



Influence of Glycerol and Clay Contents on Biodegradability of Corn Starch Nanocomposites

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ABSTRACT

In this study, biodegradation of corn starch/glycerol/Na-MMT nanocomposites by soil burial tests were carried out for up to 6 months. Films were prepared by casting method according to central composite design and response surface methodology was applied for analysis of the results. The effects of formulation on the water solubility, contact angle and tensile strength of starch films were investigated. Increasing in Na-MMT content decreased biodegradability; while presence of glycerol, increased it. The effect of nanoparticles content might have been attributed to interactions between starch and Na-MMT that further prevented enzymatic attack on the biopolymer. In addition, the films with higher initial contact angle and lower water solubility revealed slower biodegradation. The effect of glycerol is debated because higher hydrophilicity and weaker mechanical properties enhanced the amylase attack on the samples. Furthermore, weight loss due to soluble matters caused an increase to account of the biodegradability. By increasing in glycerol amount as a water holding agent, the films could store sufficient water content for microbial activities around the buried samples. According to FTIR spectra for partially biodegraded films, the intensity of the peaks at 1150 and 1040 cm^{-1} associated with starch glycosidic linkages decreased which indicates the action of alpha-amylase produced by soil microorganisms.

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1. INTRODUCTION

Petroleum-based polymers, due to their desirable mechanical properties and cheap production costs are widely used. Most of these polymers could not be degraded and are one of the most sources of solid wastes. Using biodegradable polymers is the best solution to overcome this problem which decompose easily into carbon dioxide, water, methane, inorganic compounds and biomass via microbial activities [1]. Applications of biopolymers as environmental friendly packaging materials were studied extensively [2, 3]. One of the most investigated biopolymers is starch, owing to its wide availability, good biodegradability as well as low cost [4]. Starch is the main energy reserve in plants such as corn, wheat, potato and cassava, which accumulated in storage organs [5, 6]. Despite of aforementioned merits, films prepared from starch are

very sensitive to water which limits their applications [7] and also exhibit low mechanical properties and high permeability for gases and water vapor [8, 9]. One of the suitable solutions which recently draws many attentions is the application of nanoparticles for aforementioned purpose [10, 11]. The most common nanoparticle employed in this field is Na-MMT which significantly increased resistance of biopolymers against water, improved mechanical properties and decreased permeability [12, 13]. Soil burial test is a commonly used method to evaluate biodegradability of the polymers [14-16]. Although, biodegradability have been measured and reported using other methods such as image processing [17] and respirometric method [1]. Living microorganisms of the soil are the main cause of biodegradation, which produce enzymes that permit degradation of biodegradable materials [18]. The burial test conditions i.e. pH, temperature and humidity are important in view point of the optimum living conditions for the main microorganism which consume the polymer [19].

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In some related published papers, effects of Cloisite 30B [20] and attapulgite clay[15] nanoparticles have been studied on the biodegradation of the nanocomposites using soil burial test method. The objectives of this study were: (i) to evaluate the biodegradability of corn starch films, (ii) to investigate the role of Na-MMT and glycerol contents on biodegradability and (iii) to study effects of mechanical properties, initial contact angle and total soluble matter on biodegradability using soil burial tests.

2. MATERIALS AND METHODS

2. 1. Materials Corn starch (11% moisture) was obtained from Glucosan (Ghazvin, Iran). Na-MMT Cloisite® (Moisture: 4-9%, Color: Off White, d001= 1.17 nm) was purchased from Southern Clay Products (USA). Food grade glycerol as a plasticizer was prepared from Merck Company.

2. 2. Experimental Design and Statistical Analysis The Design Expert software (version 7.0.0, Stat-Ease, Inc., Minneapolis, MN) was used for the statistical design of experiments and data analysis. A faced center CCD combined with RSM was constructed on three levels of two independent variables, i.e. Na-MMT content and glycerol content. The amounts of plasticizer and nanoparticle were presented in Table 1. RSM is a technique to design experiments, evaluating the effects of operation conditions and achieving the best conditions for desirable responses with a limited number of planned experiments. Recently RSM has been applied in some research related to biopolymers [21-23]. A regression design was used to model a response as a mathematical function of factors. Each response can be expressed as second-order polynomial, according to Equation (1).

$$R = \beta_0 + \sum_{i=1}^k \beta_i x_i + \sum_{i=1}^k \sum_{j=i+1}^k \beta_{ij} x_i x_j + \sum_{i=1}^k \beta_{ii} x_i^2 \quad (1)$$

where, R is the predicted response used as a dependent variable; k is the number of independent variables (factors) which is 2 here; x (i = 1, 2) is the independent factors; β_0 is the constant coefficient, and β_i , β_{ij} and β_{ii} are the coefficients of linear, interaction and quadratic terms, respectively. There are a lot of researches which confirm that the quadratic polynomial equation is the best model [24, 25]. Design Expert was also used to find the 3-D surfaces and perturbation plots of the response models. Analysis of variances (ANOVA) was used for analyses of the data to obtain the interaction between the process variables and the responses. The proper and accurate models were selected based on p-value of the model less than 0.05 to have a significant

model. The quality of the fitting model was expressed by the coefficient of determination R^2 and Adj R^2 . In parallel, ANOVA was applied on the results using the statistical program SPSS 19.0.0 (IBM, Chicago IL, USA) and the Duncan test was used to evaluate average differences. The films were prepared and analyzed triple times in one block.

2. 3. Film Preparation Films were prepared by casting method. The concentration of plasticizer was set at 25 to 35% to have a desirable flexibility. The content of glycerol depends on required applications [26]. The minimum concentration of plasticizer was set at 25% because for the films with glycerol amounts smaller than 25%, good flexibility was not observed. In addition, films with 40% glycerol revealed very weak mechanical properties and very high hydrophilicity. The minimum and maximum amounts of clay were set to zero and 5%, respectively. The clay amount more than 5% caused the casting solution very viscous which release of bubbles was impossible without using a vacuum apparatus. The specified amount of Na-MMT according to Table 1, was added to 99.5 g of distilled water under rigorous mixing for 20 min at 80 °C. Then, the resulted aqueous suspension homogenized for 20 min at 80°C via sonication method to make a uniform solution. The proper amount of glycerol according to Table 1, with 5 g of pristine corn starch was added to the mixture and mixed by stirrer for 10 min and sonicated for 20 min at room temperature. The final mixture placed in water bath (80 ± 0.2 °C) under stirring for 30 min. The casting solution poured into polystyrene petri dishes and placed on a flat surface at the laboratory. After 36 hours, films were carefully separated and stored in poly ethylene bags. The prepared samples was designated with a label such as G30C2.5, where G and C referred to glycerol and clay, respectively and the number 30 indicated 30% glycerol and 2.5 referred to 2.5% Na-MMT.

2. 4. Total Soluble Matter Water solubility of the films was determined according to Romero-Bestida and others [27]. To determine the total soluble matter (TSM), pieces of films of 2 × 3 cm were cut from each sample and were stored in a dessicator with silica gel during 7 days. The dried samples were weighed to the nearest 0.0001 g. Then, samples were placed into beakers with 80 ml dionized water and samples were maintained under agitation for 1 h at room temperature (23 ± 2 °C). The remained pieces of film were collected by filtration and dried at 60 °C to constant weight. The TSM was calculated as follows:

$$\text{TSM(\%)} = \frac{\text{Initial dry weight} - \text{Final dry weight}}{\text{Initial dry weight}} \times 100 \quad (2)$$

TABLE 1. Variables and their levels for the CCD.

Variable levels	Clay % (dry starch basis)	Plasticizer % (dry starch basis)
-1	0	25
0	2.5	30
+1	5	35

2. 5. Wettability To study the effects of glycerol and Na-MMT on the wettability, one droplet of dionized water was placed on the surface of the films. The close view photo was captured using an optical microscope and the pictures of both sides of water droplet were taken. Contact angles were measured using Adobe Photoshop CS5 (Adobe Systems, Inc., San Jose, CA) software. The initial contact angles in three replications were measured.

2. 6. Mechanical Properties The tensile strength of the films were evaluated using a texture analyzer (HIWA, Iran) in accordance with ASTM method D 882 [28]. Samples were conditioned in laboratory (23 ± 2 °C, 52 ± 3 % RH) for three days.

2. 7. Soil Preparation Soils for burial tests were obtained from the yard of Sharif University of Technology in June 2012. Soils were grinded by hand and most of the undesirable components such as metals, plastics and glasses were removed. Film samples were prepared and placed in $9 \times 6 \times 3$ pots, where 9 stands for nine obtained samples, 3 is because of three replications and 6 is related to six measuring times according to the ASTM D6400 standard which requires 60% biodegradation within 180 days [29]. Depths of pots were 10 cm and 4 cm of soils were considered for both top and bottom of the buried samples to ensure the living of microorganisms for aerobic biodegradation and oxidative reactions. In order to not measuring the water content, every 3 days, the control pot weighted and the amount of weight lost was maked up by water to the experimental pots and of course the control pot to achieving the initial water content.

2. 8. Soil Burial Tests Film samples with almost same thickness were placed between two stainless steel sheets through mesh equal to 200. All margins of the sheets contained polymer samples were punched carefully to inhibit the soil entrance. At specified interval times, the steel cages were extracted from the pots and the attached soils were cleaned with a smooth brush. Then cages were opened and remained films were carefully gathered and weighted. This step is very time consuming and might cause the big errors in measuring of the biodegradability. The

biodegradability was calculated using the following equation:

$$\text{Biodegradability}(\%) = \frac{W_0 - W_t}{W_t} \times 100 \quad (3)$$

where, W_0 and W_t are weight of initial sample and weight of sample at interval, respectively.

2. 9. FTIR Analysis FTIR spectra of samples in the form of KBr pellets were recorded using an ABB Bomem MB-100 FTIR spectrophotometer.

3. RESULTS AND DISCUSSION

3. 1. Total Solubility Test By increasing the plasticizer, water solubility was increased. Glycerol has three hydroxyl groups which gives high tendency to absorb the water molecules. As observed, increase in Na-MMT and glycerol will result in decreasing and increasing the water solubility, respectively. The obtained results with standard deviations are summarized in Table 2 and averages are compared with Duncan test. The linear form of water solubility as function of glycerol and Na-MMT contents, had a good correlated function with $R^2=98.92\%$ and $p\text{-value} < 0.0001$ is presented in Equation (4) which G and C are coded amounts of glycerol and Na-MMT contents.

$$\text{Water solubility} = 22.66 + 4.08G - 2.48C + 0.18C \times G \quad (4)$$

($-1 \leq C \leq +1$, $-1 \leq G \leq +1$)

In addition, because of the p-value smaller than 0.05, this model is significant and could be used to study and compare the importance of each factor on the studied domain. Model indicated that effect of G is more sensible to C parameter. The final equation in terms of actual factors is presented in Table 3 and details of analysis of variance (ANOVA) for the models is presented in Table 4. Response surface of the water solubility of the nanocomposites as a function of glycerol and nanoparticles content is presented in Figure 1(a). Increasing in Na-MMT content decreased solubility; while presence of glycerol, increased it.

3. 2. Wettability By increasing the glycerol content, the initial contact angle decreased. It means that presence of this plasticizer made the surface more hydrophilic. Glycerol is more hydrophilic than starch and is sensitive to the relative humidity [30]. By presence of Na-MMT in the polymer matrix, the surface of biopolymer became hydrophobic and the contact angle will increase. By changing the formulation of films according to Table 1, contact angle will vary from the 35.27 to 69.93 ° related to G35C0 and G25C5, respectively.

TABLE 2. Results of the water solubility, contact angle, tensile strength and biodegradability in 30 days.

Sample	WS (%)	TS (MPa)	CA(°)	BD in 30 days (%)
G25C0	21.27±0.84 ^{a,A}	7.30±0.75 ^{a,A}	48.87±1.34 ^{a,A}	51.05±1.24 ^{a,A}
G25C2.5	17.47±1.10 ^{b,A}	8.14±0.61 ^{a,A}	64.83±1.27 ^{b,A}	37.93±1.33 ^{b,A}
G25C5	16.11±0.72 ^{b,A}	8.51±0.77 ^{a,A}	69.93±1.68 ^{c,A}	32.64±1.11 ^{c,A}
G30C0	25.24±0.63 ^{a,B}	4.60±0.39 ^{a,B}	44.07±0.57 ^{a,β}	56.00±1.84 ^{a,B}
G30C2.5	22.59±1.03 ^{b,B}	6.04±0.75 ^{ab,B}	53.53±1.37 ^{b,B}	39.92±1.28 ^{b,AB}
G30C5	19.97±1.17 ^{c,B}	6.45±1.03 ^{b,B}	63.53±1.96 ^{c,B}	31.68±1.15 ^{c,AB}
G35C0	28.50±0.94 ^{a,C}	2.55±0.07 ^{a,C}	35.27±1.10 ^{a,C}	59.63±1.56 ^{a,C}
G35C2.5	27.72±0.59 ^{a,C}	3.29±0.40 ^{b,C}	44.37±2.00 ^{b,C}	42.62±1.57 ^{b,B}
G35C5	24.08±0.98 ^{b,C}	3.99±0.33 ^{c,C}	47.07±0.74 ^{b,C}	35.07±1.73 ^{c,B}

At each glycerol level, values with same lower case letter are not significantly different and at each nanoparticles level, values with the same capital case letter are not significantly different ($P > 0.05$).

TABLE 3. Fitting of second-order polynomial equation for response surfaces in actual form ($0 \leq C \leq 5$, $25 \leq G \leq 35$).

Surface	Equation	R ²	Adj-R ²
Water solubility (%)	$=4.90+0.56G-1.12C+0.02G \times C-3.66G^2-0.06C^2$	99.16%	97.77%
Contact angle (°)	$=37.82+1.93G+9.58C-0.14G \times C-0.06G^2-0.35 C^2$	98.91%	97.08%
Tensile strength (MPa)	$=16.93-0.32G+0.37C+4.60E-3G \times C-2.66E-3G^2-0.04C^2$	99.53%	98.74%
Biodegradability in 30 days (%)	$=21.14+1.10G-0.82C-0.14G \times C-3.47E-3G^2+0.29 C^2$	100%	99.99%

TABLE 4. Details of analysis of variance (ANOVA) for the models

Response 1 Water Solubility						Response 2 Contact angle					
ANOVA for Response Surface Quadratic Model						ANOVA for Response Surface Quadratic Model					
Analysis of variance table [Partial sum of squares - Type III]						Analysis of variance table [Partial sum of squares - Type III]					
Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Model	136.9	5	27.37	71.09	0.0026 significant	Model	1016	5	203.2	54.21	0.0039 significant
G	99.63	1	99.63	258.8	0.0005	G	526.4	1	526.4	140.4	0.0013
C	36.75	1	36.75	95.45	0.0023	C	462.9	1	462.9	123.5	0.0016
C×G	0.137	1	0.137	0.356	0.5930	C×G	12.96	1	12.96	3.458	0.1599
G ²	0.017	1	0.017	0.044	0.8479	G ²	4.109	1	4.109	1.096	0.3720
C ²	0.317	1	0.317	0.824	0.4309	C ²	9.534	1	9.534	2.544	0.2090
Residual	1.155	3	0.385			Residual	11.24	3	3.748		
Cor Total	138	8				Cor Total	1027	8			

Response 3 Tensile strength						Response 4 Biodegradability-30days					
ANOVA for Response Surface Quadratic Model						ANOVA for Response Surface Quadratic Model					
Analysis of variance table [Partial sum of squares - Type III]						Analysis of variance table [Partial sum of squares - Type III]					
Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Model	36.76	5	7.352	126.7	0.0011 significant	Model	563.9	5	112.8	22934	0.0001 significant
G	33.23	1	33.23	572.9	0.0002	G	42.56	1	42.56	8655	< 0.0001
C	3.375	1	3.375	58.18	0.0047	C	502.2	1	502.2	1E+05	< 0.0001
C×G	0.013	1	0.013	0.228	0.6656	C×G	12.71	1	12.71	2584	< 0.0001
G ²	0.009	1	0.009	0.153	0.7216	G ²	0.015	1	0.015	3.055	0.1788
C ²	0.132	1	0.132	2.271	0.2289	C ²	6.468	1	6.468	1315	< 0.0001
Residual	0.174	3	0.058			Residual	0.015	3	0.005		
Cor Total	36.93	8				Cor Total	563.9	8			

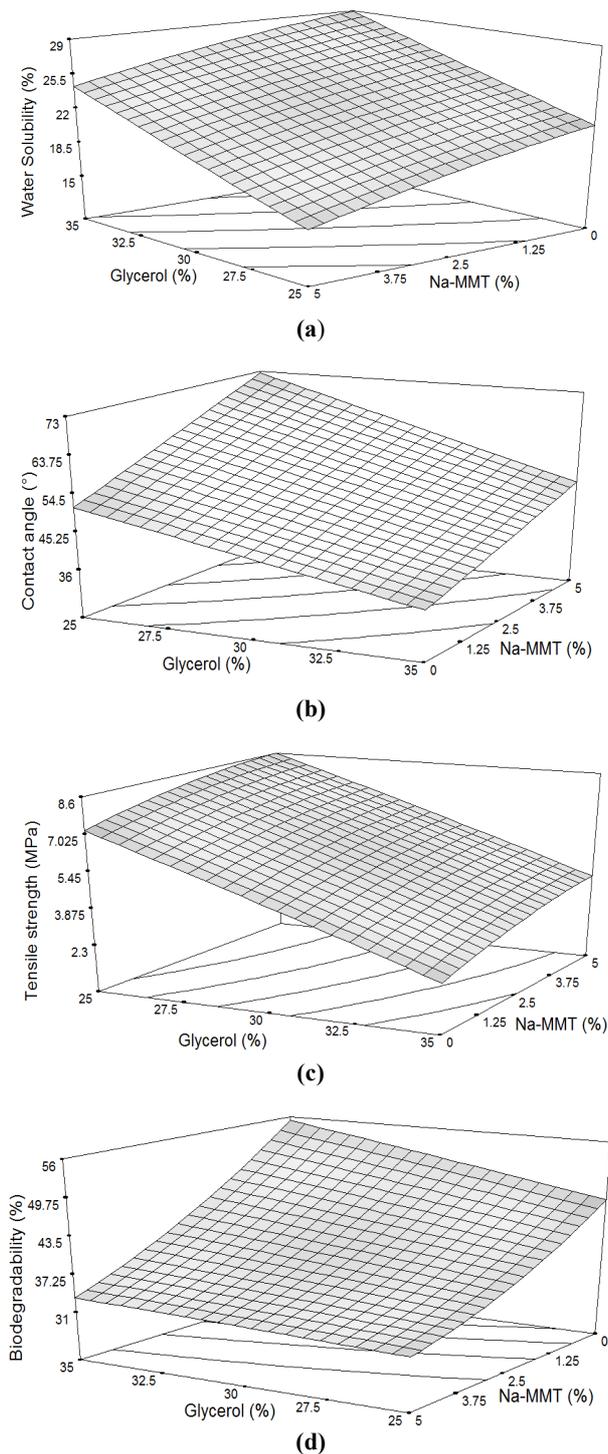


Figure 1. Response surface for, (a): water solubility, (b): contact angle, (c): tensile strength and (d): biodegradability in 30 days.

When a water droplet is placed on a polymer surface, an attraction occurs between the molecules in the water and polymer surface. A lower contact angle means that polymer and water have a strong attraction.

Therefore, they adhere better to each other [31]. The contact angle had a good correlated function with $R^2=97.58\%$ and is significant in the linear coded form with $p\text{-value}=0.0002$. Linear form presented in Equation (5):

$$\text{Contact angle}=52.58-9.37G+8.78C-1.80C\times G \quad (5)$$

$$(-1\leq C\leq+1, -1\leq G\leq+1)$$

The presented linear form indicates that effect of Na-MMT is almost equal to glycerol effect on hydrophilicity or hydrophobicity of the polymer surface. According to Table 2, the effects of both glycerol and clay contents on contact angle of surface are significant, except at the highest level of glycerol which there is no differences between 2.5 and 5% of Na-MMT. The final equation in terms of actual factors is presented in Table 3 and the response surface of the contact angle of the nanocomposites as a function of glycerol and nanoparticles content is presented in Figure 1(b). Details of ANOVA for the models is presented in Table 4. The maximum of contact angle will occur in the highest level of Na-Montmorillonite and lowest level of glycerol.

3. 3. Mechanical Properties The results showed increasing in plasticizer content resulted in decrease in tensile strength. Be noted, glycerol reduced the intramolecular attraction between the starch chains by forming hydrogen bonds between plasticizer and starch molecules. In harmony, plasticizer reduces the formation of hydrogen bonds between the starch chains and allows greater flexibility and subsequently decreases the tensile strength. The obtained results are in accordance with the research publication [31-33].

The effect of glycerol as a plasticizer in tensile strength reduction will be amplified due to the role of water as a plasticizer [33]. The same effect of water content on mechanical properties might be observed by an increase in clay content. Although clay improves the mechanical properties, because presence of water and its plasticizing effect, part of tensile strength will be reduced. According to Table 2, the effect of nanoparticles in 25% of glycerol on tensile strength is not significant and by increasing the plasticizer up to 35%, its effect becomes more significant. It might be because of the low mobility of the polymer chains in the lower glycerol contents, while by increase in plasticizer, the tensile will decrease sharply. Thus, the role of Na-MMT contents on tensile strength will be more significant. Moreover, the effect of plasticizer contents in all levels of nanoparticles amount were significant. For the tensile strength, linear form of correlated function with $R^2=99.15\%$ and $p\text{-value}=0.0001$ in coded form presented in Equation (6):

$$\text{Tensile strength}=5.65-2.35G+0.75C+0.057C\times G \quad (6)$$

$$(-1\leq C\leq+1, -1\leq G\leq+1)$$

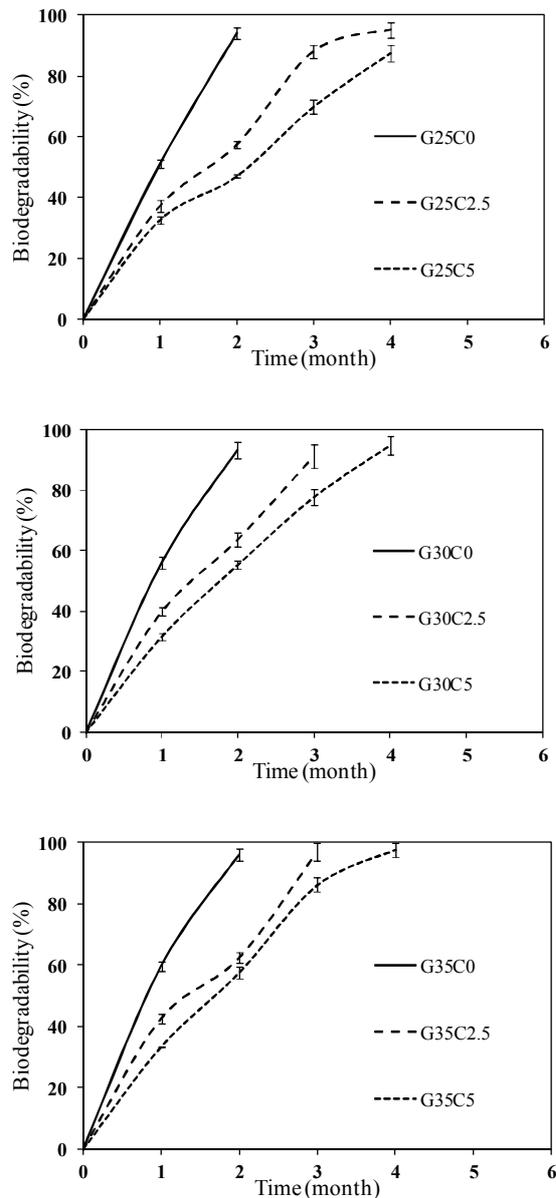


Figure 2. Biodegradability of samples in soil.

According to Equation (6), the effect of G is more sensible in tensile strength related to C. The final equation in terms of actual factors is presented in Table 3 and details of ANOVA for the models is presented in Table 4. Response surface for the tensile strength of nanocomposites as a function of glycerol and nanoparticles content is presented in Figure 1(c). Increasing in Na-MMT content increased tensile strength; while presence of glycerol, decreased it.

3. 4. Biodegradability At interval times, metal containers were excavated from the pots and containers were opened carefully. According to eye view, only very small amounts of soil entrance were observed for

some containers. Because of high density of soil in comparison to polymers, these small portions will cause an overestimation of the remaining biopolymer which makes a considerable under estimation of biodegradability. Although using stainless steel mesh 200 with very small holes may restrict the proper contact between soil and polymers, but almost all of the remaining pieces of polymer could be gathered and weighted. Scattering the small polymer pieces in soil causes an overestimation of biodegradability. Weight of polymer at each intervals are affected by weight of polymer consumed by microorganisms, polymer lost during collecting the remained polymer pieces, weight of the attached soil particles and weight of colonies of the microorganisms. The remaining biopolymers were easily crumbled when touched, probably because of diminishing the tensile strength due to the result of biodegradation.

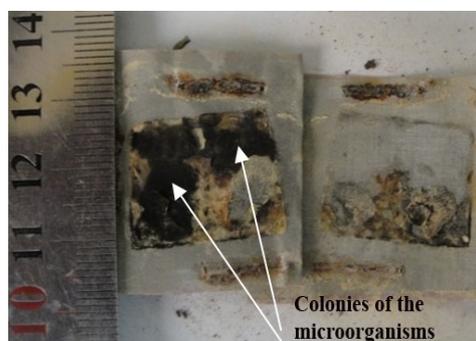
As result, it was very hard to remove the colonies of the microorganisms from the surface without losing the polymer. Therefore, the extracted polymers was cleaned to remove only the soil particles. The remained colonies will cause underestimation of biodegradability. The role of both attached soil particles and missed polymer pieces would cause undesirable behavior for biodegradation or cause very large error for the results [17]. The obtained data for biodegradability are shown in Figure 2. The latest experimental points on each graph is related to the last measuring biodegradability and the points for fully degraded polymers are not reported on the figures because collecting the remained polymers was impossible. By preventing the mentioned sources of errors, the maximum standard deviation for our experiments were only 3.87%. As shown, by increasing in glycerol content, the rate of biodegradability increased while; all samples without Na-MMT were completely biodegraded in three months. The part of increasing in biodegradability by increasing of glycerol could be discussed by releasing of soluble matters to the soil. Moreover, glycerol as a good water holding agent prepared needing humidity for microorganism's activities around the buried films. Also by increasing the glycerol content, surface became more Hydrophilic due to the excess of the -OH group that facilitates the penetration of enzymes to the films, subsequently enhancing the amylase attack on the surface of the samples [34]. By increasing in nanoparticles content, slope of biodegradability versus time reduced and samples containing Na-MMT were biodegraded in four up to five months under the same conditions. As discussed above, with increasing in clay content made the polymer surface more hydrophobe, so penetration of water and/or enzymes produced by microorganisms could be reduced. The linear form of biodegradability based upon the coded values had a valid correlated

function with $R^2=98.85\%$ and $p\text{-value}<0.0001$ and presented in Equation (7).

$$\text{Biodegradability} = 41.13 + 2.66G - 9.15C - 1.78C \times G \quad (7)$$

$(-1 \leq C \leq +1, -1 \leq G \leq +1)$

The model indicated that the effect of C is more sensible related to G. The final correlated equation in terms of actual factors is presented in Table 3. Details of ANOVA for the models is presented in Table 4. Response surface of the biodegradability of the nanocomposites as a function of glycerol and nanoparticles contents were presented in Figure 1(d). Increasing in Na-MMT content decreased biodegradability; while presence of glycerol, increased it. The maximum of biodegradability will occurs in the highest level of glycerol and lowest level of Na-Montmorillonite. These results were consistent with our visual observation changes of samples. In Figure 3, images for sample G30C5 after soil burial of 1 month (a) and for 5 months (b) are presented and the colonies of the microorganisms should be observed by black color on the surface of polymers. According to Figure 1, it was found that the maximum of hydrophilicity, biodegradability and total soluble matter of were obtained by minimization of clay and maximization of plasticizer in the considered range, which resulted in minimum of tensile strength.



(a)



(b)

Figure 3. Photographs for biodegradability of sample G30C5 after soil burial, (a): for 1 month and (b): for 5 months.

The whole picture of the current investigation is similar to Magalhães and Andrade study [20]. They studied the effects of glycerol and nanoparticles contents on biodegradability using soil burial test, which their films prepared using melt extrusion method and also used Na-MMT and Cloisite[®] 30B nanoparticles. In contrary to our results, they found by adding Cloisite[®] 30B, the rate of biodegradability was increased and the nanocomposites were fully degraded in the shorter time than their samples without nanoparticles. The first glance to describe the delaying in biodegradability which observed in our experiments was antimicrobial role of Na-MMT. No antimicrobial activity of Na-MMT against *Salmonella typhimurium*, *Listeria monocytogenes*, *Staphylococcus aureus* and *Escherichia coli* were reported, which means that the role of these nanoparticles on postponing the biodegradability was not related to its probable antimicrobial effects against available microorganisms of soil. It should be clarify, that Cloisite[®] 30B revealed antimicrobial activity against *Listeria monocytogenes* [35].

To have a better understanding, perturbation plots for water solubility, contact angle, tensile strength and biodegradability were presented in Figure 4. Results are in good harmony to each other for effects of Na-MMT and glycerol contents on studied responses. Negative or positive slopes of these diagrams are related to effect and importance of each factor. For example, according to Figure 4 (d), effect of Na-MMT on reducing the biodegradability is more important than the role of glycerol on increasing the biodegradability. Furthermore, reverse behavior of water solubility and contact angle was reported for ZnO nanoparticles [36]. By increase in Na-MMT content, tensile strength was increased and caused the reduction in biodegradability of the samples. As presented in the Figure 1(d) and Figure 4 (d), it was found that the maximum of biodegradability will occurs in the highest level of glycerol (35%) and lowest level of Na-Montmorillonite (0%). Enzymatic degradation, using α -amylase and amyloglucosidase produced by microorganisms, is the reason to explain the biodegradability of starch based polymers. Starch is composed of two types of complex carbohydrate polymers of glucose, namely amylose and amylopectin depended on the source of the plants. Amylose is a linear polymer of glucose units linke with α -1,4-bonds. Amylopectin, which is an extremely high molecular weight polymer similar to amylose, but with many α -1,6-linked branch points [37, 38]. Amylases are the class of glucosidase hydrolyzing enzymes that cleave the α -1,4 and/or α -1,6 glucosidic linkages in starch and other polysaccharides. Some investigation on the enzymatic hydrolysis of starch blends by the above mentioned enzymes preformed in the literature [39].

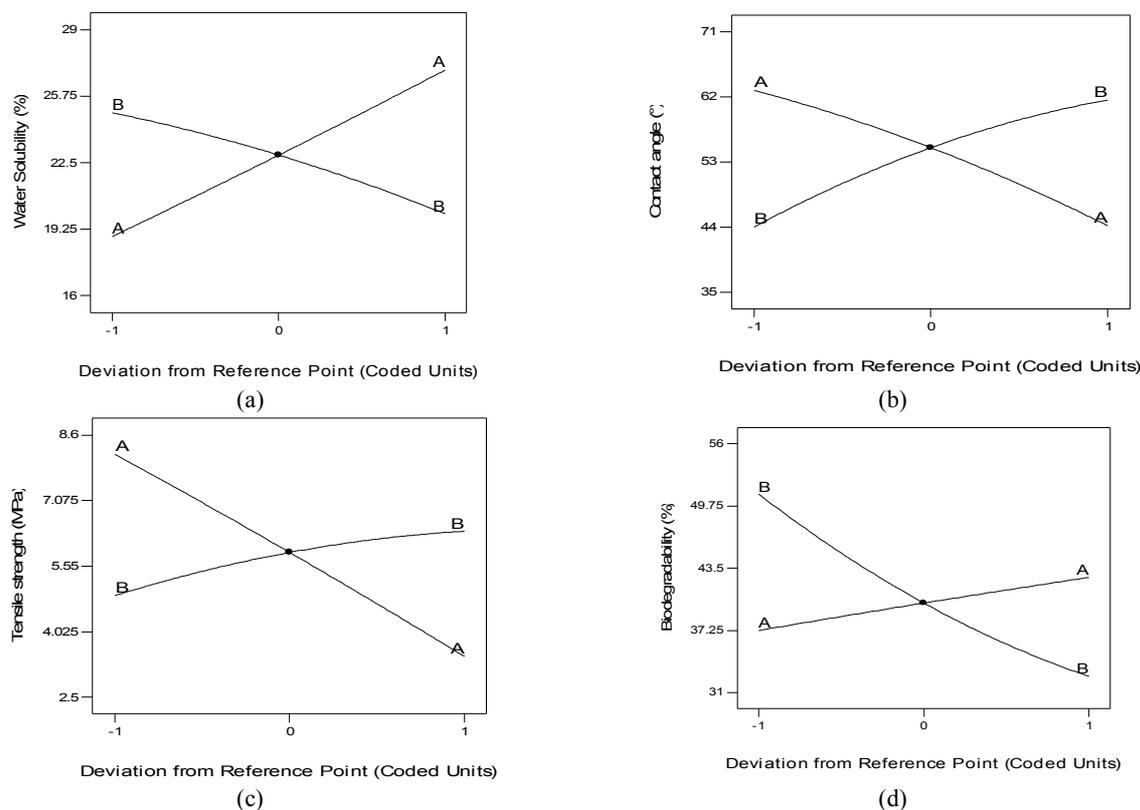


Figure 4. Perturbation plots for, (a): water solubility, (b): contact angle, (c): tensile strength and (d): biodegradability in 30 days (for A: Glycerol and B: Na-MMT).

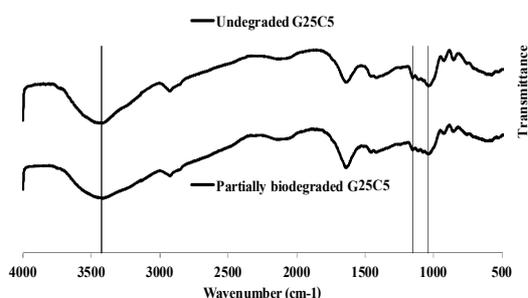


Figure 5. FTIR spectrum of undegraded and partially biodegraded G25C5 sample.

By an increasing in contact angle weather by increasing the nanoparticles or reducing glycerol contents, the rate of enzymatic degradation will be reduced. Effects of Na-MMT and SiO₂ nanoparticles on reducing starch hydrolysis were reported for some nanocomposites [40, 41]. It should be explained by reduction in water absorption and lower tendency between liquid and solid phases to adhere together. The films with higher water solubility revealed an increasing the biodegradability, because water and enzyme would penetrate easier in the matrix of polymer. In addition, solubility of the soluble matters

in soil will reduce the weight of polymer and causes the higher degree of biodegradation. Finally, by increasing in tensile strength utilizing the nanoparticles, the biodegradability was decreased. This phenomena should be clarified via stronger interactions between nanoparticles while by adding the glycerol, intra- and intermolecular interactions caused through hydroxyl groups of glycerol which makes weaker bonds in polymer matrix [8].

3. 5. FTIR Analysis The colonies of microorganisms were removed carefully from the partially biodegraded sample containing 25% glycerol and 5% Na-MMT after one month, then polymer was washed gently with distilled water and dried in room temperature. The FTIR spectrum of undegraded and partially biodegraded G25C5 sample are shown in Figure 5. The broad band in the region of 3430 cm⁻¹ is due to the O-H stretching vibration which is related to hydroxyl groups presented on starch-glycerol films [42].

After one month of biodegradation in soil, the intensity of the peaks at 1150 and 1040 cm⁻¹ decreased which are related to glycosidic linkages of starch. These reductions could be explained because of the action of α -amylase produced by soil microorganisms in cleaving the mentioned linkages [43]. In addition, the intensity of the peak at 3430 cm⁻¹ decreased which

means that by consumption and degradation of starch by microorganisms, the amount of water and glycerol in the undegraded films will be diminished. Part of reduction in this peak is also related to the leaching of glycerol [18].

4. CONCLUSION

The effects of clay and glycerol contents on the water solubility, tensile strength, wetability and biodegradation of corn starch biopolymers were evaluated. Films with high content of glycerol and low content of Na-MMT indicated more water solubility and biodegradability. Moreover, to have the films with higher contact angle and tensile strength, nanoparticles and plasticizer must be determined on their higher and lower contents, respectively. Na-MMT nanoparticles might have no anti microbial effect against available microorganisms of soil. Therefore, reduction in biodegradability was because of reinforcement of polymer by nanoparticles and interactions between starch and Na-MMT that further prevented enzymatic attack on the biopolymer. According to FTIR results, the action of alpha-amylase produced by soil microorganisms and leaching of the soluble matters to the soil are the main reasons for the biodegradation.

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Influence of Glycerol and Clay Contents on Biodegradability of Corn Starch Nanocomposites

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در این تحقیق، زیست تخریب پذیری نانوکامپوزیتهای نشاسته ذرت-گلیسرول-سدیم مونت موریلونیت به روش دفن در خاک برای مدت شش ماه مورد بررسی قرار گرفت. فیلمها به روش تبخیر حلال بر اساس روش طرح مرکب مرکزی ساخته شدند و نتایج با روش سطح پاسخ آنالیز گردید. تاثیر فرمولاسیون بر میزان حلالیت در آب، زاویه تماس و استحکام کششی بررسی گردید. افزایش سدیم مونت موریلونیت، زیست تخریب پذیری را کاهش داده درحالیکه گلیسرول باعث افزایش آن می شود. تاثیر میزان نانوذرات می تواند مرتبط با برهم کنش های بین نانوذرات و نشاسته باشد که از حمله آنزیمی به سطح بیوپلیمر جلوگیری می کند. به علاوه، فیلمهای با زاویه تماس بیشتر و حلالیت در آب کمتر زیست تخریب پذیری کمتری نشان دادند. تاثیر گلیسرول می تواند بواسطه آبدوستی بیشتر فیلم و خواص مکانیکی ضعیف تر باشد که باعث افزایش آبکافت آنزیمی نمونه ها می شود. همچنین میزان کاهش وزن بواسطه حلالیت در آب نیز یکی از دلایل افزایش تخمین زیست تخریب پذیری می باشد. با افزایش میزان گلیسرول به عنوان یک عامل نگهدارنده آب، فیلمها می توانند آب مورد نیاز برای فعالیت های میکروبی در اطراف فیلم های دفن شده را نگهداری کنند. براساس طیف های تبدیل فوریه مادون قرمز برای نمونه های تخریب شده جزئی، شدت پیک های مرتبط با پیوندهای گلیکوزیدی در طول موجهای ۱۱۵۰ و ۱۰۴۰ کاهش یافته است که نشان دهنده فعالیت آمیلازی تولید شده توسط میکروارگانیسم های خاک می باشد.

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