

Kinetic Studies of Polycaprolactone Production Using Lipase Enzyme as Catalyst

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Abstract. Enzymatic polymerization is one of the fastest progressing fields to produce biodegradable polymers like polycaprolactone. Poly (ϵ -caprolactone) (PCL) is attractive aliphatic polyester due to its favorable biodegradability, biocompatibility, and permeability characteristics. In this paper, kinetic studies on enzymatic catalyzed polymerization process of converting ϵ -caprolactone to polycaprolactone with commercial lipase enzyme (Novozym435) as catalyst using ring opening polymerization (ROP) in a bioreactor was carried out. The influence of temperature and reactor impeller speed on molecular weight was determined using the Michaelis menten equation and Arrhenius equation. The kinetic model was developed to represent the relationship of product concentration with time at various temperatures and various impeller speeds.

Keywords: Enzymatic polymerization, Lipase enzyme, kinetics, Michaelis menten equation, Product concentration.

1. Introduction

Enzymatic polymerization is one of the fastest progressing fields to produce biodegradable polymers like polycaprolactone. Poly (ϵ -caprolactone) (PCL) is attractive aliphatic polyester due to its favorable biodegradability, biocompatibility, and permeability characteristics. The seven-membered unsubstituted lactone ϵ -CL is the most extensively studied with respect to lipase-catalyzed ring-opening polymerization. Researchers focusing on the kinetics of enzymatic polymerization process include Mee et al [1] carried out the kinetic studies where in the Michaelis-Menten constants K_M and V_{max} were measured for various lactones, which revealed that the K_M was more or less independent of the ring size, suggesting similar affinities of the lipase for all lactones, while no obvious trend could be discerned for V_{max} . Jiutong ma et al [2] carried out the ring-opening polymerization of ϵ -caprolactone catalyzed by a novel thermophilic esterase from the archaeon *Archaeoglobus fulgidus* (*AFEST*) successfully conducted in organic solvents. Mei et al [3] performed the studies of the kinetics and mechanism of *Candida antarctica* Lipase B (CALB) catalyzed ϵ -caprolactone (ϵ -CL) polymerizations in toluene where in effect of enzyme water content on the time course of ϵ -CL polymerization was determined. Even though there have been researches on kinetic aspects not many have focused upon studying the effect of temperature, reactor impeller speed etc. with that of the output of the reaction which is the molecular weight. In this paper, kinetic studies on enzymatic catalyzed polymerization process of converting ϵ -caprolactone to polycaprolactone with commercial lipase enzyme (Novozym435) as catalyst using ring opening polymerization (ROP) was carried out. Investigations were carried out on the influence of temperature on the rate of formation of the product and rate of consumption of the monomer using the Michaelis Menten equation and Arrhenius equation.

2. Materials and Methods

Novozyme-435 (specified activity 7000 PLU/g) was purchased from Science Technics Pte. Ltd. Polymerization grade ϵ -caprolactone purchased from Merck Pte. Ltd, was first dried over calcium hydride

and then distilled under reduced pressure in nitrogen atmosphere. Chloroform and toluene, were purchased from Merck Pte. Ltd. Toluene was dried over calcium hydride, and distilled under nitrogen atmosphere.

The **Reactor Control Unit** includes a chiller unit, involving a chiller (L), cold tank (F) and hot water tank (B) filled with water. Water inlet valve (M) and water outlet valve (O) were opened. The valve to control the water flow rate from the chiller and back to the chiller was regulated. The hose of the water inlet (J) and outlet (K) to the jacketed glass reactor was connected. The water pump at the control panel (G) was turned on. The chiller unit (L) was switched on and temperature was set to the desired value. The heater was turned on and temperature was set to the desired value.

The solvent (Toluene) to monomer (ϵ -caprolactone) to catalyst ratio (Novozym 435) taken up for the experiment is 2:1:10 (v/v/wt). The effective volume of the reactor is 2 litres which is the total volume for solvent and monomer. The chemicals and enzyme were fed into the reactor. The reactor was started and the reactor temperature was allowed to rise to the desired value. The stirrer was started and set to the desired speed. As the mixture starts to react and the sample were removed every 1 hr until 7th hr.

3. Development of the kinetic Model for Biopolymerization of ϵ -Caprolactone to Polycaprolactone

The reaction mechanisms have to be interpreted into conventional reversible reactions appearances. So a set of reversible reactions representing the biopolymerization process can be written as below [4],



[M] = Monomer, [E] = Enzyme, [EM] = Enzyme monomer complex, [HA] = hydroxyl carboxylic acid, [P] = Product. But in order to carry out kinetic studies there is a need to assume that the process is irreversible. Therefore to obtain the kinetic model for the existing process the Michaelis-Menten equation is used.

3.1. Michaelis-Menten Kinetics

The Michaelis-Menten mechanism for basic one-substrate enzyme reactions consists of three elementary reactions steps:



E= Enzyme, S=substrate, ES= Enzyme substrate complex, P = product

The standard Michaelis-Menten equation, based on one-substrate- one product is given as:

$$-r_s = \frac{k'_3 [E_t] [S]}{[S] + K_m} \quad (5)$$

where [S] is the substrate concentration (mol/cm³), [E_t] is the total enzyme concentration (mol/cm³). K_m represents Michaelis-Menten constant (mol/cm³) and k'₃ = k₃[W] is the rate constant (s⁻¹).

Let V_{max} represent the maximum rate of reaction, for a given total enzyme concentration, [E_t]. Hence,

$$V_{max} = k'_3 [E_t] \quad (6)$$

From eq.(1) the Michaelis Menten equation can simply be rewritten as:

$$-r_s = \frac{V_{\max}[S]}{[S] + K_m} \quad (7)$$

Considering the biocatalytic reaction, substrate A was converted into product B through an enzymatic reaction. Therefore, the reaction rate, based on the Michaelis-Menten equation derived previously (in respective to this particular biocatalyst reaction for the decomposing rate of substrate A) is given by:

$$r_A = \frac{k_3' C_A C_{Et}}{C_A + K_m} \quad (8)$$

where r_A represents the moles of substrate A decomposing per hour per cubic meter of reacting mixture, C_A shows the concentration of A in the reacting mixture, C_{Et} is the total enzyme concentration, and K_m represents the Michaelis-Menten constant. Applying the Arrhenius equation for the rate constant k_3'

$$k_3' = A \cdot e^{-E/RT} \quad (9)$$

Equation (4) gives us the rate of the reaction which is the rate of change of the product dP/dt . On integration of Equation (4) the value of P, the concentration of the polymer can be obtained.

4. Result and Discussion

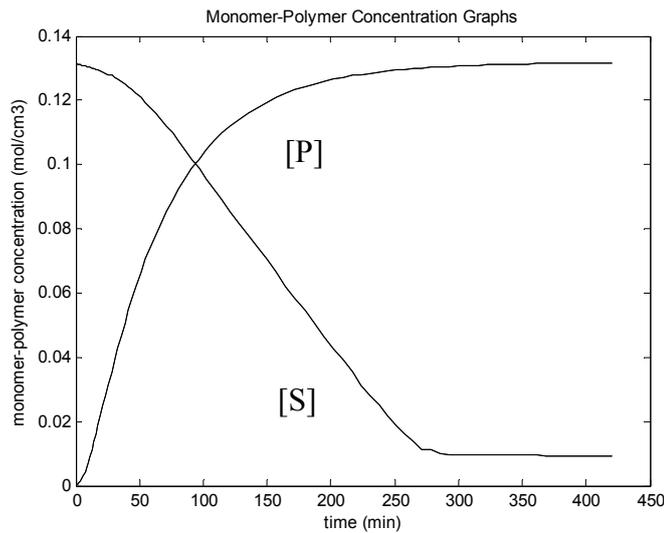


Fig 1: Monomer (substrate)-polymer concentration plot from mechanistic model

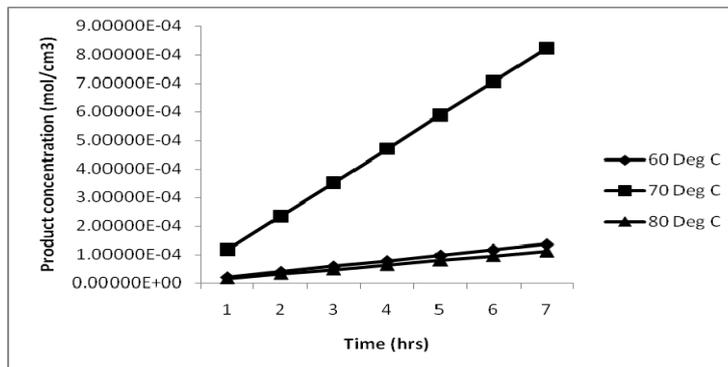


Fig 2: Effect of Temperature on product concentration at 250 RPM

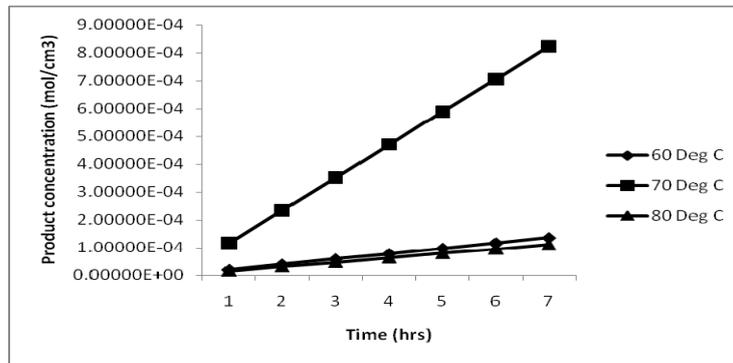


Fig. 3: Effect of Temperature on product concentration at 500 RPM

With the availability of k values, the differential equations that represent elements in biopolymerization process can be solved. The pieces of the equations are brought together and the equations show the results are really worthwhile. By plugging in all the k values, the trend of the plots is observed. By right, the trend of the plots should exhibit the same trend as in Figure 1 which is the representation of time versus monomer-polymer concentration for the mechanistic model where in it is found that as the time varies between 1 hr to 7 hrs the product concentration increases and the monomer concentration decreases. If an increasing trend is followed by the product concentration, then it is assumed that the kinetic model is acceptable. The following plots represent the relationship between the impeller speed and time at various temperatures and also temperature and time at various impeller speeds. Fig 2, 3, 4, 5 are the plots which show the effect of temperatures 60 °C, 70° C, 80 ° on product concentration at C impeller speeds 250, 500, 750,1000 RPM. The increasing profile of product concentration with time indicates that kinetic model is validating the experimental results and the mechanistic model. The initial monomer concentration is 0.00278 mol/cm³.

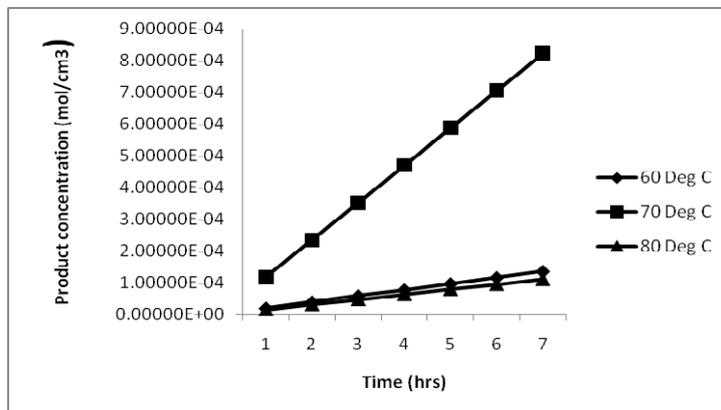


Fig. 4: Effect of Temperature on product concentration at 750 RPM

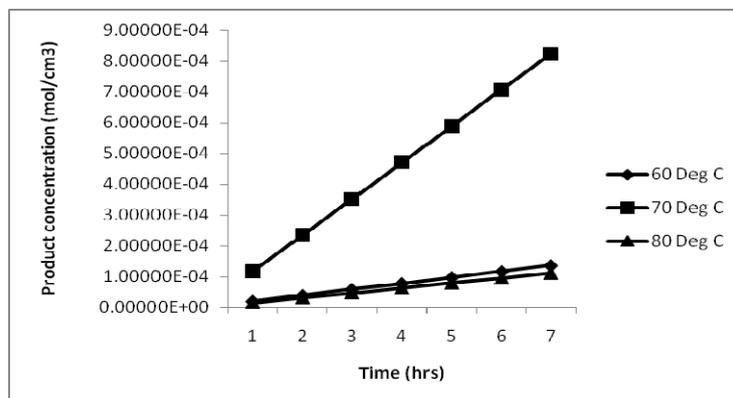


Fig. 5: Effect of Temperature on product concentration at 1000 RPM

4.1. Effect of Impeller Speed on Monomer Concentration at 60°C, 70°C, 80 °C

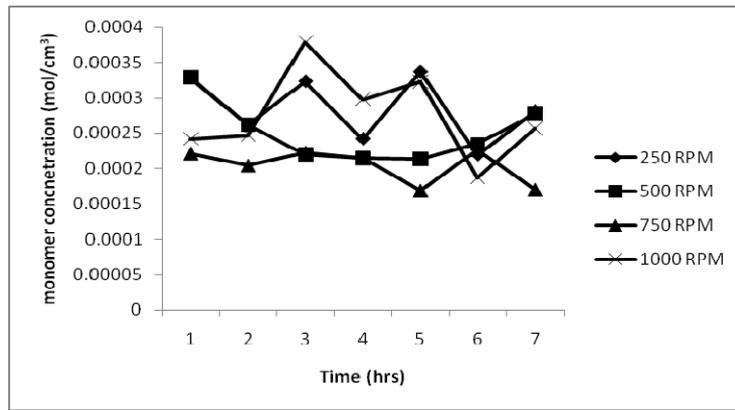


Fig. 6: Effect of Impeller speed on monomer concentration at 60°C

Fig 6,7,8 indicated that even though there is not a linear decrease of the monomer concentration as one would expect from the reference plot of fig 1, there is a uniform trend that is followed in each of the figure showing that the behaviour of the system in terms of monomer concentration is similar for all the temperatures 60°C, 70°C, and 80 °C. The decreasing profile of monomer concentration with time indicates that kinetic model is validating the experimental results and the mechanistic model.

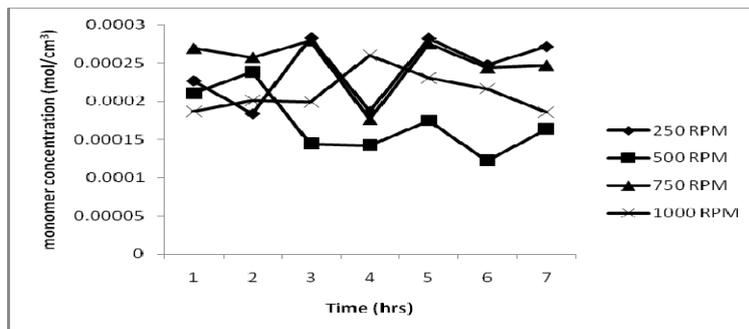


Fig. 7: Effect of Impeller speed on monomer concentration at 70°C

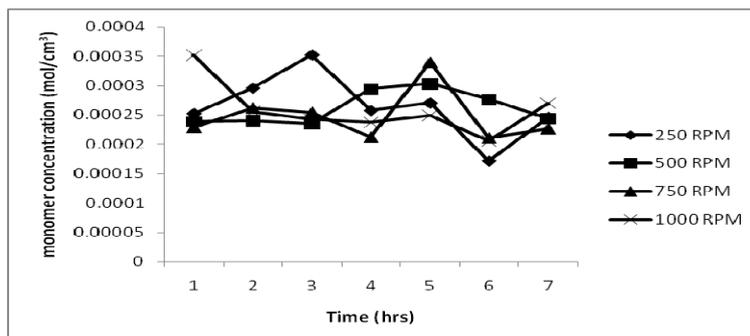


Fig. 8: Effect of Impeller speed on monomer concentration at 80 °C

5. Conclusion

In this paper a kinetic model is developed based on the Michalis menten equation and Arrhenius equation in order to obtain a relationship between the product concentration and the impeller speed and temperature for the enzymatic catalyzed polymerization process of converting ϵ -caprolactone to polycaprolactone with commercial lipase enzyme (Novozym435) as catalyst using ring opening polymerization (ROP) in a bioreactor.

6. Acknowledgement

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7. References

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