Short Communication

The Impact of Hydraulic Retention Time on the Biomethane Production from Palm Oil Mill Effluent (POME) in Two-Stage Anaerobic Fluidized Bed Reactor

Laily Isna Ramadhani[a], Sri Ismiyati Damayanti[a,b], Hanifrahmawan Sudibyo[a,c], Muhammad Mufti Azis[a], Wiratni Budhijanto[a,c*]

[a]Department of Chemical Engineering, Faculty of Engineering, Universitas Gadjah Mada, Yogyakarta, 55281, Indonesia

[b]Department of Chemical Engineering, Faculty of Engineering, Universitas Lampung, Lampung, 35145, Indonesia

[c] Center for Energy Studies, Universitas Gadjah Mada, Yogyakarta, 55281, Indonesia

ABSTRACT. Indonesia is currently the most significant crude palm oil (CPO) producer in the world. In the production of CPO, 0.7 m^3 of Palm Oil Mill Effluent (POME) is emitted as the wastewater for every ton of fresh fruit bunches processed in the palm oil mill. With the increasing amount of CPO production, an effective POME treatment system is urgently required to prevent severe environmental damage. The high organic content in the POME is a potential substrate for bio-methane production. The biomethane production is carried out by two groups of microbes, i.e., acidogenic and methanogenic microbes. Each group of bacteria performs optimally at different optimum conditions. To optimize the biomethane production, POME was treated sequentially by separating the acidogenic and methanogenic microbes into two stages of anaerobic fluidized bed reactors (AFBR). The steps were optimized differently according to the favorable conditions of each group of bacteria. Although perfect separation cannot be achieved, this study showed that pH control could split the domination of the bacteria, i.e., the first stage (maintained at pH 4-5) was dominated by the acidogenic microbes and the second stage (kept neutral) was governed by methanogens. In addition to the pH control, natural zeolite was added as microbial immobilization media in the AFBR to improve the performance of the microorganisms, especially in preventing microbial wash out at short hydraulic retention time (HRT). This study was focused on the understanding of the effect of HRT on the performance of steady-state continuous AFBR. The first stage as the acidogenic reactor was run under acidic conditions (pH 4-5) at five different HRTs. In comparison, the second stage as the methanogenic reactor was run under the neutral condition at four different HRTs. In this work, short HRT (5 days) resulted in better performance in both acidogenic AFBR and methanogenic AFBR. The immobilization media was hence essential to reduce the risk of washout at such a short HRT. The two-stage system also resulted in quite a high percentage of soluble chemical oxygen demand (sCOD) removal, which was as much as 96.06% sCOD.

Keywords: Anaerobic Fluidized Bed Reactor; Hydraulic Retention Time; Immobilization Media; Natural Zeolite; Palm Oil Mill Effluent

1. Introduction

Palm Oil Mill Effluent (POME) is highly polluting wastewater, which causes severe problems in the environment due to the high pollutant content. For every ton of fresh fruit bunches processed in the palm oil mills, there will be 120-200 kg of crude palm oil (CPO), 230-250 kg of empty fruit bunches, 130-150 kg of fiber, 60-65 kg of kernels, and 0.7 m^3 of wastewater effluent. Due to its simplicity and easiness, an open lagoon system was usually preferred in most palm oil mills in Indonesia to accumulate POME. Unfortunately, the effectiveness of this system is being questioned since it leads to another problem of releasing CO_2 dan CH_4 directly to the atmosphere in addition to the long retention time and wide-area needed. On the other hand, the high organic content of POME is very potential to be converted into bio-methane under anaerobic process.

One of the efforts to improve the efficiency of pollutant conversion into biomethane is optimizing the reactor design. The vertical column contained small particles of zeolite called anaerobic fluidized bed reactor (AFBR) was used in this work because of its effectiveness in processing high organic loading rate (OLR) wastewater with shorter hydraulic retention time (HRT). A fluidized bed reactor exhibits several advantages that make it useful for the

treatment of high-strength wastewaters. The fluidized particles provide extensive surface areas for biomass attachment (Borja *et al.* 2001), which leads to possible higher OLR and shorter HRTs during operation (Garcia *et al.* 1998; Sowmeyan & Swaminathan, 2008). The fluidization system had been proven as an effective way to optimize an anaerobic process, as it was shown by methane purity of 63.164% v/v, which was higher than the non-fluidization system (Ramadhani *et al.* 2018). Wash-out phenomenon is the most common problem in a short HRT bioreactor. Natural zeolite was added as immobilization media to support AFBR performance, especially on preventing washout phenomena and enhancing methane formation. Zeolite addition in anaerobic digestion could increase the organic removal rate, stabilize Volatile Fatty Acid (VFA) fluctuation while maintaining high bio-methane production (Halim *et al.* 2017). The immobilization medium was then fluidized to maximize the contact area between substrate and microorganism (Nicolella *et al.* 2000).

The anaerobic process comprises two major processes, i.e., acidogenesis and methanogenesis processes. The value of optimum pH for acidogenic bacteria is 5.2-6.5 and 6.7-7.5 for methane forming-microorganism. Methanogenic bacteria are too sensitive to pH changes. POME was treated sequentially in this work by separating acidogenesis and methanogenesis based on its optimum pH level. The primary function of the first stage as the acidogenic reactor was to convert organic materials, which were measured as sCOD, into volatile fatty acid (VFA) under acidic conditions. The VFA, as the product of acidogenic reactor, was then transferred into the second stage to be converted into methane under neutral pH condition.

It is essential to adjust the pH value in the second stage to be higher than that in the first stage of a two-stage biomethane plant (Deublein & Steinhauser, 2008). A previous study Ayu *et al.* (2017) indicated that the methanogenic microorganism activity inside the bioreactor was deficient due to the inhibition of high VFA concentration. The two-stage system was also preferred due to the high fat and oil content in POME. Two-phase anaerobic digestion system had been recommended for the treatment of wastewater containing high VFA content such as dairy waste (Demirer & Chen, 2005), ice cream factory effluents (Borja & Banks, 1995), fish meal processing waste (Guerrero *et al.* 1999), slaughterhouse waste (Wangs & Banks, 2003), and olive mill solid waste (Córdoba et al. 2008). This research aims to study the effectiveness of separating the acidogenic and methanogenic processing on sCOD removal for the acidogenic reactor and methane production for the methanogenic reactor. The optimization was conducted in terms of hydraulic retention time (HRT), which gave optimum conditions.

2. Materials and Methods

2.1 Materials

The materials used in this research were palm oil mill effluent (POME) taken from PTPN VII Lampung, natural zeolite from Tasikmalaya as the immobilization media,

and digested biodiesel waste from an active bio-methane reactor treating biodiesel plant waste as the starter inoculum. The POME was obtained from PTPN VII, Lampung, Indonesia with total solid (TS) of 11,900 mg/L, soluble Chemical Oxygen Demand (sCOD) value as much as ±8,000 mg/L, oil/grease of 115 mg/L, and pH value of 4.48. Digested biodiesel as inoculum starter waste was taken from the biodiesel industry operating in East Java with the COD of 1,980 mg/L and pH of 7.53.

2.2 Two-Stage Anaerobic Fluidized Bed Reactor (AFBR)

Two anaerobic fluidized bed reactors made of the acrylic vertical column were used in this study. The first AFBR was a 15 L acidogenic bioreactor, and the second one was a 10 L one, equipped with a close loop recirculation system for fluidization (Figure 1). As much as 150 gram of natural zeolite was added as microbial immobilization media in each bioreactor.

The acidogenic bioreactor was designed to be larger than the methanogenic bioreactor because the workload in the acidogenic reactor was not only the acidogenesis process but also the hydrolysis process by another group of bacteria. The POME contained about 100-150 mg/L oil/grease so that it took more time for the complex materials to be broken down into simpler compounds consumable for acidogenic bacteria in the bioreactor.

In Figure 1, R1 and R2 are the acidogenic and the methanogenic reactors. Tank 1, Tank 2, and Tank 3 are the influent tank, intermediate tank, and effluent tank. P1 and P4 are the dosing pump. The other pumps, P2 and P5, are the circulating pump, with the same function as P3 and P6. Valves are represented by V1/V3 (controlling valve for circulating stream) and V2/V4 (controlling valve for effluent stream). Gas meter is symbolized as GM1/GM2 and flowmeter as FM1/FM2.

The first AFBR was run under acidic condition (pH 5-5.5) to prevent the growth of methanogenic bacteria, while a neutral state (pH 7) was maintained for the second stage AFBR. The pH inside the second AFBR was adjusted by adding sodium hydroxide in the started up phase of methanogenic bioreactor. AFBR was operated in a continuous mode of operation by feeding fresh POME intermittently eight times per day. Feeding volume was adjusted to obtain the variation of HRTs presented in Table 1.
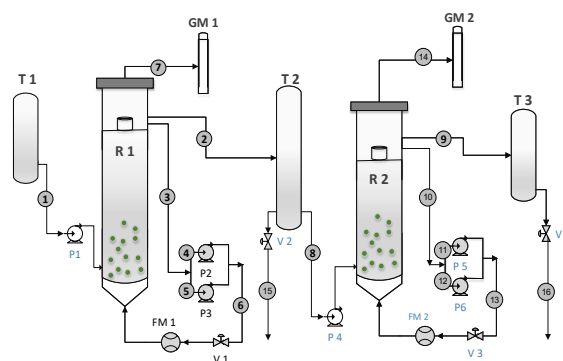

Fig 1. Experimental setup

Table 1
Feeding volume of continuous AFBR at difference HRT

Hydraulic Retention Time (day)	Feeding Volume (mL/day)	
	Acidogenic Reactor	Methanogenic Reactor
20	750	500
15	1,000	750
10	1,500	1,000
5	3,000	2,000
2	7,500	-

2.3 Analysis of sCOD, VFA, and CH₄

In this work, the parameter used to represent the available substrate for bio-methane production was Volatile Fatty Acid (VFA) while soluble Chemical Oxygen Demand (sCOD) was used to represent all the organic matter besides acids organic. The sCOD measurement gave the total sCOD value, which included VFA. The values of sCOD presented in this manuscript is excluded the VFA, i.e. the total sCOD value substracted by the VFA value. The analysis of sCOD and VFA followed the standard procedure by APHA (Cleseri *et al.* 2005). The sCOD analysis was conducted with closed reflux colorimetric method. The VFA analysis used the titrimetric method. The gas volume was measured by using the gasometer method (Walker *et al.* 2009), while the methane content was analyzed by using Gas Chromatography (GC) Shimadzu GC 8A.

3. Results and Discussion

### 3.1 sCOD Removal and VFA Formation in Acidogenic Anaerobic Fluidized Bed Reactor

The acidogenic AFBR was run with continuous mode operation in four variations of the HRTs, started from 20 days HRT and followed by the HRT of 15 days, 10 days, and 5 days. Since this work is aimed for scale-up purposes, chemical addition for pH controlling was being avoided to reduce operating costs. Figure 2 shows the comparison between the sCOD concentrations in the influent and effluent streams.

Significant sCOD removals were observed for all HRTs tested in this study, as presented in Table 2. However, not all of the sCOD was converted into methane. Figure 3 shows that only a minimal amount of the sCOD was converted into methane in the acidogenic reactor. Methane productivities were quite low for all HRTs, which was as expected in an acidogenic AFBR. The purpose of acidogenic AFBR is to maximize the production of VFA to feed the methanogenic AFBR. High sCOD removal, high VFA production, and low methane production in the acidogenic AFBR were good signs of the successful process in the acidogenic AFBR. At the HRT of 5 days, methane production was the lowest compared to other HRTs. This

phenomenon indicates that methanogenic microbes were almost totally washed out at the HRT of 5 days.

The purpose of the acidogenic stage was to supply the substrate for the methanogenic stage in the form of Volatile Fatty Acid (VFA) which was the result of sCOD conversion. The sCOD conversion into other compound was termed "removal" in this study. All of the sCOD removed was converted into VFA, with a small amount of methane production. The maximum percentage of sCOD removal in this work was 96.06% at 20 days of HRT. This result was higher compared to a similar experiment using double stage AFBR with one-time feeding/day, which was about 75% (Prasetyo *et al.* 2017). Intermittent feeding system was applied in this work to maximize the contact time between the microorganism and substrate. This feeding technique also minimized the potential inhibition of long chain fatty acids in the POME. The intermittent feeding has been indeed revealed as the key strategy for a long-term operation, even in the absence of adapted sludge (Gonçalves et al. 2009).
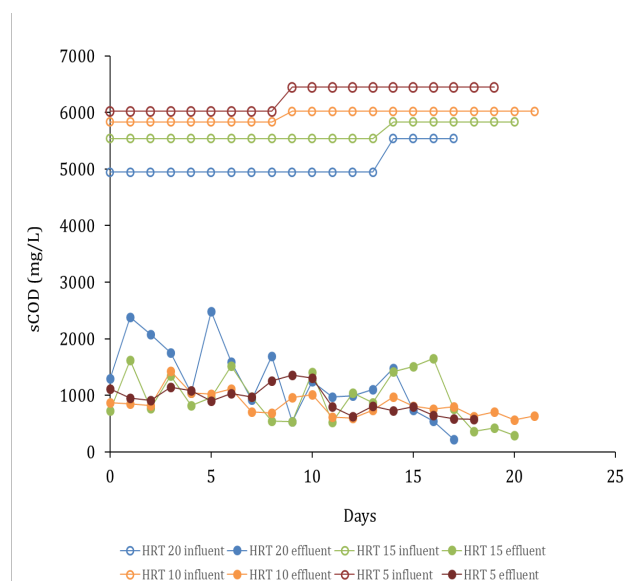
Fig 2. The sCOD profile in the influent and effluent of the acidogenic reactor with HRT variations
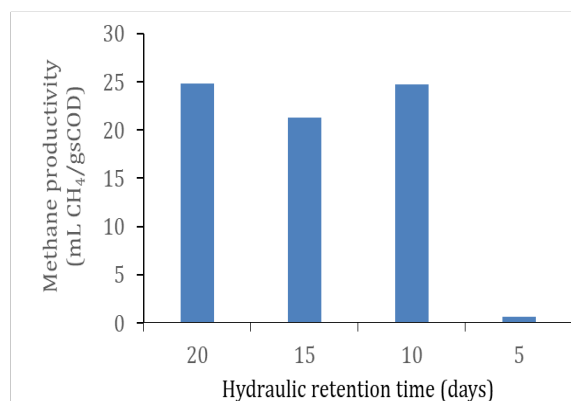
Fig 3. Comparison of methane productivity among various HRT in the acidogenic reactor

Table 2
Soluble COD (sCOD) removal for all HRTs in acidogenic reactor

Hydraulic Retention Time (day)	% sCOD Removal
20	96.06
15	94.96
10	89.48
5	91.13
2	92.81
1	84.40

Lower HRT (HRT of 10 days and 5 days) gave a better performance to minimize methane formation (Figure 3). VFA concentration increased along the time and this indicated that the acidogenesis process was dominating the system at lower the HRTs of 10 and 5 days (Figure 4). This confirmed the previous sCOD result that acidogenic bacteria were well adapted at 10 days and 5 days of HRT. The experimental duration of 20 days was considered quite short for the biofilm to stabilize. Longer duration for a continuous reactor gave a more stable process compared to batch reactor since the microbes had been well acclimated (Soetopo *et al.* 2011) and hence better results could be expected.

Since the first stage was intended for maximizing VFA formation, acidogenic bacteria were supposed to dominate the system. From Figure 4, VFA concentration in the effluent was lower than VFA concentration in the influent stream. The VFA effluent concentration shows the tendency to decrease at the beginning of 20 days and 15 days of HRT (Figure 4). Beyond the 15 days of the experiment, VFA concentration remained relatively constant. This phenomenon indicated that the acidogenesis process was quite successful, with only a small amount of methane produced (Figure 3).
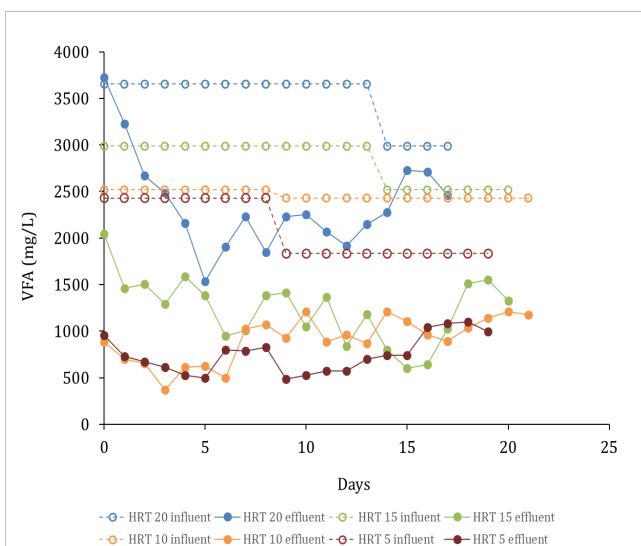
Fig 4. The VFA profile in the influent and effluent of the acidogenic reactor at various HRT
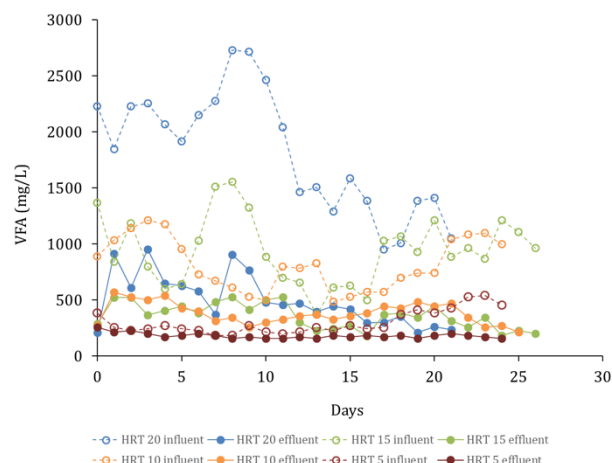
Fig 5. The VFA profile of methanogenic reactor at various HRTs

3.2 Concentration Profile of VFA in Methanogenic Anaerobic Fluidized Bed Reactor

Four different HRTs were applied in the methanogenic stage, started from HRT of 20 days, 15 days, 10 days, and 5 days. Neutral condition (pH 7) was set in this stage to maximize the growth rate of methanogenic bacteria that would be indicated by the high VFA conversion into bio-methane. The substrate measured in the methanogenic reactor was VFA produced from the acidogenic stage.

Figure 5 shows that the VFA concentration profile at the effluent stream was always lower than the influent. It confirmed that VFA conversion by the methanogenesis process was more dominating than VFA formation. The microbial population preparation in the batch system before the continuous operation had an important role to acclimate methanogenic cells in this stage (Ramadhani *et al.* 2018). The methanogenic bioreactor was run for 21 days in the batch mode operation at pH 7 until the biofilm was well developed before it was switched into a continuous system.

The good performance of this methanogenic stage was also shown by the stable concentration of VFA effluent for all different HRT. Generally, VFA reduction is caused by two pathways, i.e., the biomethane production and the maintenance of the cells.

Table 3.
Volatile Fatty Acid (*VFA) removal for all HRTs*

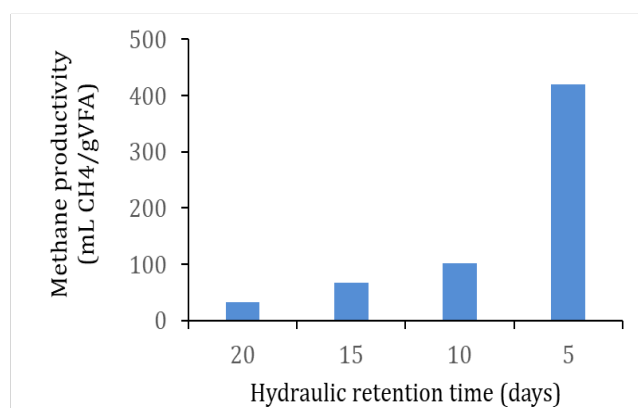| Hydraulic Retention Time (day)	% VFA Removal
20	83.33
15	81.93
10	78.57
5	71.05

Fig 6. Comparison of methane productivity among various HRT in the methanogenic reactor

The stable concentration of VFA in the effluent (200-500 mg/L) for all HRTs was defined as the minimum substrate concentration for the microorganism to survive. Methanogenic cells required specific minimum substrate concentrations to live (Prasetyo *et al.* 2017). When this minimum concentration was reached, the production of biomethane ceased because the substrate was all used for cell maintenance.

Among the four HRTs used in this methanogenic stage, 20 days of HRT gave a better performance in removing VFA (83.33%) as shown in Table 3. At lower HRTs (HRT of 15 days, 10 days, and 5 days), the high amount of substrate loaded to the system reducing the contact time between the substrate and the microorganisms, and this led to the reduction in the VFA removal. Interestingly, biomethane productivity, i.e., the amount of bio-methane produced per unit mass of VFA removal, was increasing as the HRT is decreasing, as shown in Figure 6.

3.3 Bio-methane Productivity in Methanogenic Anaerobic Fluidized Bed Reactor

Figure 6 showed the trend of biomethane formation/g VFA removed for all HRTs. Biomethane productivity was increasing as the HRT was decreasing. At lower HRT, there was the risk of the washout of methanogenic bacteria, so the minimum HRT should be carefully determined to avoid the reactor collapse.
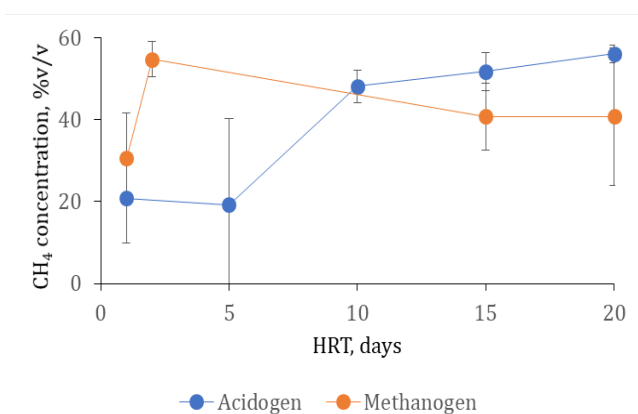

Fig 7. Comparison of methane concentrations at various HRTs in the acidogenic and methanogenic bioreactors

Nevertheless, when the reactor was operated in the safe range of HRTs, lower HRT gave a higher substrate feeding rate, which was preferable for the methanogenic bacteria. Methanogenic bacteria require a big portion of the substrate for cell maintenance so that a higher rate of substrate supply stabilized the population of these high-maintenance bacteria.

The calculation of the VFA removal in the methanogenic bioreactor was based on the difference between the VFA in the reactor input and its output. As VFA was the intermediate product between the original substrate (POME) and the biomethane, there was a possibility that there were acidogenic bacteria existed in the methanogenic bioreactor. In other word, the separation between acidogenic and methanogenic bacteria was not clean cut. They were still co-existing to some extent. With the existence of acidogenic bacteria in the methanogenic bioreactor, when the methanogenic bioreactor obtained the overflow from acidogenic bioreactor operated at the lower HRT (still contained quite high sCOD), then there might be a significant amount of VFA produced in the methanogenic bioreactor, too. Hence the VFA removal was measured lower in this case, while the actual amount of VFA converted into biomethane was actually high and the biomethane production was measured higher.

Figure 7 presents the methane concentration in both acidogenic and methanogenic bioreactors. The highest methane purity achieved in this continuous operation was 55.05%. It was slightly lower compared to methane purity in batch operation which was about 63.16%. This lower methane concentration in the continuous AFBR might be due to the duration of the reactor operation that was not sufficiently long in each HRT to stabilize the biofilm. The microbes in the biofilm could be shocked when the HRT was switched to shorter HRT. Optimization of stabilization duration and other operating conditions are suggested for further research to achieve higher methane purity.

4. Conclusion

In acidogenic AFBR, the acidogenic bacteria grew better than the methanogenic ones, as indicated by the sCOD and VFA profiles. At 5 days of HRT, methanogenic bacteria were almost totally washed out so that methane production was negligible, and the VFA accumulation was much better. By applying automatic pH control, the growth of acidogenic microbes could be further enhanced. Intermittent feeding system was proven to be an effective method to maximize sCOD removal in this work, which was as high as 96.06%. With the successful repression on the methanogenic activity, all of the removed sCOD was converted into VFA to be fed to the next stage, i.e. methanogenic reactor.

Neutral pH in the methanogenic reactor successfully minimized acidogenic bacteria and enhanced the methanogenic bacteria. Methanogenic AFBR exhibited the highest methane productivity when the reactor was operated at the lowest HRT, i.e. 5 days. Nonetheless, extra precautions should be taken in determining the minimum HRT to avoid methanogenic washout. The optimization of biofilm stabilization duration and other operating conditions should be considered further in the next study

to maximize the methane purity in the methanogenic AFBR.

Acknowledgments

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