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THE EFFECT OF SYNTHETIC JET ON ENZYME ACTIVITY

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Abstract

Mixing of shear sensitive materials such as enzyme and microorganisms is applied in a number of biological and pharmaceutical processes. Conventional mixing process is normally associated with high shear stresses that can introduce changes at the molecular level of the shear sensitive materials, which can have a far reaching effect on their activity and functionality. In addition, it draws relatively high energy as induction motor with a gearbox is usually used. For instance, when high shear rates or high operation temperatures are applied, the enzyme and microorganisms tend to denaturalise and degrade, respectively. The objective of current study is to use the synthetic jet mixer to reduce the rate of denaturalisation of enzyme in aqueous form and power consumption for optimum mixing to enhance the rate of enzymatic reaction. A novel flow pulsing mechanism to generate a mixing jet termed 'synthetic jet' using solenoid actuator is used in this study. The later one is attached to Teflon diaphragm at the bottom of predesigned 3L reactor to reduce power consumption in bench-top synthetic jet apparatus. The effect of the displacement, oscillation frequency, input signal and operating temperature on the enzyme denaturalisation rate is studied to optimise the enzymatic reaction rate. The results gives that synthetic jet mixer is better mixing compared with conventional overhead stirrer. It has 10.3% higher enzymatic reaction rate, 10.9% lesser of enzyme denaturalization rate as well as 14.5% less power consumption compared.

Keywords: Synthetic Jet, Enzyme, Mixing, Denaturalization rate, Kinetics.

1. Introduction

Mixing of enzyme and microorganisms is applied in pharmaceutical and biological processes. Conventional mixing process is normally involved with high shear stresses which able to deteriorate the protein structure of enzyme [1]. Besides that, it consumes high electrical power. Other than that, when high shear rates is applied, enzyme and microorganisms tend to denaturalize and degrade, respectively [1, 2].

In engineering fluid systems, researchers have investigated the synthetic jet application for different geometries to improve airframe efficiency and reduced jet noise. Active flow control on jet flow is traditionally using the mechanical systems such as movable surfaces and gimballed nozzles. However, there is advantage for fluidic jet flow control which involves zero mass flux if compared with the traditional mechanical system which has a higher response rates, precise control as well as improved lifetime. These fluidic systems include synthetic jets, voice coil actuator and piezoelectric actuators [3].

In jet mixing, an actuator (a device vibrates an elastic diaphragm at a certain amplitude and frequency) could be mounted to Polytetrafluoroethylene (PTFE) or Teflon diaphragm on one side of the cavity below a mixing compartment. On the other side of the cavity, there will be an orifice where a jet of fluid generated to increase the mixing rate as seen in Fig. 1.

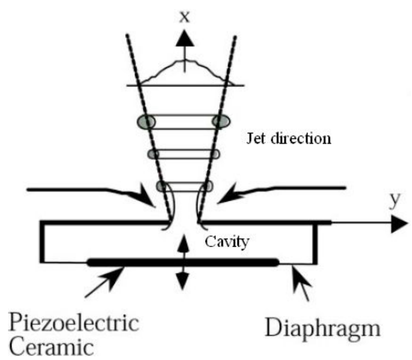

Fig. 1. Schematic Diagram of a Synthetic Jet Actuator [4].

When the actuator pulls the diaphragm from its original position, it draws the fluid back to the cavity due to pressure change. In a complete cycle, the jet gives a net flow of fluid jet momentum into the synthetic jet mixer while the total net mass in the cavity is zero as it is called Zero-Net-Mass-Flux jet [5].

In this study, the synthetic jet mixer will be used to reduce the external diffusion limitations for an enzymatic reaction [6-8]. The power consumption, enzyme kinetics and rate of enzyme denaturalization were also studied. In addition to that, the proposed mixing method is compared with the conventional overhead stirrer bioreactor, also known as Stirred Tank Reactor (STR), where the applied shear from the rotating blades of the propeller will be dispensed to the enzyme solution [9].

2. Methodology

To test the effectiveness of proposed mixing device, a biological hydrolysis reaction using lipase as a catalyst to measure the rate of butyric acid production for different mixing operating parameters. Some of the studied parameters were stroke amplitude (0.5 and 1.0 mm) and diaphragm oscillation frequency (5-15 Hz). In addition to that, the power consumption was also measured and compared with that of overhead stirrer. Samples were collected from the mixed vessel in predetermined intervals; pH was measured and controlled to 7.0 by neutralising reaction with 0.05 M NaOH [10]. The results were compared with the counterpart mixing technique (conventional mixing) as control to monitor the difference.

2.1. Experimental design

The Synthetic Jet Mixer, hereof defined as the assembly of a transparent tube (150 mm inside diameter and 170 mm height) made from Poly (methyl methacrylate), a cavity volume 1.00×10^{-4} m^3, 0.5 mm thick Polytetrafluoroethylene (PTFE) as well as the solenoid actuator. The experimental set-up is shown in Fig.2.

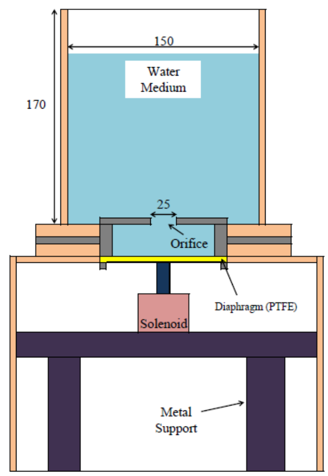

Fig. 2. Experimental Set-up with Dimensions (mm).
All Material Made from 5 mm Thickness Acrylic Glass.

2.2. Materials

Autotitrator, Titrino Plus 848, was obtained from Metrohm, Switzerland, data acquisition device NI USB 6009 was obtained National Instrument Malaysia, homogenizer Ultra-Turrax T18, IKA, Germany to generate the emulsion. To measure the power consumption, current transducer HXS 50-NP, was obtained from LEM, USA.

Lipase from *Aspergillus niger* (Lipozyme CALB-L) with activity of 100 kLU/ml was obtained as a testing sample from Novozyme, Denmark. Analytical grade of tributyrin oil (98%) is obtained from Fisher Scientific (M) Sdn. Bhd. Anhydrous glycerine (99.5 v/v%) and sodium hydroxide pellet were obtained from sigma Aldrich, Malaysia.

2.3. Experimental procedure

2.3.1. Substrate emulsion

Dissolve 8.95 g of NaCl and 0.2 g of KH_2PO_4 in 230 ml distilled water with 270 ml of glycerol. Under vigorous stirring, 3.0 g of Gum Arabic was added to the mixture until it was homogenized. Under vigorous stirring, mix 50 ml of emulsifying reagent with 3.0 ml of tributyrin oil and 298.0 ml of distilled water [10]. A fresh emulsion was mixed every time before start the experiment.

2.3.2. Enzyme kinetics

Each mixing experiment, 2.00 liters of substrate emulsion solution was placed in the synthetic jet mixer (reactor). Half millilitre of pure lipase with initial activity of 100 kLU/ml was added into the synthetic jet mixer. At the same time, the actuator is activated on predetermined stroke amplitude and oscillation frequency as appear in section 2. On 10 minutes sampling frequency, 20 ml of the samples was taken and heated with Bunsen burner for 5 minutes to deactivate the enzyme in the sample and hence, stop the reaction in the sample bottle permanently. Titration was performed with an autotitrator and the total volume of NaOH (0.05 M) required was recorded for achieving neutralise the sample to pH 7.00. The enzyme kinetics will be compared with conventional overhead stirred mixer [5].

2.3.3. Enzyme deactivation

The reactor was charged with 2.00 liters of distilled water and 500 μL of pure lipase (initial enzyme activity was 100 kLU/ml). Samples were collected for activity test to find the amount of enzyme activity lost as a function of time for specific operation condition. Concurrently, the results were compared with that of conventional overhead stirrer [6, 11].

2.3.4. Power consumption

The power consumption is the product of voltage and current. The measurement of voltage and current was made with data acquisition instrument (NI USB-6009, National Instrument, Malaysia) where the device was to monitor the real time voltage flow. The current flow was measured with current transducer (HXS 50-NP, LEM, USA). The current transducer was able to convert current flow to analog voltage output. Software programming with National Instrument LabView 2012 was done with the voltage and current monitoring to calculate the power consumption by the mixer.

3. Results and Discussion

3.1. Effect of mixing on enzyme kinetics

Enzyme kinetics is affected by several factors such as enzyme concentration, substrate concentration, operating temperature as well as the mixing effectiveness of the stirrer (effect on the external mas transfer and convective molecular diffusion). In this study, the operating conditions were kept constant as following: enzyme concentration at 25 LU/ml, change of substrate concentration was negligible as the initial substrate concentration was set to 0.85% (v/v) and finally the operating temperature at 25^{o}C. Hence the mixing effectiveness of the proposed mixer was tested based on the production of butyric acid inside the reactor as a function of time. The effect of oscillating frequency ranged 5 - 15 Hz and stroke length (amplitude of oscillating diaphragm; 0.5 mm and 1.0 mm) on total moles of substrate consumed in other word total moles of butyric acid produced was examined.

Change of frequency and amplitude resulted in almost constant production rate of the acid. This indicates that there is no improve on the diffusion limitation and the mixing was almost maintained constant. The results of synthetic jet mixing for 5 Hz oscillation frequency and 1 mm amplitude was plotted with that of conventional overhead stirring on 600 rpm in Fig. 3. It is clear in the figure the production of acid was 53.9 millimoles in the jet mixing compared with conventional overhead stirrer at 600 rpm operation with only 48.8 millimoles.

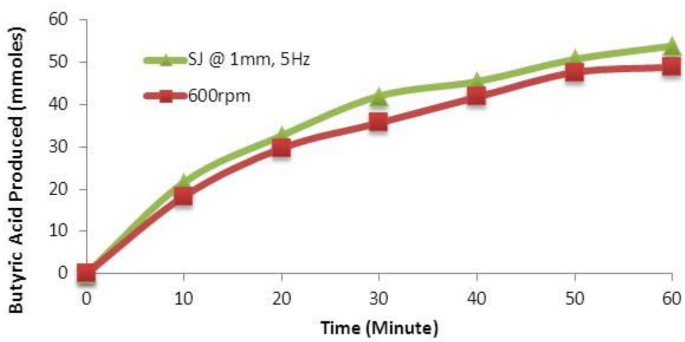

Fig. 3. Effect of Synthetic Jet Mixing and Conventional Overhead Stirring on the Production of Butyric Acid Production. Operating Temperature 25^{o}C.

From the acid production, the synthetic jet created slightly more turbulence than that created by overhead stirrer at 600 rpm. This indicates that eddies generated by the jet mixer were effectively mixing the solution as that generated by energy dispensed from the propeller to the solution from the overhead stirrer at the above mentioned revolution per minute [9].

3.2. Enzyme denaturalization

The effect of synthetic jet mixer and overhead stirrer mixer on the rate of denaturalization of enzyme was investigated for 2.5 hours. One milliliter sample was taken from the solution every 30 minutes and tested for the loss of enzyme activity. Enzyme solution at the same concentration without any mixing was used as a control for this test.

The results are shown in Fig. 4 indicating the loss of enzyme activity using synthetic jest was slightly higher than that without mixing (Control), as 10.1% and 8.1%, respectively. This gives a clear image that the jet created using this test has minimum effect on the macromolecules (enzyme) inside the mixing vessel over the overhead stirrer, where the later one reduced the enzyme activity 19.0% when it stirs at 600 rpm. It was reported earlier that shear came from stirring can deactivate enzyme in the solution [12] indicating high loss of the enzyme activity at high shear rate.

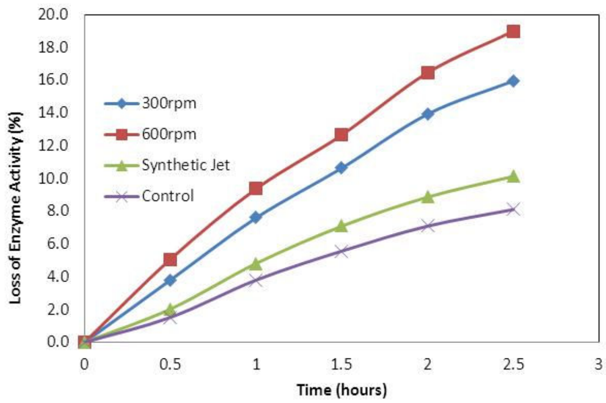

Fig. 4. Enzyme Activity Loss of Over Time during Application of Shear.

3.3. Power consumption

The power consumption of the synthetic jet mixer at 5 Hz and 1.0 mm oscillating amplitude was compared to the conventional overhead stirrer at 600 rpm. Table 1 shows the voltage, current and power consumption of the mixer.

Table 1. Voltage, Current and Power Consumption of the Mixer Expressed as Root Mean Square Value (rms).

Device	Voltage (V)	Current (A)	Power (W)
Overhead Stirrer @ 600 rpm	240.0	0.111	26.64
Synthetic Jet @ 5Hz, 1.0 mm	11.0	0.350	3.85

The results above indicating that the power consumption for the proposed mixer is about 6 times less than that of the power consumed using the conventional overhead stirrer. This will open the door to propose using renewable energy such as solar energy to replace the conventional mixers with synthetic jet mixers.

4. Conclusions and Future Work

Synthetic jet mixer has been proven with better performance in the aqueous enzymatic reaction, where conventional overhead stirrer at 600 rpm operation has total production rate of 48.8 millimoles compared to 53.9 millimoles of butyric acid in a synthetic jet mixer. The later one runs with 1.0 mm amplitude and 5 Hz oscillating frequency. Besides that, the loss of enzyme activity associated with previous operation was found to be 10.1% and 19.0% for synthetic jet and overhead stirring at 600 rpm, respectively. Interestingly, the loss of activity in a non-mixed system did not differ much from that for synthetic jet mixing where only 8.1% of enzyme activity was lost.

For power consumption, synthetic jet mixer only draws 3.85 W of power while conventional overhead stirrer draws 22.51 W for 300 rpm operation and 26.64 W for 600 rpm operation when stirring the enzyme – substrate solution.

Future work can focus on the optimization of the oscillating amplitude, frequency as well as the change of the waveform of the oscillation. The change of enzyme concentration with varying temperature can be considered where both of these are one of the main factor that affecting the enzyme kinetics. From the results, the suggested ranges for the parameter of oscillating amplitude and frequency will be in between 0-1.0 mm and 0-10 Hz.

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