Enzymatic Degradation of Polycarbonates: Response Surface Methodology (RSM) based approach.
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Abstract: Polycarbonate is a tough polymer known for its extreme toughness, inertness and transparency and is considered to be chemically resistant. Polycarbonates are susceptible to photo degradation and thermal degradation. The mechanism followed in the degradation of similar polymeric compounds is found to be mostly hydrolysis reactions. Reactions of Bisphenol A polycarbonate with the lipase Candida rugosa were carried out over a period of 72 hours at different temperatures ranging from 25°C to 65°C and at different lipase activities of 400 U/ml, 800 U/ml, 1200 U/ml and 1600 U/ml. The weight loss of polycarbonate was studied against various factors. It is found that there is a rapid loss of polycarbonate around the time period above 48 hrs and at the temperature 55°C for above enzyme activities. The supernatant was subjected to FTIR and the presence of the Bisphenol A, a monomer was found. The results were subjected to the statistical tool, Design of Experiments, in which the fitness of the results were statistically analyzed and the interactions between the parameters studied. The Response surface methodology (RSM) and the ANOVA analysis were performed on the experimental data and the parameters were found to be non interactive. The model equation for the degradation kinetics is obtained from the coefficients of the ANOVA analysis and the fitness of the model data with the actual obtained experimental data is found to be close and similar over the parameters.

Keywords: Biodegradation, polycarbonates, ANOVA, Response surface methodology.

INTRODUCTION

Degradation of polymers is proving to be a major issue on the environmental front, because of their physicochemical properties. Polycarbonates are extremely useful class of high heat polymers, considered to be chemically resistant, known for their toughness, inertness, transparency and are widely used in compact discs, helmets, feeding bottles and shatterproof glasses required for bus shelters, phone booths, and car headlamps. In the digitalized world of today, electronic items have become indispensable and omnipresent resulting in electronic wastes (Mattsson et al. (2015), Islam et al. (2019)). The vast majority of polycarbonates are based on bisphenol A (BPA) and sold under the trade names Lexan (GE), Makrolon (Bayer), Caliber (Dow), Panlite (Teijin) and Iupilon (Mitsubishi).

Polycarbonates have susceptibility towards the photo and thermal degradation (Darwis et al. (1998); Giorgio et al. (2002); Labow et al. (2001); Lee et al. (2004)). Due to their structural bonding, polycarbonates are tough to break down chemically under mild conditions (Palm et al. (2019). Biodegradation of aliphatic polycarbonates are reported (Ming et al. 1999; Suyama and Tokiwa (1997); Tang et al. (2003); Suyama et al., (1998) but they lack the statistical approach which throws light onto the kinetics of the degradation (Chang et al. (2011), Perez et al. (2017)).

Statistical Design of Experiments

Experiment has always been a major tool in tackling practical problems and testing theoretical hypotheses in basic and applied sciences. A statistically designed experiment is usually a systematically tailored series of experiments by which a meaningful result is obtained by changing the input variables in a designed fashion. Application of statistical design of experiments in process development can lead to improved yield of a process, reduced process variability, reduced development time and overall costs. One of the major strengths of the statistical design of experiments is the scientific approach, which helps in drawing valid, meaningful and objective conclusions.
Response Surface Methodology

Response surface methodology (RSM) is the combination of mathematical and statistical techniques used in empirical study of relationships and optimization, where several independent variables influence a dependent variable or response, the goal being to secure the optimal response. By definition, Response Surface Methodology (RSM) (Montgomery, 1991; Khuri and Cornell, 1987) is an empirical technique, which employs multiple regression analysis of the quantitative data obtained from properly designed experiments to simultaneously solve multivariate equations. The graphical representations of these equations are called response surface contours, which describe the cumulative interactions of the test variables on the response.

It involves the following stages, (i) design of experiments that will yield adequate and reliable measurements of the response, (ii) detailed analysis of the results obtained to formulate adequate mathematical model(s), (iii) check for fits of these models with experimental data using suitable hypothesis and (iv) verification of the optimum points obtained.

A central composite design coupled with full quadratic polynomial model is a very powerful combination that provides an adequate representation of most continuous response surfaces without expending much of resources. A usually complex and costly experimental situation is easily resolved with Design of Experiments (DOE). All factors are considered in a minimal number of experiments, and the results are verified with recognized statistical methods. The DOE techniques are formal techniques, which support the design and analysis of experiments. The Statistical analysis of the data in the form of ANOVA for main effects can be judged from the low P value and the high F value.

The overall aim of this study was to understand the biodegradability of polycarbonates and to evaluate the same using statistical design. Accordingly the following objectives are formulated.

- To study the extent of hydrolytic degradation of polycarbonates using lipases.
- To ascertain the effect of parameters on the degradation and the analysis of end products using FTIR.
- To check the fitness of the obtained results with the help of statistical tool Design of Experiments and study the interactions between the various parameters with Response Surface Methodology (RSM).

MATERIALS AND METHODS

Polycarbonate powder was obtained from G.E India. The Lipase CRL – Candida rugosa was obtained from SIGMA, chemicals. Other reagents used in the experiments are of analytical grade. The polymer degradation is characterized mainly by the breaking up of the high molecular weight compounds to low molecular weight ones and this can be studied by weight loss, IR spectra.

Enzymatic reaction method

The procedure given by Darwis et al. (1998) was followed to study the degradation of polycarbonate. Every 2.5 ml of the reaction solution contained 1.66 ml of 0.2 M Phosphate buffer at pH 7.0, 0.4 ml of 0.1 % MgCl$_2$ solution, 0.4 ml Enzyme solution of different activities for different experiments and 0.02 g of 0.1% hypochlorite solution treated polycarbonate. The solutions were sterilized before use.

Enzyme solutions of different concentrations (1600 U/ml, 1200 U/ml, 800 U/ml, 400 U/ml) were prepared (according to the experiment) by adding (1.104 mg, 0.857 mg, 0.571 mg, 0.285 mg respectively) to 1ml of double distilled, sterilized water (Toyama et al. (2009)).

Polycarbonate samples of 0.02 g were taken in 15ml test tubes and the reaction solution was added according to the procedure to each of the test tube to make the final volume to 6.0 ml. The reaction was carried out at different temperatures (25, 35, 45, 55, 65°C) for different lengths of time (0, 12, 24, 36, 48, 60 and 72 Hrs) according to the requirement in a shaker incubator. The reactions were carried out at a shaking speed of 150 rpm. After the required time, the reaction was stopped by adding 10 ml of absolute ethanol to each of the test tube.
Weight reduction
The polycarbonate was filtered to study the weight loss. The polycarbonate powder obtained after filtration was dried to constant weight in a hot air oven at 600°C and then observed for the weight loss. The percentage weight loss was calculated using the formula:

\[
\text{Fractional wt. loss} = \frac{\text{initial weight} - \text{final weight}}{\text{initial weight}}.
\]

\[
\% \text{ wt. loss} = \text{Fractional wt. loss} \times 100
\]

The reaction was carried in duplicates to enhance the accuracy. The weight loss was determined and the % wt. loss was calculated.

RESULTS AND DISCUSSION

Selection of Enzymes
In polycarbonates, the carbonate bonds are susceptible for hydrolysis and lipases are proved hydrolases. Lipases are enzymes that catalyze hydrolysis of fatty acid ester bond in triacylglycerol (TAG) thus releasing free fatty acids. Most organic substrates are insoluble in water and this creates problem for the enzymologist as enzymes require water for structure (bound water). Lipases have to work on emulsions (water-in-oil emulsion), and enzymes act at the oil-water interface. This presents problems in assessing the reaction parameters. Various factors affect the stability of the emulsion and therefore the rate of reaction. Most lipases have an alkaline pH range of 8-9. So it seems that lipases were simple carboxylic esterases that have evolved a special binding site that recognizes insoluble fats binds to them and then switches on the active site.

Effect of Parameters
In the enzymatic reactions conducted, the parameters like the temperature, enzyme activity, and time against the weight reduction were analyzed. The observations were made at different temperatures for enzyme activities of 400, 800, 1200 and 1600 U/ml over a time period of 72 hours. The enzyme was ineffective at 25°C on the polycarbonate even for 72 hours. At 35°C and 45°C, around 2% and 3.5% respectively of weight loss observed. At temperature above 60°C, the steepness of the curves reduces and this shows that the loss of the polymer is reduced at this temperature.

From the Time vs % weight reduction profile in Figure 1, it can be deduced that the enzymatic reaction is active after 20 hours. Up to 20 hours there is no or little weight loss at all temperatures. At the temperature of 55°C there is very rapid loss of polycarbonates after 20 hours and reaches its peak at the time period of 50 hours after which the rate of weight loss is reduced. At 65°C, the weight loss is very similar to that at 55°C.
Effect of Temperature on the Weight loss

Figure 2 shows the profile of Temperature against percentage weight reduction for different enzyme activities, it is seen that there is considerable weight loss only at the temperature of 55°C for all activities and that at lower temperatures the weight loss is very less. The weight loss is zero for all enzyme activities at 25°C, showing that the enzyme is not effective at that temperature.

![Figure 2: Percentage Weight reduction after 72 hrs at different Enzyme activities](image)

At temperatures 35°C and 45°C, the weight loss is very minimal and similar for all activity levels, showing the weak response induced by the enzyme on the polymer. At the temperature of 55°C, there is rapid weight loss for all enzyme activities, reaches 25 to 60 %. But this trend is not seemed to be continuous for the temperature 65°C, where there is small increase in the weight loss. It can be deduced that the enzyme is active around the temperature of 50°C and is very low at lower temperatures and unresponsive at higher temperatures.

FTIR Analysis

The supernatant solution after filtration and removal of the polymer was collected and subjected to FTIR analysis. The reaction was quenched using sufficient amount of ethanol and the FTIR spectra was recorded using neat sampling technique.

Figure 3 shows the IR spectra of pure polycarbonate sample. Polycarbonates have a strong C=O stretching band at 1772 cm⁻¹, and strong C–O stretching bands at 1195 and 1232 cm⁻¹, distinguishing them from polyesters. The amount of phenol end groups can be determined from the O–H absorption at 3400 cm⁻¹. Since the phenolic groups are in trace amounts and are towards the end of the polymer chain, the bands present in this range show relatively weak absorbance. This can be studied in detail using NMR studies.
Figure 3: FTIR spectra of pure Bisphenol A polycarbonate

Figure 4: FTIR spectra of pure Bisphenol A
Figure 5: FTIR spectra of supernatant for reaction at 55 °C and enzyme activity of 1600 U/ml

Figure 6: FTIR spectra of supernatant for reaction at 45 °C and enzyme activity of 1600 U/ml

Figure 4 shows the IR spectra of pure Bisphenol A. Pure Bisphenol A sample (SRL Chemicals) was dissolved in methanol and the FTIR spectra was collected in the range 400 – 4000 cm⁻¹. Spectra shows the presence of a broad
OH peak around 3340 cm⁻¹ and an aromatic C=C stretching around 1640 cm⁻¹. A peak around 2968 cm⁻¹ for CH₃ of isopropyl group and C-O stretch of 1231 cm⁻¹, 1178 cm⁻¹ are also noted.

The spectra for the aqueous product of the reaction were obtained from FTIR analysis. Figure 5 shows the IR spectra of the supernatant (Lipase degraded substrate) from the reaction at 55°C for 72 hours at 1600 U/ml enzyme activity. The supernatant spectra contains absorbance C=C (aromatic) around 1655 cm⁻¹. A broad OH peak around 3392 cm⁻¹ was also obtained. The absence of any ester bonds which are present in the pure polycarbonate sample can be identified. The polycarbonate has a strong peak absorbance of C=O (carbonyl bond) around 1770 cm⁻¹. The fingerprint region of degraded product contains several absorbance peaks which are similar to that of BPA spectrum. Hence the presence of BPA in the supernatant was confirmed.

Figure 6 shows the IR spectra of the supernatant (Lipase degraded substrate) from the reaction at 45°C for 72 hours at 1600 U/ml enzyme activity.

**Design of Experiments**

The statistical analysis was carried out with the help of the software Minitab, which is a statistical tool available for the statistical analysis and has Response Surface methodology (RSM) tools. The results of the effect of enzyme activity on the % weight reduction at different temperatures for the time period of 72 hours were tabulated and the ANOVA analysis was performed. The response surface analysis and the ANOVA results are given below:

Response Surface Regression I: The analysis was done using uncoded units and is shown in Table 1 and 2.

### Table 1: Analysis of Variance for percentage weight reduction after 72 hrs

| Source         | DF | Seq SS  | Adj SS  | Adj MS   | F       | P     |
|----------------|----|---------|---------|----------|---------|-------|
| Regression     | 3  | 7287.10 | 7287.10 | 2429.03  | 18.89   | 0.000 |
| Linear         | 2  | 6682.07 | 3158.46 | 1579.23  | 12.28   | 0.001 |
| Interaction    | 1  | 605.04  | 605.04  | 605.04   | 4.70    | 0.051 |
| Residual Error | 12 | 1543.42 | 1543.42 | 128.62   |         |       |
| Total          | 15 | 8830.52 |         |          |         |       |

S = 11.34 \[ R^2 = 82.5\% \quad R^2 (adj) = 78.2\% \]

### Table 2: Estimated Regression Coefficients for percentage weight reduction after 72 hrs

| Term      | Constant | Enzyme A | Temp | Enzyme A*Temp |
|-----------|----------|----------|------|---------------|
| Coef      | -17.27   | -0.04    | 0.47 | 0.00          |
| St Dev    | 31.8256  | 0.0291   | 0.6212 | 0.0006        |
| T         | -0.543   | -1.546   | 0.762 | 2.169         |
| P         | 0.597    | 0.148    | 0.461 | 0.051         |

For the linear analysis, the high F value and the P value of zero indicates the goodness of the data and the R² value of 82.5% is very reasonable and an excellent indicator of the fitness. But for the interaction analysis, the F value is relatively low and the P value is 0.051, relatively higher than for linear one. Thus the reaction parameters are more linear in nature than interactive between them. The change in one parameter affects only one of the other
parameters; here weight reduction, and not the others. The contour plot and the response plot (wire plot) were obtained and they show that the parameters are linear in nature and that there is no or very less interaction between the parameters, given by the absence of any curve in the wire plot and the absence of circular waves in the contour plot.

![Contour Plot of % Wt red](image)

**Figure 7:** Surface plot for RSM analysis for interactions between enzyme activity, temperature and weight reduction after 72 hrs

![Contour Plot of % Wt red](image)

**Figure 8:** Contour plot for RSM analysis for interactions between enzyme activity, temperature and weight reduction after 72 hrs

Response Surface Regression II: Response surface analysis and the ANOVA analysis for the % weight reduction, time and Enzyme activity at 55°C were also done. The results of the analysis are given in Table 3.
Table 3: Analysis of Variance for percentage weight reduction at 55°C

| Source          | DF | Seq SS   | Adj SS   | Adj MS   | F       | P     |
|-----------------|----|----------|----------|----------|---------|-------|
| Regression      | 3  | 10349.4  | 10349.38 | 3449.79  | 148.89  | 0.000 |
| Linear          | 2  | 9529.1   | 9529.12  | 4764.56  | 205.63  | 0.000 |
| Interaction     | 1  | 820.3    | 820.26   | 820.26   | 35.40   | 0.000 |
| Residual Error  | 24 | 556.1    | 556.10   | 23.17    |         |       |
| Total           | 27 | 10905.5  |          |          |         |       |

The analysis was done using coded units and the coefficients are given in Table 4

Table 4: Estimated Regression Coefficients for percentage weight reduction at 55°C

| Term             | Coef | time | conc | time*conc |
|------------------|------|------|------|-----------|
| Constant         | 19.90| 24.63| 11.29| 10.89     |
| St Dev           | 0.9097| 1.3645| 1.2205| 1.8307    |
| T                | 21.878| 18.047| 9.250| 5.950     |
| P                | 0.000| 0.000| 0.000| 0.000     |

S = 4.814  \quad R^2 = 94.9\%  \quad R^2 (adj) = 94.3\%

Model Equation

From the ANOVA analysis, the coefficients for the model equation are obtained. The ANOVA analysis of this data was also found to be good, as seen from the high F values and the zero values of the P. Also the \( R^2 \) value, an important parameter of ANOVA is as high as 94.9\% which is typical for a good fitness of the data. Here also, for the linear analysis the P value is zero and F value is high. But for the interaction analysis, the F value is relatively low, although the P value is zero. This is evident from the absence of smooth curve or bending of the wire plot. The response (wire) plot and the contour plots of the analysis are given below:

The coefficients given by the ANOVA are the coefficients for the parameters time (\( X_1 \)), enzyme activity (conc.) (\( X_2 \)). From the given values of the ANOVA analysis, the model equation for the estimation of the % weight reduction is given as

\[ Y = 19.90 + 24.63X_1 + 11.29X_2 + 10.89X_1X_2 \]

where \( Y = \) Weight reduction Term

\( X_1 = \) Time factor \( = (T-36)/36 \), where \( T = \) actual Time in hrs,

\( X_2 = \) Enzyme activity factor \( = (C-1000)/600 \), where \( C = \) actual enzyme activity in U/ml
Figure 9: Surface plot for RSM analysis giving the interaction between percentage weight reductions, time and enzyme activities (conc.) at 55°C

Figure 10: Contour plot for RSM analysis giving the interaction between percentage weight reductions, time and enzyme activities (conc.) at 55°C

Figure 11: Actual Vs Model predicted Weight reduction at 55°C and enzyme activity = 1600 U/ml
From the plot Fig 11, the actual % weight reduction Vs the ANOVA statistical predicted % weight reduction, it can be seen that the actual experimental data are in accordance with the predicted, over all enzymatic activities, thus showing the high fitness of the experimental data obtained.

CONCLUSION

From this study it was found that Bisphenol A Polycarbonate can be degraded by the lipase Candida rugosa. The maximum % weight reduction of the polymer was found to be 61.55% at 55°C and time period of 48 hours. FTIR analysis of degraded product confirms it to be Bisphenol A, a monomer of BPA polycarbonate. The Response Surface Methodology analysis carried out on the experimental data and the parameters were found to be non interactive and are linear in nature. Further analysis with RSM will give a better insight into the kinetics of the degradation.

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