Department of Chemical Engineering

PRETREATMENT OF WASTEWATER CONTAINING FATS AND OILS USING AN IMMOBILIZED ENZYME

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"This thesis is presented as part of the requirements for the award of the Degree of Doctor of Philosophy of the Curtin University of Technology"

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DECLARATION

I certify that, this thesis contains no material which has been accepted for the award of any other degree or diploma in any university. To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgement has been made. This thesis is the result of my own work and contains nothing which is the outcome of work done in collaboration.

Huanfei JIA

28 January 2002

ABSTRACT

This thesis investigates an application of immobilized lipase for pre-treating wastewater containing fats and oils, which is difficult to treat practically. The kinetics of soluble lipase was studied for establishing background of the lipase. The immobilization of lipase was adopted in order to repeatedly use the expensive lipase. The developed immobilization methods were based on the characteristics of carriers, but covalent bonding of lipase was preferred because of strong adsorption nature. Three types of materials, nylon membrane and polystyrene-divinylbenzene and silica gel beads, were used for studying the lipase immobilization characteristics. The lipase from *Canada rugosa* was chosen because of its relatively high catalytic activity and commercial availability. The oily wastewater sources used were a simulated mixture of olive oil and distilled water as well as actual restaurant oily wastewater. A packed bed reactor packed with immobilized lipase was suitable for the study. Moreover, a comparative study of anaerobic digestion of lipase treated and un-treated oily wastewater was undertaken to evaluate the efficiency of the lipase pre-treatment method due to lack of the relevant literature in the enzymatic wastewater treatment field.

The kinetics of lipase catalyzed hydrolysis reactions was investigated in a stirred tank reactor. The experimental results confirmed that the lipase catalyzed reaction obeyed Michaelis-Menten model. The optimal pH and temperature of the lipase catalysed hydrolysis reaction were 7 and 37 °C, respectively. The conversion of oil to fatty acid was dependent on the reaction time and mass of the enzyme used. The lipase activities depended on the concentrations of some selected additives. Calcium ion improved lipase activity significantly amongst the additives used.

The immobilization of lipase was carried out using different materials, nylon membranes, polystyrene-divinylbenzene beads, and silica gel. Covalent adsorption was simple and successful for immobilizing the lipase onto nylon membrane which was pre-treated with HCl solution for releasing amino groups. The adsorption of lipase was completed after only a 2-hour reaction time. It was much more practical

for this shorter adsorption time (2 hours) rather than the 24 hours required for physical capillary adsorption of lipase.

The properties of the immobilized lipase and the performance of the reactors were compared amongst the soluble and immobilized lipase forms. The immobilization, particularly for covalent bonding, made lipase more resistant to thermal deactivation. It was evident that the optimum temperature was shifted from 37 °C for the soluble lipase to 45 and 40 °C for immobilized lipase adsorbed onto nylon and polystyrene-divinylbenzene beads, respectively. The immobilized lipase could be used repeatedly with only little activity loss. The repeatedly operational stability made the reuse of the immobilized lipase possible.

Comparison was also made between two types of beads, polystyrene-divinylbenzene beads and silica gels. Though polystyrene-divinylbenzene beads showed higher lipase activity and shorter adsorption time when compared to silica gels, the former beads were not suggested for large scale study because of high cost of the beads. One improvement achieved in this work was that the 24 hours required for silanization of silica gel was reduced to only a few hours using evaporating 3-APTES in acetone instead of refluxing 3-APTES in toluene.

It is worthwhile to point out that much higher enzyme activity was obtained using the packed bed reactor as against the membrane reactor when aqueous oil emulsion was fed into the reactors. The lipase activity was 64.2 % of soluble lipase activity for the immobilized lipase in the packed reactor but its activity was hardly detectable in the membrane reactor. Moreover, the operation of the packed bed reactor solved the oil separating problem that severely hampered the lipase catalytic activity in the membrane reactor in aqueous phase. This result suggests that the packed bed reactor with the immobilized lipase is applicable in treating oily wastewater.

The intrinsic parameters, V_{max} and K_m, were evaluated to study the internal diffusional effects of the porous spherical silica gel on the immobilized lipase. The changes of V_{max} and K_m for the immobilized lipase from those of the soluble lipase indicated that some alteration in the lipase intrinsic properties was caused by the immobilization of lipase. However, the magnitude of Thiele modulus suggested that the immobilized lipase was most likely reaction controlling. In addition, good agreement for V_{max} and K_m from experiments and numerical model estimations seemed to suggest that the numerical model could be used for estimating V_{max} and K_m for the immobilized lipase.

An application was tried for conducting the hydrolysis of oily restaurant wastewater by soluble and the immobilized lipase. Enzyme activity of both forms was severely inhibited by the oily wastewater. The enzymatic activity was only 20 % and 15 % for soluble and the immobilized lipase, respectively, when compared to the initial activity value for the hydrolysis of olive oil by soluble lipase.

Evaluation of the efficiency for the proposed lipase pre-treatment method was carried out by monitoring the performance of two anaerobic digesters. These two digesters were fed with lipase treated and untreated restaurant wastewater that was neutralised with KOH solution prior to feeding. The oil-floating problem was minimised by this saponification of fatty acids with potassium hydroxide. However, there was no clear sign of an improvement for the treatment efficiency of the anaerobic digesters in terms of COD removal and methane production rate resulted in digesting lipase treated oily wastewater when compared to the one without lipase pre-treatment.

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B_I	Biot number
BOD	biological oxygen demand (mg/l)
COD	chemical oxygen demand (mg/l)
D_e	effective diffusivity (m^2s^{-1})
E	free enzyme
ES	enzyme and substrate complex
G	geometric factor; 1 for slab, 2 for cylinder and 3 for sphere
K_m	intrinsic Michaelis-Menten constant (kg m^{-3})
N	number of internal collocation points
n	number of data sets
R	radius of cylinder or sphere (m)
r	iteration number
S_o	initial substrate concentrate in a support (kg m^{-3})
S_b	substrate concentration in a support (kg m^{-3})
V_{max}	intrinsic maximum reaction rate (kg $m^{-3}s^{-1}$)
V_o	initial reaction rate (kg $m^{-3}s^{-1}$)
V	experimentally measured initial reaction rate (kg $m^{-3}s^{-1}$)
$\hat{V}$	Theoretical calculated initial reaction rate (kg $m^{-3}s^{-1}$)
VSS	volatile suspended solid (mg/l)
y	dimensionless substrate concentration in a porous support
z	dimensionless coordinate from centre of a support
β_b	dimensionless substrate concentration in a bulk liquid, $=S_b/K_m$
η	effectiveness factor
ϕ	Thiele modulus, $= \frac{R}{3}\sqrt{V_{max}/D_e K_m}$

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