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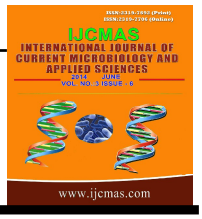


Original Research Article

Biodegradation of Citric Acid based synthetic polymer

P.Aruna[1], Maragaret Marie[2] and Priya Iyer[1]*

[1]PG and Research Department of Biotechnology, Women`s Christian College, Chennai, India
[2]Department of Chemistry, Women`s Christian College, Chennai, India

**Corresponding author*

A B S T R A C T

Keywords

Citric acid Polymers, *Bacillus* spp, Biodegradation Synthetic polymer

Synthetic degradable polymers are play a major role in tissue engineering andcitric acid based man made polymers can also be used as a polymeric scaffold for tissue expansion in the body Microbes were isolated from differentplaces like open drainage region, sewage,effluent water from chemical industry etc and identified. The isolated organisms were used to degrade PP7, PP8, PP9 and PP12 synthetic citric acid polymers. The release of citric acid was estimated spectrophotometrically and cytotoxicity test of the polymer was performed using VERO cell lines.

Introduction

A variety of natural, synthetic, and biosynthetic polymers are bio and environmentally degradable. A polymer based on a C-C backbone tends to resist degradation, whereas heteroatom-containing polymer backbones confer biodegradability. Biodegradability can, therefore, be engineered into polymers by the judicious addition of chemical linkages such as anhydride, ester, or amide bonds, among others. The usual mechanism for degradation is by hydrolysis or enzymatic cleavage of the labile heteroatom bonds, resulting in a scission of the polymer backbone (Senthil Kumar, et a., 2010).

Biodegradable polymers with hydrolysable chemical bonds are researched extensively for biomedical, pharmaceutical, agricultural, and packaging applications (Senthil Kaliloor et al., 2012). Biodegradation is the decay or breakdown of biopolymers materials that occurs when microorganisms use an organic substance as a source of carbon and energy*(Sonil Nanda Smiti et al.,2010)*. For example, sewage flows to the wastewater treatment plant where many of the organic compounds are broken down; some compounds are simply biotransformed (changed), others are completely mineralized**.** These biodegradation processes are essential to recycle wastes so that the elements in them can be used again (Aamer Ali Shah, *et.al*.,2011).

Materials and Methods

Isolation and identification of organisms degrading polymer

The samples were collected from two different region, one from open drainage collection &sewage dump region at Villivakkam and from chemical industry of effluent water.Minimal media with the polymer were used to detect the degradation ability of the organisms in the samples,where the polymer that as to be degraded will serve as a carbon source. The organism degrading the polymers were streaked on Nutrient Agar and the colony and biochemical characteristics.

Degradation of polymer without organisms and with organisms

Polymer degradation in phosphate buffer saline (PBS) solution was carried out in two different concentration of PBS solution. The concentrations were 1X PBS &10 X PBS solutions. Minimal media with the polymer was used for the degradation using organisms.

Estimation of citric acid

The presence of organic acid (citric acid) was detected by paper chromatography and estimated spectrophotometerically at 340 nm.

Toxicity test on the polymers

The polymer was tested for its toxic effect using VERO cell lines and then MTT Assay. The effect of the samples on the proliferation of VERO cells was expressed as the % cell viability, using the following formula:

Percentage of cell viability = A570 of treated cells / A570 of control cells × 100%

Results and Discussion

Isolation and identification of organisms degrading polymer:The samples were collected from two regions are open drainage collection in Villivakkum & effluent water from a chemical industry and used as source for isolating microorganisms are ability to degrade these polymers degrading microbes were cultivated in minimal media. Four types of bacterial stains were identified, which includes: *Bacillus sp, Staphylococcus aureus, Serratia marcescens, Bacillus subtilis* were isolated from sewage sludge (open drainage +effluent H_20). The Bacterial consortium was obtained from dirty water, having ability to grow on synthetic citric acid polymer films. The bacterial strains were purified in the nutrient agar medium. The dominant bacterial strains were named as OPD-7, EFF-8, OPD-9& EFF-12.

Biochemical test method was performed to each of the isolate to identify the organisms (Table I). Strain OPDS-7 was identified as *Bacillus* spp. The strain of EEF-8 was confirmed as *Staphylococcus aureus* while the biochemical characteristic of OPDS-9 was similar to *Serratia marcscens.* Strain EFF-9 showed similar characteristics as *Bacillus subtilis* there by bacterial strain was confirmed as *Bacillus subtilis*. The results were similar to the one reported earlier (Holt *et al.*, 1994). The biochemical tests were performed to identify these microorganisms. Four microorganisms were identified as polymer degrading strains by biochemical characterization method these are includes *Bacillus spp, Staphylococcus aureus, Serratia marcescens* and *Bacillus subtilis.* Degradation of polymer with and without organisms: Citric acid derived synthetic polymers also showed degradation in PBS

solution without addition of organisms and in minimal media with organisms. The citric acid released was confirmed by paper chromatography. The paper chromatography was performed along with standard citric acid in the ratio of n-butanol: acetic acid :water(10:13:10) the spot was identified as yellow colour with help of bromophenol blue yellow colour spots indicated the presence of citric acid. Degradation of the polymers was found to be fast in 1 XPBS & Minimal media than in 10 XPBS.

Estimation of citric acid: Citric acid based polymer when degraded leaves citric acid as a byproduct (D.G. Barrett Muhammad et.al., 2009) which was quantified by UV Spectroscopy. The Table III shows the amount of citric acid released in different concentration of PBS and minimal media. The degradation mechanism and rate of biodegradable polymers can be affected by many factors Among the factors which affect degradation are: molecular weight, structure and content of co-monomer unit, crys reported (J.C Middleton & A.J.Tipton, 2000) that when catalytic molecules or substances such as enzymes and alkalis are present in the degradation media or environment, the degradation of polymer-materials proceeds via a surface erosion mechanism.

In the surface erosion mechanism, catalytic molecules or ions act only on the surface of materials and will not diffuse into the material. As a result, the material is eroded from the surface while the core part of the material remains unchanged. On the other hand, the degradation of biodegradable polymers takes place via a bulk erosion mechanism in the absence of catalytic molecules or ions as in a phosphate-buffered solution. It was also reported that the hydrolytic degradation mechanisms depends on the thickness of biodegradable materials(Naznin Sultana,2013) based on above result & dissection the polymer in minimal media along with microorganism degraded via surface erotion but in PBS solution it was fond to be bulk erotion because the polymer which imbibe the buffer & had swelled.

Toxicity test on the polymers

B.subtilis, B.polymyxa, B.cereus, B.firmus, Streptococcus spp, Staphylococcus spp, Miccrococcussppp, la & Pseudomonas spp, Rhodococcus ruber C208 and fungal strain *Aspergillus glaucus* which are able to degrade polyethylene bags& plastic cups bags in mangrove soil (Kathiresan,2003; Gilan *et al.,*2004). The same micro organisms were isolated from open drainage, sewage collection &effluent water collectecd from respective areas which are includes *Bacillus spp* from OPDS-7, *Staphylococcous aureus* from EFF-8, S*erratia marcescens* from OPDS-9 & *B.subtilis* from *EEF-12*.under other 3 spp are common plastic degradable bacteria except *S.marcescens* which is different bacteria found as plastic degradable bacteria under our lab condition Citric acid has been detected based on general technique of paper chromatography the spot was identified as yellow which indicates the presence of citric acid in different concentration (Figure I, II, III and IV).

The citric acid estimation was done by UV spectroscopy and reading was extrapolated in graph sheet. from the extrapolation the concentration of citric acid was calculated. 1-1.5 mm thickness of specimens are put into 10 ml of PBS solution at 37°C upto 26 weeks, the sample removed frm PBS and dried for 1 week then subtract the initial weight final from weigh (Jian Yang Antonio R *et.al., 2012*).

Table.1 Biochemical tests

S.No.	Biochemical test performed Biochemical test	OPD 7 Results	EFF 8 Results	OPD 9 Results	EFF 12
1	Simple stain	Rods	Cocci	Rods	Rods
2	Gram stain	Gram Positive	Gram positive	Gram negative	Gram positive
3	Mannitol motility test	Motile	Non motile	Motile	Motile
4	Indole	_	_	_	_
5	Methyl red	+	+	+	_
6	Vogues Proskauer	_	-	+	+
7	Citrate Utilisation	+	+	+	+
8	Gelatin	_	_	+	+
9	Triple sugar iron	Acid and gas	Acid no gas	_	_
10	Oxidase	+	+	+	+
11	Urease	+	+	_	+
12	Starch hydrolysis	_	_	_	+

Table.2 Time for degradation (Complete degradation)

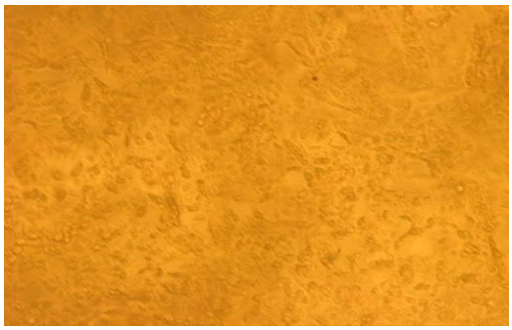
S.No	Polymers	10 X PBS	1XPBS
1	PP7	35 days	22 days
2	PP8	42 days	38days
3	PP9	29 days	21days
4	PP12	15 days	13days

Table.3 Concentration of Citric acid released

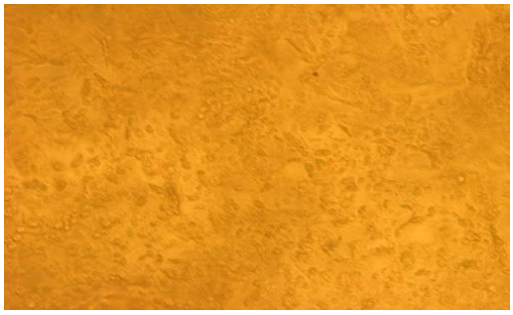
S.No	Polymer	Citric acid concentration in mg/ml		
		10 XPBS	1XPBS	Minimal
1	PP7	2	3.5	4.8
2	PP8	6.5	3.6	2.6
3	PP9	3	2.2	3
4	PP12	8.5	8.3	7.7

Fig.I Toxicity Test - Cytotoxicity Effect of sample –PP7- on VERO cell line

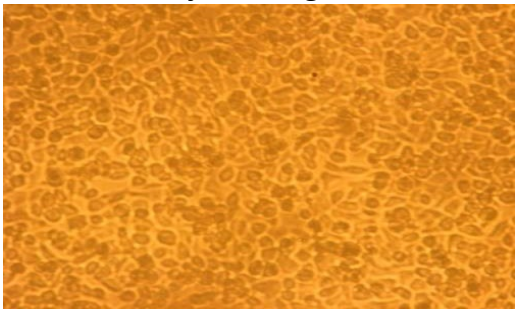
Toxicity- 10mg/ml



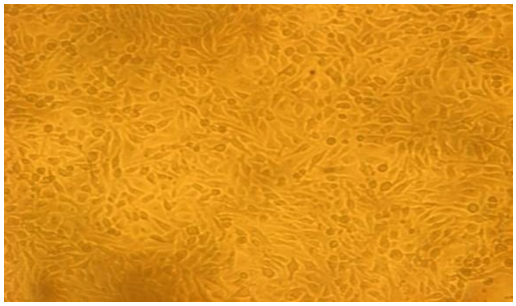
Toxicity- 1.25mg/ml



Toxicity- 0.625mg/ml



Toxicity- 0.312mg/ml



Normal Vero Cells

Fig.II Viability test of sample PP7 - on VERO cell lines

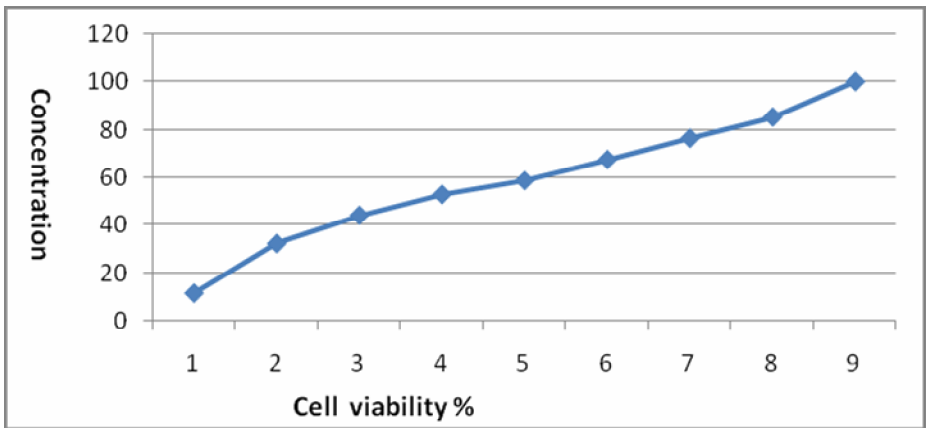


Fig.III Viability test of sample PP8- on VERO cell lines

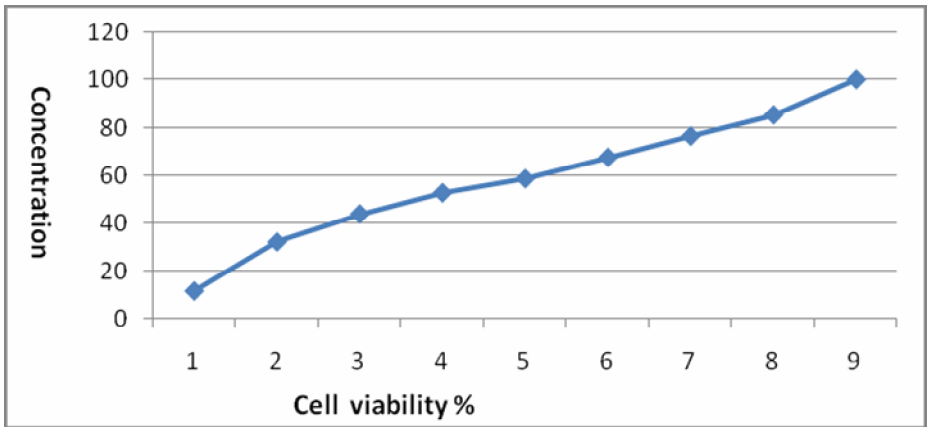
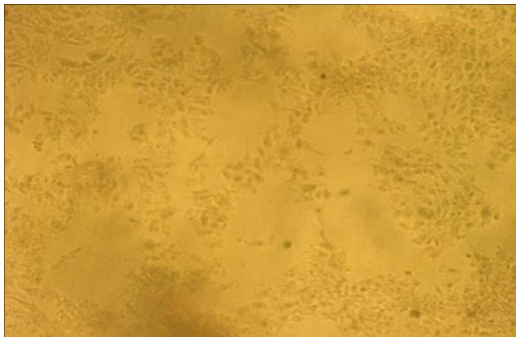
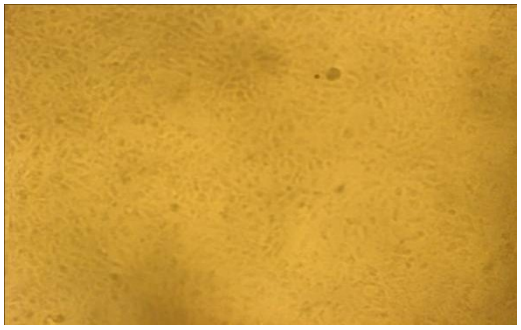


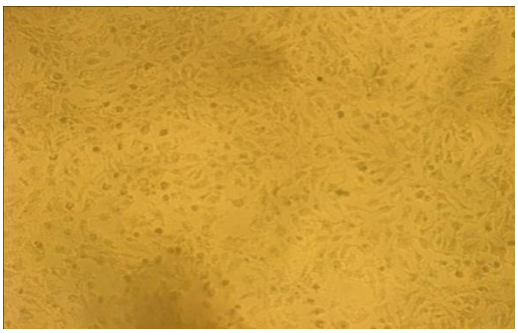
Fig.IV Cytotoxicity Effect of sample –PP8- on VERO cell lines



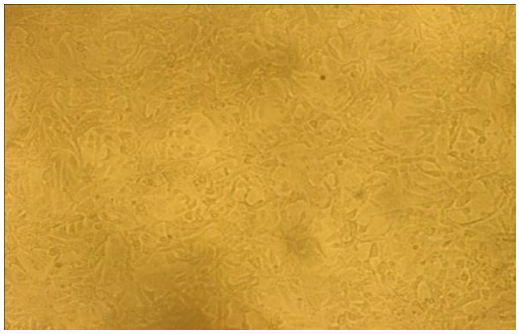
Toxicity- 10mg/ml



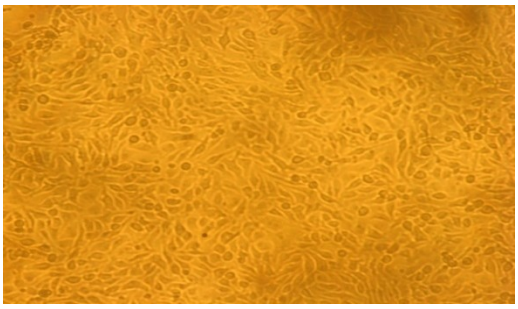
Toxicity- 1.25mg/ml



Toxicity- 0.625mg/ml



Toxicity- 0.312mg/ml



Normal VERO cell line

But due to rapid degradable capacity of PP7,8,9&12 polymers we monitor the complete degradation. Among these polymers PP 7,8,9&12 P12 has rapid degradation capacity than others .The degradation rates are found to de PP12 > PP9 > PP7 >PP8. The polymer toxicitity test was formed with Human aortic smooth muscle cells and endothelial cells (Clonetics, Wakesrille,MD) with SmGM-2EBM-2 culture medium the result showed the morphologically attachment cells (Jian Yang *et. al*) But in VERO cell line the polymers PP7 showed the cell viability is 52.94% in 1.25 mg/ml & PP8 showed the cell viability is 50% in conc of 0.625mg.

The recalcitrant properties of synthetic man –made polymers tough to biodegradation .From the last few years the research focused on designing biodegradable polymers for various purposes. In other hand very rapid established field of tissue engineering in medical area. Synthetic degradable polymers are play a major role in tissue engineering. From the above result discusion on experiment these citric acid based man made polymers can also be used as a polymeric scaffold for tissue expansion in the body. Because the citric acids are involved in energy metabolism (Kreb's cycle). The issue of synthetic plastic utility and their waste ending up in the environment can be partly resolved by developing and subsequently applying biodegradable material.

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