*Malays. Appl. Biol. (2018) 47(2): 119–124*

# CHARACTERISATION OF THE OSCILLATORY FLOW REACTOR FOR THE FERMENTATION OF *Escherichia coli*

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Accepted 20 April 2018, Published online 25 May 2018

## ABSTRACT

Oscillatory Flow Reactor (OFR) is a novel type of tubular reactor consists of an equally spaced orifice baffles fitted inside the column of the reactor. The mixing in the OFR can be controlled by a combination of geometrical (*i.e*. baffles and orifice diameter) and operational parameters (*i.e.* oscillation frequency). The aim of this work was to develop and characterizes the novel OFR as a bioreactor. Upon completion of the OFR development, the reactor was subjected to a certain experiment for bioreactor characterization. The parameters studied were temperature profile, volumetric mass transfer coefficient ($k_La$), water loss due to evaporation and sterilization hold. The results showed the OFR was able to maintain the set temperature and its sterility throughout the experiment duration of 24 hours. The water loss due to evaporation inside the OFR was negligible as it was less than 4%. The highest $k_La$ obtained was 33.6 $h^{-1}$ at an oscillation frequency of 5 Hz. The OFR has shown its suitability as a bioreactor through various experiments conducted and will be tested for the fermentation of *E. coli* in the future.

**Key words:** Oscillatory flow reactor; bioreactor, characterization, temperature profile, sterilization hold

## INTRODUCTION

The oscillatory flow reactor (OFR) is a type of tubular reactor fitted with equally spaced doughnut baffles plate (Harvey & Stonestreet, 2001). Mixing inside the reactor is provided by an oscillatory motion superimposed on the net flow of the process liquid, creating a flow pattern with similar radial and axial velocity.

Researchers have suggested that there are many potential applications of the oscillatory flow reactor in the process and product enhancement (Azhari *et al.*, 2008). An early application of the OFR in the biological process was the fermentation of poly-β-hydroxybutyrate using *Alcaligenes eutrophus* H16 (Harrison & Mackley, 1992). Here, they demonstrated the ability of the OFR for the cultivation of rapidly growing, oxygen-demanding microorganism. Other applications of the OFR in biological process include production of biobutanol from fermentation of *Clostridium acetobutylicum* (Takriff *et al.*, 2009), production of biopolymer from fermentation of *pululla*n (Gaidhani *et al.*, 2005), and fermentation of *Sachharomyces cerevisiae* by using a novel oscillatory flow micro-bioreactor (Reis *et al.*, 2006).

In this paper, the novel OFR was designed specifically for fermentation of aerobic micro-organism, *Escherichia coli*. *E. coli* plays an important role in biological engineering and industrial microbiology such as a host for recombinant proteins. This is due to its rapid growth rate, easier genetic manipulations and high level of recombinant protein synthesis rates (Baeshen *et al.*, 2015). Since biological process is often deal with less viscous liquids, low and uniform shear rate provided by the OFR makes it more suitable for shear-sensitive organisms and large molecules than that other conventional reactor (Abbott *et al.*, 2013). Hence, OFR was characterized based on requirements for *E. coli* fermentation. The characterizations include temperature hold, water loss due to evaporation, sterilization hold and mass transfer coefficient.

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## MATERIALS AND METHODS

The OFR was developed and fabricated by the author in Faculty of Chemical and Natural Resources Engineering workshop in Universiti Malaysia Pahang. The main parts of the reactor (top plate, main column, baffle train) are made from 304 stainless steel column. The reactor column has 60 mm internal diameter and a wall thickness of 2 mm. The height of the reactor column is 500 mm. Six orifice plate baffles are arranged at an equal distance of 90 mm between each plate inside the reactor column. Two pieces of stainless steel rods with 3 mm diameter are used to hold the baffles plate to produce baffle train (Figure 1a). The oscillation of the liquid in the OFR is provided by the oscillation system as shown in Figure 1(b). The system used a diaphragm which can provide an oscillation frequency up to 5 Hz with fix amplitude of 0.8 cm. The whole OFR setup is installed vertically as shown in Figure 1(c).

The OFR will be used for *E. coli* fermentation which has an optimum growth temperature of 37ºC and incubation period of 24 hours. In order for the OFR to comply with this condition, a seven-meter-long silicone tube with 10 mm internal diameter is coiled along the reactor column length. The circulated water bath is used to supply water at 40ºC through the silicone tube to bring the temperature inside the column to 37ºC. The temperature was measured using a 450 mm long thermocouple which basically read the temperature at three different points as shown in Figure 2.

Liquid loss from the OFR was determined by measuring the liquid volume using measuring cylinder before the fermentation simulation. Then, the liquid was once again measured using the same technique thereafter and the volume change was determined. The OFR was operated with initial liquid volume of 1500 mL and hold for 24 hours at 37ºC. The oscillation was set to frequency of 0.25 Hz with amplitude of 0.8 cm. Aeration inside the

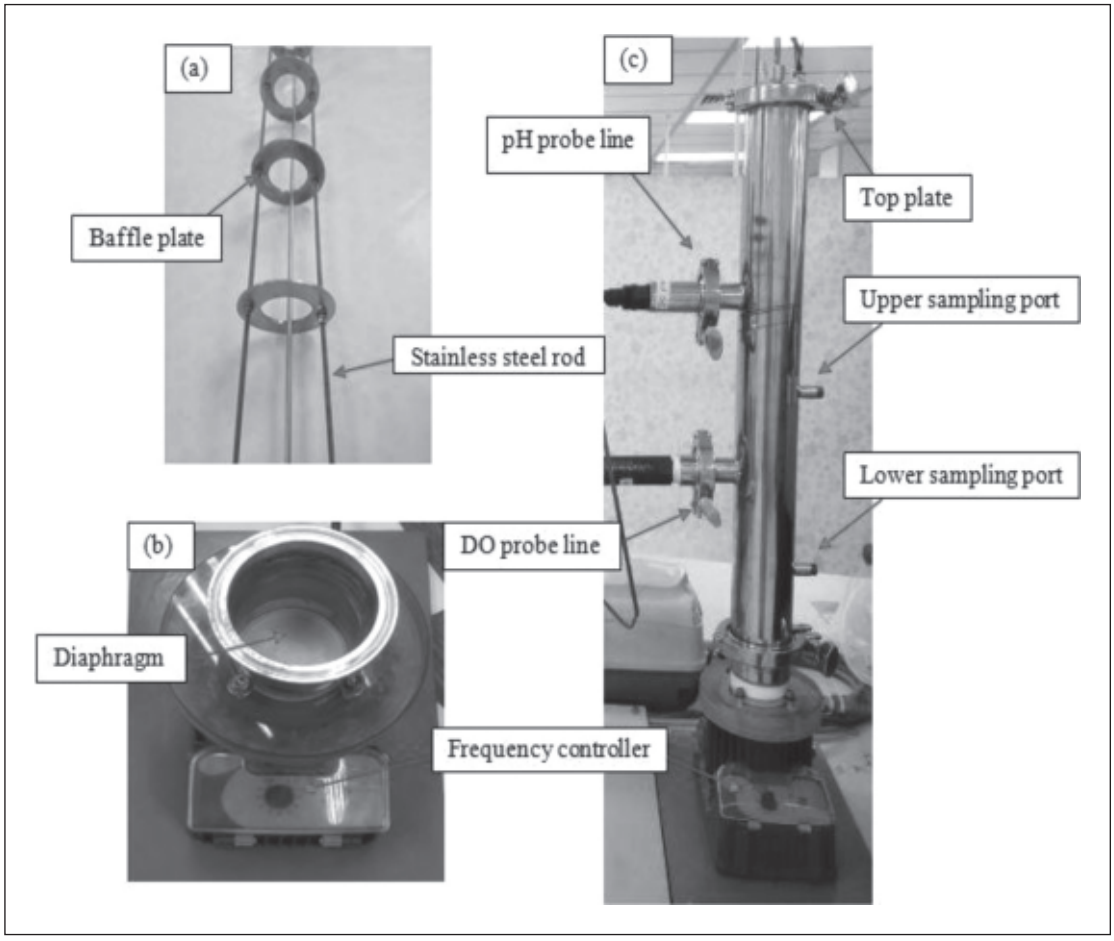


**Fig. 1.** (a) Baffles train supported by stainless steel rods, (b) Oscillation system of the OFR consists of diaphragm and frequency controller, (c) Complete OFR setup showing the main parts: top plate; reactor column; oscillation system; probes and sampling ports.

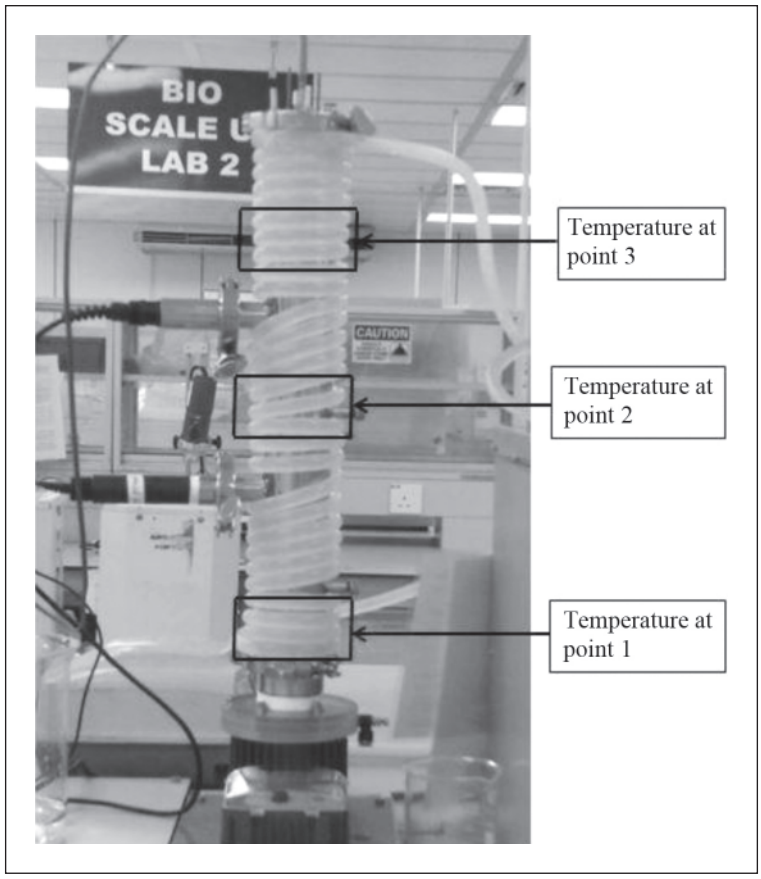


**Fig. 2.** Three points of temperature taken along the OFR column.

reactor was set to 1 $Lmin^{-1}$ using air. The final liquid volume was measured once the fermentation was finished.

Sterilization of the OFR was conducted using two methods, *ex situ* moist heat sterilization and *in situ* chemical sterilization. Initially, the main parts of the reactor (top plate, reactor column and baffles train) were sterilized using moist heat at 130ºC for 20 minutes. Then, the reactor was reassembled before *in situ* chemical sterilization took place using 70% ethanol. The OFR was soaked in 70% ethanol for 24 hours and rinsed twice using sterile distilled water. Next, nutrient broth was poured aseptically into the OFR and the reactor was hold for 24 hours at 37ºC for sterilization holding. Samples were taken using 3 mL syringe from upper and lower sampling port (as shown in Figure 1c). About 20 µL of the sample was streaked on nutrient agar plate. The observation on the presence of microorganism on agar plates was made after incubation period at 37ºC for 24 hours.

## RESULTS AND DISCUSSION

A good bioreactor is able to maintain its fixed temperature throughout the fermentation course. Efficient distribution of heat inside the reactor is another main feature for a good bioreactor. Figure 3 shows the temperature reading inside the OFR taken from three different points along the length of the column.

The result shows the temperature inside the OFR was successfully maintained at 37ºC throughout 24 hours. The warm water coiled outside the OFR column was initially set at 60ºC to rapidly increase the internal temperature to the set point. It took an hour for the internal temperature to reach the set point of 37ºC, as shown in Figure 3. On the second hour, the outside water coiled temperature slowly decreased to 40ºC in order to maintain the internal temperature set point. All three points of the temperature measurement inside the OFR shows the same temperature reading throughout the OFR column and able to maintain the temperature at 37ºC. This was due to the oscillation provided from the bottom plate of the OFR (Figure 1b). Oscillations help to distribute the heat evenly along the length of the OFR column. The presence of baffles also helps in heat distribution from the wall to the centre of the OFR column. The result was in general agreement with those given by Mackley *et al.* (1990) where the presences of baffles in the oscillatory flow column results in significant

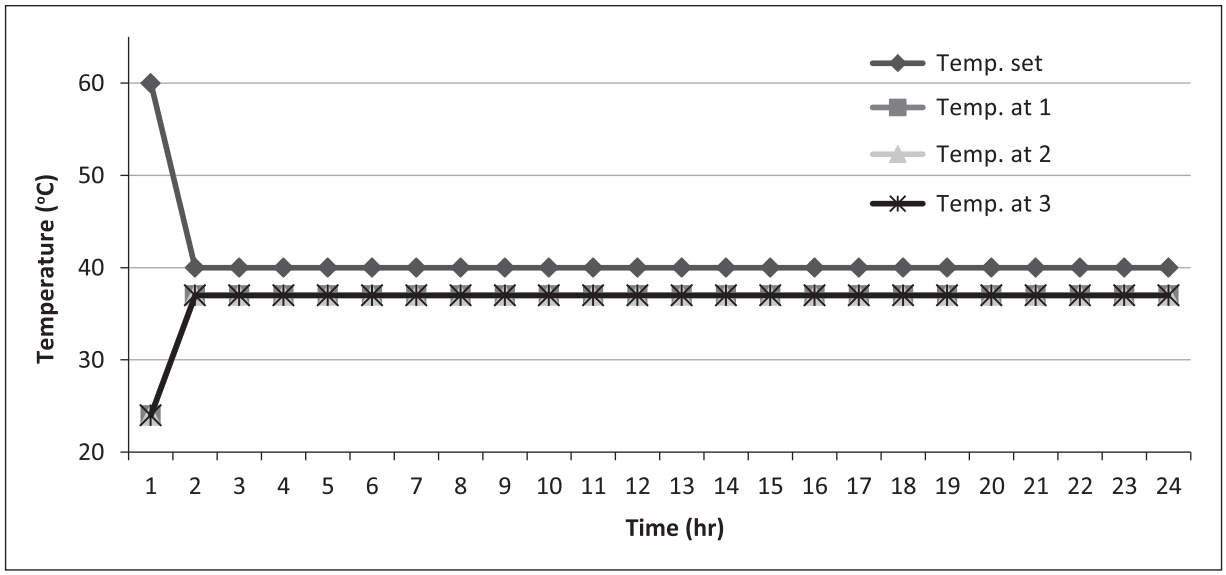


**Fig. 3.** Results on temperature profile in three points along the OFR column throughout the fermentation course.

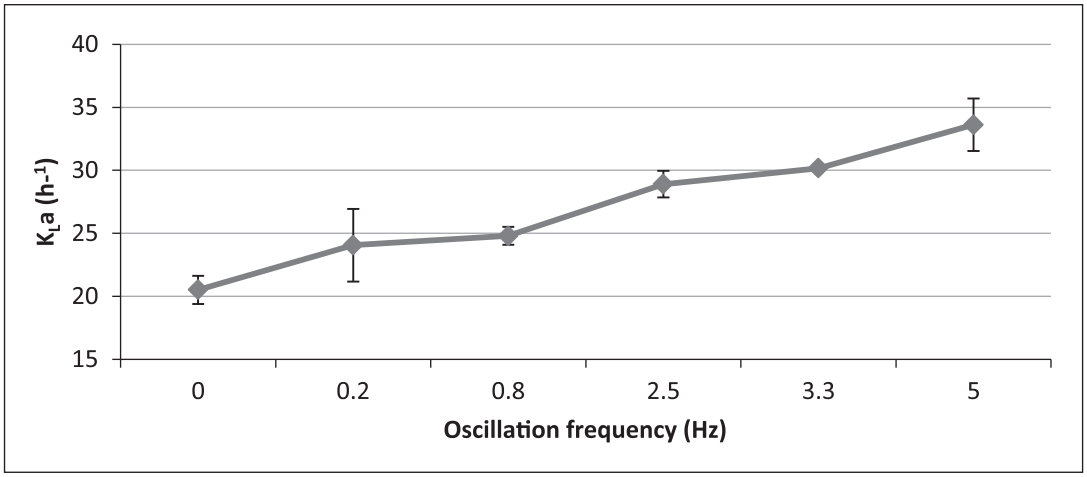


**Fig. 4.** Mass transfer coefficient values in the OFR at different oscillations frequency.

improvement in heat transfer performance. The greatest enhancement was obtained where both baffles and oscillations were present on the reactor (Mackley & Stonestreet, 1995).

Fermentation of aerobic microorganism, in this case *E. coli*, requires efficient gas-liquid mixing to ensure sufficient oxygen transfer from gas to the liquid phase. The oscillatory flow reactor is typically consisting of a pipe fitted with a sharp-edge baffled along the column and it was operates with an oscillatory liquid motion which creates recirculating flow and eddies. This flow enables the efficient mixing of not only in heat transfer but also mass transfer between gas and liquid in the reactor (Mazubert *et al.*, 2016). In the OFR, gas bubbles were generated by direct air sparging to supply the air to the culture medium with the help of oscillations. A 480 mm long sparging line was attached to the top plate of the OFR and pass through each orifice baffles plate along the length of the column so that the air bubbles will rise from the bottom of the OFR, these will give the air bubbles longer retention time. Figure 4 shows the result of mass transfer coefficient, $k_La$ in the OFR at different oscillations frequency (0, 0.2, 0.8, 2.5, 3.3 and 5 Hz). Nutrient broth media (Merck) was used at an aeration rate of 1 vvm and temperature of 37°C. The condition was representing *E. coli* fermentation condition.

Figure 4 shows the increasing trend of $k_La$ as the oscillations increases up until 5 Hz. The $k_La$ value was 33.6 $h^{-1}$ at maximum oscillations of the OFR (5 Hz). It was found that the mass transfer was strongly dependent on the oscillation frequency

since the $k_La$ values increases as the oscillations increases. The result correlates with the findings by Mackley and Stonestreet (1995) where the mass and heat transfer rate was strongly dependent on the product of frequency and amplitude of oscillations. Mackley and Stonestreet (1995) also stated that by choosing a particular frequency and amplitude, the precise control of mass and heat transfer enhancement can be obtained.

Throughout the fermentation time of 24 hours at 37ºC, the OFR lost 4.4% of the liquid water from the initial volume of 1500 mL. Based on the observation, the water loss was due to several reasons. Firstly it was due to leak at the drainage line of the OFR. Secondly, some of the water was left behind between the baffles plate and inside the probes lines after the water was drained out from the OFR. Lastly, some of the water droplets came out from the exhaust line at the top plate when the oscillation was present. Due to insufficient head-space, the gas bubble created during oscillation was forced out through the exhaust line creating the water droplets. Hence, actions need to be taken to overcome these problems. The leaking drainage line was fixed to ensure water inside the OFR was properly drained at all times. The working volume inside the OFR was reduced to 70% of the OFR total volume in which equals to 1125 mL, thus creating sufficient headspace which prevented the water droplets out of the exhaust line. The results show that, after all the actions were taken, the water loss due to evaporation was none and assumed to be negligible.

Sterilization is a process that destroys or eliminates all forms of microbial life (Rutala *et al.*, 2008). The term was intended to convey an absolute meaning where the thing is sterile over a given period of time or in certain condition (Masngut, 2013). In the OFR sterilization hold, the reactor sterilization is considered successful if no micro-organism growth is observed on the nutrient agar plate after 24 hours of incubation.

It was observed that the agar plate maintained clear after 24 hours showing that the sterilization technique used, moist heat and chemical sterilization are effective to sterilize the OFR. In the future, the OFR will be *in situ* sterilized using 70% ethanol before fermentation takes place.

## CONCLUSION

As a conclusion, the OFR managed to maintain its sterility and set temperature along the reactor column throughout the experiment duration of 24 hours at 37ºC. The OFR has shown an efficient heat distribution along the length of its column due to the contribution of the short-lived vortices as the result of continuous oscillation. The $k_La$ value was 33.6 $h^{-1}$ at maximum oscillations of the OFR. The water loss due to evaporation seems negligible as it was mainly due to OFR technical problems. Full actions have been taken to overcome these problems. Thus, it can be seen that the OFR is suitable to be applied as a bioreactor to carry out the fermentation of *E. coli*.

## ACKNOWLEDGEMENTS

The authors would like to give credits to the Universiti Malaysia Pahang for providing laboratory facilities and the Ministry of Higher Education for funding the research work under Fundamental Research Grant Scheme (FRGS) with grant number, RDU 150117.

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