Engineering,  
Federal University of Technology, Akure, Ondo, Nigeria.*

<sup>3</sup> *Department of Chemical, Metallurgical and Material Engineering,  
University of Witwatersrand, Johannesburg, South Africa.*

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### Abstract

Synthetic plastics pose one of the biggest threats to the environment and a promising solution is biodegradable polymers. This study investigates the properties of biofilms prepared using starch/keratin blend with and without formaldehyde. Some starch properties in percentage are; moisture content 0.27, hydration capacity 189.66, amylopectin content 65.79 and amylose content 34.21. From the water testing results, thickness swelling, water absorption capacity and linear expansion of biofilm without formaldehyde after 10 s of soaking in water were 28.59%, 8.89% and 4.90% respectively and 65.30%, 91.33% and 46.29% respectively after 40 s. But, higher values are recorded for those biofilms made with addition of formaldehyde. Thus using water effect on the properties of the biofilms as the performance index, the research indicates that biofilms without formaldehyde had better performance than those with formaldehyde.

**Keywords:** starch, chicken-feather, keratin, biofilm, formaldehyde

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### 1. Introduction

Anthropogenic environmental contamination, though of minimal gravity has been in existence since the creation of man. However, due to exponential increase in population and technological development, environmental contamination has become a full-fledged threatening menace for the whole world. Today, over 60% of municipal waste comprises of packaging materials which mostly are non-degradable synthetic petroleum-based polymers (Marsh and Bugusu, 2007; Garreau *et al.*, 2002). This is a strong indication that development of bio-degradable packaging materials of renewable source is

a major path to environmental sustainability (Chiellini and Solaro, 1996).

In this direction, increased numbers of scientists have been researching into preparation of bio-plastics and bio-films in the last decades (Lange and Wyser, 2003). Bioplastic is a plastic that is made partly or wholly from polymers derived from biological sources such as sugar cane, potato starch or the cellulose from trees, straw and cotton. Some bioplastics degrade in the open air, others are made so that they compost in an industrial composting plant, aided by fungi, bacteria and enzymes (Sita *et al.*, 2012; Koushal *et al.*, 2014). Biodegradable plastics can be broken down in either aerobic or anaerobic environments depending on how they are manufactured. Biopolymers, from which bioplastics can be produced, include polypeptides (e.g keratin) and polysaccharides e.g: cellulose and starch (Stepto 2003).

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\* Corresponding author.

Email address: [oluwasinagbenga@yahoo.com](mailto:oluwasinagbenga@yahoo.com)

In the quest for complete biodegradable plastics, starch being a natural polymer coupled with its low cost, renewability, unlimited availability and biodegradability has been considered as a promising raw material. In view of this, many efforts have been exerted to develop starch-based polymers for conserving the petrochemical resources and reducing environmental impact (Stepito 2003; Lu *et al.*, 2009). Starch is an organic food reserve which occurs as granules in the chloroplasts of green leaves and other photosynthetic cells, and in the amyloplasts of non photosynthetic storage organs such as seeds, roots, and tubers (Ellis *et al.*, 1998). The starch granule is essentially composed of two main polysaccharides: amylose and amylopectin with some minor components such as lipids and proteins (Buleon *et al.*, 1998).

The most common sources of food starch are corn, potato, wheat, cassava/tapioca and rice. Cassava has been proved to be a viable, relatively cheap and readily available plant source of starch (Akpa and Dagde, 2012). Cassava (*Manihot esculenta* Crantz, also known as manioc or yucca) is one of the leading food and feed plants in the world: it ranks fourth among staple crops with a global production of about 160 million tons per year (Ukwuru and Egbonu, 2013). Cassava is a renewable, almost unlimited resource and one of the most abundant substances in nature. It is one of the most important starchy root crops of the tropics used for food and industrial purposes. In Nigeria, it is consumed raw or cooked as garri, starch flour and a variety of other items (Tonukari, 2004). In Nigeria, cassava is a staple food for both rural and urban areas and in recent years it has been transformed from being a subsistence crop to an industrial cash crop (Akpa and Dagde, 2012).

Apart from starch, another biopolymer of significance in production of bioplastics and biofilm is keratin (Stepito, 2003). Keratin is a protein that can give durability to plastics which out-performs other plastics of biological products like starch and plant proteins (King'ori, 2012). Thinking of suitable source of keratin, chicken feather immediately comes to mind. Chicken feathers are waste products that are normally thrown out. They are inexpensive, abundant and readily available in all countries that eat poultry (Schmidt and Barone, 2004). Chicken feathers have been investigated for a number of potential applications ranging from reinforcement in plastics to microchips (Menandro, 2010; Khot *et al.*, 2001).

Physical properties like water absorption of biofilms and bioplastics are of great importance, especially for those intended for application in food packaging as they must keep the content dry. The water absorption properties also reflect on the mechanical properties of the product (Khazaei, 2008). Urea formaldehyde (UF) resin serves as particle binder in wood particleboard and it was reported that the addition of UF in the particleboard reduced the particleboard's capacity for absorbing water (Tay, 2014).

The aim of this study was to utilize starch from *Manihot esculenta* Crantz and keratin (from chicken feather) materials considered as waste for the production of eco-

friendly biofilm. This would be achieved by isolation of starch from cassava, determining the physicochemical properties of starch flour, isolating keratin from chicken feather, producing starch-keratin biofilm with and without formaldehyde and determining the water-soaking properties of the biofilm.

## 2. Materials and Methods

### 2.1 Chicken feathers and cassava starch

The chicken feathers were obtained by slaughtering a 28-week old broiler. They were thoroughly washed with detergent to remove blood and stains, then sun dried. The chicken downy fibers were then stripped off the barbs and dried inside the oven at 105°C to constant 6% moisture content before being used. Cassava tubers were obtained from Agbani farm plantation, Agbada Ekiti Gbonyin LGA, Arunpale from Alfa Jimoh's compound in Ekiti State, Nigeria.

### 2.2 Dissolution of chicken feathers

The downy barbs of chicken feather which weighing 118 g were added to 320 mL of 1:1 solution of 6% NaOH and 0.5 M Na<sub>2</sub>S i.e. 160 mL of NaOH and 160 mL of Na<sub>2</sub>S and was then mixed together under reflux for 4 hours at 40°C. The solution was later cooled and then centrifuged for 10 min at 3000 rpm. The sediment was then discarded and the supernatant collected.

### 2.3 Extraction of keratin

To isolate keratin from the collected supernatants after the dissolution of chicken feather, 775 mL of 2.5M (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> was added and mixed thoroughly. This was then centrifuged for 10 min at 3000 rpm. After centrifuging, the precipitated keratin was then isolated and dried at 40°C to constant weight.

### 2.4 Extraction of starch from cassava

The harvested roots were washed with water to remove soil particles. The cleaned roots were peeled, sliced and ground in a milling machine with small volumes of water. The resultant slurry was allowed to settle for eight hours before the supernatant was decanted. The sediment washed several by re-suspending in distilled water and allowed to precipitate after some time. The starch mash was dried at 50°C to constant weight. The starch flour obtained was sieved through mesh size of 212 to 249 µm, packed in polythene bags and stored at room temperature for further use.

### 2.5 Bioplastic formulation

In order to prepare biofilm, 3% starch (water based) was gelatinized at 85°C with constant stirring for 5 min. This was allowed to cool. Also, 40 % keratin was prepared using

Table 1. Bioplastic formulation

Formulates	3% starch	40% keratin	40% sorbitol	Formaldehyde
1	20g	3g	1g	0.5g
2	20g	3g	1g	1.0g
3	20g	3g	1g	1.5g
4	20g	3g	1g	2.0g
5	20g	3g	1g	Nil

60 g of 0.05M NaOH and 1 mL of ethanol, and then heated to dissolve the keratin. Biofilm was then prepared by mixing thoroughly 20 g of the 3% gelatinized starch, 3 g of 40% keratin and 1 g of 40% sorbitol. To this formulation was added 0 g, 0.5 g, 1 g, 1.5 g and 2 g (Table 1) formaldehyde separately.

## 2.6 Determination of moisture content of starch

The method of (Olayemi *et al.*, 2008) was used: 2 g of the sample was weighed and then dried in an oven at 105°C for about 3 h and then weighed again until a constant weight was obtained and the experiment done in triplicate. The percentage weight loss on drying was calculated as Moisture Content (M.C);

$$M.C = \frac{w_i - w_f}{w_i} \times 100\% \quad (1)$$

where,  $w_i$  is the initial weight of the sample before drying,  $w_f$  is the final weight of the sample after drying.

## 2.7 Gelatinization temperature

About 1 g of the starch sample was put into a 20 mL beaker and 10 mL of distilled water was added. The dispersion was heated on a hot plate. The gelatinization temperature was taken with a thermometer suspended in the starch slurry immediately the starch forms gel.

## 2.8 Determination of starch hydration capacity

About 1 g of the sample was placed in a 20 mL tarred stopper centrifuge tube. The weight of the tube was taken, 10 mL of distilled water was added and shaken vigorously for 2 min. It was then allowed to stand for 10 min during which it was mixed by inverting the tube three times at the end of 5 and 10 min. The sample was centrifuged at 3000 rpm for 10 min. The aqueous supernatant was then carefully removed and the tube with the sediment was re-weighed. The hydration capacity was calculated as the ratio of the weight of the sediment to the initial weight of dry powder. The procedure was done in triplicate and the mean value taken.

## 2.9 Determination of swelling capacity of the starch

The swelling capacity of the starch powder was

determined by the method of Iwuagwu and Okoli (1992). The tapped volume occupied by 5 g of the powder in the 100 ml measuring cylinder  $V_x$ , was noted. The powder was then dispersed in 85 mL of distilled water and the volume made up to 100 mL with more water. The experiment was done in triplicate. After 24 h of standing, the volume of the sediment,  $V_v$  was determined and the swelling capacity was computed as;

$$\text{Swelling capacity} = \frac{V_v}{V_x} \quad (2)$$

where:  $V_v$  is the volume of the sediment,  $V_x$  is the tapped volume of the starch.

## 2.10 Amylose content determination of starch

Approximately 0.1 g of the sample was weighed into a 100 mL volumetric flask and 1 mL of 100 % (v/v) ethanol and 9 mL of 1 N sodium hydroxide was carefully added and the mouth of the flask covered with foil and the content thoroughly mixed. The sample was heated for 10 min in a boiling water bath to gelatinize the starch. The sample was then removed from the water bath and allowed to cool. The flask was then filled up to the mark with distilled water and shaken well. About 5 mL of the mixture was then pipetted into another 100 mL volumetric flask. 10ml of 1 N Acetic acid and 2 mL of iodine solution were added and topped up to the mark with distilled water. Absorbance A was then read using a spectrophotometer at 620 nm wavelength.

The amylose content was then calculated as;

$$\text{Amylose content (\%)} = (3.06)(A)(20) \quad (4)$$

Where, A is the absorbance value

## 2.11 Amylopectin content

$$(\%) = 100\% - \text{amylose content} \quad (5)$$

## 2.12 Moisture sorption capacity

The method of Ohwoavworhwa *et al.*, (2004) was used. About 2 g of the starch (**W**) was weighed and put into a tarred petri dish. The sample was then placed in a desiccator containing distilled water at room temperature and the weight gained by the exposed samples at the end of a five-day period

(Wg) was recorded and the amount of water absorbed (Wa) was calculated from the weight difference as;

$$Wa = Wg - W \quad (6)$$

where,  $Wa$  is the amount of water absorbed

$W$  is the weight of starch before the experiment

$Wg$  is the weight gained by starch after 5- days

### 2.13 XRD analysis

Starch powder was analyzed by X-ray diffraction using Siemen D 5000 X-ray diffractometer.

### 2.14 Measurement of thickness

The film thickness with dimension 2 x 2cm was measured using a hand held electronic digital micrometer at fifteen different positions on the film and thickness calculated using the formula (Oluwasina *et al.*, 2014).

$$\text{Thickness}(t_0) = \frac{\text{sum of measured values before soaking}}{15} \quad (7)$$

### 2.15 Biofilm thickness swelling

To determine thickness swelling of the film, the material was maintained in the condition of the laboratory at 65% relative humidity and 25°C. The film dimension was 2 x 2 cm and the test specimen was soaked to a depth of 4 cm in 200 mL water (distilled) in 250 mL beaker. Soaking was continued for 10, 20, 30 and 40 s, after which the specimens were removed and suspended to drain for 10 min to remove the excess water. After draining, the thickness was measured at fifteen different points and the average obtained, after which the thickness swelling (TS) was determined (Oluwasina *et al.*, 2014).

$$t_{15} = \left[ \frac{\text{sum of the measured values after soaking}}{15} \right]$$

$$TS = \left[ \frac{(t_{15} - t_0)}{t_0} \right] \times 100 \quad (8)$$

where TS is the thickness swelling (%),

$t_0$  is the thickness before soaking

$t_{15}$  is the thickness after soaking.

### 2.16 Biofilm water absorption capacity

For water absorption, initial weight ( $W_0$ ) of the specimen was noted before soaking and the final weight ( $W_t$ ) determined after draining.

$$WA(t) = \left[ \frac{w(t) - w_0}{w_0} \right] \times 100 \quad (9)$$

### 2.17 Biofilm linear expansion (LE)

Linear expansion which is the test that measures the dimensional stability of a composite to changes in moisture content was determined by measuring the length of the specimen before soaking and after draining (Oluwasina *et al.*, 2014).

$$LE(\%) = \frac{L_f - L_i}{L_i} \times 100 \quad (10)$$

where,  $L_f$  = length after draining

$L_i$  = length of material before draining

## 3. Results and Discussion

### 3.1 Physicochemical analysis of the starch

#### 3.1.1 Moisture content

The moisture content of starch is the amount of moisture present in it. The higher the moisture content the lower the amount of dry solids in the flour. The maximum allowable limit for moisture in starch flour is 14% (Austin, 1984) and 12% according to African Organization for Standardization of cassava starch. Higher values cause caking of the starch; affects its texture; and also promotes growth of microorganisms which cause odours and off-flavour.

Moisture content of the cassava starch was found to be 0.27% (Table 2). Apart from being within acceptable limit, the moisture content recorded in this work was lower than kaffir potato starch of 17.16% (Muazu *et al.*, 2012), rice starch with moisture content of 6% and maize starch with moisture content of 4% (Olayemi *et al.*, 2008). This indicates a higher solid content and that this cassava starch could stay longer under storage as compared with those other starches (kaffir potato, rice and maize). The low moisture content of cassava starch makes it easy to store at room temperature and less prone to fungal and microorganism infections, making them amenable for utilization in low moisture content starch applications.

Table 2. Result of physicochemical properties of cassava starch

Parameters	Starch
Moisture content (%)	0.27 ± 0.03
Amylopectin (%)	65.79 ± 4.77
Amylose (%)	34.21 ± 4.77
Hydration capacity (%)	189.66 ± 13.86
Swelling capacity (%)	24.41 ± 17.27
Moisture sorption (%)	27.00 ± 1.00
Gelatinization temperature (°C)	75.66 ± 0.57

Mean ± standard deviation (n=3)

### 3.1.2 Moisture sorption capacity

Moisture sorption capacity is used in determining the measure of moisture sensitivity of the cassava starch and it reflects the relative physical stability of the bioplastic formulated with the starch when stored under humid conditions (Ohwoavworhu *et al.*, 2004). Cassava starch absorbed moisture value of 2% (Table 2), which is significantly higher compared with rice starch with 1.86%, wheat starch 2.55% and maize starch 4.87% (Ohwoavworhu *et al.*, 2004).

There would be need for modification of the cassava starch used in this current research for the production of good bioplastic because of its high moisture sorption.

### 3.1.3 Amylose and amylopectin content

The amylose content of cassava starch in the current study was 34.21% which is higher than 23.01-26.98% and 19.69-26.63% respectively, reported by Moorthy *et al.* (1993) on parent and progenies of the cassava starch. The differences in these results may be attributable place of harvest, storage effect and possibly be experimental error. A higher amylose content of cassava starch implies decreased crystallinity and gel stickiness; increased amorphous regions, gel firmness, higher pasting temperature and amylose aggregation / retrogradation (spoilage) tendencies.

Also, high amylose cassava starch have an increased tendency for water absorption, although, the stability of resulting starch water mixtures is low and the visco-elastic properties are lower coupled with their high tendency to retrograde (Soh *et al.*, 2003). The result obtained for the amylopectin content is 65.79%. Amylose content decrease with an increase in amylopectin, meaning that one is a function of the other and both properties are important in food preparation and development.

### 3.1.4 Hydration capacity

The starch hydration capacity was 189.66%, which implies that the cassava starch has a high hydration capacity. It is assumed that the hydration of starch represents the water absorbed by the particle or the particle surface (Ohwoavworhu *et al.*, 2004). As observed earlier, the smaller the particle size, the larger the relative surface area for water absorption.

### 3.1.5 Gelatinization temperature

The gelatinization temperature of the cassava starch is 75.66°C as shown in Table 2, this is higher than 71.69°C and lower than 76.84°C reported for starch and flour from bambarra groundnut (Sirivongpaisal, 2008). The gelatinization temperature of starch is an important factor in the production of high quality maltodextrin as it will affect the efficiency of starch hydrolysis either using enzyme or acid modification technique. At the starch gelatinization stage, the starch polymeric material is dispersed by swelling and

gelatinization in water will result in significant hydrolytic cleavage of 1,4-glycosidic bonds in the starch.

### 3.1.6 X-Ray diffraction of keratin

X-ray diffraction of starch (Figure 1) showed diffraction peaks at  $2\theta$  equal to 6.18° and 8.30°. These peaks are not very strong or well defined. The diffraction peak pattern in general was irregular without any sharp distinguished peak, an indicator of the starch amorphosity. This diffraction pattern showed that the starch is amorphous in nature. X-ray diffraction of keratin (Figure 2), showed a peak at  $2\theta$  equal is 8°, much more irregular like the starch diffraction pattern. The diffraction has no sharp signal and on the whole the diffraction pattern was very irregular- an indication that the keratin is amorphous in nature.

### 3.1.7 Biofilm thickness swelling

Figure 3 shows that an increase in formaldehyde content of biofilm led to an increase in the thickness swelling irrespective of the contact time or time of immersion. The biofilm sample with no formaldehyde showed the least thickness swelling in this respect. However, for each particular biofilm sample of specified formaldehyde content, swelling was more pronounced as immersion time increased. That is, swelling of biofilm sample with 2 g formaldehyde was greater at 30 s than at 20 s, which was also greater than that observed at 10 s.

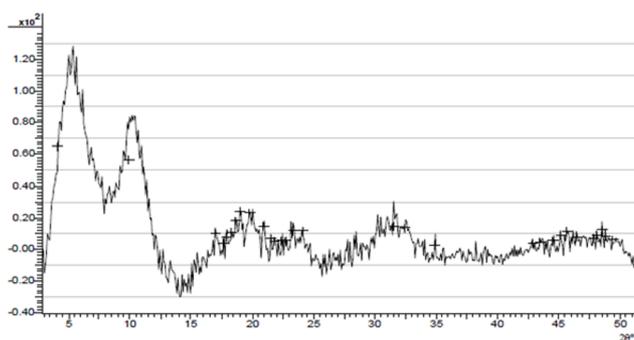


Figure 1. X-ray diffraction of cassava starch

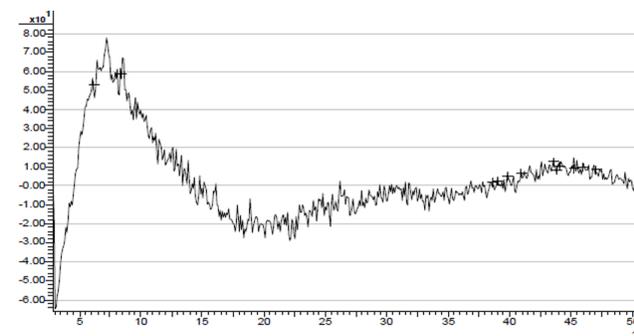


Figure 2. X-ray diffraction on keratin

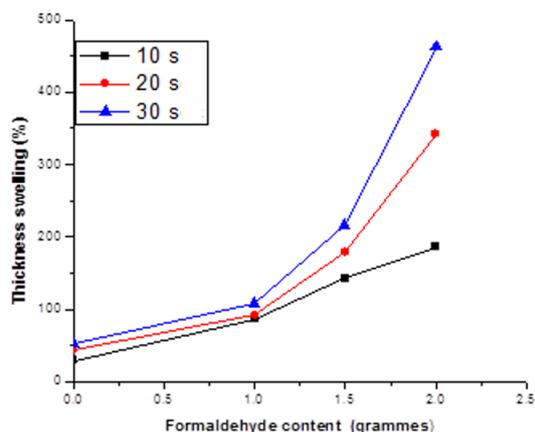


Figure 3. Effect of formaldehyde on thickness swelling profile

No result was obtained for biofilms prepared with 0.5 g and 1.0 g formaldehyde after 40 s of soaking in water because they swelled too much and became torn. The sample without formaldehyde and those containing 1.5 g, 2.0 g of formaldehyde did not get torn until about 40 s after immersion in water. This implies that they are stronger than those containing 0.5 g and 1.0 g of formaldehyde, even after 40 sec of immersion in water. The tearing noticed is consistent with the observation reported by Tay *et al.* (2014) of micro cracking of brittle thermosetting resin when swollen.

### 3.1.8 Biofilm linear expansion

Similar to the observed trend in the thickness swelling, linear expansion (increase in length) became more pronounced as formaldehyde content increased, up to 1.5 g formaldehyde content. Beyond this point, further increase in formaldehyde content led to a sharp decrease in the linear expansion (Figure 4). This linear expansion is used in determining the dimensional stability of the biofilms to changes in moisture content. Hence, the biofilm sample containing no formaldehyde, which showed least linear expansion, could be referred to as the most dimensionally stable. However, for applications in which linear expansion is desirable, the sample with high formaldehyde content would be appropriate.

### 3.1.9 Biofilm water absorption

Generally, water absorption increases with immersion time (Tay *et al.*, 2014) and the biofilm is expected to absorb water as the cassava starch and keratin used in its production is highly amorphous (as evidence from the x-ray diffraction) and has high hydration capacity. Figure 5 shows that water absorption increased with increasing formaldehyde content. Also 1.5 g formaldehyde gave the maximum water absorption and further increase in formaldehyde content led to decrease in water absorption similar to the observations on linear expansion.

## 4. Conclusions

Biofilm was successfully produced from waste starch and keratin obtained from waste chicken feather. Also the results from the analysis of biofilm revealed that biofilm without formaldehyde performed better than those with formaldehyde. The study therefore showed that starch, which is abundantly available in most wild-underutilized tuber, and keratin from feathers, a common environmental pollutant, could be used with proper modification to produce bioplastics. This could serve as replacement for synthetic polymers that have been polluting the environment. The result obtained from the analysis of the biofilm shows that biofilms produced with formaldehyde addition absorbed water; this could be because of the hydrophilic nature of both the starch and the keratin as reflected by their X-ray diffraction. Since amorphous material would absorb much more water than the crystalline material. Also, the swollen performance of the bioplastic with formaldehyde is an indication that with adjustment of the reaction parameter, hydrogel could be produced from the mixture of starch, keratin and formaldehyde. Thus, it is recommended that further studies on chemical modification of both the starch and keratin with

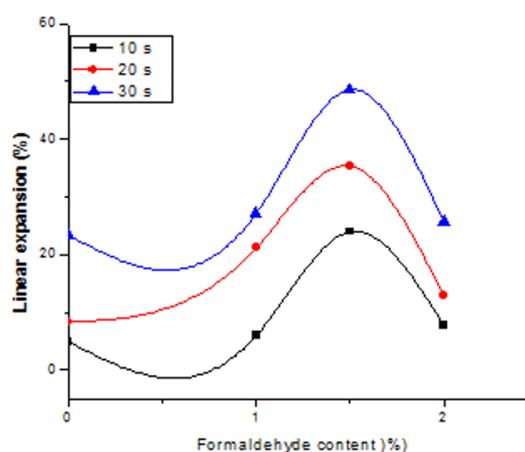


Figure 4. Effect of formaldehyde on linear expansion

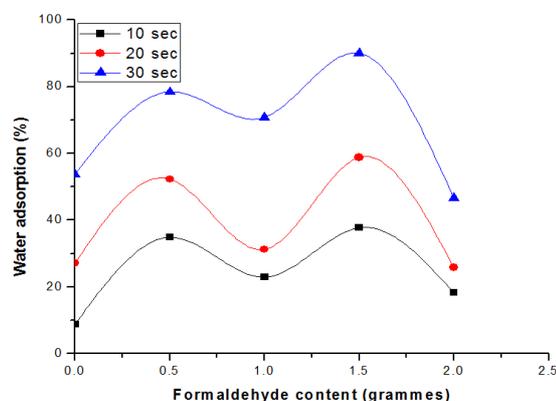


Figure 5. Effect of formaldehyde on water absorption

hydrophobic chemical group(s) to improve the water properties of the biofilm be carried out and that hydrogel performance of the biofilm produced in this study would be attempted.

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