Experimental Investigation of Reaction Kinetics and Mass Transfer data for Hydrolysis of Penicillin

Amin Ismaili, Keyur Thaker, Shuchen B. Thakore, Umesh Bhardwaj, Jalpa Shah and Gopal H Chaudhari.
E-mail: sbthakore@yahoo.co.in, jalpa6019@gmail.com,gopalhc2006@yahoo.co.in,

Abstract

A bio-catalytic hydrolysis of penicillin-G potash salt (PenGK) has been studied experimentally for reaction kinetic and mass transfer data. Effect of various process parameters on rate constant (k) & reaction order (n) has been studied. In a study carried out in kinetically controlled regime, it was observed that "k" increased with increase in temperature & catalyst loading, while "n" was decreased marginally. No significant effect of PenGK concentration was found on reaction kinetics. On other end "k" was increased significantly with increase in RPM & decrease in particle size, while operated reaction in mass transfer controlled regime. Estimated value of mass transfer co-efficient (ks_L) was increased with increase in RPM below "Just suspension speed Njs". Approach was further extended for reactor design. Various reactor schemes of CSTRs and PFRs in series has been evaluated. It was identified that two CSTR in series found to be most efficient reactor configuration with highest level of conversion.

Keywords: Penicillin-G ,Penicillin amidase, Hydrolysis, 6-APA, Reaction kinetics.

1. Introduction

One of the most important and established processes in the pharmaceutical industry is the enzymatic production of 6 Aminopenicillanic Acid (6-APA). Immobilization of penicillin-G amidase enzymes to form 6-APA in the synthesis of semi synthetic penicillins such as ampicillin, amoxicillin, cloxacillin, dicloxacillin etc. forms one of the most applied processes in the industry. 6-APA is a semi synthetic antibiotic with a broad spectrum of bactericidal activity against gram positive and gram negative micro-organisms and is one of the major β-lactam antibiotics as a bulk formulated drug. Conventionally the enzyme used is immobilized on various solid supports [1,2]. It is always important to operate the process economically, i.e. utilization of enzyme in every batch cycles. Ospina [3] has shown that Penicillin reactors operate at a much lower temperature than the optimal value, but with twice the stability in terms of half-life of the enzymes.

The most critical parameter concerning the stability of the enzymatic reaction has been known to be reaction pH. Formation of phenyl acetic acid (PAA) during hydrolysis leads to decrease in pH. If the pH drops below 4.5 to 5, inactivation of enzyme is accelerated [4].

Various other factors that also affects the reaction conversion or efficiency, i.e. Reaction temperature, Reaction batch time, Penicillin-G Substrate concentration, % Enzyme Loading, Microbial contamination to reaction water or enzymes, Enzyme properties & Enzyme biological activity, Presence of enzyme inhibitors, Mode of Operations (Batch & Continuous), applied reaction technologies, and kinetics of substrate and product degradation.

Pangarkar, et. al. [5], discussed the effect of various process parameters affecting conversion of 6-APA., i.e. reaction pH, catalyst loading and substrate concentration. They operated hydrolysis in batch mode by varying enzyme concentrations. They found that there was a sharp increase in the reaction rate & penicillin conversion (56 % to 96 %) with increase in enzyme concentration (8 % to 10 % w/w of Penicillin). On other experiments carried out to study the effect of substrate concentration, they found that with increase in substrate concentration (1 % to 5 % w/w), substrate inhibition and byproduct (PAA) formation increases which will lead

to lower final conversion (97 % to 50 %). They concluded that at least 8-10 % w/w of penicillin concentration is economical for industrial manufacturing processes. Some experiments carried out in semibatch mode, they found that at lower reaction pH, the rate of the enzymatic reaction decreases. In a set of trials carried out with different Alamine 336 concentration, they found that with increasing Alamine 336 concentration (15 to 25 % w/w), increases the tendency of organic solvent to extract PAA. Marconi et. al. [6], operated 6-APA hydrolysis in continuous mode by using a Fibers reactor. In which penicillin acylase enzymes was physically entrapped into cellulosic fibers. Hydrolysis was operated by using 12 % w/w Penicillin G solution , while pH was adjusted by using NaOH solution. Hydrolysis was operated by (46.5 to 360 lit/hr) .Kinetic data estimated showed that about 90 % hydrolysis completed without any diffusion problem. Hasal, et.al. [7], studied an Electro-Membrane reactor for continuous 6-APA preparation. Hydrolysis of penicillin-G was carried out in a flow-through; miniature electro-membrane reactor where penicillin acylase enzymes (5 % w/v) was entrapped in a polyacrylamide gel slab , charged with an electric field . Hydrolysis was carried out by using 2 % w/v substrate concentration, while pH was adjusted to 8.0 by using 50 mM Phosphate buffer at temperature 30 °C. The conversion of penicillin G increased from 0.15 to 0.5 by applying electric current density -600 to +600 A/m^2 for batch residence time of 60 minutes. They concluded that the apply of electric field in reaction zone can enhance the electrophoretic transport of the H+ and OH- ions within the reaction slab , which was easily controllable with reference to reaction pH in course of reaction. On other way Bossi et.al. [8], applied electric filed to enzymes directly for the hydrolysis of penicillin G. The enzymes were trapped within an isoe-lectric membrane chamber (pI 5 and pI 9 atmospheric). A novel class of zwitterionic buffers was used to control reaction pH in range of 3.0 to 10.0. A doubly discontinuous mode of operation could achieve 96% conversion within 4-8 mins. Wandrey et.al [9], operated penicillin hydrolysis process in continuous mode, where the pH was controlled by using NaOH solution.. A combination of continues stirred tank reactor (CSTR) followed by a plug flow reactor (PFR) was used with lower substrate concentration (2.6 % w/v) to achieve higher conversion of 6-APA (CSTR – 95.5 % and PFR – 98.0 %). Product 6-APA was further concentrated by reverse osmosis (RO) to shift iso-

electric point at higher values for 6-APA precipitation. Debnath et.al [10], operated for continuous production of 6-APA in a packed column reactor by using agarose immobilized penicillin-G acylase as a block polymer. In which penicillin acylase was physically entrapped on agarose gel. and immobilized enzyme was packed in the column reactor. It reduces the accumulation of 6-APA and has an inhibitory affect on penicillin acylase. They observed that Conversion of 6-APA obtained is maximum at temperature (37°C) and pH (8) with substrate concentration (2% w). Dennen et. al. [11], investigated critically the degradation kinetics of 6-APA due to rupture of β-lactam ring. Degradation is followed pseudo first-order kinetics with initial concentration of 0.05 – 0.92 and in range of pH (5.8-6.6) and temperature in range of (35-90 ° C). The degradation rates were of a higher order with increasing pH. The maximum stability of 6-APA at all temperatures was at pH 8.0. Sudhakaran1 et.al [12], identified that a re-circulated packed bed reactor filled with immobilized penicillin G acylase can be used for Penicllin G hydrolysis. Reaction pH was controlled in an external circulation loop .They observed that the flow rate of penicillin-G solution was a rate limiting step for its hydrolysis and the maximum rate of hydrolysis of penicillin G was obtained at a flow rate of 3.0 L/min. Xia. et. al [13], operated Penicillin G hydrolysis in three phase liquid system. The method used dodecane as organic solvent and PEO-PPO-PEO (polymer complex) as a covalent phase. In Reaction mass , a three phase system i.e. Top (upper) organic phase contains extracted PAA , bottom (lower) phase hold enzymes covalently in polymeric system while, middle phase contain aqueous 6-APA at higher pH conditions. Method reduce the degradation of enzymes at lower pH conditions and improve the reaction kinetics in the direction of 6-APA conversion. Wang et.al [14], investigated a direct extraction of PAA from hydrolysis of Penicillin–G. They used different non ionic surfactants – which on addition riches to requisite cloud point temperature of reaction, which was favorable to extract PAA from reaction zone without pH adjustment by using any alkali. These cloud point extraction decreases the inhibition of enzymes by the acidity generated due to PAA and shifts the reaction equilibrium towards further hydrolysis. They observed that the conversion of 6-APA increases steadily with increasing surfactant concentration. Pangarkar, et.al [15], used an alternative method to neutralized Phenyl acetic acid (PAA) generated during the

hydrolysis of Penicillin G. Alamine 336 (diluted with kerosene) was used to remove PAA from reaction mass, extracted PAA in the organic phase was back extracted with stronger & low molecular weight Di-Methyl Amine (DMA). Advantage was the shifting of reaction equilibrium towards desired product of 6-APA.

In the present work, effect of reaction kinetic parameters such as temperature, % catalyst loading and substrate concentration have been evaluated. Also the effect of mass transfer parameters such as intensity of mixing and enzyme particle size has been studied.

2. Experimental Setup

The enzymatic reactor is a Stirred Tank Reactor (Figure 1) having 2000 ml working & 2300 ml total capacity. A glass cylindrical shell having internal diameter (A) of 124 mm and clear liquid height of 163 mm is placed with a 400 mesh size SS-316 screen (B) at the bottom of reactor to hold fine enzyme catalysts in vessel. The equipment is facilitated with a central SS-316 shaft (O), bolted with two nos. of SS-316 impellers (45° Down-flow Pitch blade Turbine)(C)(Figure 2) located apart to keep enzymes in uniform suspension. Agitation system is provided with a dry mechanical seal (D) and an induction motor (E) (¼ HP, 1440 rpm, 230 Volts, 1 Ø , Make: CROMPTON GREAVES). A digital RPM controller (F) (Make: DELTA) can facilitate the alteration of agitation level potentiometrically in range of 50 to 1200 rpm. A facility of top insertion of pH electrode (G) can measure & control the reaction pH in range of (7.8–8.2) with a pH controller (H) (Make: PROMINENT, Model: PHD Dulcometer). Reaction acidity due to generation of Phenyl Acetic acid is neutralized continuously by dosing of 4 % w/w aqueous ammonia (I) solution by diaphragm type dosing pump (J) (Make: PROMINENT, Model: C 10.006PP). Reaction temperature was maintained by circulating hot water through reactor jacket (HWI & HWO) by using a digital temperature controller (Make: KUMAR, Model: KI33AE02) (Not shown in Figures). Reaction temperature was accurately measured by a RTD (K) (3 Wire , Pt -100 , Teflon coated ,

Make : ALTOP) having measurement precision of ± 0.01° C. Reaction mass samples for HPLC were collected in glass vials (10 ml) from a sampling valve (N) located at the bottom of reactor. Reactor temperature, Hot water utility temperature, pH of Reaction Mass and RPM of reactor was continually monitored by using a Data Acquisition System (L) (DAS).(Make : NIPPON , Model:UNSC-16). Said process parameters were logged in a computer (M) through a software "NippoLog R2" with real time. (Make: NIPPON).

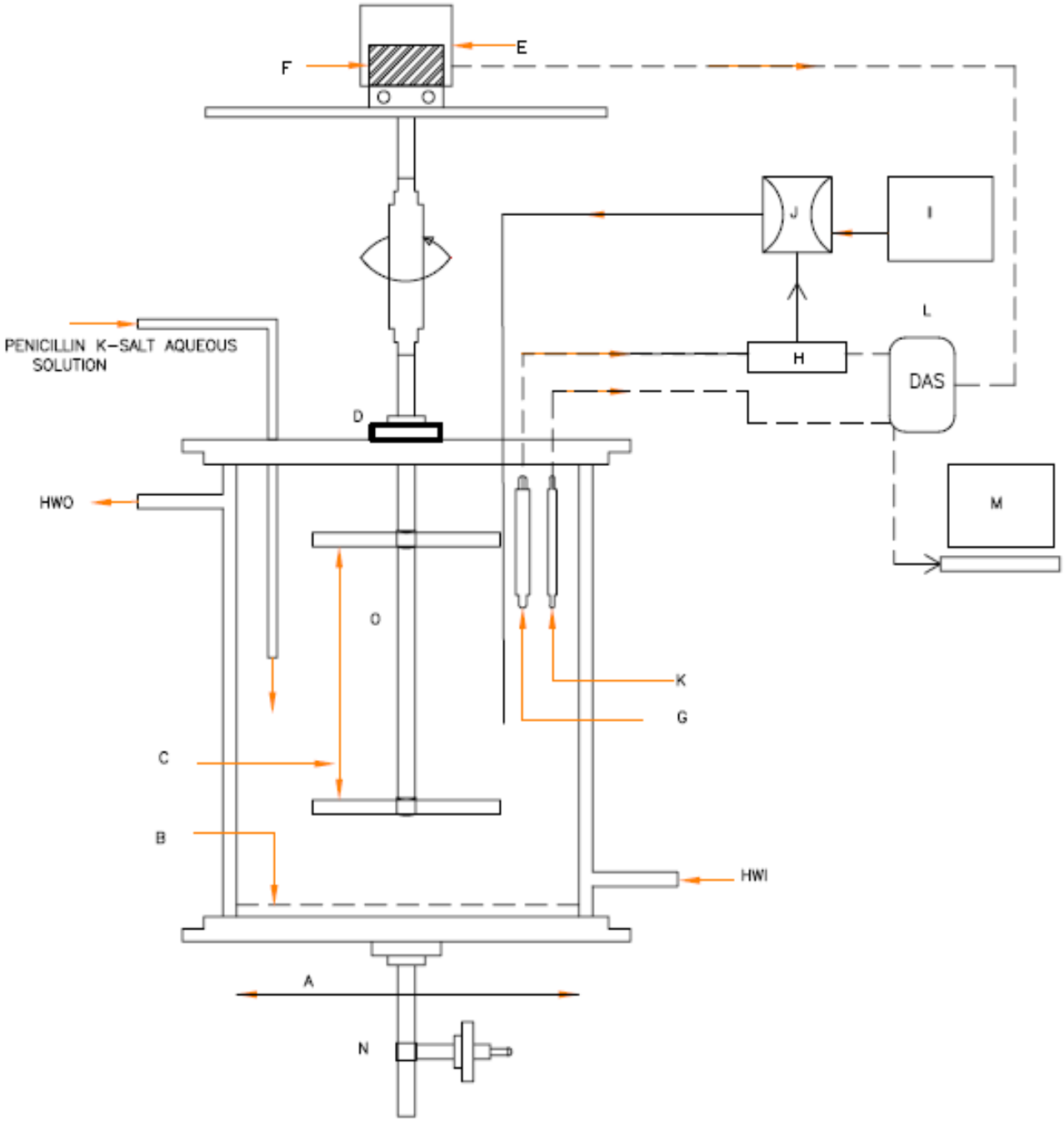

Fig.1 Schematic Diagram of Enzymatic reactor.
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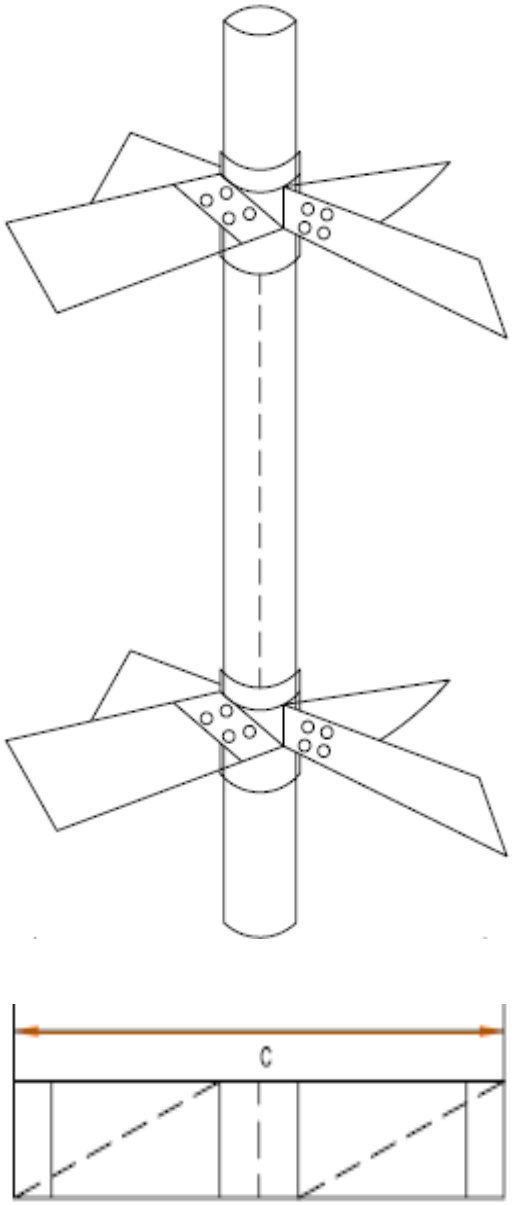

Fig.2 45°C Deg Pitched Blade Downflow Turbine.
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3. Materials & Method

3.1 Experimental Procedure

Aqueous solution (4 % to 12 % w/w) of Penicillin-G - Potash salt (KPG) had been prepared by dissolving required quantity of KPG in De-mineralized Water.Enzymatic reactor was pre-filled with Enzymes (Penicillin Amidase – Immobilized on polyamide beads). Solid Enzymes have (Bulk density about 0.9 gm/ml, Particle size in range of 150 microns, and biological activity NLT 150 Units/mg). Required temperature in range of 28-38 deg C is maintained in reactor by circulating hot water through jacket of Enzymatic Reactor. During Enzymatic reaction RPM is kept in range of 312 to 512 to keep enzymes in complete suspension. Generated PAA is continuously neutralized & pH is maintained in range of 7.8-8.2 by dosing 4 % w/w aqueous ammonia (100-120 ml) solution through pH controller through out the reaction.

Chemical Reaction [Bio-catalytic De-Acylation of Penicillin G Potash salt]**.**

3.2 Chemical Reaction

Bio-Catalytic De-Acylation of Penicillin G Potash salt is shown in Fig.3.

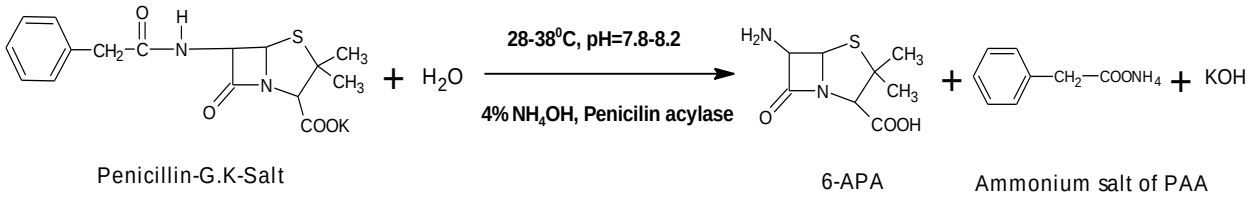

Fig.3 Enzymatic Hydrolysis of Penicillin –G K Salt .
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3.3 Concentration Analysis Method

The Concentration of reaction components were determined by HPLC using a Lichrospher-100 RP- C18 Column (4mm i.d × 250 mm) with 0.163 M phosphate buffer, pH 4.15 /methanol (74.78 %v/v)/ Acetonitrile (08.69 %v/v) as mobile phase at 1.2 ml min-1 at ambient temperature with evaluate monitored at 220 nm. Isocratic elution pump followed by gradient elution column with 5µ packing beads at 200 psi pressure was used for analysis.

3.3 Calculations

3.3.1 Estimation of Activation Energy and Frequency Factor (ArheniusTheory)

Sr.	Temperautre (° C)	k (Rate constant)
1	30	0.8150
2	33	0.9480
3	36	1.0160
4	38	1.1206

Method Used : Multivable non-linear regress analysis – leaset square fitting

Regression co-efficient: 0.98

Ea = 7056.278 cal/mol
K_0 = 100904.38

3.3.2 Mass-Transfer Coefficient Determination

Data :

Type of Impeller : 45 C Pitch blade turbine
Impeller speed : 315 RPM
Vessel diameter(T) : 125 mm =12.5 cm
Impeller diameter(Di) : 72 mm = 7.5 cm
Viscosity of Solution(µ) : 1 cp
Density of Solution : 1 g/cm^3
Diffusivity of Solute in Solution (D_A) : $2 * 10^{-5}$ cm^2/sec
Power input per unit mass of liquid (ϵ_p)
Catalyst particle size : 100 µ

Equation:

$$\frac{k_{sL} d_p}{D_A} = 2 + 0.47\left(\frac{dp^{4/3} \in_p^{1/3}}{\nu}\right)^{0.62}\left(\frac{Di}{T}\right)^{0.17}\left(\frac{\mu_L}{\rho_L D_A}\right)^{0.36} \quad \text{---------- (1)}$$

So,

$$k_{sL} = \frac{D_A}{d_p}\left[2 + 0.47\left(\frac{d_p^{4/3} \in_p^{1/3}}{\nu}\right)^{0.62}\left(\frac{Di}{T}\right)^{0.17}\left(\frac{\mu_L}{\rho_L D_A}\right)\right]^{0.36}$$

Where,

$k_{sL} = Mass - transfer - coefficient$

$D_A = Diffusivity - of - So.lute - in - solution$

$dp = Catalys - particle - size$

$\in_p = The - power - input - per - unit - mass - of - liquid$

$\nu = Kinematic - viscosity$

$Di = Diameter - of - \mathrm{Im}peller$

$T = Diameter - of - vessel$

$\mu_L = Viscosity - of - solution$

$\rho_L = Density - of - Solution$

Power Input:

$$p = N_p \rho_L N^3 Di^5$$

$$N_p = Power - number$$

Impeller speed: N

$$N = \frac{315}{60} rps$$

$$N = 5.25 rps$$

Power Number for Pitch blade turbine Np = 1.2
Density of Solution = 1 g/cm^3
Impeller diameter = 7.5 cm

$$p = N_p \rho_L N^3 Di^5$$

$$Power - Number - P = \left(1.2 \times 1 \times 5.25^3 \times 7.5^5\right)$$

The Power Input per unit mass of liquid ($\in$p):

$$\in_p = \frac{p}{(\pi/4)d^2 \times H \times \rho_L}$$

$$P = Power - No$$

$$d = Diameter - of - vessel = 12.5cm$$

$$H = Height = 14cm$$

$$\rho_L = Density - of - solution = 1 - g/cm^3$$

$$\in_p = \frac{4.073\times10^6}{(\pi/4)(12.5)^2(14)(1)}$$

$$\in_p = 2370.69 - erg/g.\sec$$

$$\in_p = 2.370\times10^3 - erg/g.\sec$$

$$catalyst - particle - diameter - dp = 100\mu = 1\times10^{-2}\,cm$$

$$viscosity - of - solution = 1\times10^{-2}\,cm^2/\sec$$

$$Diameter - of - \mathrm{Im}\,peller = 7.5cm$$

$$Diameter - of - vessel = 12.5cm$$

So from the equation-(1) we can calculate the value of Mass-Transfer Coefficient (ks_L):

$$k_{sL} = \frac{D_A}{d_p}\left[2 + 0.47\left(\frac{d_p{}^{4/3} \in_p{}^{1/3}}{\nu}\right)^{0.62}\left(\frac{Di}{T}\right)^{0.17}\left(\frac{\mu_L}{\rho_L D_A}\right)\right]^{0.36}$$

$$k_{sL} = \frac{2\times10^{-5}}{1\times10^{-2}}\left[2 + 0.47\left(\frac{(1\times10^{-2})^{4/3}(2.370\times10^3)^{1/3}}{1\times10^{-2}}\right)^{0.62}\left(\frac{7.5}{12.5}\right)^{0.17}\left(\frac{1\times10^{-2}}{1\times2\times10^{-5}}\right)^{0.36}\right]$$

$$k_{sL} = 0.002\,(4.75775)$$

$$k_{sL} = 0.0195\,cm/\sec$$

$$k_{sL} = 1.95\times10^{-2}\,cm/\sec$$

3.3.3 Just Suspension speed (N_{js}) Determination

Theoretically if we increase the RPM of CSTR above the Just suspension speed then there will be no effect of RPM because the particle are already suspended at its just suspended speed. So above the just suspension speed of particles there will be no effect of mass transfer effect on reaction [16].

Concentration of Pen-G Solution: 8 %

Enzyme particle size : 250 µ

CSTR	Total Enzyme Taken	% of Total Enzyme	Final Enzyme Taken
CSTR-I	200 g	20 %(520)	104 g
CSTR-II	200 g	30% (520)	156 g
CSTR-II	120 g	50% (520)	260 g
	520 g	100 %	520 g

Calculation for CSTR-I :

Enzyme taken: 200 g
Pen-G taken: 8%
Temp: 29 C

Estimation of Just Suspension Speed (N_{js}):

$$N_{js} = \frac{s\nu^{0.1} d_p^{0.2} (g\Delta\rho/\rho_L)^{0.45} (X)^{0.13}}{Di^{0.85}}$$

Where,

$S = Constant$

$\nu = Kinematic - viscosity, m^2/\sec$

$g = gravitational - constant, m/\sec^2$

$\rho_L = Density - of - Solution - water, kg/m^3$

$\rho_s = Densityof - Enzyme, Kg/m^3$

$X = Weight - percentage - of - Solids.$

$T = Reactor - InsideDiameter, m$

$Di = Impeller - Diameter, m$

$d_p = Particle - Size, \mu$

Solution:

$\text{Re}\,actor - Dia = 0.125m$

$\text{Im}\,pellerDia = 0.072m$

$$s = 2\left(\frac{T}{Di}\right)^{1.33}$$

$$s = 2\left(\frac{0.125}{0.072}\right)^{1.33}$$

$$s = 4.165$$

$$\nu = Kinematicvis\cos ity$$

$$Absolute - Vis\cos ity - of - water = 10^{-3} kg/m.\sec$$

$$Density - of - Solution - water = 1000\, kg/m^3$$

$$Density - of - Enzyme = 900\, kg/m^3$$

$$\nu = \frac{Absolute - vis\cos ity}{Density}$$

$$\nu = \frac{\mu}{\rho}$$

$$\nu = \frac{10^{-3} kg/m.\sec}{1000 kg/m^3}$$

$$\nu = 10^{-6} m^2/\sec$$

$$g = gravitational - cons\tan t = 9.81 m/\sec^2$$

$$\Delta\rho = p_L - \rho_s = 1000 - 900 = 100 kg/m^3$$

$$weight - of - Enzyme = 200g$$

$$volume - of - solution(overflow) = 2300ml$$

$$X = (\frac{wt}{wt + vol}) \times 100$$

$$X = (\frac{0.2}{0.2 + 2.3}) \times 100$$

$$X = 8$$

$$d_p = Particlesize = 250 \times 10^{-6} m$$

$$N_{js} = \frac{s\nu^{0.1} d_p^{0.2} (g\Delta\rho / \rho_L)^{0.45} (X)^{0.13}}{Di^{0.85}}$$

$$N_{js} = \frac{(4.165)(10^{-6})^{0.1}(250\times10^{-6})^{0.2}\left(\frac{9.81\times100}{1000}\right)^{0.45}(8)^{0.13}}{(0.072)^{0.85}}$$

$$N_{js} = \frac{(4.165)(0.251)(0.190)(0.9914)(1.32104)}{(0.1068)}$$

$$N_{js} = 2.4357 - rev/\sec$$

$$N_{js} = 2.4357\times60 - rev/\min(rpm)$$

$$N_{js} = 146 - rev/\min(rpm)$$

3.3.4 Selection of best reactor configuration for serious of Contactors:

1st Contactor	2nd Contactor	Final Conversion
CSTR	CSTR	0.999
PFR	CSTR	0.756
PFR	PFR	0.978

4. Results and Discussion

In the experimental investigation of reaction kinetics and mass transfer data; the results obtained, show that with increase in temperature in range of 28-38° C, Reaction rate constant (k) increases from (0.86 to 1.12) and order of reaction decrease marginally (0.22 to 0.19). No significant effect of temperature on conversion is observed after 36° C. It is observed that with increase in % catalyst loading (Concentration of Enzymes) in range of 0.5 times to 2 times of Substrate concentration Reaction rate constant (k) increases from (0.52 to 0.87) and order of reaction (n) decreases marginally (0. 32 to 0.29). It shows there is a significant effect of catalyst loading on conversion of Penicillin & conversion increases with increasing catalyst loading. It shows that there is no significant effect of Penicillin G Concentration on reaction rate constant (k) & order of reaction (n).

Mass transfer effects were also studied by lowering the kinetic resistance of hydrolysis reaction. i.e. (Temperature – 38° C, Catalyst concentration = 2.0 Times of penicillin G and Substrate concentration 4 % w/w). It appears that with increase RPM in range of (150 to 600), Reaction rate constant (k) increases from (0.74 to 0.87) and order of reaction increases marginally (0.22 to 0.36). There is no significant effect of RPM on conversion observed above the just suspension speed.It was observed that as we decrease the particle size in range of (250 μ to 100 μ), conversion of Penicillin-G was increases.

A Multivariable non linear regression approach was used to fit the rate constant data with reaction temperature. Estimated the values of Activation energy - ΔEa (7056.278 cal/mol) and kinetic frequency factor A_0 (100904.38) (as per Arrhenius theory).Calculation is shown as per 3.3.1. From the experimental data it was found that there is certain mass transfer effects which drive the reaction kinetics. Estimated value of mass transfer co-efficient (ks_L = 1.95 x 10^{-2} cm/sec). 3.3.2. Minimum suspension speed was also estimated, Just suspension speed – N_{js} (146 rpm), Calculations areas shown as per 3.3.3. For a continuous process various reactor schemes of different configuration (CSTR and PFR), has been studied and identified that two CSTR in series can result in best conversion 3.3.4

5. Mathematical Modeling - Simulation

6. Conclusion

In the present work the results clearly indicate that the kinetic data are dominating over the mass transfer data. Hydrolysis of Penicillin G is observed to be a kinetically controlled reaction as the conversion of Penicillin G increases with temperature and catalyst concentration directly. The effect of mass transfer has also been observed .while increase in RPM and decreases in particle size of enzyme catalyst.

In the set of calculations carried out for the determination of best reactor sequence it was observed that two CSTR in series is the best option for the kind of reaction to be performed. However in the packed bed reactor there is the limitation of controlling pH and Temperature in desired range, which can be overcome by using sophisticated instrumentation in closed circulation loop.

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