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Performance of Oscillatory Flow Reactor and Stir Tank Reactor in Solvent Fermentation from Palm Oil Mill Effluent

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Abstract

Advance in mixing technology has developed a new way of mixing fluids by introducing an oscillatory motion to replace the conventional mechanical agitation or an air bubble displacement. This new way of mixing breakthrough has been implemented in an Oscillatory Flow Reactor (OFR). This research will be focus on the performance of OFR as a bioreactor by comparing with Stir Tank Reactor (STR), which is the traditional device in fermentation. The experimental work was conducted in an OFR and a STR with a working volume of 1.5 l. Solvent production strain, Clostridium acetobutylicum NCIMB 13357 was grown in OFR and STR, using fresh Palm Oil Mill Effluent (POME) as growth medium. All of the experiments were conducted anaerobically under batch mode for 72 hours at constant temperature of 35°C. Comparisons of the growth trend and solvent fermentation performance for both devices were investigated. Total solvents (acetone, butanol and ethanol) produced in an OFR was comparable with that of STR. Total solvents production in OFR is 1.8 times higher than that of STR resulted in total 1.6 g/l of solvents. The results of this investigation showed that OFR has an excellent potential as an alternative device in fermentation processes.

Keywords: Oscillatory Flow Reactor (OFR), Stir Tank Reactor (STR), solvent fermentation, C. acetobutylicum.

1.0 Introduction

Recent work has shown that it is possible to apply oscillatory flow mixer as fermentation device. Ni et al. [1] showed that pulsed baffle system produced higher mass transfer rate than the stir tank fermenter for both yeast re-suspension and culture. Harrisons and Mackley also reported that baffled pulsatile bioreactor is suitable for the cultivation of rapidly growing, oxygen demanding microorganisms [2]. The oscillatory flow reactor has no moving parts except for a the pair of piston at each ends and such situation is very conducive for the growth of shear sensitive microorganisms. The system can be operated to yield gentle yet uniform mixing that enhanced radial transport thus resulted in efficient mass and heat transfer and also providing a route towards obtaining a near plug flow residence time distribution within a flow channel [3, 4].

The dynamic nature of oscillatory flow in a baffled tube can be characterized by the following three dimensionless groups. The oscillatory flow can be described by oscillatory Reynolds number, Reo, where:

$$\mathrm{Re}_o = \frac{D\omega x_o}{\nu} \qquad (1)$$

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where D is the tube diameter (m), ω is the angular frequency of the oscillator drive ($rads^{-1}$), χo is the oscillatory amplitude measured from center-to-peak (mm) and ν is the kinematic viscosity (m^2s^{-1}). The oscillatory Reynolds number describes the oscillation intensity that applies to the fluid system. A second group is the Strouhal number, *St*, where

$$St = \frac{D}{4\pi x_o} \tag{2}$$

The Strouhal number represent the amplitude ratio of oscillation, the larger the *St*, the smaller the amplitude. A third group is the net flow Reynolds number, Re_n, where

$$\mathrm{Re}_n = \frac{DU}{\nu} \tag{3}$$

where U is the superficial net flow velocity through the tube (m/s) [5]. The fluid mixing is generated primarily from the oscillatory motion and the mixing can be decoupled from any flow that might be applied through the tube. These factors suggest that the system has useful applications in a number of process engineering situations [6]. It is also possible to generate a range of mixing intensity for oscillatory flow in baffled tube. If Re_o ~300 and *St* ~ 1, gentle uniform mixing can be achieved where the fluid oscillation is sufficient for each interbaffle region to operated as a stirred tank. It is also possible to achieve intense eddy mixing if Re_o is increased to say 10^3-10^4. Therefore, oscillatory flow reactor configuration is viable for appliaction to both delicate and intense agitation condition [2].

Previous investigations on oscillatory flow were mainly directed on the behavior inside the tube. Oscillatory flow in baffled tube manage to give a significant effect on heat transfer resulted in identification of viable device for energy-efficient and compact heat exchanger [4]. Hewgill et al. [7] showed that this device operates more efficiently than a gas-sparged stirred. tank. It has excellent potential for application in fermentation technology with its capability to mix two phases, it would be useful in gas-liquid reaction, where presently facing with limited diffusion control and scale up difficulties.

The objective of this research is to introduce oscillatory flow reactor as an alternative fermentation device that is able to utilize agro industry waste (POME) as a feedstock to produce solvent. The performance of oscillatory flow reactor as a fermenter was also investigated to illustrate its potential compared to stir tank reactor. This fermentation will also attract interest in an alternative and easy to obtain substrate for the production of solvents, especially in the depletion of today's petroleum stocks, from which these solvents are currently produced [8].

2.0 Materials & Method

The oscillatory flow reactor that was utilized in this work is shown in Fig. 1. The device consisted of a U tube made of stainless steel with 52.2 mm diameter and 1376 mm length, The OFR is equipped with three ports for inoculation, sampling and nitrogen inlet and

exhaust. The working volume for this investigation was 1.5 L. The U tube is place inside the heating bath for inert temperature control to achieve the desired condition by adjusting the heater temperature. A series of orifice type stainless steel baffles with a diameter 31.32 mm and spacing 1.5 times the tube diameter were welded to the tube wall. The baffle spacing was 1.5 times the tube diameter as suggested by Brunold et al. [3] to achieve effective mixing over broad range of oscillation amplitudes and frequencies. Both ends of the tubes were attached to an oscillation unit that consists of a pair of pistons. Pneumatically driven pistons that work in a push and pull sequence were used to oscillate the fluid inside the OFR. The OFR has frequencies range up to 0.78 Hz and oscillation amplitudes from 0 to 12.5 mm.

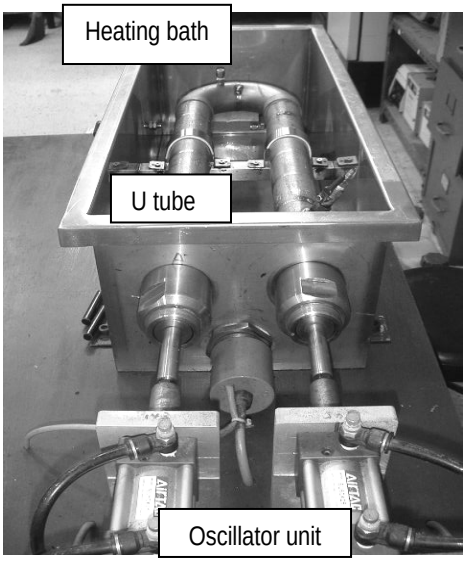

Figure 1. Oscillatory Flow Reactor

A 2-litre stir tank reactor from Ko Biotech in Korea was used for solvent fermentation using *C. acetobutylicum*. Details of the fermenter is shown in Table 1. The working volume for this investigation was 1.5 l.

Table.1 Details of the stir tank fermenter

Details	Values
Fermenter capacity	2.5 l
Operating volume	1.5 l
Liquid height	10.5 cm
Impeller type	Rushton turbine
Number of impellers	1
Impeller diameter	6.9 cm
Number of baffles	3
Sparger type	Ring sparger

Clostridium acetobutylicum NCIMB 13357 was used in this study. Laboratory stocks of *C. acetobutylicum* were routinely maintained as spore suspensions of sterile deoxygenated Reinforced Clostridial Medium, Difco (RCM) at 4^{o}C under anaerobic condition. Inoculum was prepared in a 250ml Schott bottle that consist of deoxygenated RCM medium and 10% by volume of spore suspension and was incubated at 35^{o}C for 18 hr anaerobically in stationary mode.

POME medium was prepared using an overnight sediment fresh POME taken from Sri Ulu Langat Palm Oil Mill, Dengkil, Selangor. Initial pH of the POME was adjusted to pH 5.8 by the addition of 5M NaOH. RCM medium was prepared by dissolving 38 g of the powder into 1 l distilled water. Both mediums were autoclaved at 121^{o}C for 20 min.

The details of the experimental work is presented in Table 2. An anaerobic condition was achieved by purified N_2 sparging for 1 hr before the inoculation and continuously during the 72 hr fermentation. pH culture was monitored and samples were collected at twelve hour interval time for determination of solvents (ABE), acids, cells and glucose concentration. *In-situ* sterilization using dry-heat sterilization method was performed on the OFR before each run [9] whereas sterilization of the STR was done in the autoclave. Solvent (acetone, butanol and ethanol) concentrations were determined using 5890 Hewlett-Packard gas chromatography equipped with FID detector. pH reading was recorded using Corning pH meter 440 (Corning, New York). The cell concentration in the culture was determined using colony forming unit method (CFU, cell/ml). Total glucose consumption concentration was assayed using the DNS method.

Table.2 Operating condition for the oscillatory flow and stir tank reactor

Details	OFR	STR
Fermenter capacity	1.9 l	2.5 l
Operating volume	1.5 l	1.5 l
Agitation type	Baffles + oscillation	Rushton disc turbine
Agitation rate	0.45 Hz, 12.5 mm	100 rpm
	0.78 Hz, 7.5 mm	250 rpm
Impeller diameter	NA	0.069 m
Number of baffles	15	3
Diameter/width of baffle	40 mm	14 mm
Sparger type	Single orifice	Ring sparger
Inoculum volume (v/v)	10%	10%
pH level	5.8 for POME	5.8 for POME
Temperature	35 ^{o}C	35 ^{o}C

3.0 Results and Discussion

Growth profile of *C. acetobutylicum* utilizing POME grown in a stationary flask is shown in Fig. 2. Fresh POME contains two kinds of major carbon source, lipids and sugar [10]. The growth curve of *C. acetobutylicum* utilized both carbon source can be divided into two phases, acidogenic phase and solventogenic phase. An acidogenic phase where organic acid (acetic and butyric acid) were actively produced was observed during the first 12 hr of fermentation which cause the reduction in culture pH. At the same time glucose is also actively consumed to accommodate the high growth rate in the culture between 0 to 36 hr fermentation. The cell growth reached it maximum concentration at 36 hr with value of $1.5x10^7$ cell/ml. Fermentation entered the solventogenic phase as growth curve reached a deceleration phase after 36 hr. During this phase, the metabolism of cells undergoes a shift to produce solvent by reasimilation of organic acid (acetic and butyric acids) caused a slightly

pH increased in the culture medium. The glucose consumption continues to maintain the cell viability in the culture. Total of 0.74 g/l of solvent was produced in flask fermentation indicate that POME is suitable medium for solvent fermentation by *C. acetobutylicum* .

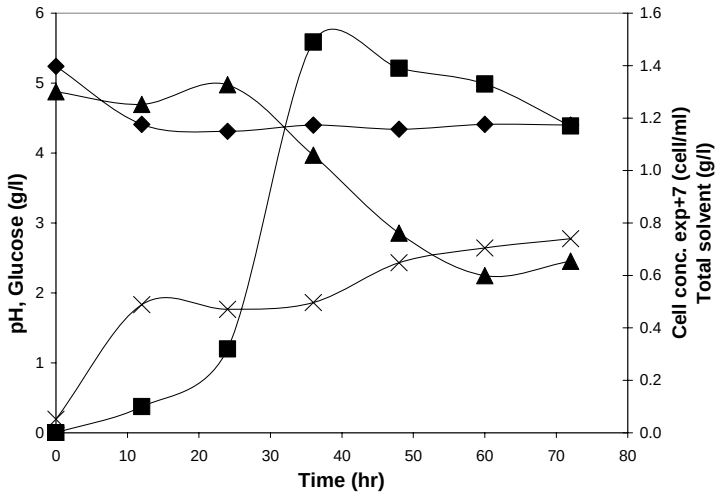

Figure 2 Growth profile of C. acetobutylicum in flask utilized POME as growth medium. ♦pH; ■ cell concentration; ▲ glucose consumption; × total solvent.

Subsequent investigations looks into the effect of mixing in OFR and STR on solvent production. The mixing intensities were varied (OFR: 0.45 Hz and 0.78 Hz; STR: 100 rpm and 250 rpm). Results obtain from these experiments were presented in Figure 3. In the OFR, glucose was consumed more rapidly at higher frequency of the OFR (0.78 Hz) at the early stage of fermentation and abruptly slows as the cells growth reaches its maximum concentration after 24 hr. At lower frequency (0.45 Hz), glucose was gradually consumed until 48 hr fermentation as the cells growth reached its maximum concentration. Higher glucose consumption rate (0.26 g/l/hr) was observed in frequency of 0.78 Hz due to its high cell concentration production. By increasing oscillation frequency from 0.45 Hz to 0.78 Hz, the maximum colony forming unit (CFU) of *C. acetobutylicum* is 99% increased from 0.0105×10^{10} cell/ml to 1160×10^{10} cell/ml. An acidogenic phase was observed during the first 24 hr of fermentation in both frequencies where *C. acetobutylicum* grew rapidly with high production of acids (acetic and butyric) which cause the reduction in pH culture.

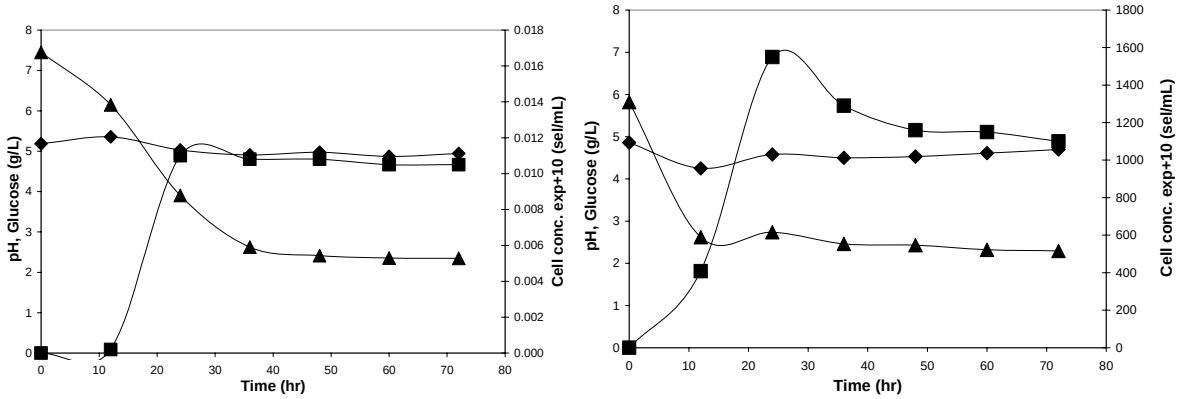

Figure 3 Growth profile of *C. acetobutylicum* in OFR at different oscillation intensity (a) low frequency: 0.45 Hz (b) high frequency: 0.78 Hz.♦ pH; ■ cell concentration; ▲ glucose concentration.

The fermentation entered the solventogenic phase when growth reached a deceleration phase after 24 hr. During this phase, the metabolism of cells undergoes a shift to produce solvent by reasimilation of organic acid (acetic and butyric acids) causes slightly increased in pH culture. Solvent production in the OFR increased from 1.09 g/l to 1.6 g/l when oscillation intensity increased from frequency of 0.45 Hz to 0.78 Hz (Fig. 4).

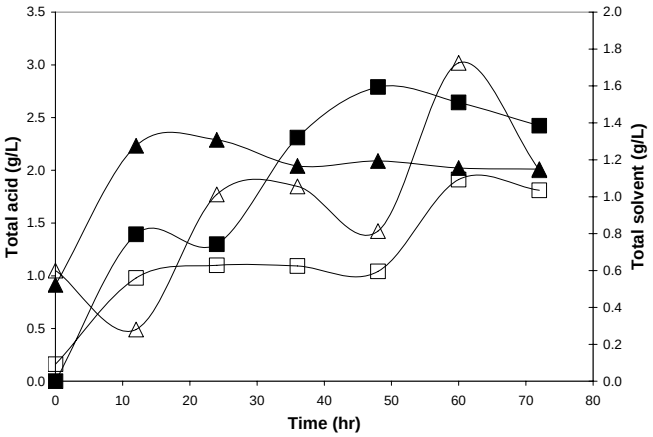

Figure 4 Fermentation of POME to solvent by *C. acetobutylicum* NCIMB 13357 over time course in OFR at different frequency. □ total solvent (0.45 Hz); ■ total solvent (0.78 Hz); Δ total acid (0.45Hz); ▲ total acid (0.78 Hz).

This result has been expected since higher frequency will gave better mass transfer rate and will produce higher cell concentration as well as solvent production. Organic acid production was observed during the fermentation with highest production in 0.45 Hz after 60 hr incubation with value of 3 g/l.

Fig. 5 shows *C. acetobutylicum* growth profile in STR at different agitation speed. The STR performance in high agitation speed (250 rpm) was better than in low agitation speed (100 rpm). As the agitation speed increased from 100 rpm to 250 rpm, the maximum cell concentration (CFU) increased 83% from 1.86×10^7 cell/ml to 11×10^7 cell/ml after 24 hr cultivation. Although the glucose consumption rate was similar (0.225 g/l/hr) for both agitation speed, but total solvent production reach its maximum concentration at different hour as agitation at high speed (250 rpm) reach 0.86 g/l of total solvent after 60 hr cultivation, whereas at low agitation speed of 100 rpm, maximum total solvent of 0.83 g/l was achieved after72 hr fermentation (Fig. 6), demonstrating a clear effect of mixing intensity on solvent production.

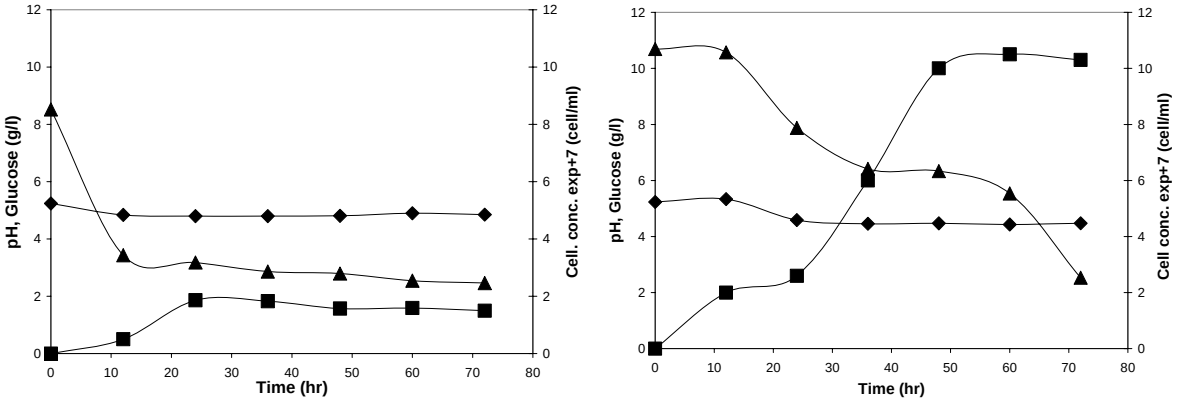

Figure 5 Growth profile of *C. acetobutylicum* in STR at different agitation intensity (a) low agitation: 100 rpm (b) high agitation: 250 rpm.♦ pH; ■ cell concentration; ▲ glucose concentration.

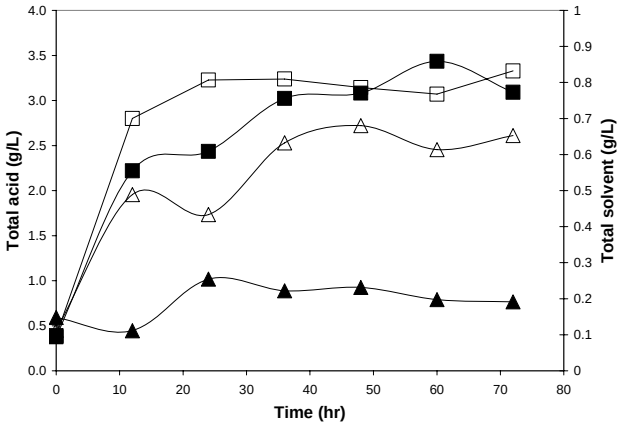

Figure 6 Fermentation of POME to solvent by *C. acetobutylicum* NCIMB 13357 over time course in STR at different agitation speed. □ total solvent (100 rpm); ■ total solvent (250 rpm); Δ total acid (100 rpm); ▲ total acid (250 rpm.

High organic acid concentration was also observed in low agitation speed which caused low solvent production due to low reasimilation of acid to produce more solvent. In general, cultures maintained at a relatively high pH produced more acid and less solvent than cultures maintained at low pH [11].

Table 3 shows direct comparison of the effect of different mixing method in solvent fermentation utilized POME. The kinetic parameters in the STR were different from those in the OFR. Total solvent production, glucose consumption rate and solvent production rate, in OFR were higher than in the STR. Table 3 shows that OFR has a better productivity than in the STR. High butanol concentration (1.6 g/l) was produced in OFR that was 50% more than in the STR. There was no ethanol production and very little acetone produced (0.05 g/l) in fermentation utilizing POME as substrate which implied that there is possibility that OFR have the ability to produce just single solvent. Successful of solvent fermentation in the OFR, and laboratory yields better than the STR, confirm that the OFR is a valid fermentation device. The importance of mixing intensity suggests that there are benefits in maintaining a uniform mixing environment on reactor scale-up. The OFR offers an advantage: it is easier to scale than a STR if the intention to obtain uniformity of mixing at process scale [12].

Table 3. The performance of *C. acetobutylicum* fermentation in OFR and STR utilized POME as growth medium.

Performance	OFR	STR
	0.78 Hz	250 rpm
Max. cell concentration (cell/ml)	$1160x10^{10}$	$11x10^{7}$
Total organic acid concentration. (g/l)	2.30	1.74
Final culture pH.	4.6	4.5
Max. acetone concentration. (g/l)	0.05	0.13
Max. butanol concentration. (g/l)	1.54	0.50
Max. ethanol concentration. (g/l)	0	0.24
Total solvent concentration. (g/l)	1.59	0.86
Ratio of A:B:E	0.03:1:0	0.3:1:0.5
Fermentation time (hr)*	48	60
Glucose consumption rate (g/l/hr)	0.260	0.225
Solvent production rate (g/l/hr)	0.033	0.014

**Fermentation time is a time taken to reach a maximum total solvent concentration.*

4.0 Conclusion

The results of this work indicate that POME is a suitable fermentation medium for solvent production. POME which is rich with natural carbon source (BOD higher than 20000 mg/l) and dissolve complex substance may require efficient mixing to enhance substrate interface with the microorganisms to produced high yield. The results of this work show that cell growth, glucose consumption and solvent production are better than in stir tank reactor. The combination of gentle mixing and efficient mass transfer proved that oscillatory flow reactor is a viable fermenter for solvent production.

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