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FORMULATION AND CHARACTERIZATION OF STARCH BASED BIOPOLYMERS FOR DRUG DELIVERY

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ABSTRACT:

This investigation was carried out to evaluate the potential of biopolymers membrane synthesized using starch and CMC. Strengthening of the membrane was treated with various solvents (acetone, chloroforms, acetic acid, water).the bio degradability of the membrane was proven using soil burial test, which observed that 75% in red soil & 69.9% in garden soil. These bio polymers have good flexibility that makes them a suitable antibiotic control membrane. These bio membranes are used for wound healing and facial mask for cleaning oil, dust and infection.

Keywords: Biopolymer, SEM, Biofilm.

INTRODUCTION:

The term 'plastic' is defined as any of numerous organic synthetic or processed materials that are mostly thermoplastic or thermosetting polymers of high molecular mass and that can be made into objects, films or filaments (Merriam–Webster Dictionary definition). The most commonly used plastic materials such as polyethylene, polyvinylchloride (PVC), polystyrene and polypropylene are synthesized from petrochemicals and are often referred to as traditional plastics (Mooney, 2009).

Traditional plastics can release toxic byproducts into the environment. For example, PVC plastics are used to make food containers and contain plasticizers called phthalates that can leach out into food (Meeker *et al.*, 2009). Phthalates, bisphenol A or polybrominated diphenyl ethers, as well as other chemicals found in plastics, may be harmful to humans by altering endocrine function or interfering with other biological mechanisms.

Toxicity, depleting petroleum resources as well as waste accumulation has made it imperative to rethink synthetic polymers. Biopolymer-based plastics (bioplastics) can mitigate many of these problems. For example, a major concern with traditional plastic usage is green house gas emissions; 2-3 kg CO2 emissions are estimated to be produced per kg of resin used while polyhydroxy alkanoate (PHA), a biopolymer-based plastic, only causes about 0.45kg of CO2 emissions. Therefore, using bioplastics like PHA can lead to about 80% reduction in global warming potential. Traditional plastics not only use fossil fuels as feedstock but also as an energy source for their manufacture. Bioplastics would reduce reliance on fossil fuels; PHA requires 0.44MJ less fossil fuel in its processing compared to its petroleum-based counterparts (Yu and Chen, 2009).

Another concern is plastic disposal; traditional plastics are designed to be stable in different conditions and to persist in the environment for many years. The ubiquitous use of plastics has contributed to about 12% of the 227 metric tons of municipal waste produced annually in the United States. About 30% of plastic is recovered from the waste stream by recycling, but that still allows plastics to accumulate in the environment at a rate of more than metric tons per year (Mooney, 2009).

Bioplastic are mainly means two types of plastic, compostable plastic and bio based plastics. The compostable plastics are made up of renewable and non-renewable resourceswhereas bio based plastic are made up of renewable resources. Bioplastic are a new generation of plastics which are biodegradable and compostable. They are manufactured generally from renewable raw materials like starch from e.g. corn, potato, or plants as a whole is used as a feedstock for bioplastic to get starch, cellulose, lactic acid etc. which are not hazardous in production and decompose back into carbon dioxide.

Plants produce enormous amounts of biomass by carbon dioxide fixation during photosynthesis. Plant biomass synthesis is estimated to be about 140 billion tons annually. Biomass includes materials such as lignin, cellulose and starch which are produced continuously and are almost in exhaustible, unlike fossil fuels. Lignocelluloses and starch have been used in various ways in the industry, the most notable of which are food, paper, textile and adhesives (Flieger *et al.*, 2003). It is not surprising that plastics are also being made from plant polymers since the first known man-made plastic material (in 1862) was cellulose nitrate and another one of the earliest plastics used in the mid-nineteenth century, colloidal, was made with cotton cellulose (van Beilen and Poirier, 2007). More recently, starch has emerged as one of the primaryraw materials for eco-friendly plastics. Our aim is to synthesis a bioplastics or membrane with corn starch and Carboxy methyl cellulose materials. To strengthening the bioplastic materials with various solvents (acetone, chloroform, acetic acid and water).To analyze the stability of drug with biomembrane by antimicrobial activity test and to study the biodegradability by soil buried test.

MATERIALS AND METHODS

Materials used to produce the bioplastic sheets or membranes were Corn starch, Carboxy methyl cellulose, glycerol (99.5%), water and vinegar. Glycerol was used as a plasticizer. Acetone ethanol was used as a solvent for solubility testing.

1. Preparation of Films

3gms of corn Starch was properly dry mixed in a beaker; 7 ml of acetic acid and 7 ml of glycerol were added and mixed properly. 30 ml of water was added to the mixture and stirring at room temperature for 10-15 minutes at a moderate speed. The beaker along with its content was maintained at 65^{0}C on the heater with continuous stirring for 15-20 minutes.

The gelatin-like solution was formed and spread in a mold to make a film. The film prepared was kept in a room temperature for 24 -48 hours to obtain a dry film. The film was further treated with various solvents to make the thin and stability of the film surface.

2. Preparation of CMC bioplastic

3gms of carboxy methyl cellulose was properly dry mixed in a beaker; 7 ml of acetic acid and 7 ml of glycerol were added and mixed properly. 50 ml of water was added to the mixture and stirring at room temperature for 10-15 minutes at a moderate speed. The beaker along withits content was maintained at 65^0C on the heater with continuous stirring for 15-20 minutes. The gelatin-like solution was formed and spread in a mold to make a film. The film prepared waskept in a room temperature for 24 -48 hours to obtain a dry film. The film was further treated with various solvents to make the thin and stability of the film surface.

3. Solubility studies

Samples of bioplastic were cut into small pieces and were inserted into a test tube containing different solvents (Glacial acetic acid, chloroform, acetone and water).

4. Biodegradation Behaviors under Soil

The degradation tendency of the films under soil was studied. The soils were collected from the garden and red soil. The treatment of soil was done in the laboratory. The microorganism present were explored to know which microorganisms (bacteria) were responsible for the decay of the films. The humidity of the soil was maintained at approximately20%. The samples were buried 7cm below under the soil. In twenty five days, films were taken away from the soil. Later than clean-up through water and exposure to air at room temperature, changes in weight were calculated. Changes of weight (%) were calculated using the following equation:

$$\% \, Wg = \frac{Wi - Wf}{Wi} \; 100$$

Where Wi and Wf be the weight of the samples before and after the soil action.

The appearances of physical changes were also determined by distinguishing the pictures of thefilm surface before and after soil treatment.

5. Preparation of Antibiotic coated biofilm

Corn starch and Carboxy methyl cellulose was properly dry mixed in a separate beaker; some amount of acetic acid and glycerol were added and mixed properly. Enough water was added to the mixture and stirring at room temperature for 10-15 minutes at a moderate speed.The beaker along with its content was maintained at 65^0Con the heater with continuous stirring for 15-20 minutes.

The gelatin-like solution was formed. After hand bearable heat (45^{o}C), add100 microgram/ml of streptomycin powder and spread in a mold to make a film. The film prepared was kept in a room temperature for 24 -48 hours to obtain a dry film. The film was further treated with various solvents to make the thin and stability of the film surface.

6. Antimicrobial activity studies (well method)

Muller Hinton agar medium was prepared. The medium was smeared with *E.coli, Streptococcus pyogenes, Micrococcus sp* and *Klebsiella sp*. The wells were made by well cutter in the agar plates. Different biofilm were cutted by well cutter and placed in the well. All the plates were incubated at 37^{o}C for 24 hours. After incubation, zone of clearance was measured and tabulated.

7. Haemolytic activity studies

Blood agar medium was prepared. Small piece of different biofilm were kept on theblood agar medium and incubated at 37^{o}C for 24 hours. After incubation, observed the haemolytic activity of biofilm.

8. Preparation of colored biofilm

3gms of carboxy methyl cellulose was properly dry mixed in a beaker; 7 ml of acetic acidand 7 ml of glycerol were added and mixed properly. 30 ml of water was added to the mixture and stirring at room temperature for 10-15 minutes at a moderate speed. The beaker along with its content was maintained at 65^{0}C on the heater with continuous stirring for 15- 20 minutes. 20 micro liters of 100% safranin dye and methylene blue dye. The gelatin-like solution was formed and spread in a mold to make a film. The film prepared was kept in a room temperature for 24 - 48 hours to obtain a dry film. The film was further treated with various solvents to make the thin and stability of the film surface. The dye color degradation or color disappearance study was performed.

RESULTS:

Nowadays, due to the depletion of petroleum sources and environmental effects caused by the conventional plastics, bioplastics film are being studied extensively as an alternative to conventional plastics because of their excellent biodegradability, biocompatibility and edibility.

In this study, corn starch and carboxy methyl cellulose were used in water –soft gel –like jelly which clumped together in the solution. Corn starch materials are very cheap and easily available as in large quantity. Bioplastic are prepared from corn starch with acetic acid, glycerol and water (Plate-1).

Plate-2 shows that thin bioplastic was prepared from Carboxy methyl cellulose material.
This is highly elastic, flexible and transparent in nature.

Plate-3 shows that the prepared bioplastic materials were treated with acetone solvent. It will increase the thickness and white in color. This solvent treatment was used to improve the gel (polymerization) properties.

Plate-4 shows that the prepared CMC bioplastic are treated with acetone solvent. It was improve only the elastic nature of membrane not thickness.

Two different colored biomembrane were synthesized by using Methylene blue and safranin dyes. The dyes did not decolorize after long time (Plate-5).

Plate-6 shows that antimicrobial sensitivity tests of ingredient used in biofilm formation by agar well method (control).

Table -1 and plate-7 shows that antibiotic activity test of different biofilm coated with streptomycin antibiotic against bacterial pathogens (*E.coli*, *Klebsiella sp*, *Micrococcus sp* and *Streptococcus sp*. The zone of inhibition was obtained in the range of 2mm-12mm.

Hemolytic activity of the biofilm was performed by using blood agar hemolysis method (Plate-8)

Plate-9a shows the biodegradability activity of the different polymer by using garden soil.

Plate- 9b shows the biodegradability activity of the different polymer by using red soil.

Table-9a and table 9b shows that Soil burial test was carried out to determine the biodegradability of corn and CMC bioplastic film in two types of soil (garden and red). Corn bioplastic film was able to 75% decompose after 25 days in red soil but in garden soil 69.9%.

Table -2 shows that antimicrobial activity of solvent treated and untreated biofilm was performed. Maximum zone of inhibition was occurred in Untreated CMC biofilm (17 mm) against *E.coil* and corn biofilm (12 mm) against *Klebsiella sp*.

Solubility tests of biofilm were analyzed in different solvents such as water, acetone, chloroform and acetic acid (Table-3).

DISCUSSION:

Biodegradation is a chemical process during which the tiny organism or microorganismthat is present in the environment convert materials into natural substances such as water, carbondioxide, and compost that predominantly depend on the surrounding environmental conditions. [Gregory, 2009 and Barnes *et al.*, 2009] One of the most undeniable and long-lasting recentchanges to our planet is the accumulation and fragmentation of plastics.

The most infamous talk on plastics causing cancer is one to be noted. At least some amount of the cancer population has been affected by plastics. The chemicals present in them or their abundant use in an unhealthy way could be one of the causes. Hence, bioplastics can also analternative healthy solution.

Starch is one of the prime materials used to make bioplastics. The commonly used bioplastic globally as of right now is the polyacetic acid. The total amount of plastic waste arising every year is estimated to be 5.9 million tones [Shaxson *et al.*, 2009]. All these plastics are dumped intentionally or unintentionally and cause major environmental hazards. They are not biodegradable and harms the ecosystem [Smith,2005]. Bioplastics do not cause any environmental and health hazards. [Modebelu and Edward, 2014] and are eco-friendly. [Maheshwari *et al.,* 2013; Hester and Harriso, 2015] Plastics can be recycled and reused, but bioplastics are even reversible.[Kumar *et al.,* 2016] .However, lightweight and durable, plastics have became a main causative of environmental pollution, thus bioplastics can be used in the place of synthetic plastics.[Ganesh *et al.,* 2017]

In our study, the raw materials for bioplastics are commercial corn starch and CMC powder (plate-1 and 2). After preparation of bioplastic, the materials are strengthening by using alcohol and acetone. These solvents are helpful to thicken for polymerization properties in corn starch and CMC based plastics. It will increase the whitening of the sample, so it may be actingas a bleaching agent (plate-3 and 4).

Maulida et al., (2017) reported that the bioplastics are made from mango seed starch. Theresulting starch grayish powder form with a particle size 100 mesh. The results starch extraction from the mango seeds yield of starch obtained by 43.2 %, which of 100 g dried starch mango seeds gained as much as 43.2 g. The composite produced from banana peels was thin, papery and showed good tensile strength. The composite with potato starch was thin and transparent but brittle. Composite from corn starch was thick, flexible, opaque and hard. Agar gave the toughest material, which was like a transparent film and tensile strength was greater than other composites.

In this work, we focused on the preparation of bio film with different colors for attracting the materials preparation. The color has to retain for long periods of time. That is, there is no color change. Both corn and CMC based bio film are flexible in nature (Plate-5).

Our aim is to synthesis a medically important bio material such as wound healing membrane and facial mask for cleaning the oil and dust. For that, the corn and CMC membrane are mixed with antibiotic compounds (Streptomycin). Antimicrobial activity of different biofilm against bacterial pathogens was performed (Plate-7, Table -1and 2).

For the preparation of bioplastic, the main ingredients are water, corn starch, acetic acid and glycerol (Plate-1). water is the primary plasticizer in binding starch molecules but the thermoplastic produced using only water as the plasticizing agent are highly rigid and usually appears to have various tears in formation as a result of shrinkage as water rapidly evaporate from the material during drying [Nafchi *et al*., 2013].

Glycerol as an oil is less evaporative and allows better inter-aliased of the starch molecules during plasticization. In this experiment, the tensile strength values are higher with higher glycerol content. The response shows the highest value of 11 MPa at 30% glycerol content with similar higher tensile strength value from 20% and above glycerol content. This value is close to those obtained in the works of [de M. *et al*., 2012], [González-Gutiérrez *et al*., 2011], [Oladayo *et al*., 2016]. The results do not quite agree with the recent work of [Ezeoha *et al*., 2013] who quoted higher tensile strength value of above 20 MPa but similar elongation at break.

One of the most important properties of bioplastics is its ability to be degraded naturally in the environment so that the damage caused by the use of synthetic plastic does not occur [González-Gutiérrez *et al*., 2011]. Biodegradation is one parameter observation that might indicate that bioplastics are environmentally friendly or not. The simplest quantitative method to characterize the occurrence of a polymer biodegradation is to determine the loss of mass during testing.

Tests carried out on each treatment with three replications. The biodegradation testing conducted on a sample size of 2x3 cm. Each sample is inserted into a plastic tray that has a length of 34 cm, width 28 cm and depth of 13 cm. Land that is used as a medium of biodegradation taken in the area KhatibSulaiman, Padang. Before being used as a medium of biodegradable bio-plastics, soil is measured its water content and pH. The water content of the

soil used was 60.47% and had a pH of 6.2. Sample mass reduction is seen every week for one month at room temperature.

Analysis the biodegradable of bio-plastics value was performed in soil buried test, it can be seen in Plate-9a, b and Table-4. TutyAnggraini (2017) reported that the starch polymer and chitosan used in bioplastics proved to be biodegradable, as seen in the first week, losing mass ranges bio-plastics 15.71% - 22.03%. In the second week, the mass loss ranging bioplastics dueto the burial is at 31.52% - 43.45%.

The mass reduction of bioplastics continues to increase the length of time the biodegradation testing. It shows the bio-plastic could be degraded in soil media. But the biodegradation testing week 3 and 4 show that the presence of a significant mass does not occur in each treatment bio-plastics. This is presumably because the soil moisture content is used as a medium of biodegradation decreases; the absence of controlling soil moisture content is used as a medium of mass loss causes biodegradable bioplastics at weeks 3 and 4 are not so significant.

The degradation in soil burial method was mainly caused due to the presence of microorganism in the soil. To identify the microorganism responsible for the degradation of the polymer films, the soil testing was carried out in the laboratory.

The microorganism wascharacterized depending upon their shapes. The rod like shape indicates the presence of *Bacillus*, *Vibrios* are comma shaped curved rods; coccus represents spherical or oval cells

SEM analysis shows the main dominating microorganism present in the soil was *Bacillus* and was reported. Besides *Bacillus* species other microorganism present in the soil was *Vibrios*, coccus and fungi. These microorganisms were responsible for the degradation of film surface. The degradation of starch/ sucrose, starch/ sucrose/ glycerol and starch/ glycerol films under the

7 cm layer soil. The starch/sucrose film is easily degraded under the soil and losses about 88% weight in 30 days.

Azahari *et al.*, (2011) studied that the interaction of microorganism on starch and sucrose molecules increased initially which leads to faster degradation rate. As soon as the starch and sucrose molecules was almost fully degraded, the glycerol molecules was promote to degrade which shows slower degradation behavior. The F3composite film losses about 70% weight in 40 days whereas F5 (starch/glycerol) film losses about 47% weight in 40 days.

The commonly produced polymer types of bioplastics includes bio-polyethylene (bio- PE), polylactic acid (PLA), polyhydroxyalkanoate (PHA), bio-polyethylene terephthalate (bio- PET), and bio degradable polyester among others. In the non degradable bioplastics segment, growth is expected to be led by bio-PE and bio-PET on account of the wide application scope of these plastics. In the biodegradable segment, polyhydroxyalkanoate (PHA) and polylactic acid (PLA) are expected to witness fastest growth over the next seven years Azahari *et al*., 2011).

Starch from different resources such as potato, corn or rice is combined with biodegradable polymer precursors to form elastomers with engineered mechanical properties.The elastomers also show exceptional water proof properties. In this technology, starch is not destructed compared to quite fast whereas in the case of oregano waste the polymerization is slower. For the process to be effective, all the plant waste should be ground to fine powder in order to increase their surface area for reaction. The elastomerization can be controlled in orderto obtain highly or partially stretchable bioelastomers depending on the precursor chemistry as well as the amount of pulverized vegetable waste used. In general, up to 85% of vegetable waste can be used while maintaining a good degree of stretching.

CONCLUSION:

Corn starch and CMC based biopolymer were prepared. The main ingredient of biopolymer is sugary materials and it could be easily degradable one. Strengthening of corn starch biopolymer by acetone increases the thickness of polymer. Strengthening of CMC biopolymer by acetone. It is improved the elastic in nature. Preparation of biopolymer with uses the different dyes as methylene blue and safranin. The dyes did not decolorize after long time. So we can prepare colored bioplastics. Antimicrobial activity of ingredients used in biofilm formation by Agar well method Antibiotic activity test of different biofilm coated with streptomycin antibiotic against bacterial pathogens (*E.coli*, *Klebsiella sp*, *Streptococcus sp*, *Micrococcussp.)*Antimicrobial activity of solvent treated and untreated biofilm was performed. Haemolytic activity of the biofilm was performed by using blood agar hemolysis method.Testing of polymer solubility were analyzed with different solvents such as water,acetic acid, acetone, chloroform. Soil burial test was carried out to determine the biodegradability of corn andCMC bioplastic in two types of soil (Red and garden).

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Table.1 Antimicrobial activity of Biofilm

S.No	Type of biofilms	Test organisms	Zone of incubation (mm)		
			Day 1	Day 2	Day 3
1	Corn + acetic acid +streptomycin	*E.coli*	5	8	12
		Klebsiella sp	2	2	2
		Streptococcus sp	8	8	8
		Micrococcus sp	10	10	10
2	Corn + acetic acid eptomycin +acetone	*E.coli*	8	10	13
		Klebsiella sp	3	3	3
		Streptococcus sp	6	6	6
		Micrococcus sp	9	9	9
3	CMC + acetic acid + streptomycin	*E.coli*	3	7	8
		Klebsiella sp	3	3	2
		Streptococcus sp	4	4	4
		Micrococcus sp	5	5	5
4	CMC + acetic acid eptomycin +acetone	*E.coli*	2	5	7
		Klebsiella sp	4	4	3
		Streptococcus sp	3	3	3
		Micrococcus sp	4	4	4
5	IC + glycerol + streptomycin	*E.coli*	1	1	1
		Klebsiella sp	1	0	0

		Streptococcus sp	1	0	0
		Micrococcus sp	1	0	0
6	CMC + glycerol + streptomycin + acetone	*E.coli*	2	2	2
		Klebsiella sp	2	2	0
		Streptococcus sp	2	0	0
		Micrococcus sp	2	0	0

Table 2.Antimicrobial activity of solvent treated and un treated different Biofilm

S.No	Test organisms	Types of biofilm	Zone of inhibition (mm)
1	*E.Coli*	Corn	9
2	*Klebsiella sp*		12
3	*E.Coli*	Corn+ acetone	5
4	*Klebsiella sp*		9
5	*E.Coli*	CMC	17
6	*Klebsiella sp*		7
7	*E.Coli*	CMC+ acetone	8
8	*Klebsiella sp*		8

Table 3. Solubility of Biofilms in different solvents

S.No	Types of biofilm	solvents	Biofilm weight (1g)	Incubation time and OD value	
				1hrs	2 hrs
1	Corn strach	Water	0.32	0.01	0.01
2		Acetone	0.49	0.01	0.01
3		Chloroform	0.37	0.02	0.00
4		Acetic acid	0.38	0.02	0.03
5	CMC	Water	0.41	0.02	0.03
6		Acetone	0.27	0.00	0.01
7		Chloroform	0.34	0.01	0.00
8		Acetic acid	0.38	0.02	0.02

Table 4. Biodegradability of Bioplastic (after 25 days)

S.No	Types of bioplastic	Types of soil	Initial weight (grams)	Final weight (grams)
1	Corn starch	Red	0.9	0.17
2		Garden	1.03	0.31
3	CMC	Red	0.7	0.03
4		Garden	1.02	0.05
5	Normal plastic	Red	0.08	0.08
6		Garden	0.09	0.09

Plate 1. Preparation of bioplastic from corn starch

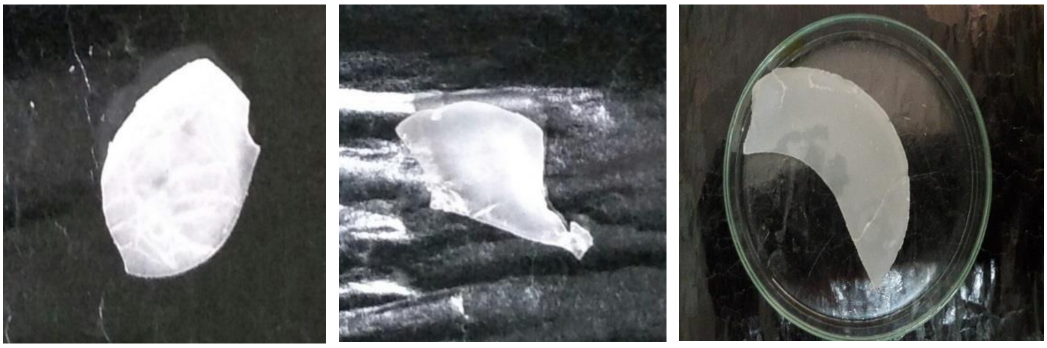


Plate 2. Preparation of biopastic from CMC

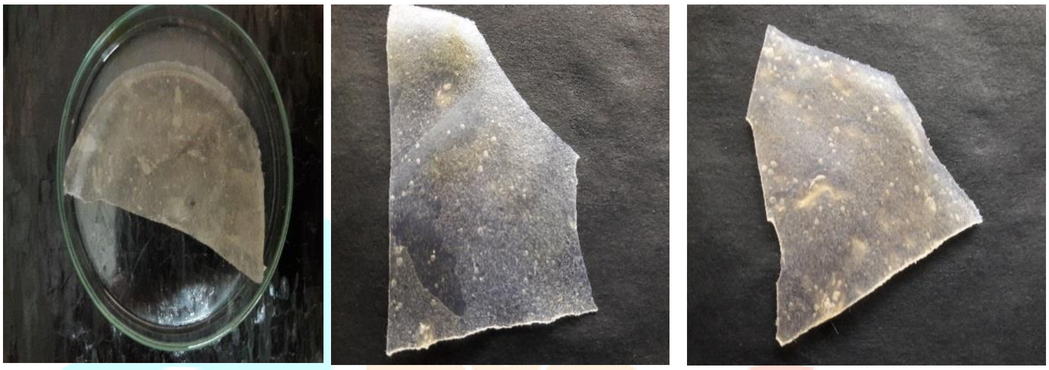


Plate 3.Preparation of corn starch bioplastic treated with acetone

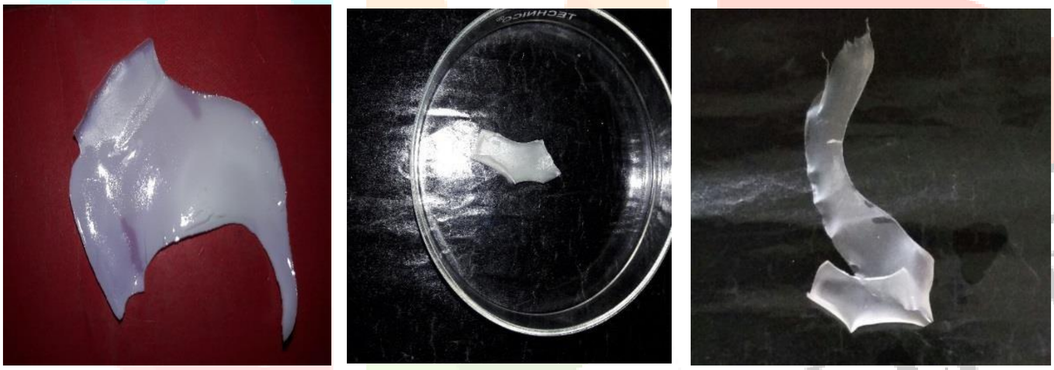
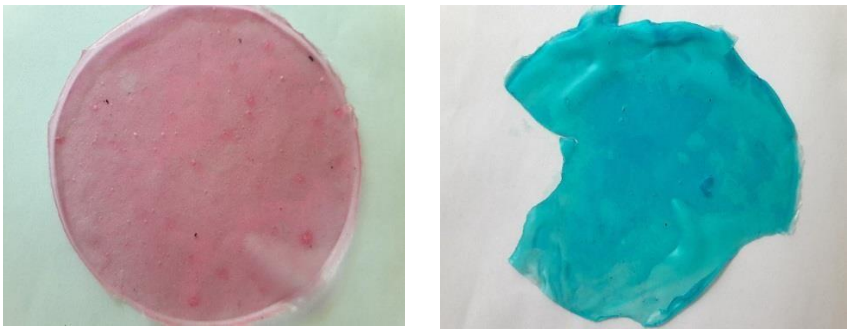


Plate 4.Preparation of CMC bioplastic treated with acetone

Plate 5. Preparation bioplastic with dyes

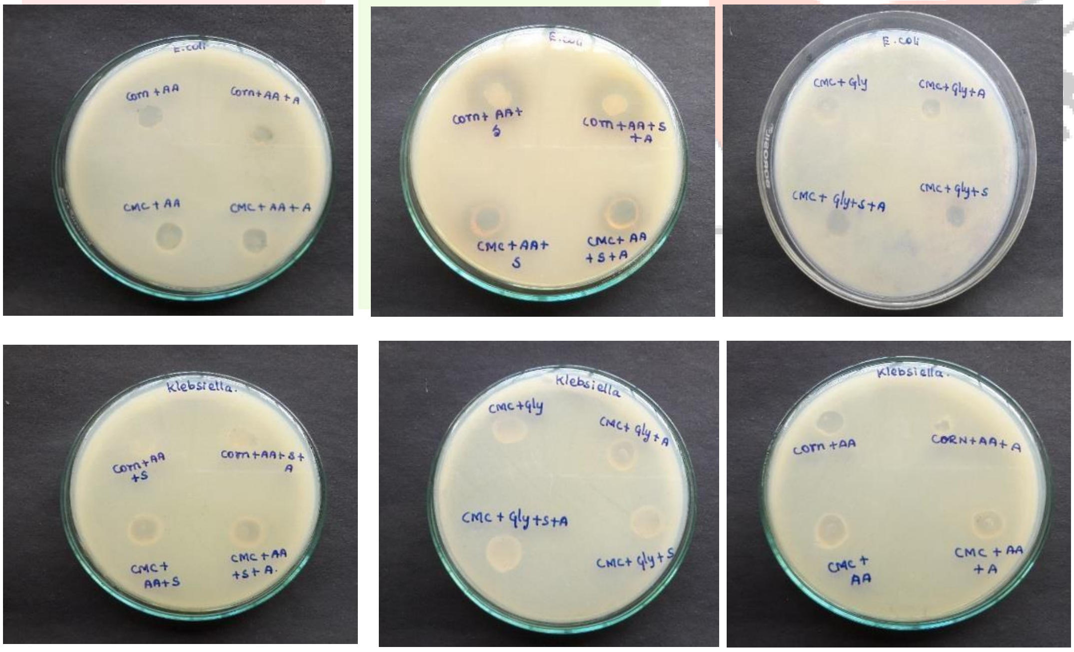


CMC + safranin corn starch+ methylene blue

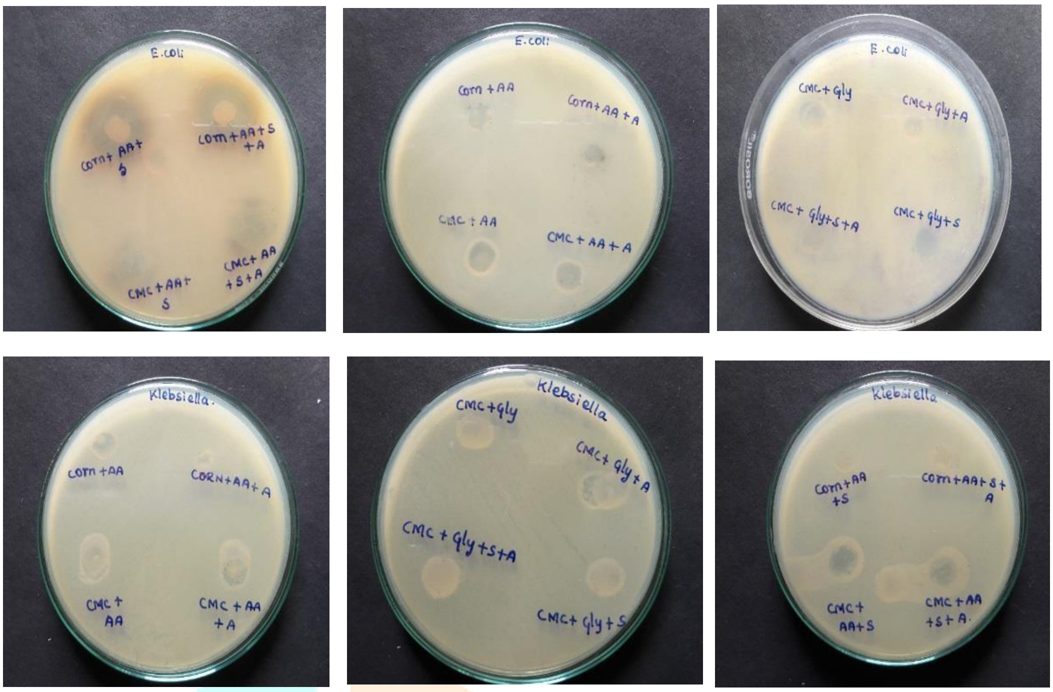
Plate 6. Antimicrobial sensitivity test of ingredients used in biofilm formation by Agarwell method

Plate 7. Antibiotic activity test of different biofilm coated with streptomycin antibioticagainst bacterial pathogens

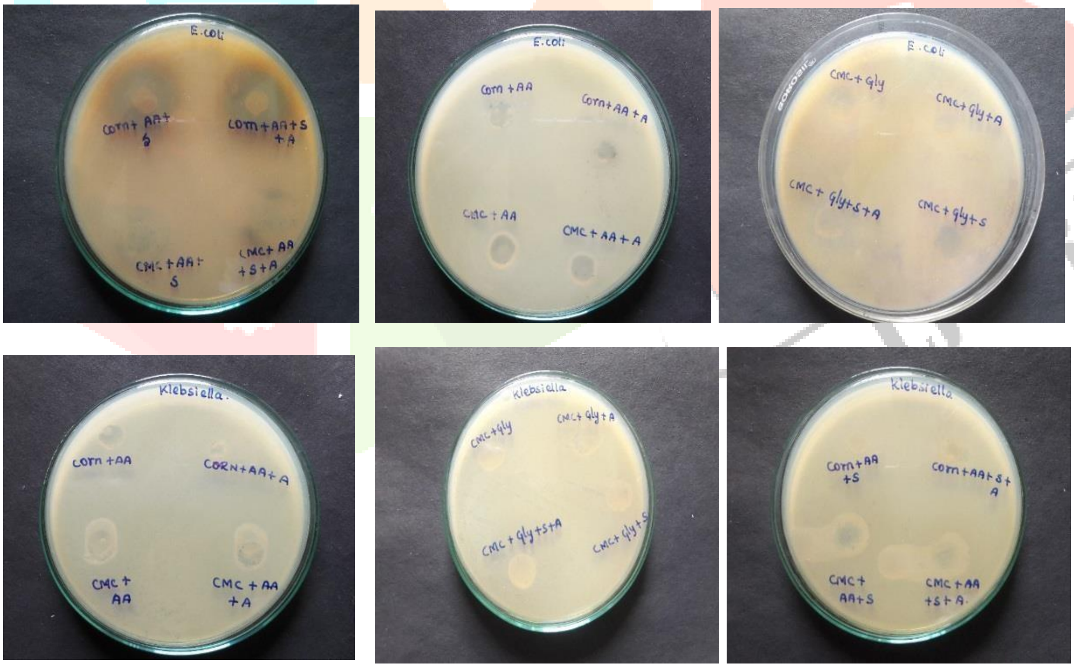
After 24 hrs incubation



After 48 hrs incubation



After 72 hrs incubation



Gram positive:

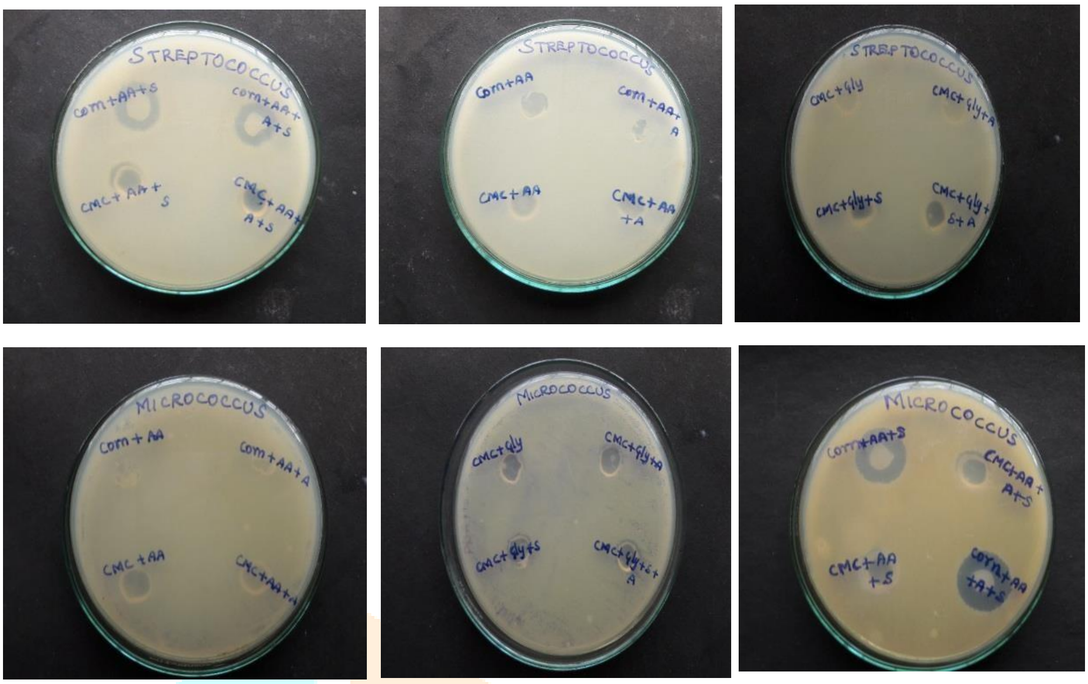
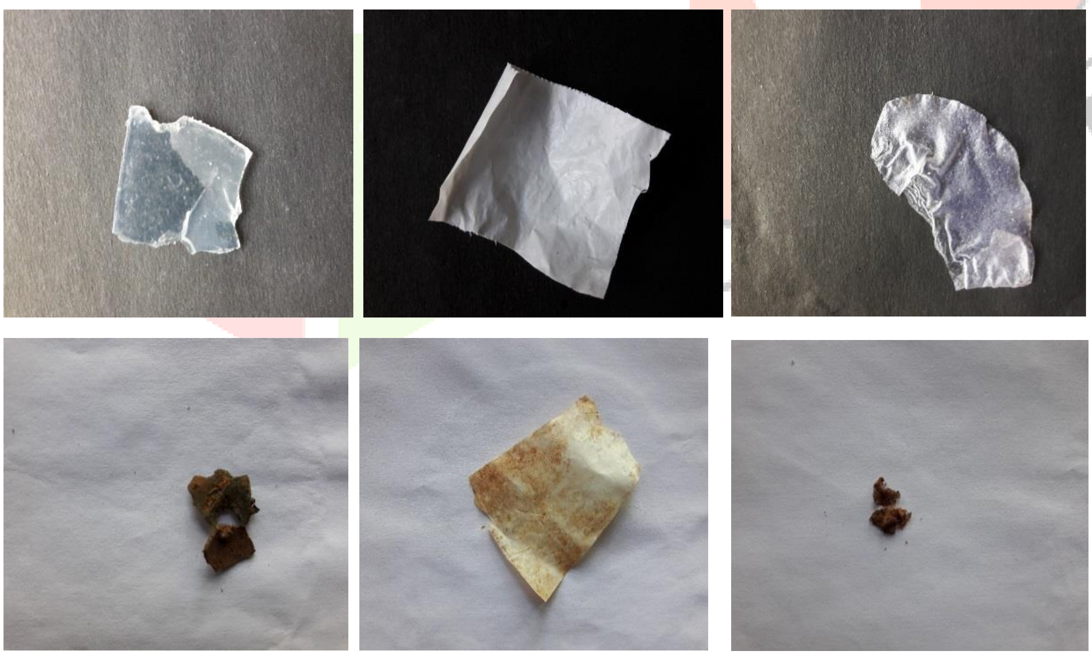


Plate 8. Haemolytic activity of different biofilm

Plate 9a. Bio-degradability of biofilm in garden soil

Corn starch Normal plastic CMC

Plate 9b. Bio-degradability of bioplastics in Red soil



Corn starch Normal plastic CMC