



Enzymatic Degradation of Nanocomposites Carboxymethyl Cellulose and Poly (ϵ -Caprolactone) and Containing Sodium Montmorillonite Clay by Cellulase

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ABSTRACT

The aims of the study were to investigate the effect of poly(ϵ -caprolactone) (PCL) and Sodium Montmorillonite (MMT-Na) within the thermoplastic Carboxymethyl cellulose (CMC) blends on the rate and extent of CMC enzymatic hydrolysis using enzymes cellulase. The results of this study have revealed that blends with a MMT-Na content at 6 wt% exhibited a significantly reduced rate and extent of CMC hydrolysis. The results suggest that this may have been attributed to interactions between CMC and MMT-Na that further prevented enzymatic attack on the remaining CMC phases within the blend. The total solids that remained after 3000 min were 44.8 wt.% (CMC: PCL); 56.5 wt.% (CMC: PCL: 2% MMT-Na); 58.2 wt.% (CMC: PCL: 4% MMT-Na); 61.4 wt.% (CMC: PCL: 6% MMT-Na). Enzymatic degradation behaviour of CMC: PCL: MMT-Na was based on the determinations of Water resistance, Weight loss and the Reducing sugars.

KEYWORDS: Nanocomposites; Polymer composites; Biodegradable polymers; Water resistance; Reducing sugars

INTRODUCTION

Biodegradable polymers have been extensively investigated since the 1970s in order to protect the environment from non-biodegradable plastic wastes [1, 2]. The use of synthetic degradable polymers as biomaterials implies they are biocompatible by themselves and the use of particular additives and/or processing technologies should not interfere with the biocompatible behaviour [3]. Among biodegradable polymers, poly (ϵ -caprolactone) (PCL), a synthetic aliphatic polyester, has been widely used in medical, packaging and agricultural applications because of its excellent mechanical properties, including its flexibility. The major disadvantage of PCL is its price, which limits its wider use as a substitute for conventional polymers. Polymeric blends, i.e., mixtures of two or more polymers that may or may not be biodegradable, are commonly used in the plastic industry [4]. In particular, blends of PCL and natural materials, such as starch and cellulose derivatives [5] have been extensively studied because of their lower cost compared to other materials [6]. Cellulose, the most abundantly available carbohydrate polymer in nature, is continually replenished by the photosynthetic reduction of CO₂ catalyzed by sunlight—a mechanism that is highly beneficial in mitigating environmental problems resulting from increased atmospheric CO₂. The annual net yield of photosynthesis is 1.8 trillion tones of biodegradable substances, about 40% of which is estimated to be cellulose [7]. However, only a small fraction of available cellulosic resources is currently utilized, in products such as lumber, fuel, textiles, paper, and plastics. As is well known, cellulose is a polymer composed. Cellulose is the most abundant renewable organic material and can be converted into cellulose derivatives and regenerated materials [9]. However, its poor solubility in common solvents restricts its application. Recently, a nontoxic, inexpensive, and recyclable cellulose solvents based on cold aqueous alkali-urea/thiourea solution was developed [10]. These solvent system gives highly transparent cellulose hydrogels in desired forms by manipulating the coagulation procedure [11]. Enzymatic hydrolysis of cellulose has been extensively studied in the past decades since the utilization of cellulosic biomass as a renewable resource has great potential for reducing emissions of carbon dioxide and thereby prevents global warming [12, 13]. Cellulase, a multicomponent enzyme consisting of three different enzymes (endocellulases, cellobiohydrolase, and β -glucosidases) is responsible for the bioconversion of cellulose into soluble sugar [14]. Taghizadeh and Abbasi [15] reported that TPS:PVA blends was degraded by amylase. Similarly, Taghizadeh et al. [16] and Abbasi [17] also studied the effect of enzyme on nanocomposites. Braganca [18] reported that hydrolysis was the principal mechanism for the biodegradation of Cellulose acetate (CA), with the first step as depolymerization and being in contact with extracellular microbial enzymes; the resulting oligomers were then easily phagocytosed by the cells, followed by mineralization. Abbasi et al. [19] described a system to study the biodegradation of Cellulose and MMT-Na by using a Cellulase.

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The current paper studies the cellulase action on CMC: PCL composite film containing MMT-Na at temperature 50 °C. The modifications induced by the enzymatic treatment were evidenced by determination of weight loss, water absorption capacity, sugars released during biodegradation, as well as by UV spectroscopy and Total sugars were estimated by dinitrosalicylic acid (DNS) method.

EXPERIMENTAL

Materials

Carboxy methyl cellulose sodium salt, with an average molecular weight of M_n 295225 =was purchased from Fluka company and PCL (type P-767) was supplied in pellet form by Dow Qui´mica S.A. (Cubata˜o, SP, Brazil). The melt flow at 80 °C was 1.970.3 g/10 min (ASTM D-1238), with a density of 1,145 kg/m³ and a average molecular weight (Mw) of 50,000. The water used was distilled and deionized water. Cellulase from *Aspergillus niger* (specific activity; 111U mg⁻¹) and hydroxide soduim provided by Floka company. Sodium montmorillonite (Cloisite Na⁺ with a cation exchange capacity (CEC) of 92.6 mequiv. /100 g clay was supplied by Nanocor Inc. (Arlington Heights, IL) and Reagent DNS was used for determination sugars released during degradation.

Film preparation

The nanocomposite of PCL with CMC containing sodium montmorillonite clay were prepared by casting . The nanocomposite have been prepared from 50 wt% PCL–50 wt% CMC containing small amounts of plasticizers, stabilizers and destructuring agents (stabilizers or destructuring agents such as sodium montmorillonite clay and plasticizer such as glycerol).The solutions were prepared by dissolving the material in 10% (w/v) acetone, with stirring at 60± 5 °C for 5 h. The mixtures were then poured into culture dishes and the solvent was allowed to evaporate in an atmosphere saturated with acetone.

Enzymatic degradation test

Each sample was placed in a vial filled with 20 ml of 0.1 M acetat buffer, pH 6.9, containing 1.0 mg of cellulase, and then incubated in a thermostatted oven at 50 °C. The buffer/enzyme system was changed for every 24 h during the evaluation period in order to maintain the original level of enzymatic activity. For every 48 h, the samples were removed from the incubation medium, washed with distilled water, wiped dry, weighed, and examined by light microscopy before being returned to the incubation medium. The controls consisted of samples incubated in buffer without enzyme. The dried samples were cut into 5 cm× 5 cm square specimens, weighed, and immersed in the conical flasks. The flasks were placed in a shaking incubator (*Fanavaran Sahand Azar Co. ISH 554D, Iran*) with a rate of 150 rpm for 50 h at 50 °C. After 1, 2, 3, 5, 7, 9, 12, 18, 24, 29, 36, 40, 45 and 50 h, the samples were removed and rinsed with distilled water to remove the enzymes, dried and weighed, respectively. The degree of enzymatic degradation (DED) was calculated as:

$$DED \% = (W_0 - W_1) / W_0 \times 100$$

where W_0 represents the initial weight of a specimen and W_1 is the weight of a specimen after degradation.

Water absorption test

Pieces of the films were placed in a freeze dryer (*Pishtaz Engineering Co, FD-4, Iran*) and dried for least 24 h. then samples were weighed for the dry weight, and then placed in a bath in distilled water at room temperature. After 1, 2, 3, 5, 7, 9, 12, 18, 24, 29, 36, 40, 45, 50 h, the samples were removed from distilled water and weighed. The water absorption capability (WAC) was calculated with the equation below:

$$WAC \% = (W_{wet} - W_{dry}) / W_{dry} \times 100$$

Where W_{wet} represents the weight of the wet specimen and W_{dry} represents the weight of the dry specimen.

Detection of reducing sugars

The reducing sugars in the degradation solutions were quantified by the dinitrosalicylic acid method: 1 ml of reagent DNS was added to 1 ml of the sample to be analysed. using 1 mg/ ml glucose stock solution as a standard. At the same time, the blank was prepared using 1 ml of control sample. The mixture was heated at 90-100 °C for 10 min. After cooling to room temperature, 5ml of distilled water was added, and the absorbance at 540 nm was measured. The respective carbohydrate concentration was obtained by comparison with a standard curve.

Scanning electronic microscopy (SEM)

The morphology of the surface of the films, before and after biodegradation, was investigated using a scanning electronic microscope of XL30 type (*Netherland*). The films were covered with pure metallic Ag. The laying down of Ag was carried out using evaporation of the metal under a high vacuum, to give a thickness of around 100 Å .

RESULTS AND DISCUSSION

Degradability of polymers is a critical functionality for their application. Currently, no official standard method was established in determining biodegradability of polymers. The enzyme method [20] the microbiological method [21] and the soil burial method [22] have been used by different researchers. Moreover, the biodegradability was also recorded by diverse indexes even in the same method. The current paper studies the cellulase action on CMC: PCL composite film containing MMT-Na at temperature 50 °C. Bajpai and Shrivastava [23] who studied the biodegradation of carboxymethyl-cellulose/ starch blends, found that, at small amounts of starch in the blend, a high percent of weight loss occurred while, at high starch contents, the weight loss was lower. This variation was explained in the first case, by the increase of the number of starch molecules contacting the α -amylase, so that the amount of degraded starch was higher. At high starch contents, the material becomes much more compact, which hinders the α -amylase diffusion in the polymer film.

Weight loss and Water Uptake:

The water absorption capacity and the degradability are the most important properties for biodegradable materials. The water absorption capacities of the CMC: PCL: MMT-Na blend film were found to have significant difference. The increase of nanoparticle leads to the decrease of both weight loss and Water uptake Figure 1 and Figure 2 clearly show that degradation is much more pronounced when the WAC % is high. A comparison between the variation of the DED % and WAC % with respect to MMT-Na clearly show that degradation is much more pronounced when the water sorption is high. The total solids that remained after 3000 min were 44.8 wt.% (CMC: PCL); 56.5 wt.% (CMC: PCL: 2% MMT-Na); 58.2 wt.% (CMC: PCL: 4% MMT-Na); 61.4 wt.% (CMC: PCL: 6% MMT-Na). CMC: PCL exhibited both a high water sorption and the most significant weight loss.

Rate and extent of glucose production:

The rate and extent hydrolysis by the action of cellulase was measured using the DNS method glucose assay of four blends of varying MMT-Na. The production of glucose was used as a measure of CMC hydrolysis. Figure 3. Shows the extent of glucose over a 50 h hydrolysis time for each substrate. Figure 4. illustratess the initial rate of glucose production by each substrate up to a hydrolysis time 5 h. The rate of glucose production was calculated; refer to Table 1. by assuming a linear relationship between the concentration of glucose and time for the first 5 h of hydrolysis. The rates of glucose production from each composite substrates, were most rapid for the substrate without MMT-Na and decreased with the addition of MMT-Na, for CMC: PCL blend (492 $\mu\text{g}/\text{ml}\cdot\text{h}$), 398 $\mu\text{g}/\text{ml}\cdot\text{h}$ (CMC: PCL: 2% MMT-Na), 321 $\mu\text{g}/\text{ml}\cdot\text{h}$ (CMC: PCL: 4% MMT-Na), 263 $\mu\text{g}/\text{ml}\cdot\text{h}$ (CMC: PCL: 6% MMT-Na). The rate of CMC hydrolysis was most rapid for the substrate CMC:PCL and decreased with the addition of MMT-Na. The amount of reducing sugars in the degradation solutions, reduced by dinitrosalicylic acid, increased since the beginning until the end of the assay the relative amount of reducing sugars in the degradation solutions in similar assays without enzymes was about 130 times lower. One of the routes of biodegradation is by hydrolysis, and the enzymatic hydrolysis of CMC is accompanied by the release of glucose. Figure 4 shows the release of glucose ($\mu\text{g}/\text{ml}$) during exposure to cellulase. The amount of free glucose increased with time for the blends showed a peak release of glucose at 5 h, followed by a decline. Apparently, the MMT-Na has a stabilizing effect against the enzymatic attack, even after increasing the content of insoluble fraction.

Scanning electronic microscopy (SEM)

Several scanning electronic microscopy images of Nano composites are given in Figure 5. One may observe that the films are considerably destroyed, although during degradation a much more stable fibrillar fraction is revealed.

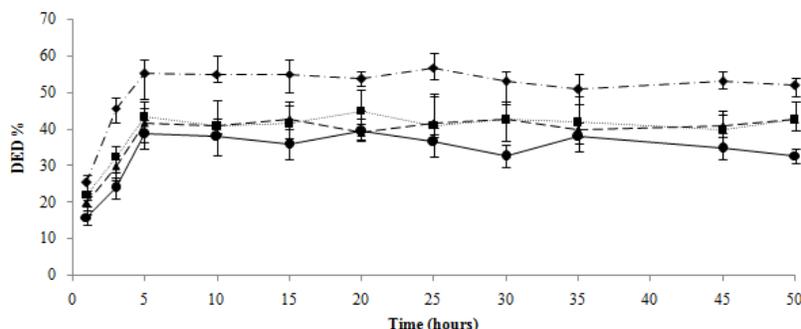


Fig.1. Enzymatic degradability of the CMC: PCL (♦),CMC: PCL: 2% MMT-Na (■),CMC: PCL: 4% MMT-Na (▲),CMC: PCL: 6% MMT-Na(●).

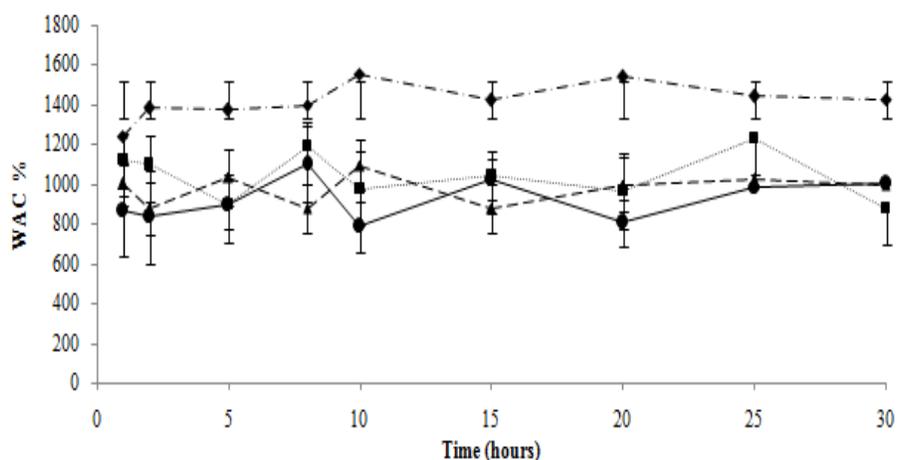


Fig.2. Water Absorption Capability (WAC) of the CMC: PCL (◆),CMC: PCL: 2% MMT-Na (■),CMC: PCL: 4% MMT-Na (▲),CMC: PCL: 6% MMT-Na (●).

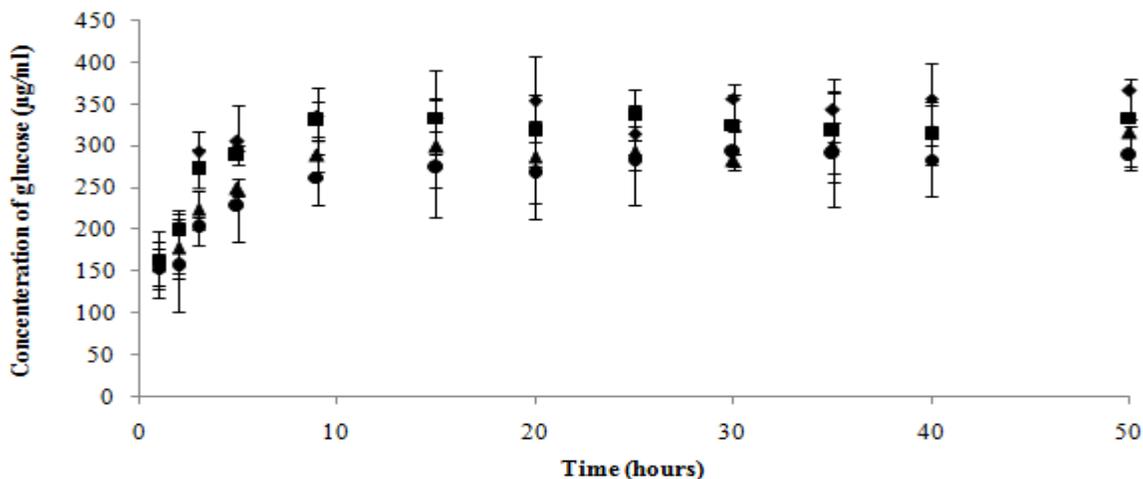


Fig.3. Concentration of glucose produced for nanocomposite films in the 50 h of enzymatic degradation due to the action of cellulase. CMC: PCL (◆),CMC: PCL: 2% MMT-Na (■),CMC: PCL: 4% MMT-Na (▲),CMC: PCL: 6% MMT-Na(●).

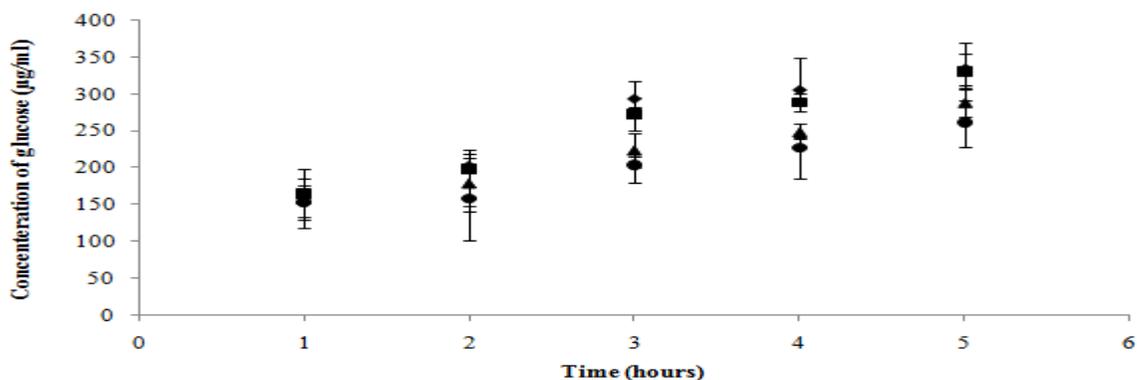


Fig.4. Concentration of glucose produced for nanocomposite films in the first 5 h of enzymatic degradation due to the action of cellulase. CMC: PCL (◆),CMC: PCL: 2% MMT-Na (■),CMC: PCL: 4% MMT-Na (▲),CMC: PCL: 6% MMT-Na(●).

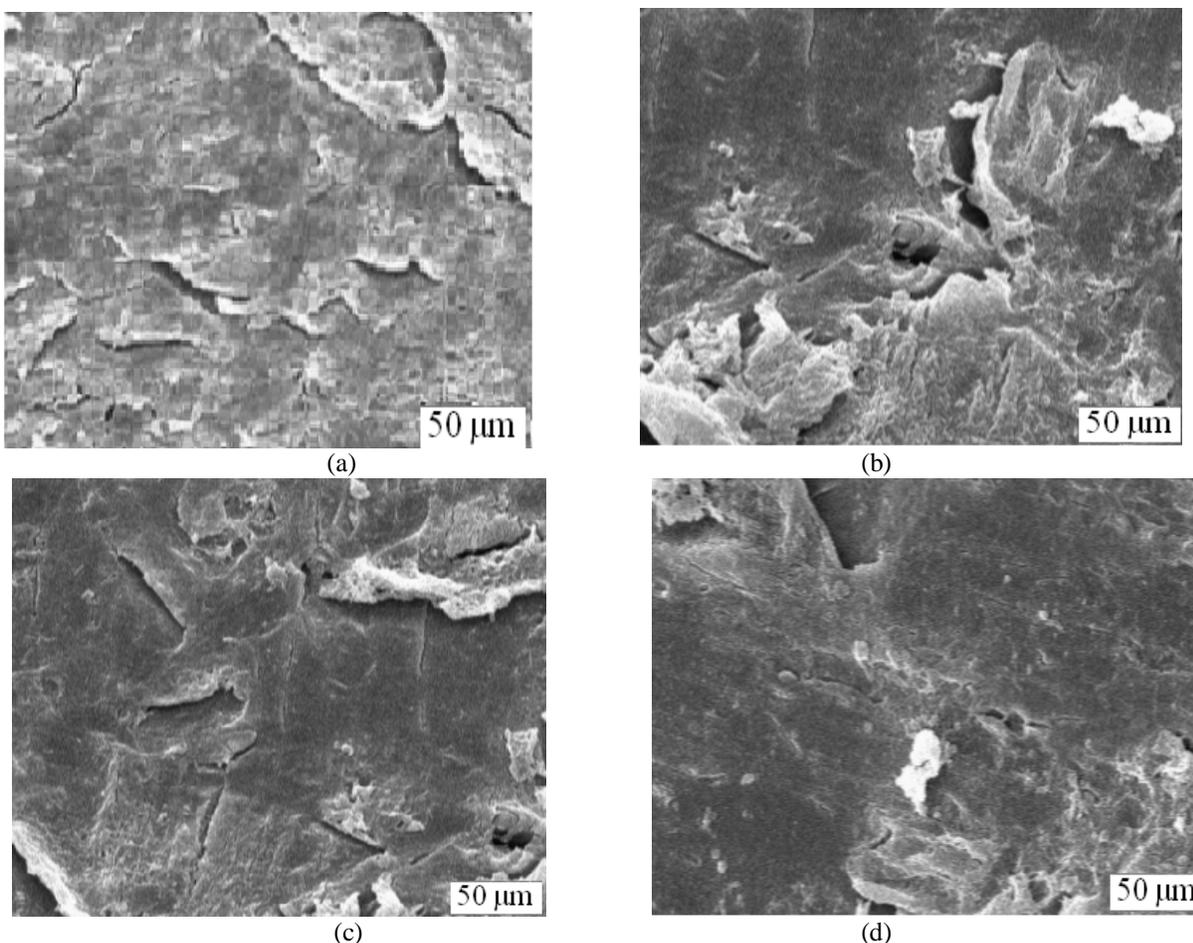


Fig.5. Scanning electron micrographs of degradable films in 50 h of enzymatic degradation due to the action of cellulase: (a) CMC: PCL degraded; (b) CMC: PCL with 2 wt% MMT-Na degraded; (c) CMC: PCL with 4 wt% MMT-Na degraded; (d) CMC: PCL with 6 wt% MMT-Na degraded.

Table 1: A summary of the rates of glucose production due to the action 1.0 mg of cellulase from each substrates

<i>substrate</i>	<i>Rate (µg/ ml. h)</i>	<i>R²</i>
CMC: PCL	492	0.989
CMC: PCL: 2% MMT-Na	398	0.997
CMC: PCL: 4% MMT-Na	321	0.998
CMC: PCL: 6% MMT-Na	263	0.997

CONCLUSIONS

The present study shows the role of cellulase nanocomposites degradation. The MMT-Na content significantly impacted on the rate of starch solubilisation. The decrease of the degradation rate observed in the final stage can be explained to the lower degradability of the MMT- PCL domains that remain in the material. After 5-50 hour, the variation is almost negligible, nearly zero, as no saccharides and other compounds leached to the solution, as demonstrated before. The reduction of the degradation rate is also influenced by the water uptake ability of these polymers.

REFERENCES

- [1] Doi Y, Fukuda K. Biodegradable plastics and polymers. Amsterdam: Elsevier; 1994.
- [2] Krochta JM, De Mulder-Johnston CLC. Biodegradable polymers from agricultural products. ACS Symp Ser 1996;647:120–40.

- [3] A.M. Thayer. Plastics recycling efforts spurred by concerns about solid waste. In: Glass JE, Swift G, editors. *Agricultural and synthetic polymers, biodegradability and utilization*. American Chemical Society; 1990. p. 38–51.
- [4] U.S. shiaku, K.W. Pang, W.S. Lee, Z.A.M. Ishak. *European Polymer Journal*. 38 (2002) 393.
- [5] C. Bastioli, A. Cerutti, I. Guanella, G.C. Romano, M. Tosin. *J Environ Polym Degrad*. 3 (1995) 81.
- [6] C. Bastioli. *Polym Degrad Stab*. 59 (1998) 263
- [7] P.A Wagner, B.J Little, K.R Hart and R.I Ray. “Biodegradation of composite materials”. *Int Biodeterior Biodegrad*, 38:125–32.1996.
- [8] M Vikman, M Itaevaara and k Poutanen. “Biodegradation of starch-based materials”. *Plastics engineering*, vol. 29. New York: *Degradable Polymers, Recycling, and Plastics Waste Management*; 1995. pp. 271–4.
- [9] M T. Taghizadeh and A Mehrdad. “Calculation of the rate constant for the ultrasonic degradation of aqueous solutions of polyvinyl alcohol by viscometry”. *Ultrason Sonochem*.10:309–13.2003.
- [10] T Ouchi, S Ichimura and Y Ohya. “Synthesis of branched poly(lactide) using polyglycol acid thermal, mechanical properties of its solution-cast film”. *Polymer*. 47:429–34. 2006.
- [11] N Follain, C Joly, P Dole, C Bliard. “Properties of starch based blends. Part 2. Influence of poly vinyl alcohol addition and photocrosslinking on starch based materials mechanical properties”. *Carbohydr Polym*. 60:185–92. 2005.
- [12] T Shangwen, Z Peng, X Hanguo and T Huali.” Effect of nano-SiO₂ on the performance of starch/polyvinyl alcohol blend films”. *Carbohydr Polym*. 72:521–6. 2008.
- [13] K Pal, A K Banthia and D K Majumdar. “Preparation of transparent starch based hydrogel membrane with potential application as wound dressing”. *Trends Biomater Artif Organs* 20(1):59–67. 2006.
- [14] M. Alikarami, Z. Abbasi, V. Moradi.”Study of enzymatic degradation and water absorption of composites carboxymethyl cellulose and poly (ϵ -caprolactone) containing SiO₂ nanoparticle by cellulase. *Journal of Environmental Science and Health, Part A* 48, 1516–1521. 2013.
- [15] M. T. Taghizadeh, Z. Abbasi. “The effect of temperature and water absorption on enzymatic degradation of starch / polyvinyl alcohol blend film by α -Amylase”. *J. Iran. Chem. Res*. 4, 77-85. 2011.
- [16] M. T. Taghizadeh, Z. Abbasi and Z. Nasrolahzede, “Study of enzymatic degradation and water absorption of nanocomposites starch/ polyvinyl alcohol and sodium montmorillonite clay”. *Journal of the Taiwan Institute of Chemical Engineers*, 43. 120–124. 2012.
- [17] Z. Abbasi. Water resistance, weight loss and enzymatic degradation of blends starch/polyvinyl alcohol containing SiO₂ nanoparticle *Journal of the Taiwan Institute of Chemical Engineers* 43, 264–268. 2012.
- [18] F.C. Braganca. Universidade Saõ Francisco, Itatiba, 2003.
- [19] Z. Abbasi, M. Alikarami, M.T. Taghizadeh, N. Saouri and F. Raoufi. *Journal of Chemical Science and Technology*, 2012, Vol. 1 Iss. 3, PP.70-73.
- [20] C.V. Benediet, W.J. Cook, P. Jarrett, S.J. Huang, J.P. Bell. *Fungal degradation of polycaprolactones*. *J Appl Polym Sci* 1983;28:327–34.
- [21] M.Huskie, I.Brnardie,M.Zigon, M.Ivankovie. *Modification of montmorillonite by quaternary polyesters*. *J Non-Cryst Solids* 2008;354:3326–31.
- [22] D.Demirgoz, C.Elvirs, J.F.Mano, A.M.Cunha, E.Piskin, R.L.Reis. *Chemical modification of starch based biodegradable polymeric blends: effects on water uptake, degradation behaviour and mechanical properties*. *Polym Degr Stab* 2000;70:161–70.
- [23] Y.X.Xu, M.A.Hanna. *Preparation and properties of biodegradable foams from starch acetate and poly (tetramethylene adipate-co-terephthalate)*. *Carbohydr Polym* 2005;59:521–9.
- [24] M.N.Kim, A.R. Lee, J.S.Yoon, I.J.Chin. *Biodegradation of poly(3-hydroxybutyrate)*. *Sky-green and Mater-Bi by fungi isolated from soils*. *Eur Polym J* 2000;36:1677–85.
- [25] A.K.Bajpai, J.Shrivasaava.. *In vitro enzymatic degradation kinetics of polymeric blends of crosslinked starch and carboxymethyl cellulose*. *Polym Int* 2005;54(11):1524–36.