

# Evaluation of power density on the bioethanol production using mesoscale oscillatory baffled reactor and stirred tank reactor

H W Yussof<sup>1</sup>, S S Bahri<sup>1,2</sup> and N A Mazlan<sup>1</sup>

<sup>1</sup> Faculty of Chemical Engineering & Natural Resources, Universiti Malaysia Pahang, 26300 Gambang, Pahang, Malaysia

E-mail: syamsutajri@gmail.com

**Abstract.** A recent development in oscillatory baffled reactor technology is down-scaling the reactor, so that it can be used for production of small-scale bioproduct. In the present study, a mesoscale oscillatory baffled reactor (MOBR) with central baffle system was developed. The reactor performance of the MOBR was compared with conventional stirred tank reactor (STR) to evaluate the performance of bioethanol fermentation using *Saccharomyces cerevisiae*. Evaluation was made at similar power density of 24.21, 57.38, 112.35 and 193.67 Wm<sup>-3</sup> by varying frequency (f), amplitude (xo) and agitation speed (rpm). It was found that the MOBR improved the mixing intensity resulted in lower glucose concentration (0.988 gL<sup>-1</sup>) and higher bioethanol concentration (38.98 gL<sup>-1</sup>) after 12 hours fermentation at power density of 193.67 Wm<sup>-3</sup>. Based on the results, the bioethanol yield obtained using MOBR was 39% higher than the maximum achieved in STR. Bioethanol production using MOBR proved to be feasible as it is not only able to compete with conventional STR but also offers advantages of straight-forward scale-up, whereas it is complicated and difficult in STR. Overall, MOBR offers great prospective over the conventional STR.

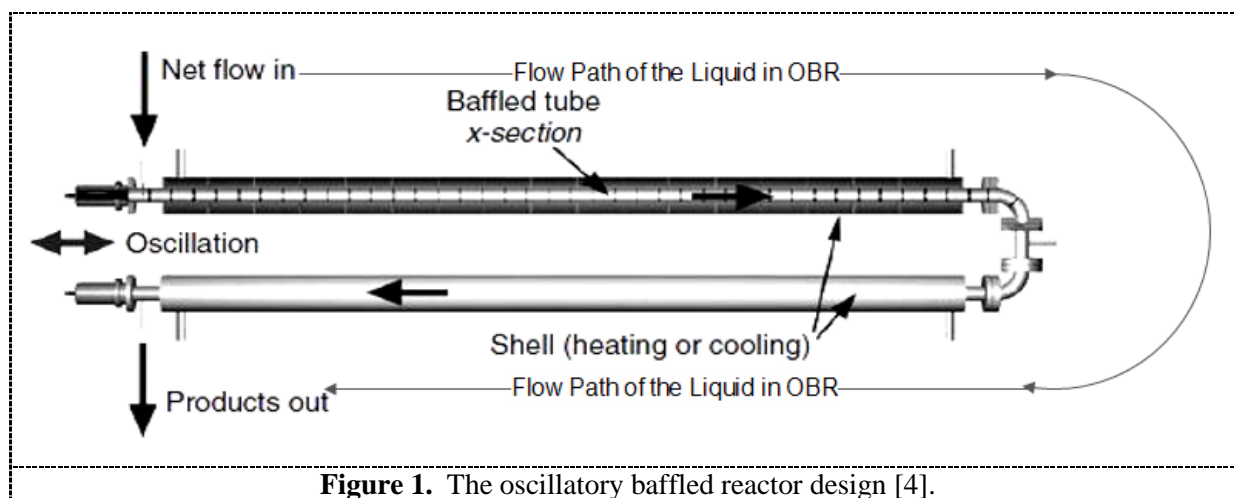
## 1. Introduction

The development of high efficiency bioreactors has been an important research objective in the field of bioprocesses. Appropriate selection and design could greatly improve the efficiency of the overall process. The oscillating reactors were firstly used in separation processes in order to enhance the contact between the phases and, consequently, to improve mass transfer rates. Since then, they have been applied to a number of systems, either chemical or biochemical, under several configurations [1]. Oscillatory baffled reactor (OBR) consists of a cylindrical column or tube as illustrated in Figure 1. It is similar to the continuous plug flow reactor. The reactor tubes fitted with equally-spaced, low constriction orifice plate baffles that have an oscillatory motion ranging from 0.5 to 10 Hz superimposed upon the net flow of the process fluid. The oscillatory flow reactor creates an oscillatory motion when the flow pumped back and forth through the orifice. Each baffle behaved like a uniformly mixed stirred tank, leads to an excellent mixing and suspension by creating vortices between orifice baffles and oscillatory fluid [2]. Moreover, eddies are generated when fluid flow passes through the baffles that enable radial motions.

<sup>2</sup> To whom any correspondence should be addressed.



The production of biofuel using OBR was first introduced using NaOH as a homogeneous catalyst [3]. The most important application of OBR is it can provide long residence time processes in continuous mode. Many such processes are currently run in batch because conventional designs of continuous reactor are impractical in term of cost, control and size [4]. In addition, the OBR offers the prospect of a compact plug flow reactor with uniform and controllable mixing. The mesoscale oscillatory baffled reactor (MOBR) was first been developed for laboratory-scale processes [5]. MOBRs are millilitre scale OBRs designed for small scale screening of reactions. These mesoreactors combine the basic capabilities of the conventional OBRs such as enhanced heat and mass transport, and product uniformity with small scale necessary for screening and production of specialist chemicals. MOBRs have received considerable attention due to their small volume and ability to operate at low flow rates, reducing reagent requirements and waste [6].



MOBR are also suitable for continuous high throughput screening, and rapid determination of reaction order and rate constant as plug flow behaviour can be easily achieved [7-8]. There have been several studies in the literature reporting that STR is complicated and difficult to scale up, as the degree of mixing and heat transfer diminish with increasing scale. This leads to substantial reductions in rate. Whereas, a straight forward scale up processes can be done by maintaining geometrical and dynamic similarity. This allows mixing and flow conditions produced at laboratory-scale to be easily replicated for pilot-scale and industrial-scale processes [9-10].

Geometric and dynamic parameters play an important role in the reactor design and operation. Scale-up can be performed directly by maintaining geometric and dynamic similarity. Geometric similarity is achieved by maintaining the baffle-spacing-to-diameter ratio and the fractional open area of the baffle while dynamic similarity is achieved by maintaining the net and oscillatory flow Reynolds numbers [11]. Reynolds number ( $Re$ ) is a dimensionless number that describes the flow conditions either laminar or turbulent. It measures the ratio of inertial forces to viscous forces for given flow conditions. The oscillatory Reynolds number ( $Re_o$ ) measures the intensity of mixing inside a column or baffled reactor [6]. It is similar to the net flow Reynolds number,  $Re_n$ , in steady flow, except that the superficial velocity ( $u$ ) has been replaced by maximum oscillatory velocity ( $2\pi f x_o$ ). A velocity ratio is introduced in order to describe the interaction between oscillatory and net flows. It is the ratio of oscillatory  $Re_o$  to net flow  $Re_n$ , and simplifies to the ratio of maximum oscillatory velocity to superficial velocity. In addition, the ratio of the two Reynolds numbers is a measure of the degree of plug flow [4]. The summary of the geometric and dynamic parameters are expressed as in Eq. (1) – Eq. (5) as below [12-14].

$$\text{Net flow Reynold number: } Re_n = \frac{\rho du}{\mu} \quad (1)$$

$$\text{Oscillatory Reynold number: } Re_o = \frac{2\pi f x_o \rho d}{\mu} \quad (2)$$

$$\text{Velocity ratio: } \psi = \frac{Re_o}{Re_n} = \frac{2\pi f x_o}{u} \quad (3)$$

$$\text{Baffle spacing: } L = 1.5d \quad (4)$$

$$\text{Open cross sectional area: } S = \left( \frac{d_o}{d} \right)^2 \quad (5)$$

where  $f$  is the frequency of oscillation (Hz);  $x_o$  is the centre-to-peak amplitude of oscillation (m);  $u$  is the superficial velocity of the liquid (m/s);  $\rho$  is density ( $\text{kg.m}^{-3}.\text{s}^{-1}$ );  $\mu$  is viscosity (Pa.s);  $d$  is tube diameter (m);  $d_o$  is orifice diameter (m) and  $L$  is baffle spacing (m). The oscillation frequency ( $f$ ) and amplitude ( $x_o$ ) are the most important operational parameters in OFR. At a given  $L$  and  $d_o$ , changing the combination of  $f$  and  $x_o$  allows control the generation of eddies and produces a range of fluid mechanical conditions as broad as required.

This work aims to evaluate the bioethanol production using MOBR and compare their performance with conventional stirred tank reactor (STR) at the same power density of 57.38, 112.35, and 193.67  $\text{Wm}^{-3}$ . The fermentation was carried out using *Saccharomyces cerevisiae* along with glucose as medium in both MOBR and STR at 30°C.

## 2. Materials and methods

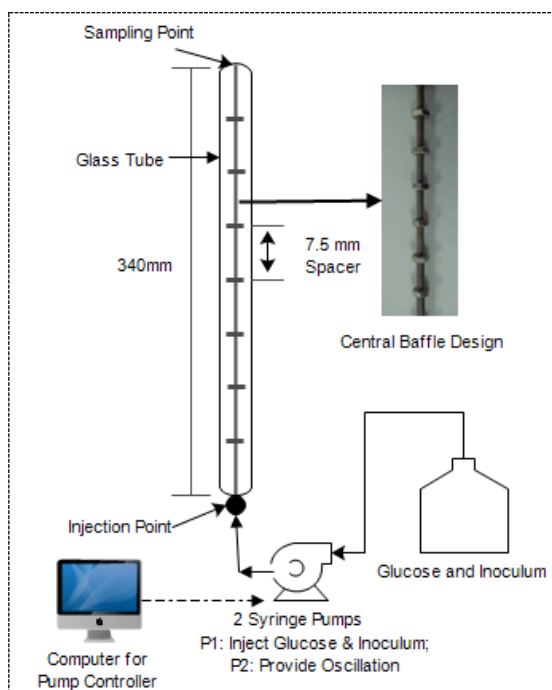
### 2.1 Experimental set-up

The set-up of MOBR consists of glass tube, one central baffle, and two syringe pumps as in Figure 2. Two syringe pumps (Eurodyne Ltd., UK) were provided to inject the inoculum inside the reactor and to oscillate the fluid inside the reactor. The MOBR was run at 8 mL working volume including inoculum. The reactor was sterilized using sodium hypochlorite (NaOCl), 5.25% prior to fermentation. Fermentations were performed at 30°C with power density value of 57.38, 112.35, 193.67  $\text{Wm}^{-3}$  in order to compare with STR by changing amplitude and frequency. Samples were taken twice at 6 and 12 hours and stored at 4°C for further analysis.

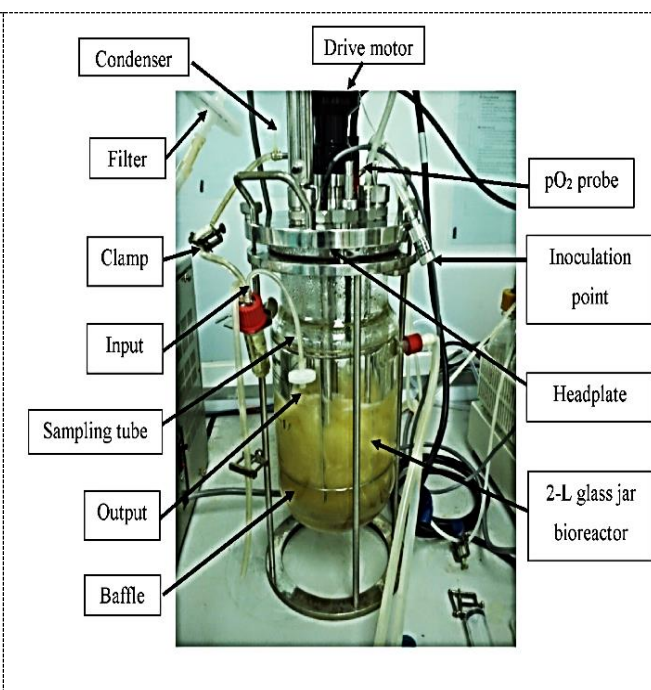
The STR was performed using a 2-L fermenter as in Figure 3. The fermenter was autoclaved at 121°C, for 15 minutes prior to fermentation with the pH and the dissolved oxygen (DO) electrodes installed. The pH and DO were always monitored but not controlled. Agitation was varied at 190, 260, 320 and 384 rpm in order to produce a power density of 57.38, 112.35, 193.67  $\text{Wm}^{-3}$  to compare with MOBR. The fermentation was maintained constantly at pH 5.0 by adding 2M sodium hydroxide solution and 2M hydrochloric acid. The working volume was approximately 1.5 L. The temperature was kept constant at 30°C. An initial sample was taken to measure their initial concentration, and then the inoculum was added to the fermenter. Samples were taken periodically every two hour for the first 12 hours and every 6 hours subsequently up to 48 hours and stored at 4°C for further analysis.

## 2.2 Sample analysis

The glucose, biomass cell concentration, and bioethanol concentrations were measured according to 3, 5-dinitrosalicylic (DNS), cell dry weight (CDW) and high performance liquid chromatograph (HPLC) methods, respectively. Glucose concentration in the fermentation broth was analysed by 3, 5-dinitrosalicylic acid (DNS) using 1% DNS reagent based on method applied in previous study [15]. The fermentation broth was centrifuged at 13,000 rpm for 10 min. One mL of the supernatant was mixed with 3 mL of 1% DNS reagent and incubated at 90°C for 10 min. After the mixture cooled to room temperature, the absorbance was measured at 540 nm, using a UV-VIS spectrophotometer (Hitachi, Japan). Standard solutions of anhydrous D-glucose containing 0.5–3 g glucose/L deionized water were prepared and 1 mL of each standard solution were mixed with 3 mL 1% DNS reagent and incubated 90°C for 10 min. A blank (deionized water) was incubated with the reagent and was used for zero adjustment of the spectrophotometer. The glucose concentration in the sample was compute by least squares linear regression, using a standard curve [16]. Biomass cell concentration was determined by the cell dry weight (CDW) at optical density of 660 nm using UV-VIS spectrophotometer. A correlation between the CDW and optical density needs to be established beforehand, that is  $CDW (g L^{-1}) = (0.407 \times OD_{600}) + 0.0014$  [16]. Bioethanol was analysed using HPLC (Shimadzu Prominence® LC-20A). The HPLC system was equipped with an auto-sampler, a Rezex ROA-Organic Acid column (7.8 x 300 mm; Rhenomenex, Torrance, CA, USA) as an analytical column, and a refractive index detector. The temperature was maintained at 60°C in a column oven and sulphuric acid (0.005 N) was used as a mobile phase at 0.6 mL/min under isocratic condition. All the samples were clarified by filtration with a 0.45 µm filter before injected to the HPLC column at 10 µL.



**Figure 2.** MOBR set-up for bioethanol production.



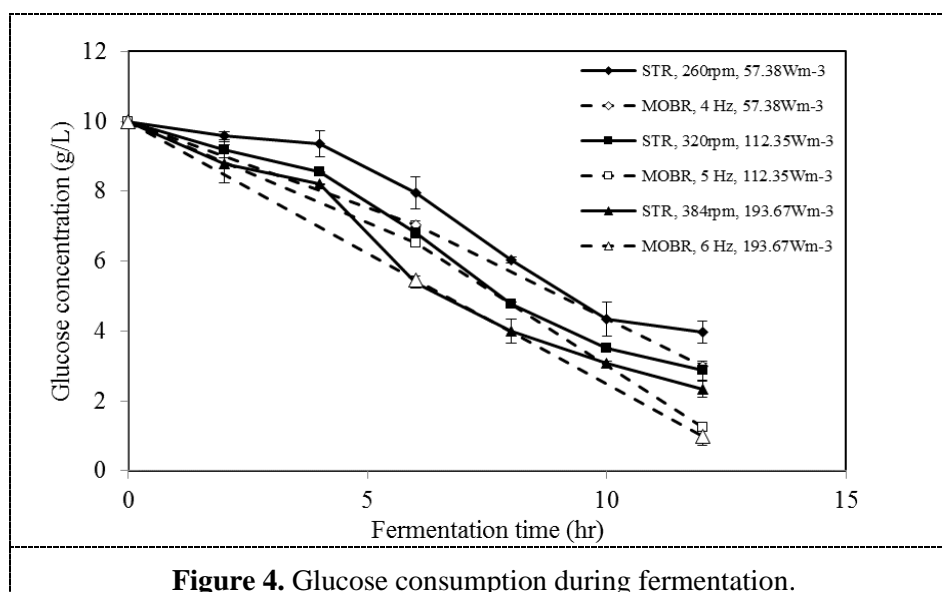
**Figure 3.** A 2-L STR setup for bioethanol production.

## 3. Results and discussion

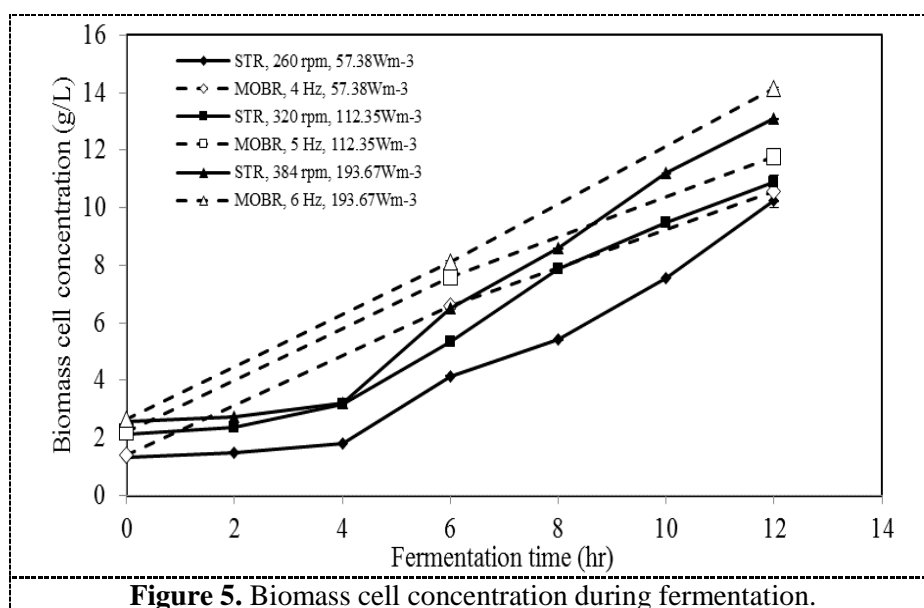
A series of batch fermentations using *Saccharomyces cerevisiae* were conducted in mesoscale oscillatory baffled reactor (MOBR) with comparison to the conventional stirred tank reactor (STR). The fermentation was conducted in order to evaluate the MOBR reactor performance and its ability to

perform anaerobic fermentations in batch culture. The comparison was made on the basis of power consumed per unit volume (power density). The fermentation was carried out at the same power density of 57.38, 112.35, and 193.67  $\text{Wm}^{-3}$  in both MOBR and STR using glucose medium at 30°C. In terms of Reynolds numbers ( $Re$ ), the fermentation in STR was performed at  $Re = 8,538$ , 10,672 and 12,787 while the fermentation in MOBR was carried out at oscillatory Reynolds numbers,  $Re_o$  of 247.59, 309.49, 372.39.

The effect of initial glucose concentration at 10  $\text{gL}^{-1}$  and cell growth (biomass cell concentration) were summarized in Figure 4 and Figure 5. It can be seen from Figure 4 that the MOBR give better glucose conversion when compared to the STR at the same power density. The lowest glucose concentration of 0.988  $\text{gL}^{-1}$  was obtained by MOBR compared to STR of 2.339  $\text{gL}^{-1}$  at 193.67  $\text{Wm}^{-3}$ . The bioethanol yield was recorded at 39.4% when using MOBR as STR only produced the highest yield of 15.4%. Nonetheless, the bioethanol yield obtained using MOBR was 39% higher than the maximum achieved in STR. This result is similar to those findings by Masngut et al. [16] where 0.22 g/L/h of biobutanol was produced from oscillatory baffled bioreactor which is 38% higher than the maximum achieved in the STR. Higher bioethanol productivity in MOBR is likely to be due to early initiation of solventogenic phase, eventually accumulating more solvent earlier than the STR [16]. In terms of Reynolds number, it is thought that both fermentation in MOBR ( $Re_o = 373$ ) and STR ( $Re = 12,787$ ) were performed at turbulent condition. It is suggested that the STR system was fully turbulent when operated at values of  $Re$  above 10,000 [17]. Even at the lowest power density of 112.35  $\text{Wm}^{-3}$  and laminar condition, the difference in glucose conversion was nearly 1  $\text{gL}^{-1}$  between the MOBR ( $Re_o = 247.59$ ) and STR ( $Re = 8,538$ ). It appears from Figure 4 that the advantages of using MOBR is not always prominent. Taking data at 6 hours, for 193.67  $\text{Wm}^{-3}$  and 112.35  $\text{Wm}^{-3}$ , the advantage of MOBR is not obviously. However for 57.38  $\text{Wm}^{-3}$ , the advantage of MOBR is clear.







**Figure 5.** Biomass cell concentration during fermentation.

This result was supported by the highest biomass cell concentration of  $14.14 \text{ gL}^{-1}$  obtained after 12 hours using MOBR at  $193.67 \text{ Wm}^{-3}$  as in Figure 5. Increase the MOBR mixing rate has increase the bioethanol concentration as in Table 1. The highest bioethanol concentration of  $38.98 \text{ gL}^{-1}$  was obtained using the MOBR at  $193.67 \text{ Wm}^{-3}$  and  $R_{eo}$  of 371.39. In contrast, the highest bioethanol concentration of  $36.11 \text{ gL}^{-1}$  was obtained using the STR at the same power density of  $193.67 \text{ Wm}^{-3}$ . The finding is consistent with findings of past studies by Abbott et al. [10] in the enzymatic saccharification of pure  $\alpha$ -cellulose using cellulase conducted in oscillatory baffled (OBR) and stirred tank (STR) reactors. Enzymatic saccharification requires a cellulase enzyme system to convert cellulose to glucose. Their finding highlights that the highest glucose conversion of  $23 \text{ gL}^{-1}$  was obtained in the OBR at a relatively low power density ( $2.36 \text{ Wm}^{-3}$ ) and highly turbulent with  $R_{eo} = 600$  compared to the STR at an impeller speed of 350 rpm ( $250 \text{ Wm}^{-3}$ ). Their work also demonstrated a 94 – 99% decrease in the required power density to achieve maximum conversion rates and showed a 12% increase in glucose consumption after 24 h at  $2.36 \text{ Wm}^{-3}$ .

In a different study, a series of pullulan fermentation experiments were carried out in OBR using *Aureobasidium pullulan*, which produces the polymer pullulan [18]. The growth rate and pattern were compared with the traditional STR, including the influence of aeration on pullulan and biomass cell concentration. The result show that the OBR gives a much higher rate of growth as well as higher yield of pullulan as compared to the STR. For example,  $11.3 \text{ gL}^{-1}$  and  $12.1 \text{ gL}^{-1}$  pullulan were produced in the STR after 96 and 144 hours fermentation, respectively compared to 37 and 39 hours were achieved when using OBF. Overall, most of these studies give an indication that the MOBR (including OBR) performance significantly better than the conventional STR when compared at the same power density.

#### 4. Conclusion

A series of batch fermentations using *Saccharomyces cerevisiae* were performed in MOBR and compared with the conventional STR in term of power consumed per unit volume (power density). This study has shown that the MOBR give better glucose conversion when compared to the STR at the same power density. The lowest glucose concentration of  $0.988 \text{ gL}^{-1}$  was obtained by MOBR compared to STR of  $2.339 \text{ gL}^{-1}$  at  $193.67 \text{ Wm}^{-3}$ , when performed at turbulent condition. This result was supported by the highest biomass cell concentration of  $14.14 \text{ gL}^{-1}$  and the highest bioethanol concentration of  $38.98 \text{ gL}^{-1}$  obtained after 12 hours using MOBR at  $193.67 \text{ Wm}^{-3}$  and  $R_{eo}$  of 371.39. In contrast, the highest bioethanol concentration of  $36.11 \text{ gL}^{-1}$  was obtained using the STR at the same

power density of  $193.67 \text{ Wm}^{-3}$ . Overall, findings from this study suggests that the MOBR is suitable for performing fermentation in a power efficient manner compared to conventional STR. Results from this study provided essential data on power input to allow any economic assessment on the bioethanol production processes based on both MOBR and STR later. In addition, MOBR may offer potential for more biological application i.e. animal cell where expensive chemical usage can be significantly reduced. However, more research needs to be done to prove this in practice, but this possible benefit offers great prospective over the conventional STR.

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