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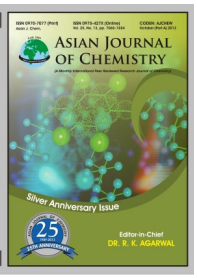

Biodegradation of Poly(vinyl alcohol) using *Pseudomonas alcaligenes*

B. BHARATHIRAJA[1,*], J. JAYAMUTHUNAGAI[2], M. JAYAKUMAR[3], M. ARUL KIRUBAKARAN[1], P. VIVEK[1], M. CHANDRAN[1] and R. PRAVEEN KUMAR[4]

[1]Department of Biotechnology, Vel Tech High Tech Dr. Rangarajan Dr. Sakunthala Engineering College, Avadi, Chennai-600 062, India
[2]Center for Biotechnology, Anna University, Chennai-600 025, India
[3]Department of Basic Engineering, Sri Ramana Maharishi College of Engineering, Thumbai Village, Cheyyar-604 407, India
[4]Department of Biotechnology, Arunai Engineering College, Tiruvannmalai-606 603, India

*Corresponding author: E-mail: btrbio@gmail.com

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Biodegradation of polymeric material has gained considerable attention in recent years, mainly because of the environmental pollution caused by the waste polymers. Several studies have been carried out on the biodegradation of polymers using polymer degrading micro-organisms. This work deals with the biodegradation of poly(vinyl alcohol) (PVA), a water soluble synthetic polymer prepared by the hydrolysis of polyvinyl acetate. Poly(vinyl alcohol) was subjected to biodegradation by the microorganism *Pseudomonas alcaligenes. P. aclaligenes* is a gram negative bacterium that can degrade polycyclic aromatic hydrocarbons. The degradation of PVA by *P. alcaligenes* was studied using batch method and flow method. The extent of degradation was studied by evaluating the effect of initial concentration of the PVA, effect of size of the inoculum, effect of pH and effect of flow rate. The studies revealed effective results of PVA degradation. In batch method the degradation rate was higher when the initial concentration of PVA was less and pH maintained under alkaline conditions. In flow method the degradation rate was greater at minimum flow rate was maintained in the upflow bioreactor. The degradation capabilities of *Pseudomonas alcaligenes* have been brought out using effective degradation techniques. The results of this research would help in minimizing the pollution caused by poly(vinyl alcohol) and poly(vinyl alcohol) based materials.

Key Words: Biodegradation, Poly(vinyl alcohol), *Pseudomonas alcaligenes*, Batch method, Upflow bioreactor.

INTRODUCTION

Polymers are large molecules made up of smaller units called monomers or repeating units, covalently bonded together[1]. Only one monomer is present in most of the polymers, however, two or three different monomers may be present in some polymers[2]. Poly(vinyl alcohol) (PVA) is a water soluble synthetic polymer prepared by the hydrolysis of poly(vinyl acetate). It is widely used in adhesive, paper-coating, textile industries and as biodegradable polymer products[3]. It is the only carbon-carbon backbone polymer that is biodegradable under both aerobic and anaerobic conditions[4]. The environmental fate of PVA has mainly been investigated due to its large usage in textile and paper industries that generate considerable amounts of wastewater contaminated by PVA[5]. The degradability depends on the molecular weight, molecular form and crystalline properties. It decreases with increase in molecular weight while monomers, dimers and repeating units degrade easily[6]. Various degradation methods have been used for the removal of PVA from industrial effluents. Degradation can occur by different molecular mechanisms *viz.*, chemical, thermal, photo and biodegradation[7]. Most polymers are too large to pass through cellular membranes, so they must first be de-polymerized to small monomers before they can be absorbed and biodegraded within microbial cells[8]. Physical forces, such as heating/cooling, freezing/ thawing, or wetting/drying can cause mechanical damage such as the cracking of polymeric materials[9]. Thermal degradation is one of the physical methods of degradation. The first degradation step mainly involves the elimination reactions, while the second one is dominated by chain scission and cyclization reactions[10]. The degradation process gives rise to the release of water from the polymer matrix, accompanied by the formation of volatile degradation products such as acetic acid in partially acetylated samples[11].

Chemical transformation is the other most important parameter in the abiotic degradation. Atmospheric pollutants and agrochemicals may interact with polymers changing the macromolecule properties. Among the chemicals provoking the degradation of materials, oxygen is the most powerful. The atmospheric form of oxygen (*i.e.*, O_2 or O_3) attacks covalent bonds producing free radicals. The oxidative degradation depends on the polymer structure. These oxidations can be

concomitant or synergic to light degradation for producing free radicals[12]. Soil microbes can initiate the de-polymerization of many natural polymers such as starch, cellulose and hemicelluloses. They secrete a variety of enzymes into the soil water and these enzymes then begin the breakdown of the polymers. The reactions can also decompose synthetic polymers, such as poly(vinyl alcohol), poly acrylic acid and poly acrylamide[9]. In view of the rising pollution problems caused to environment, lots of scientific studies on the removal of PVA have been carried out. The most commonly used is the biological method. An anaerobic baffled reactor (ABR) was used for the removal of PVA[13]. The speed of degradation depends on the specific environmental conditions[9]. It was suggested that the ultimate biological fate of PVA appeared to be largely dependent upon the kind of environment it reaches. Accordingly, high levels of biodegradation were observed in aqueous environments[14]. The tracking and removal of polymeric materials from the environment using natural, biological processes such as biodegradation mediated by microorganisms and their enzymes becomes fundamentally important. The need for studying the nature, biological activity of intermediate metabolites and final products deriving from the biodegradation processes is essential[11]. At present, the complexity of biodegradation is better understood and cannot be easily summarized. Poly(vinyl alcohol) is biodegradable under aerobic conditions and partially in anaerobic environment[15]. Biodegradation of poly(vinyl alcohol) was investigated using a bacterium *Pseudomonas* O-3. This bacterium was used for treating waste water which contains PVA[16]. Almost all the PVA-degrading strains belong to the genus *Pseudomonas* although some belong to other genera such as *Alcaligenes* and *Bacillus*[5]. In the PVA-degrading strain VM15C, PQQ-dependent PVA dehydrogenase (PVADH) dehydrogenates PVA and introduces β-diketone groups into the PVA molecules. Oxidized poly(vinyl alcohol) hydrolase (OPH) then hydrolyzes these β-diketone groups. PQQ dependent PVADH was first found in VM15C. Poly(vinyl alcohol) oxidase is also present in this strain, but the oxidase is not essential for PVA utilization because a PVA oxidase-lacking mutant grows well with PVA as sole source of carbon and energy[17].

In this paper, the biodegradation of poly(vinyl alcohol) (PVA) using *Pseudomonas alcaligenes* has been studied. *Pseudomonas alcaligenes* is gram negative rod shaped bacteria that do not ferment carbohydrates. It can degrade polycyclic aromatic hydrocarbons. *P. alcaligenes* has been placed in the *P. aeruginosa* group. Using this microorganism the extent of degradation of PVA has been analyzed by studying the effect of inoculum concentration, effect of polymer concentration, effect of pH and effect of flow rate. All the above parameters have been investigated using batch method and up-flow bioreactor.

EXPERIMENTAL

Poly(vinyl alcohol) (PVA) with a molecular weight of approximately 14,000 (viscosity 4 % aqueous 4-6 cP) was used. Silver sulphate and concentrated sulphuric acid were used in the preparation of acid for chemical oxygen demand (COD) analysis. Reagents such as potassium dichromate, ferrous ammonium sulphate, 1,10-phenanthroline monohydrate and sodium chloride were used in the COD analysis.

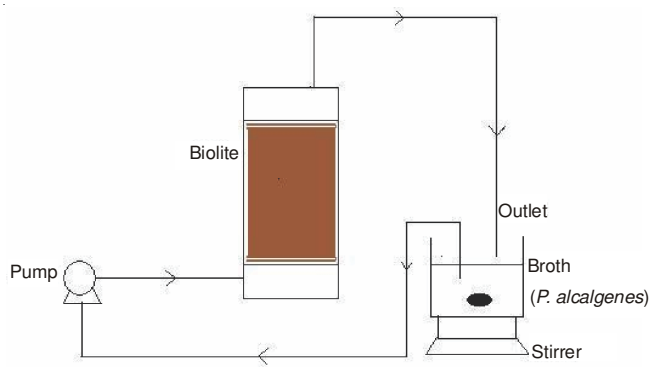
Nutrients: Nutrient medium such as nutrient agar and nutrient broth were used to grow the microorganism. The basic composition of 1 L of nutrient broth was found to contain 5 g of peptone, 3 g of yeast extract and 5 g of sodium chloride.

A COD digester was used to digest all the components in the sample at 147 ºC for 2 h. A magnetic stirrer was used for constant stirring of the sample. In flow method, a peristaltic pump was used for circulating the broth through the reactor. A pH meter to measure the pH of the sample was used to adjust acidic or alkaline conditions. And the scanning electron microscope was used to study the structural changes after the degradation of the sample.

Analytical methods: COD analysis and SEM were the two analytical methods used to study the rate and amount of degradation of poly(vinyl alcohol).

Synthetic effluent preparation: Based on the concentration required 0.5 g, 1 g or 1.5 g of PVA was dissolved in 100 mL of distilled water by heating at 100 ºC. This solution was used as the synthetic effluent.

Experimental setup: The experimental set up included batch set up with conical flasks, orbital shaker *etc.* and an up flow reactor with a peristaltic pump, magnetic stirrer, pellet *etc*. In batch method biodegradation of poly(vinyl alcohol) was tested by varying the parameters such as initial concentration of PVA, initial size of inoculum and pH. The effluent was prepared by completely dissolving PVA in distilled water and the sample was prepared by mixing the effluent with the inoculum containing *Pseudomonas alcaligenes*. This sample solution was taken in a conical flask and was kept in a rotary shaker for 5 days. The COD of the sample was measured for every 24 h time interval. In flow method an up flow bioreactor is to be used. It consists of an inlet at base of the reactor and an outlet at the top. It is packed with biolite which was properly sterilized before packaging. The reactor is connected to a peristaltic pump which helps to circulate the effluent through the reactor.



The pump helps to adjust the flow rate of the sample through the reactor. The effluent was mixed with the broth containing *Pseudomonas alcaligenes* and was allowed to circulate through the reactor. The effluent was mixed continuously with the help of a magnetic stirrer. The reactor was allowed to run continuously for 5 h and the COD was analyzed for every 1 h time interval.

Determination of COD: The sample with 2 mL of 0.25 N $K_2Cr_2O_7$ and 3 mL of H_2SO_4-$AgSO_4$ reagent was placed in a digester for at least 2 h at *ca.* 148 ºC. The sample was diluted *ca.* 150 mL and the unreacted $K_2Cr_2O_7$ was titrated with the N/10 ferrous ammonium sulphate solution using Ferroin as indicator. The colour change at the end point is from blue green to wine red. A blank experiment with distilled water instead of sample was performed.

Calculation for COD analysis:

$$\text{COD (mg/L)} = \frac{(V_1 - V_2)\times N\times 8\times 1000}{X}$$

Destruction (%): The extent of destruction of COD was calculated as follows: COD (%) removal = (COD initial – COD final/COD initial × 100).

Scanning electron microscope analysis: The scanning electron microscopy analysis was performed in order to visualize the morphological changes that have taken place due to the action of *Pseudomonas alcaligenes* on poly(vinyl alcohol). Nonconductive specimens tend to charge when scanned by the electron beam and especially in secondary electron imaging mode, this causes scanning faults and other image artefacts. Poly(vinyl alcohol) is one such nonconductive specimen. They are therefore usually coated with an ultrathin coating of electrically-conducting material, commonly gold, deposited on the sample either by low vacuum sputter coating or by high vacuum evaporation. Coating prevents the accumulation of static electric charge on the specimen during electron irradiation.

RESULTS AND DISCUSSION

Batch method: The initial concentration of PVA affected the rate of degradation to a great extent. Generally the degradation rate is higher when the PVA concentration is low[18]. Fig. 1(a) shows the percentage COD reduction at three different PVA concentrations of 0.5, 1.0 and 1.5 g. The inoculum was 10 mL of nutrient broth containing the microorganism *P. alcaligenes.* The resulting graph showed that the percentage decrease was greater with 0.5 g concentration of PVA. Whereas 1.0 and 1.5 g showed lower levels of degradation compared to 0.5 g of PVA. The COD percentage was monitored for a time interval of 24 h. When the PVA concentration was 0.5 g, the degradation rate was found to increase gradually. At 1 g concentration there was a sudden increase in the COD percentage, but it was not greater than the COD percentage of 0.5 g PVA. 1.5 g of PVA showed the least level of degradation.

Fig. 1(b) shows the percentage COD reduction at three different PVA concentrations of 0.5, 1.0 and 1.5 g, the inoculum size being 20 mL. Similar to upflow reactor, greater levels of degradation were observed at 0.5 g of PVA. The figure represents that large variation was found between 0.5 and 1.0 g of PVA. The initial COD percentage of 1.0 and 1.5 g of PVA was almost similar. After 44 h the amount of degradation was found to increase in the 1 g concentration of PVA. The percentage decrease in COD of 1.5 g PVA was very much lesser than that of 0.5 g PVA.

Effect of pH: Studies showed that the biodegradation capabilities of *Pseudomonas sp.* are highly affected by pH[19]. The initial pH of the sample was found to be neutral. The pH was adjusted to alkaline and acidic conditions using NaOH and HCl respectively. Samples at acidic, neutral and alkaline conditions were incubated in an orbital shaker for 5 days and their decrease percentage of COD was measured. The recorded values are represented in Fig. 2. It was found that the degradation rate was greater under alkaline condition. Maximum percentage of COD decrease was observed after 68 h after which there is no the COD percentage decreases. Neutral pH also showed considerable amount of degradation. At neutral pH the rate of degradation was almost stable after 20 h. Acidic pH showed lower amounts of degradation when compared to alkaline and neutral conditions. Under acidic conditions the degradation rate was initially stable and suddenly increased after 68 h.

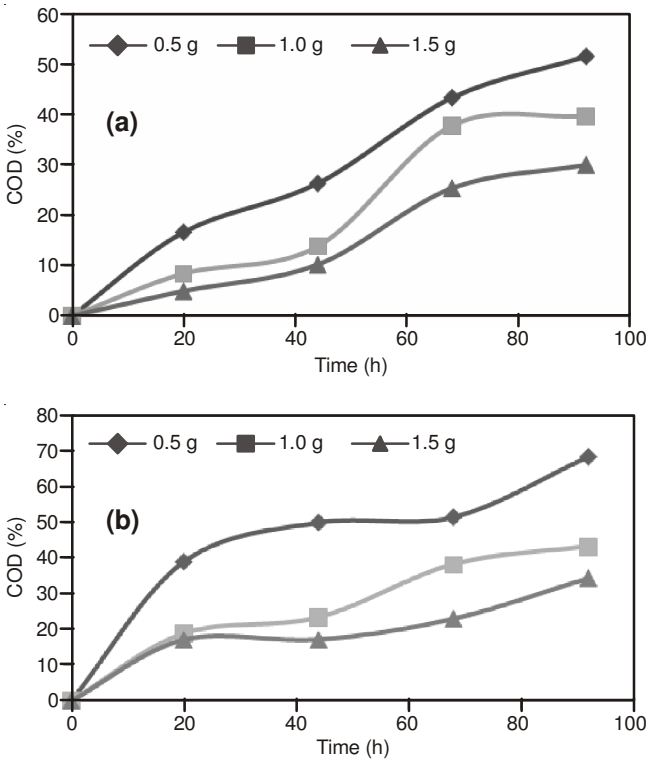


Fig. 1. (a) Effect of initial inoculum conc. of PVA, (b) effect of initial initial conc.

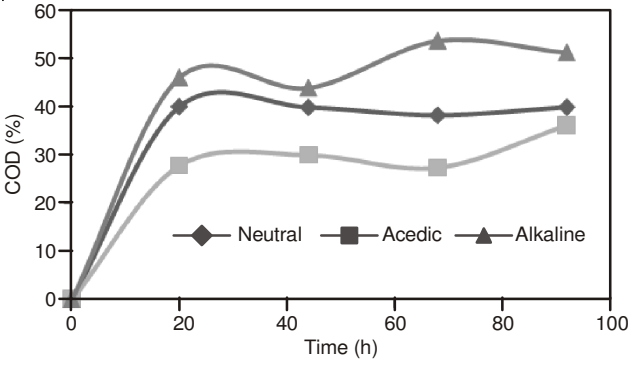


Fig. 2. Effect of pH

Effect of size of inoculums: Fig. 3 shows the effect of inoculum size on the biodegradation rate of 0.5 g PVA. The inoculum was nutrient broth containing the microorganism *Pseudomonas alcaligenes.* The sample containing 20 mL of inoculum showed higher percentage of COD decrease than

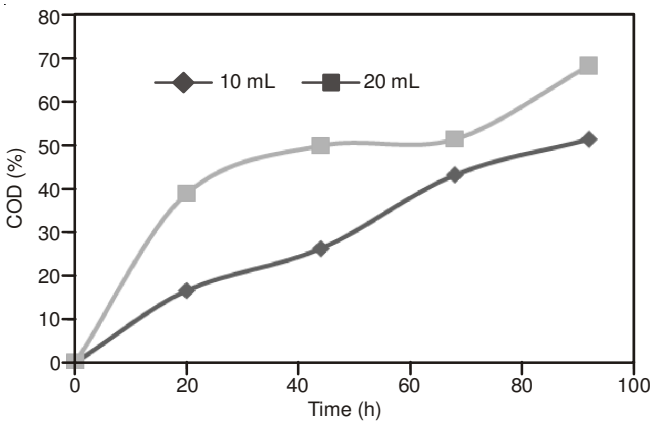


Fig. 3. Effect of size of inoculum

that of the sample containing 10 mL inoculum. The sample with 20 mL inoculum produced around 10 % higher rate of degradation. As time proceeds the rate of degradation was found to increase with a greater speed. After 68 h a sudden increase in the rate of degradation was observed. The sample with 10 mL inoculum showed a gradual rate of degradation.

Flow method

Effect of initial concentration of PVA: The effect of initial concentration of PVA was also studied by using an up-flow bioreactor. In this method the bioreactor was packed with biolite and the broth containing the 25 % of effluent (dissolved PVA) of varying concentrations was allowed to circulate through it. Samples were taken at every 1 h time interval for nearly 5 h and the COD was measured. The obtained results showed very little variation in their rate of degradation. Based on the results from the batch method degradation rate was expected to be greater when the PVA concentration was less. Similarly the Fig. 4 shows that the COD percentage was at its maximum when 0.5 g of PVA was circulated through the reactor. But the other two concentrations of 1.0 and 1.5 g did not show much variation and their final degradation rates were almost close to each other. The sample containing 1.5 g PVA showed a sudden increase in the COD percentage during the 4 h, slightly greater than the COD percentage of 1 g PVA at the same hour. But the final COD percentage of 1 g PVA was greater than that of 1.5 g of PVA.

Effect of flow rate on 0.5 g of PVA: The degradation rate was found to vary along with the changes in flow rate.

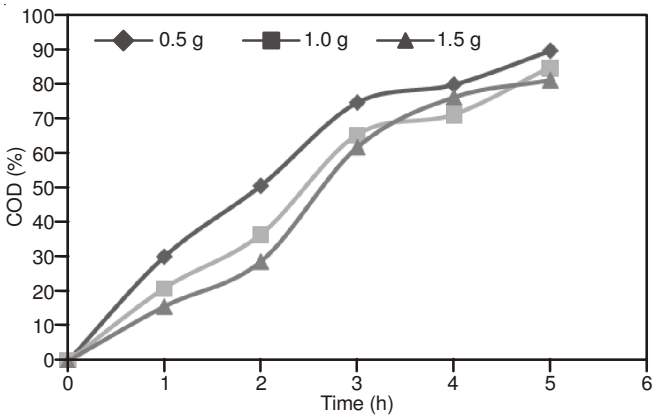


Fig. 4. Effect of initial conc. of PVA

The removal efficiency was said to be dependent on the exposure time of the effluent to the microorganism[20]. Therefore the rate of degradation of 0.5 g PVA was studied by varying the flow rate of the bioreactor. Fig. 5 shows that the COD percentage was higher when the flow rate was less. When the flow rate was 150 mL/min the COD percentage was at its peak. A maximum of 90 % degradation was achieved. As the flow rate was increased to 200 and 250 mL/min the COD % decreased considerably. Both the flow rates showed similar rates of degradation. It was found that higher flow rates were not suitable for biodegradation.

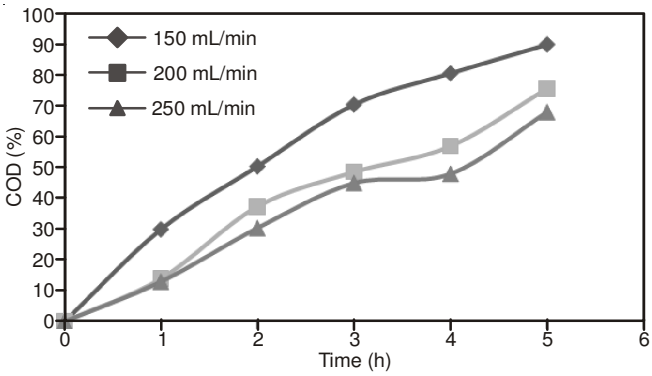


Fig. 5. Effect of flow rate

SEM analysis: The scanning elector microscopy was used to visualize the morphological changes after PVA has been subjected to degradation. In this method raw PVA which is commercially available in the crystalline form was added to the nutrient broth containing *Pseudomonas alcaligenes*. 1 g of pure PVA was added to 100 mL of broth and was incubated in an orbital shaker for 7 days. After 7 days the insoluble PVA crystals were separated from the broth and dried using a hot air oven. The dried samples were subjected to analysis under a scanning electron microscope. Fig. 6 shows the SEM image of raw PVA while Fig. 7 shows the image of degraded PVA. From both the figures it is evident that the microorganism *Pseudomonas alcaligenes* has degraded PVA over a period of time.

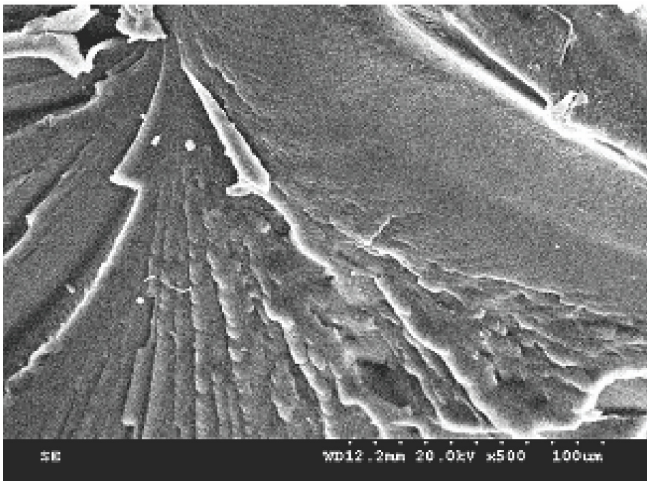


Fig. 6. SEM image of raw PVA

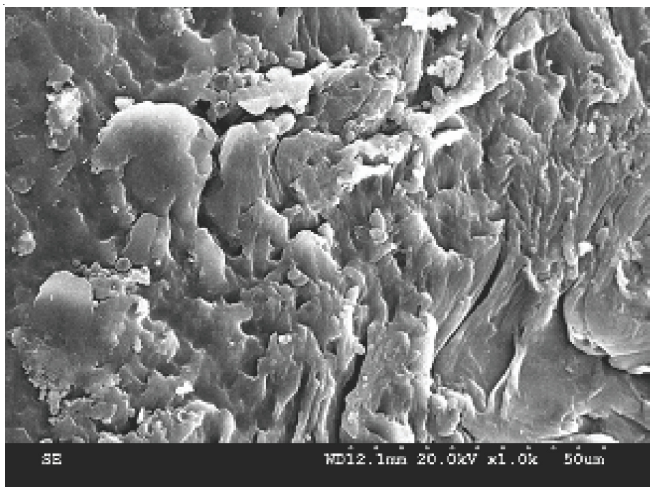


Fig. 7. SEM image of the degraded PVA

Conclusion

The biodegradation of poly(vinyl alcohol) (PVA) was studied using the microorganism *Pseudomonas alcaligenes*. The rate of biodegradation was monitored by batch and flow method. Both the methods have contributed to their own results. The results obtained lead to the following conclusions. Higher rate of degradation was achieved when the PVA concentration was less. The study showed that 0.5 g of PVA was better degraded than 1.0 and 1.5 g of PVA. The pH of the sample considerably influenced the rate and amount of degradation. Alkaline pH was found to support the degradation of PVA, whereas neutral and acidic pH showed lower rates of degradation. Depending on the size of the inoculum the degradation rates were found to vary. Greater degradation was achieved when the inoculum size was large. Considering the flow method, more biodegradation was achieved when minimum flow rate was maintained. At minimum flow rates the polymer is exposed to the action of microorganism for longer duration. Therefore the degradation rate was more. The above conclusions indicate that *Pseudomonas alcaligenes* has the ability to degrade PVA thereby helping in reducing the pollution caused by PVA based materials. The batch and the flow methods contribute to the effective degradation of PVA. Therefore it is essential for us to utilize these techniques in order to reduce pollution and preserve our planet.

REFERENCES

1. M. Kolybaba, L.G. Tabil, S. Panigrahi, W.J. Crerar, T. Powell and B. Wang, Biodegradable Polymers: Past, Present and Future - Paper Number: RRV03-0007, An ASAE Meeting Presentation (2003).
2. V. Koutsos, Introduction to Polymers-The University of Edinburgh, Division of Engineering, Session 2001-2002, Materials Science and Engineering, 1-12.
3. J. Chen, Y. Zhang, G. Du, Z. Hua and Y. Zhu, *Enzym. Microbial Technol.*, **40**, 1686 (2007).
4. V. Francis, S.R. Subin, S.G. Bhat and E.T. Thachil, Microbial Degradation studies on Linear low density poly(ethylene)-poly(vinyl alcohol) blend using Vibrio sp.- International Conference on Advances in Polymer Technology, Feb. 26-27 (2010).
5. E. Chiellini, A. Corti, G. Del Sarto and S. D'Antone, *Oxo-Biodegrad. Polym.*, **91**, 3397 (2006).
6. R. Premraj and M. Doble, *Indian J. Biotechnol*., **4**, 186 (2005).
7. Y. Orhan, J. Hrenovic and H. Büyükgüngör, *Acta Chim. Slov*., **51**, 579 (2004).
8. S. Nanda and S.S. Sahu, *New York Sci. J.*, **3**, 95 (2010).
9. D. Corning, Degradation of Polymers in Nature, Environmental Information Update, Ref. No 01-1112-01 (1998).
10. Z. Peng and L.X. Kong, *Polym. Degrad. Stab*., **92**, 1061 (2007).
11. E. Chiellini, A. Corti, S. D'Antone and R. Solaro, *Prog. Polym. Sci*., **28**, 963 (2003).
12. N. Lucas, C. Bienaime, C. Belloy, M. Queneudec, F. Silvestre and J.E. Nava-Saucedo, *Chemosphere*, **73**, 429 (2008).
13. X. Xu-Tang, X. Shuang-Shuang and L. Zhi-Hua, *J. Cent. South Univ. Technol*., **18**, 96 (2011).
14. H.Z. Zhang, *J. Polym. Environ*., **17**, 286 (2009).
15. L. Husarova, J. Ruzicka, H. Marusincova and M. Koutny, Use of temperature gradient gel electrophoresis for the investigation of poly(vinyl alcohol) biodegradation. Development, Energy, Environment, Economics, pp. 157-159 ISBN: 978-960-474-253-0.
16. R. Fukae, T. Fujii, M. Takeo, T. Yamamoto, T. Sato, Y. Maeda and O. Sangen, *Polym. J*., **26**, 1381 (1994).
17. M. Shimao, *Curr. Opin. Biotechnol.*, **12**, 242 (2001).
18. S.P. Vijayalakshmi and G. Madras, *J. Appl. Polym. Sci*., **100**, 4888 (2006).
19. M.H. El-Naas, *J. Hazard. Mater*., **164**, 720 (2009).
20. R.R. Liu, *Int. J. Environ. Sci. Technol*., **7**, 111 (2010).