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Full Length Research Paper

Isolation of polyvinyl chloride degrading bacterial strains from environmental samples using enrichment culture technique

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Plastic causes serious damage to the environment, both during its production and disposal. Biodegradation of plastic waste using microbial strain could offer a solution to the problem. Microorganisms have been a good resource for solution to this problem due to their diverse metabolic capability, adaptability to different environment and possibility of isolation using artificial growth media for utilization *in situ*. In the present investigation, potent microbial strains degrading plastic constituting polymer polyvinyl chloride (PVC) were isolated using enrichment culture technique. To increase the chances of isolating such strain which could have adapted to metabolize plastic constituting polymers, samples were collected from different environmental sites that were rich in plastic waste. These samples were used as a source of microbial culture for enrichment of potential PVC degraders. After then, some bacterial species were subsequently isolated on solid agar medium containing emulsified PVC polymer. The strain PVC 4 characterized as *Micrococcus* species was found to be more efficient among the other isolates and was chosen for further studies. The biodegradability of PVC by *Micrococcus* species with PVC as a sole carbon source was determined by their ability to release chloride from PVC polymer, increase their cell density in test media, carbon dioxide production and growth on the surface of PVC film in plate assay. The *Micrococcus* species showed 0.36% release of chloride and 8.87% mineralization measured in terms of carbon dioxide evolution respectively over a period of 70 days in PVC containing media. The increase in cell density in liquid growth media constituting PVC polymer as a sole source of carbon and growth of cells on the surface of PVC film further substantiate the potential of isolated strain for PVC utilization.

Key words: Polyvinyl chloride, biodegradation, *Micrococcus* species, enrichment culture.

INTRODUCTION

The issue of plastic waste management is a complex process and there is no simple solution for reducing this waste. Incineration, recycling, and land filling are some of the traditional methods for handling plastic waste. However, these methods are costly and often create new environmental difficulties (Mody, 2000; Mario, 2008). The best solution according to many scientists in the world is a combination of solutions that includes use of biodegradable plastic, plastic recycling, and bioremediation of plastic waste. Nowadays, bioremediation of plastic waste is gaining increased attention in environmental management of plastic waste as biological degradation is necessary for plastics that eventually enter the waste streams and can therefore neither be recycled nor incinerated (Cacciari et al.,1993; Shah et al., 2008).

In view of this, the diverse metabolic capability of microorganisms could be exploited for bioremediation of plastic waste that use microbial strains developed through selection, strain improvement, or genetic modification. The most effective microbial strain can then be produced in large scale for field application. The degradation of most synthetic plastics is a very slow process that involves environmental factors and action of wild microorganisms (Shah et al., 2009). In nature, complete mineralization of plastics to carbon dioxide and water involves succession of syntrophic association,

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between various groups of microorganisms. However systematic and reproducible study on biodegradation process necessitates isolation of microorganisms in the form of pure culture. In addition, isolation is appropriate for the identification and detailed study of microorganisms that are added with compound in the environment. However, practically, microorganisms with plastic degrading abilities are rare in environmental samples and hence their number needs to be amplified prior to isolation. Subsequently, the most potent organisms could be isolated on solid agar medium.

Enrichment culture technique is used for isolating the desired target organisms from various kinds of organisms that coexisted in nature, and is generally designed to achieve an increase in the relative numbers of particular organism by favoring growth, survival (that is, physiological competition), or its spatial separation from other members of population (Tomita et al., 2004).

The aim of this study was to isolate potent polyvinyl chloride (PVC) degrading microbial strains using enrichment culture technique from samples collected from different sites rich in plastic waste, and subsequently its isolation on solid mineral agar media incorporated with emulsified polyvinyl chloride. The work also involved preliminary characterization and study of degradation potential of selected isolated strain using different test methods.

MATERIALS AND METHODS

Collection of microbial source samples from different environmental sites

Samples from various sites were collected to be used as a microbial source for the enrichment of plastic degrading microorganisms. The sites, where plastic materials were found polluting the sites either openly or partially buried in the soil, were initially identified. Soil samples were collected from the garden area near the campus of University of Mumbai, India. In this area, it was observed that plastic was buried under the soil, and from dumping ground used to dump municipal solid waste, along with lots of plastic waste, near Kalyan city, India. Industrial effluent samples were collected from effluent drainage near Gharda Chemicals Ltd., Dombivili, India, and from sea creak, near Century Rayon Ltd., Shahad, India, where the drainage also showed large amounts of plastic bags clogging the flow of water. The sea sediments and sea water samples were collected from sites highly polluted with plastic waste from one of the beaches in Mumbai, India.

Soil burial technique for stimulation of PVC degrading microbial strains

In addition to the aforementioned sites, soil burial technique was used for obtaining samples of microbial population stimulated to PVC degradation. The method was as per the procedure used by Goheen and Wool (1991). The soil was obtained from the garden area near the University campus, Mumbai, India. It was then screened to remove large clumps, plant debris, and macro organisms, and then placed in plastic container. Soil was kept wet by frequent water spraying throughout the experiment. The container was stored in laboratory at room temperature. Commercially obtained plastic films were cut into long strips of approximately 15 × 3 cm and buried at a depth of four inches. After two months, strips were removed and washing of the strips was used as inoculums for enrichment culture.

Polymer sample

Polyvinyl chloride in a powdered form provided by Reliance Product Application and Research Centre, Mumbai, India, was used for degradation study. The molecular weight of PVC was between 31000 and 94000. Molecular weight of repeating unit of vinyl monomer was 62.5 which contains 56% by weight of chlorine

Enrichment and isolation of polyvinyl chloride (PVC) degrading microbial strains

Environmental samples collected from different sites rich in plastic waste as well as washings of the plastic strips buried in soil were used as inoculants for enrichment culture. Each solid sample of 1 to 2 g and liquid sample 1 ml was diluted to 10 and 9 ml using normal saline and used as inoculums for enrichment culture in the ratio of 1 to 100 ml of mineral salt vitamin media (MSV). The mineral salt vitamin medium (MSV) was the same as the one previously used by Pantke (1977) but with slight modification by supplementing biotin (20 mg) and vitamin B12 (10 mg). Mineral salt vitamin medium (MSV) prepared in 1000 ml distilled water contained; NH_4NO_3, 1 g; KH_2PO_4, 0.7 g; K_2HPO_4, 0.7 g; $MgSO_4.7H_2O$, 0.7 g; NaCl, 0.005 g; $FeSO_4.H_2O$, 0.002 g; $ZnSO_4.7H_2O$, 0.002 g; $MnSO_4.4H_2O$, 0.001 g; Biotin, 20 mg; Vitamin B12, 10 mg; and PVC in the form of powder, 1 g; pH-7. Flasks were then incubated in shaker incubator (Neolab, India) for at least 4 weeks. After 4 weeks, 1 ml of supernatant was transferred to the same fresh medium. Same procedure was repeated thrice. Concentration of PVC in the medium increased gradually at each repeated enrichment culture from 0.1 to 0.5%. The growth was monitored by visual assessment as increase in turbidity of the culture broth.

Isolation of PVC degrading microbial strains

A portion of enrichment culture was diluted adequately with sterile saline and spread on the nutrient agar plates. Incubation was carried out at 30°C for 48 h. Individual colonies formed on nutrient agar were picked and tested for their ability to grow on solid MSV medium containing emulsified PVC, where the medium was fortified with supplement and without any supplement such as 0.1% yeast extract and 0.1% glucose.

The solid MSV medium containing emulsified PVC was prepared by modifying the procedure previously used by Ishigaki et al. (2000). The agar plates were prepared by dissolving 0.1 g of polyvinyl chloride in 25 ml of tetrahydrofuran. The solution was then added to molten MSV agar medium at 50 to 60°C with gentle shaking and plates were poured immediately. The lids of the plate were kept partially opened for at least 30 min to allow complete evaporation of solvent. The lids of the plates were then replaced. Pure cultures of the PVC degrading bacteria were obtained by repeated sub-culturing of the isolated colonies on the same medium. The selected isolates were assigned with codes, for example, PVC 1, PVC 2 and so on, for further study. The selected isolates were characterized using Bergey's manual of determinative bacteriology (Krieg and Holt, 1984).

Optimization of media used for degradation study

For isolation and degradation studies, various compositions of mineral salt vitamin media (MSV) containing polyvinyl chloride

(PVC) as a primary source of carbon and energy were supplemented with additional carbon source as a co-substrate such as yeast extract and glucose at a final concentration of 0.1%.

Preparation of microbial cell suspension for various degradation studies

The suspension of microbial cells used in various degradation studies was grown in nutrient broth for 18 h at 30°C. The cells were harvested from culture by centrifugation at 4500 rpm for 15 min (Sorvall RC 5B Plus, Kendro, Newtown, USA). After discarding supernatant, the cell pellet was suspended in normal saline and centrifuge at 4500 rpm. The same procedure was repeated twice. The washed cell pellet was re-suspended in the medium used for degradation experiment.

Determination of polyvinyl chloride (PVC) degradation by monitoring chloride release

Polyvinyl chloride (PVC) degradation by isolated strains was determined by using the method based on chloride release as previously used by Yabannvar and Bartha (1993, 1994). The experiment was performed in 500 ml Erlenmeyer flask containing 250 ml of mineral salt vitamin medium (MSV) with 0.1% PVC and supplemented with 0.1% yeast extract (Hi media, India), 5% of washed microbial cell suspension, approximately 10^8 colony forming unit (CFU)/ml was used as inoculums for the test. The test flasks were incubated at 30°C on shaker incubator (Neolab, India) at 180 rpm along with the control flasks. Two controls were kept; one with MSV-PVC medium without test culture and the other with MSV medium without PVC inoculated with test culture. At each test intervals, 10 ml of sample was taken from each flask for analysis of chloride concentration. Samples were centrifuged at 4500 rpm for 15 min (Sorvall RC %B, Kendro, Newtown USA) for separation of cells. The resulting supernatants were filter sterilized through Sartorius filter and the filtrates were used for analysis of chloride concentrations. Two controls were kept, and were treated in the same manner. Absence of microbial contamination was checked by optical microscopy before any determination. After appropriate dilution of filtrate, chloride concentration was quantitatively determined by spectrophotometric analysis according to Bergmann and Sanik (1957). Three identical set of experiment were set and the result was given as an average of the three experimental sets.

Assessment of PVC mineralization by CO_2 evolution method

Polyvinyl chloride (PVC) biodegradation was determined as per the general guidelines of ISO 14855 (1999) and ASTM D 5338 (1998). The medium used for assay of CO_2 evolution was the same as that given in OECD (2001) guidelines for testing of chemicals. The apparatus - Biometer flask - used in the present study was as described by Reich and Bartha (1977) and Yabannavar and Bartha (1993, 1994). For measurement of CO_2, evolution the main compartment of the Biometer flask was amended with 100 ml of mineral medium with 0.1% of PVC in the form of powder along with 5% washed cell suspension of selected isolated strain. The carbon dioxide produced during metabolic activity was absorbed in a solution of barium hydroxide and subsequently determined by titration using 0.05 N HCl, where the amount of CO_2 produced was calculated from the amount of residual base remaining in the absorption tube. The mineralization was expressed as a percentage of the theoretical CO_2 ($ThCO_2$) produced, computed from the total carbon content of the samples. During the test period, flasks were incubated at room temperature in the dark. The stopcock was periodically opened for exchange of air. At each test interval (1, 7, 14, 21, 28, 35, 42, 49, 56, 63 and 70 days), $Ba(OH)_2$ from the side arm was removed for analysis of residual Ba(OH)2. The amount of un-reacted Ba(OH)2 in the sample was treated with 0.05 N HCl control, containing inoculated medium without any test compounds, was evaluated for CO2 evolution to determine endogenous metabolism of test culture. A control, containing an un-inoculated medium with test substance was also used for determining carbon dioxide evolved due to non-biological degradation. The amount of carbon dioxide evolved from control flask was subtracted from the corresponding experimental flask.The percentage biodegradation was calculated from the cumulative amount of carbon dioxide released during the entire test period. Three identical sets of experiment had been set and the result was given as an average of the three experimental sets.

Testing PVC film degradation with the selected isolated strain

Polyvinyl chloride (PVC) film was prepared in the laboratory by conventional solvent casting method as previously used by Nishida and Tokiwa (1992). PVC powder 1% (W/V) was dissolved in tetrahydrofuran. A film was cast from tetrahydrofuran by pouring a tetrahydofuran solution onto a clean glass plate. After evaporation of tetrahydrofuran, the film was left out from glass surface and kept overnight at room temperature to achieve equilibrium in crystallinity. The afore prepared PVC film was cut into strips and layered onto Whatman No. 1 filter paper covered with aluminum foil and autoclaved at 121°C for 15 min, and sterilized as per procedure given by Roberts and Davidson (1986).

The PVC film degradation study was carried out as was described by Cornell et al. (1984). Pour plate technique was used for inoculation of cell suspension into the medium. Cell suspension of culture (1 ml) was added to sterile Petri plate followed by addition of warm MSV medium maintained at 45°C in the plate. The plate was swirled and the added culture was homogeneously mixed. The PVC film was then aseptically placed on the surface of the inoculated hardened agar. The plates were sealed in polythene bag to avoid desiccation and incubated at 30°C. The plate was periodically removed and the film was observed for sign of microbial growth.

RESULTS AND DISCUSSION

Isolation of polyvinyl chloride degrading microbial strains

The samples collected from different natural environment were used as a microbial source for the enrichment of PVC degrading microorganisms. In addition to the natural environments, soil burial technique (Figure 1) in which commercially obtained PVC strips were buried in soil under laboratory condition was used for obtaining samples of microbial population, possibly stimulated to PVC degradation. Allsopp and Seal (1986) referred to this technique as a mini technique and suggested high possibility for isolating potential biodegrading agents with the use of this method.

In the present study, in order to overcome the problem of low biomass formation, that is, minute colonies on the mineral agar medium, the potential PVC degrading microbial strains from enriched samples were first cultivated on nutrient agar, which led to high cell densities, and large, as well as cultivable, colonies. From enrichment cultures

Figure 1. Soil burial technique; commercially obtained PVC films of size 3 × 15 buried in soil and incubated at room temperature under laboratory condition.

Table 1. Number of microbial strains isolated on emulsified PVC agar medium from different samples.

Microbial source	Number of microorganisms isolated on emulsified PVC MSV agar medium supplemented with			
	Total number of isolated strain	Number supplement	Yeast extract	Glucose
Garden soil	1	0	1	0
Dumping ground	1	0	0	1
Industrial effluent	0	0	0	0
Sea sediments	0	0	0	0
Soil burial technique	5	1	3	1

incorporated with PVC and incubated for 16 weeks at 30°C with different microbial sources, 20 morphologically different bacterial isolates were obtained on nutrient agar. Individual colonies were then tested for their ability to grow on emulsified polyvinyl chloride (PVC) in mineral salt vitamin agar media incorporated with and without any supplement. Among these 20 isolates, only seven strains were able to grow on emulsified PVC-MSV agar medium. Table 1 shows the number of organisms isolated on emulsified PVC-MSV agar medium with and without supplement.

As seen in Table 1, the number of PVC degrading microbial strains inhabiting in different environments is very low. However, PVC strips buried in soil harbored more strains as compared to samples collected from natural environment, possibly due to close contact of plastic material during incubation. Results also showed that co-substrates were necessary for PVC utilization by most of the strains. It was observed that only one strain showed growth on emulsified PVC agar medium without any supplements, whereas, the remaining isolates could grow either in the presence of yeast extract or glucose. The seven isolates thus obtained were designated as strain PVC 1, 2, 3, 4, 5, 6 and 7.

These isolates were further screened for their ability to release chloride in mineral salt medium incorporated with polyvinyl chloride powder. Table 2 shows the chloride releasing activity of isolates expressed in terms of µg/ml. The cell growth measured as optical density (OD_{600} nm) of culture broth was also determined. As seen in Table 2, the strain PVC 3, PVC 4, PVC 6 and PVC 7 showed the ability to release chloride from PVC. Strain PVC 4 showed considerable amount of chloride release and cell growth in MSV-PVC medium and therefore was selected for further identification and CO_2 evolution study. Figure 2 shows strain PVC 4 on emulsified PVC agar plates after incubation of 15 days at 30°C. The colonies were very minute, circular and white in color.

Table 2. Chloride releasing activity of microbial strains isolated from enrichment culture.

Isolated strains	MSV PVC medium supplemented with 0.1% yeast extract	
	Chloride release (µg/ml)	Optical density
PVC 1	0	0.12
PVC 2	0	0.72
PVC 3	46	0.98
PVC 4	115	0.92
PVC 5	0	0.22
PVC 6	65	0.66
PVC 7	31	0.49

Figure 2. Isolated colonies of strain PVC 4 on MSV PVC agar plate (plates incubated at 30°C for 15 days).

Identification of strain PVC 4

In this study, among the seven isolates, the bacterial strain PVC 4 showed the highest activity for polyvinyl chloride degradation which was measured as chloride was released. This strain was characterized as *Micrococcus luteus* on the basis of the 8th edition of Bergey's manual of Determinative Bacteriology, after studying morphological, cultural, and biochemical characteristics, and comparing it with standard strain of *M. luteus*. Cultural characteristics of strain PVC 4 were studied by following standard technique in microbiology. A pure culture of strain streaked on the nutrient agar from 1 to 2 mm. The circularly smooth, convex, mucoid, opaque and yellow pigment produced colonies on nutrient agar. Figure 3 shows isolated colonies of strain PVC -4 on nutrient agar. Table 3 shows morphological and cultural characteristics of strain PVC 4. The biochemical characteristics of the strain PVC 4 are summarized in Table 4. Strain PVC 4 was observed to be an aerobic organism and showed good growth between 28 and 37°C. The strain showed oxidative metabolism in Hugh and Leifson's media. It did not ferment lactose, galactose, mannose, arabinose, mannitol and maltose. Hydrolysis of starch and tween 80 was not observed. Hydrolysis of gelatin was observed. Urease test showed negative result. Strain was found sensitive to novobiocin and showed growth on 7.5% sodium chloride (NaCl).

Not much information was available in the literature about the ability of *M. luteus* to utilize polyvinyl chloride

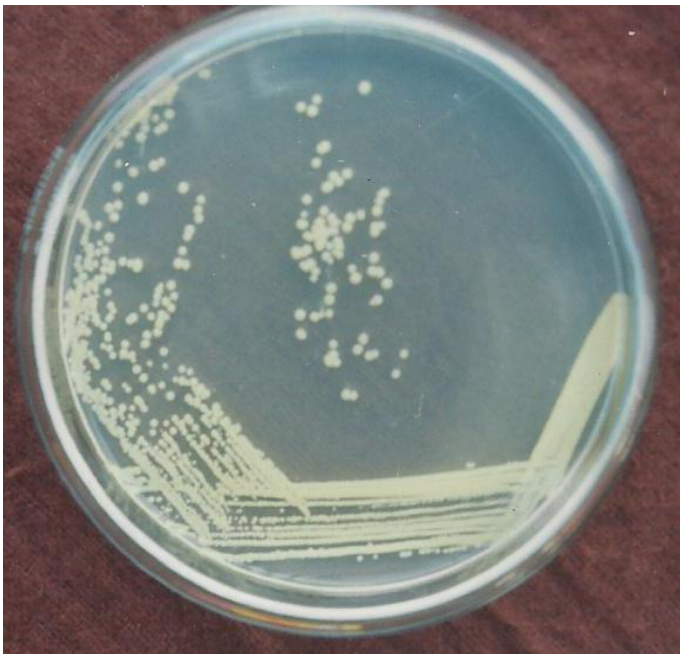

Figure 3. Isolated colonies of strain PVC 4 on nutrient agar plate (plates incubated at 30°C for 48 h).

Table 3. Morphological and cultural characteristic of strain PVC 4.

Characteristic		Strain PVC 4
Morphological characteristic	Gram staining	Gram positive
	Size	1 to 2 µm
	Form	Cocci
	Arrangement	Singles, in pairs and in tetrads
	Capsule	Absent
	Spores	Absent
	Motility	Non motile
Colony characteristic on nutrient agar (30°C/48 h)	Size	1 to 2 mm
	Shape	Circular
	Surface	Smooth
	Elevation	Convex
	Edge	Entire
	Consistency	Mucoid
	Opacity	Opaque
	color	Yellow
Growth in nutrient broth (30°C/48 h)	Surface growth	None
	Clouding	slight
	Cell Sediment	Abundant
	Type of sediment	Viscid on agitation

as a sole source of carbon and energy. However, the isolation of *Micrococcus roseus* as one of the strains which could degrade crude oil from hydrocarbon polluted streams in Lagos, Nigeria, was reported by Niranjan and Chandra (2011). Similarly, Sielicki et al. (1978) reported oxidative degradation of 1, 3,-diaphenyl butane, a compound structurally representing the smallest repeating unit of styrene by *Micrococcus* species. Ability of this

Table 4. Biochemical characteristic of strain PVC 4.

Biochemical test	Observation for strain PVC 4
Oxidation/fermentation	Oxidative
Acid from lactose	Negative
Acid from galactose	Negative
Acid from mannose	Negative
Acid from arabinose	Negative
Acid from mannitol	Negative
Acid from maltose	Negative
Starch hydrolysis	Negative
Tween-80 hydrolysis	Negative
Gelatin hydrolysis	Positive
Oxidase	Positive
Catalase	Positive
Urease	Negative
Sensitivity to novobiocine	Positive
Growth on nutrient agar with 7.5% NaCl	Positive

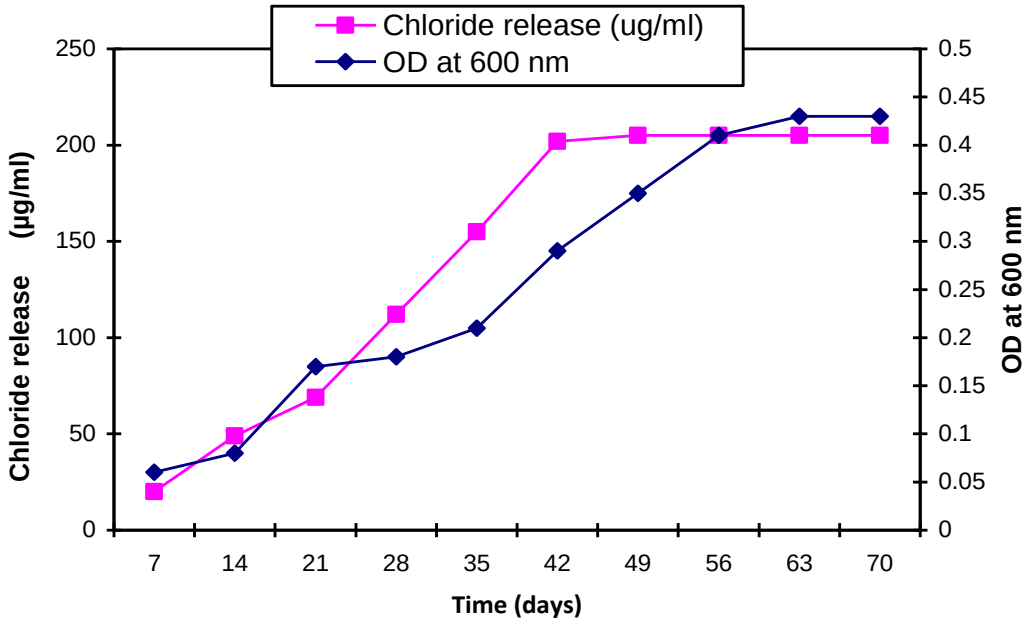

Figure 4. Cumulative release of chloride by *Micrococcus luteus* in test media inoculated with polyvinyl chloride (time course degradation study of PVC).

common soil bacterium to utilize polyvinyl chloride as a sole source of carbon and energy indicates that it might have placed a strong selection pressure on the evolution to develop an efficient catabolic enzyme for utilization of this polymer.

Time course of polyvinyl chloride degradation by strain PVC 4

The polyvinyl chloride (PVC) degradation potential of strain PVC 4 which has been characterized as *M. luteus* was evaluated by measuring increase in concentration of chloride ions release in mineral salt medium incorporated with PVC in the form of virgin PVC powder, as a measure of PVC biodegradation. The time course of PVC degradation by strain PVC 4 in MSV-PVC medium containing 0.1% PVC and 0.1% yeast extract is shown in Figure 4. The degradation of PVC resulted in release of inorganic chloride ion in the medium. Increase in chloride ion concentration during test period was quantitatively investigated spectrophotometrically. In addition to the

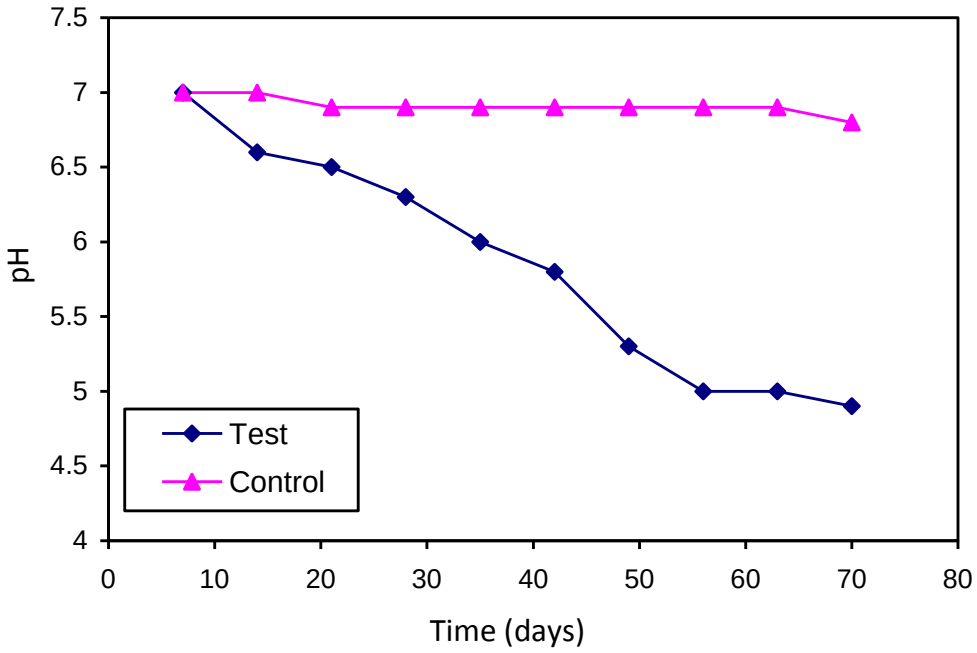

Figure 5. Change in pH of growth media inoculated with cumulative release of chloride by *Micrococcus luteus* in test media inoculated with PVC polymer.

release of chloride as a measure of PVC utilization, bacterial growth was also monitored at the same time intervals in an attempt to obtain biological measure for PVC degradation. Bacterial growth was quantitatively determined by measuring optical density of the culture media at 600 nm (OD 600 nm).

As seen in Figure 4, concentration of chloride released and cell growth were directly proportional to each other during test period. The graph of cell growth is divided into three phases; first, a lag phase of 14 days at which chloride concentration increased steadily at a value of 46 µg/ml. The lag phase was followed by an exponential phase, which ended on the 56th day during which chloride concentration increased consistently. At the end of the test period (70th day), a total of 205 µg/ml chloride released in the medium was detected. This release corresponds to 0.36% of total release of chloride from 100 mg of PVC. Figure 5 shows change in the pH of the test medium during PVC degradation. The drop in pH of culture medium may be due to formation of acidic component during biodegradation of polyvinyl chloride (PVC).

Method based on chloride release has been previously used by Yabannavar and Bartha (1993) to detect degradation of plasticized polyvinyl chloride film. Since halo substitution often has an effect on delaying or preventing biodegradation, release of chloride ion from PVC was a sure sign of biodegradation (Bartha, 1990). However, this group of scientists uses soil as a test medium, as well as a source of microorganism, and reported only marginal release of chloride which corresponds to less than 0.1% w/w of the added polyvinyl chloride (PVC). In the present investigation, degradation of polyvinyl chloride by *M. luteus* showed a release of 205 µg/ml in mineral salt vitamin medium which corresponds to 0.36% of PVC degradation, which is comparatively higher than that of earlier reported data. The good correlation between chloride release value and increase in cell growth further supports degradation of polyvinyl chloride.

Mineralization of polyvinyl chloride (PVC) by strain PVC 4

Mineralization of polyvinyl chloride (PVC) to its elemental constituent viz. CO_2 and H_2O was evaluated during 70 days of exposure with *Micrococcus* species. The complete conversion of 100 mg of PVC with 38.4 mg percent of total organic carbon (TOC) could have yielded 14.08 mg of carbon dioxide ($ThCO_2$). The net CO_2 evolution from PVC was found to be 1.25 mg in 70 days. During these 70 days of exposure, PVC underwent extensive biodegradation and up to 8.87% of their carbon was converted to carbon dioxide. Figure 6 shows cumulative CO_2 evolution during mineralization of PVC by *Micrococcus* species in the Biometer flask over a period of 70 day.In the present study, 8.87% of mineralization (ultimate biodegradation) of polyvinyl chloride was obtained during 70 days in mineral media inoculated with *M. luteus.* Yabannavar and Bartha (1993) reported 27.3% conversion of carbon from plasticized polyvinyl polymer during three months of exposure in soil. However, the

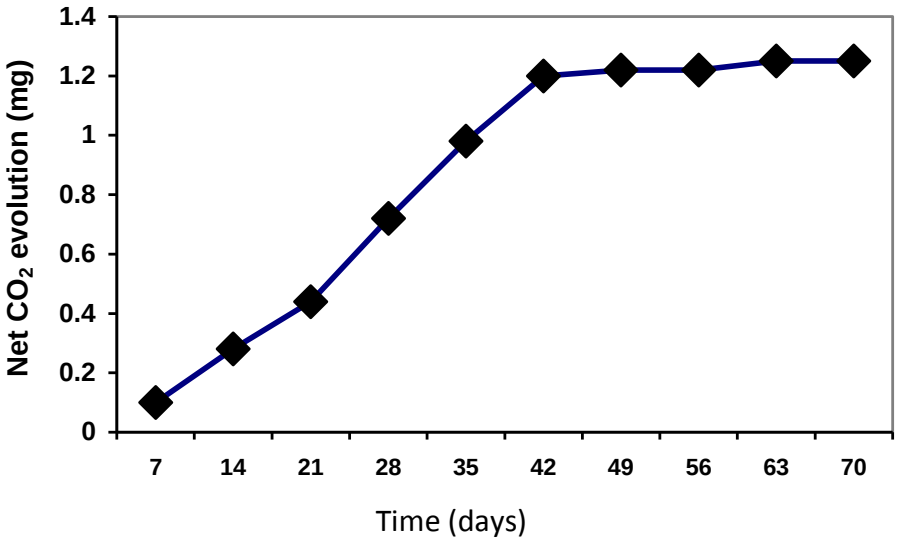

Figure 6. Cumulative CO_2 evolution during mineralization of PVC by *Micrococcus* species in the Barometer flask over a period of 70 days.

Figure 7. Growth of *Micrococcus luteus* on polyvinyl chloride film placed on mineral salt vitamin media.

polyvinyl polymer used by them was heavily plasticized with dioctyl adipate (DOA) and epoxidized soyabean oil (ESO). Later on, analysis using gas chromatography residual weight determination indicated that only the plasticizer and not the polyvinyl chloride resin were mineralized (Yabannavar and Bartha, 1993). Thus, the significance of the result obtained in the present investigation cannot be ruled out, as pure polyvinyl chloride resin was used for degradation study.

Growth of *Micrococcus* species on PVC film

Figure 7 shows growth of strain PVC 4 identified as *Micrococcus* species on polyvinyl chloride (PVC) film placed on mineral salt vitamin (MSV) agar inoculated with

Micrococcus species. The agar plate was incubated for a period of 8 weeks at 30°C. During the test period, microbial growth started appearing after one week of incubation from the edges of the film and covered the entire surface of the film within eight weeks of incubation. However, due to dehydration of culture medium, it was not possible to continue the test after that. The photographs of plates as shown in Figure 6 were taken after 8 weeks of incubation.

The results obtained showed that the isolated strain of *M. luteus* can survive on plastic film surface. Similar work was carried out by Roberts and Davidson (1986) where growth of *Aspergillus fischeri* and *Paecilomyces* species on plasticized polyvinyl film in liquid medium was reported. The growth of isolated strain on the surface of polyvinyl chloride (PVC) film gives positive intimation that the isolate can be used as potent biodegrading agent for in situ application during plastic waste remediation.

The study shows that the most recalcitrant plastic polymer polyvinyl chloride can be degraded to some extent in the appropriate environment. Isolation and screening of organisms which degrade polymers, or produce enzymes or enzyme systems that degrade polymers may prove as environmentally profitable for forthcoming research.

Conclusion

In conclusion, we successfully isolated polyvinyl chloride degrading microbial strain utilizing polyvinyl chloride using enrichment culture technique. The findings have valuable application in solving plastic waste problem through bioremediation where modern approach developed for remediation can be combined and applied with this organism. Future research will focus on various experiments to find the optimum conditions for PVC degradation process, for example, effect of varying pH of growth media, effect of different concentration of PVC on degradation, synergistic effect of different group of organism on PVC degradation, effect of different co substrate on rate of degradation, etc. Future work may also be focused on identification and isolation of enzymes involved in polyvinyl chloride degradation by the isolated strain and designing and development of small prototypic field study by applying selected isolated consortia of microbial strain for bioremediation of plastic waste.

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