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Different substrate of trickling filter on growth, survival rate, and water quality of common carp (*Cyprinus carpio*) cultivation by using an intensive recirculation system

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Different substrate of trickling filter on growth, survival rate, and water quality of common carp (*Cyprinus carpio*) cultivation by using an intensive recirculation system

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Abstract Recirculation aquaculture system is a culture technique which water conserve and reduce the waste discharge into aquatic environment. Trickling filter is the one of filter that common used for close system. Many kinds of substrate are able to use as a trickling filter to reduce the waste from aquaculture activity. The experiment aims to examine the effectivity of different substrate on growth, survival, and water quality of common carp. The different substrate as a treatment namely A) bio ball, B) bamboo, and C) gravel stone. The result showed that common carp cultured at A and B treatments on specific growth length (1.35±0.01 and 1.36±0.02%), specific growth weight (2.09±0.020 and 2.05±0.018%), and survival rate (86.67±2.44 and 87.89±1.83%) were higher than that of C treatment (0.81±0.03%, 1.74±0.075%, and 79.69±1.67%) ($P<0.05$). The biomass of common carp at A and B treatments (961.6±46.60 and 948.83±40.84 g) were higher than that of C treatment (716.17±38.23 g). Nitrite, nitrate, total ammonia, and phosphate at A and B treatments were higher compare to C treatment in term of removal efficiency ($P<0.05$). Bamboo has a potential as a substrate for trickling filter in an intensive culture using recirculating aquaculture system.

1. Introduction

Common carp is the third most widely cultivated and commercially important freshwater fish species in the world [1].Common carp is the very important freshwater fish species which is mainly cultured in Indonesia beside other species such as catfish, nile tilapia, and gourami. Fish demand has been increased with increasing human population, thus, common carp is the one fish species should be considered for food security. Common carp aquaculture production has been contributed more than 90% in Asia and 9% in the world for total finfish production [2].

In Asia, common carp is normally cultured in various aquaculture systems but the most common is the semi-intensive pond polyculture system [3]. However, common carp culture has been changed from conventional and semi-intensive to intensive system due to fulfill market demand. Most of fish farmers use an intensive because the system would be able to high stocking density and produce high fish culture. Thus, may system have been applied to increase fish production. Some effort have been conducted to improve productivity such as polyculture of common carp and Nile tilapia using deep water pond [4], probiotic[5], bio floc system [6],running water[7], that intensive system can produce high fish. However, these techniques are still using plenty of water during the operation, except for probiotic. Probiotic or bio floc utility have also found difficulty to maintain the input of material such as carbohydrate source (e.g. wheat flour) and bacteria as a probiotic during the operation. Thus, the probiotic system has to keep the number of bacteria and the C/N ratio in the water pond within the optimum condition in order to high fish production and keep the water quality stable [8].

Nowadays, the recirculation aquaculture system (RAS) has been developed in the world. This system can produce high of fish, water conserve, avoid to fish contamination from pollutant, easy to manage, and eco-friendly. RAS have many types of filtrations which one of the importance filtration is trickling filter. Media of trickling filter are rock, slag, manufacturer (plastic), and another material which is not easy to weathered. These materials attached on bacteria where bacteria have a function for degreade the pollutant or toxic compound. Thus, trickling filter would be able to reduce pollutant or toxic compound concentration[9] (EPA, 2002).Trickling filter is very efficient in terms of reducing industry waste such as COD (86.53%), BOD (95.25%), total of ammonia (69.93%), and phosphate (41.03%) [10].Trickling filter is also effective to removing manganese concentration with adding *Pseudomonas putida* bacteria on trickling filter media [11]. Trickling filter is also effective to reduce of 86.5% total nitrogen (TN) and 56.66% total [12]. The purpose of this experiment is to examine the effectivity of different substrate on growth, survival, and biomass of common carp, and water quality during the culture period.

2. Materials and methods

2.1. *Time and experimental location*

The experiment conducted from June to August 2017 at Research Station for Environment Technology and Toxicology Freshwater Aquaculture, Cibalagung, Bogor. Research Institute for Freshwater Aquaculture and Fishery Extension, Bogor. Indonesia

2.2. *Test fish and culture*

Common carp used for test fish with 4.54±0.52 cm in total length and 2.37±0.46 g in body weight in this experiment. Stocking density is 1200 fish/pond. Nine of concrete ponds with size of 4 m^3 (2 m length x 2 m width x 1 m depth) used for fish culture with completed by aeration, water pomp and trickling filter. Feeding of artificial diet was three times a day with feeding rate of 5% a day/biomass. To adjust the feeding rate during two months culture period, ten fish were taking as a sample to measuring the length and weight every ten days.

2.3. *Plastic barrel and substrate of trickling filter*

Nine of plastic barrels used for filter tank with the volume of 100l. Three kinds of substrates (i.e. bio ball, bamboo, and gravel) used for trickling filter media. Bamboo pieces with width of 2cm made by cutting of bamboo using grinding. Gravel stone with diameter of 2 cm used as a substrate. Each substrate introduced into the net with size of 60 x 60 cm and then placed in plastic barrel. The volume of substrate whether bioball, bamboo or gravel stone as media of trickling filter inside the plastic barrel with a volume of ¾ was placed. The kind of substrate depends on treatment. The plastic barrel placed on the top of table in the center of fish pond. The hole as an outlet with diameter of 1” made at the bottom of plastic barrel. The water pump (Yamano WP 3600, 20 watt, and 1000l/h flow rate) installed beneath of plastic barrel and connected by PVC with diameter of ½”. For instance, the layout of fish pond with trickling filter design (figure 1) and the implementation of trickling filter on the fish pond (Figure 2).

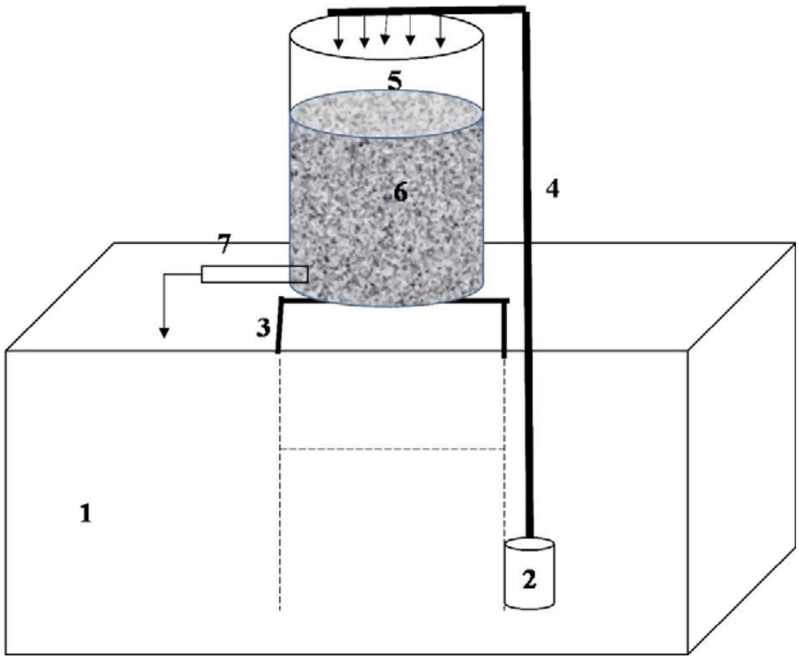


Figure 1. The layout of fish pond with trickling filter design
Remarks:
1. Concrete fish pond
2. Water pump
3. Table
4. PVC pipe ½”
5. Plastic barrel
6. Substrate of trickling filter (bioball, bamboo or gravel)
7. Outlet

Figure 2. The implementation of trickling filter on the fish pond

2.4. *Experimental design*

Block randomized design performed in this experiment. The experiment consisted of three treatments with three replications. The different substrate applied as a treatment. The treatment of different substrate as followed:

A) Recirculation pond system which completed by trickling filter with bio ball as a substrate
B) Recirculation pond system which completed by trickling filter with bamboo as a substrate
C) Recirculation pond system which completed by trickling filter with gravel stone as a substrate

2.5. *Statistical analysis*

Data analysis performed using one-way ANOVA and pos hoc multiple comparison using Tukey's test.

2.6. Parameters observed during the experimental period

Water quality parameter during culture period observed such as temperature, pH, dissolved oxygen (DO), total ammonia (TAN), nitrite (NO_2-N), nitrate (NO_3-N), and phosphate (PO_4-P) every ten days. Measured of temperature, pH, and DO using water checker where measured of TAN, nitrite, nitrate, and phosphate analyzed using Standard Nasional Indonesia (SNI) procedure [13,14,15] at the laboratory of Research Station for Environmental Technology and Toxicology Freshwater Aquaculture, Cibalagung, Bogor, west Java, Indonesia.

Calculation of biological performances data such as survival rate, specific growth length and weight, and removal efficiency of pollutant using formula as followed:
Survival rate calculated using formula:

$$SR = \left(\frac{Nt}{No}\right) \text{x } 100\% \quad (1)$$

Where: SR = Survival Rate(%)

Nt = Number of fish by the end of experiment
No =Number of fish at the beginning of the experiment

Specific growth rate of length and weght calculated using formula:

$$\propto = \left[\sqrt[t]{\frac{\bar{W}t}{\bar{W}o}} - 1 \right] \text{x } 100\% \quad (2)$$

where : α = daily growth (%)
Wt = Length (cm) or weight (g) at the end of experimentr
Wo = Length (cm) or weight (g) at the beginning of the experiment
t = culture period (day)

Biomass of fish calculated using formula:

$$B = W\ X\ N \quad (3)$$

where:
B =Biomass
W =Average of weight (g)
N =Number of population at the end of experiment

Efficiency removal calculated using formula:

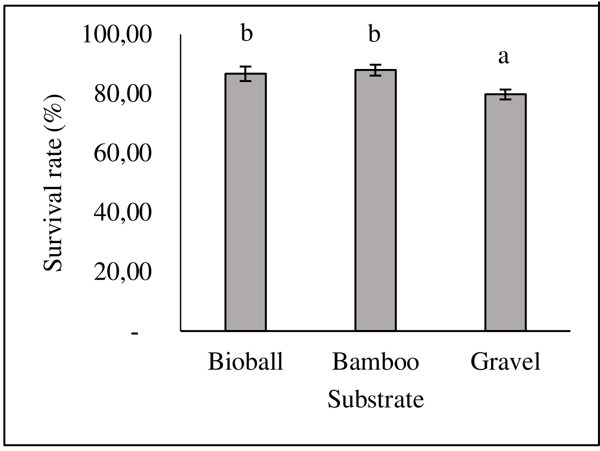
$$ER\ (\%) = C_{inlet} - C_{outlet} / C_{inlet} \text{ x } 100 \quad (4)$$

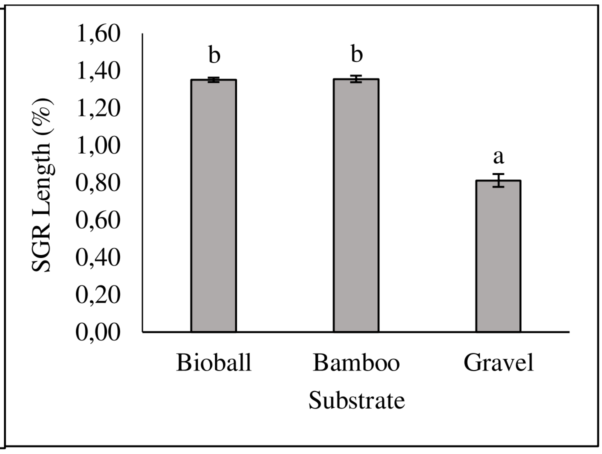
where:
ER = Efficiency removal (%)
C_{inlet} = Concentration of pollutant inlet (mg/l)
C_{outlet} = Concentration of pollutant outlet (mg/l)

3. Result

3.1. *Survival rate, specific growth rate of length and weight, and biomass of common carp*

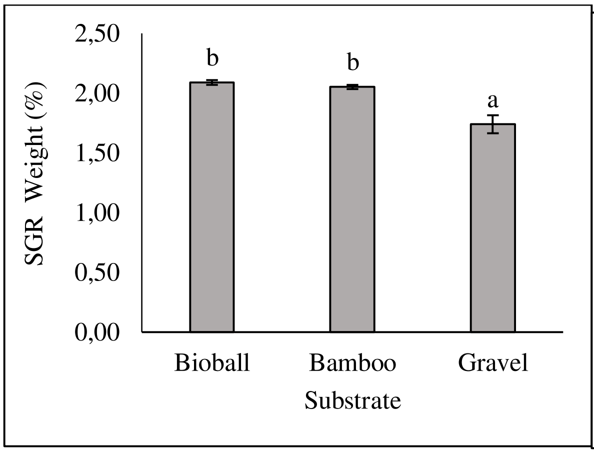
Biological performance such as survival rate, specific growth rate of length, specific growth rate of weight, and biomass of common carp cultured at different substrate of trickling filter is presented at Figure 3a-d.

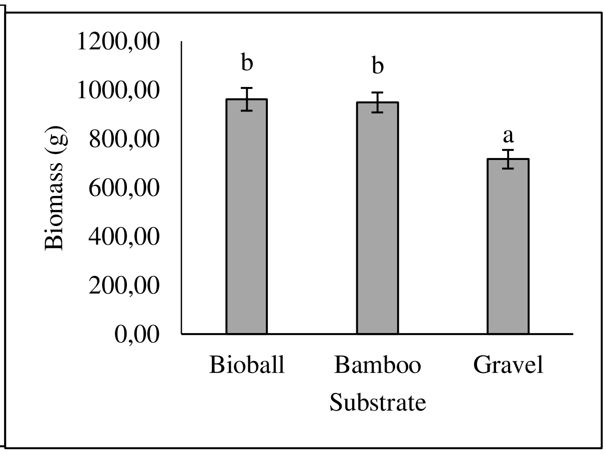




a

b





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d

Figure 3. Biological performances of common carp cultured at different substrate of trickling filter. a) Survival rate; b) SGR of length; c) SGR of weight; and d) Biomass. The bars followed the same letter are not significantly different (P>0.05)

Survival rate of common carp cultured using substrate of bamboo (87.89±1.83%) was the highest, then followed by bio ball (86.67±2.44%), and gravel (79.69±1.67). SGR of length of common carp cultured using substrate of bamboo (1.36±0.02%) was the highest, then followed by bio ball (1.35±0.01%) and gravel (0.81±0.03%). SGR of weight of common carp cultured using substrate of bio ball (2.09±0.020%) was the highest, than followed by bamboo (2.05±0.018%), and gravel (1.74±0.075%). Biomass of common carp cultured using substrate of bio ball (961.60±46.60 g) was the highest then followed by bamboo (948.83±40.48 g) and gravel (716.17±38.23 g) (Figure 3a-d). Survival rate, SGR of length, SGR of weight, and biomass of common carp cultured using substrate of bio ball and bamboo were better than that of using gravel substrate (P<0.05).

3.2. *The percentage of removal efficiency of total ammonia, (TAN), nitrite, nitrate, and phosphate*
The average of removal efficiency percentage of TAN, nitrite, nitrate, and phosphate by different substrate of trickling filter is presented at Table 1

Table 1. Removal efficiency (%) of TAN, nitrite, nitrate, and phosphate at different substrate

Parameters	Substrate		
	Bio ball	Bamboo	Gravel
TAN	40.25±18.63b	38.94±15.86b	8.98±6.89a
Nitrite (NO_2-N)	43.11±27.19b	33.29±20.08b	4.89±4.63a
Nitrate (NO_3-N)	50.81±25.20b	44.14±19.24b	6.53±3.94a
Phosphate (PO_4-P)	43.04±23.51b	38.39±18.03b	12.70±5.49a

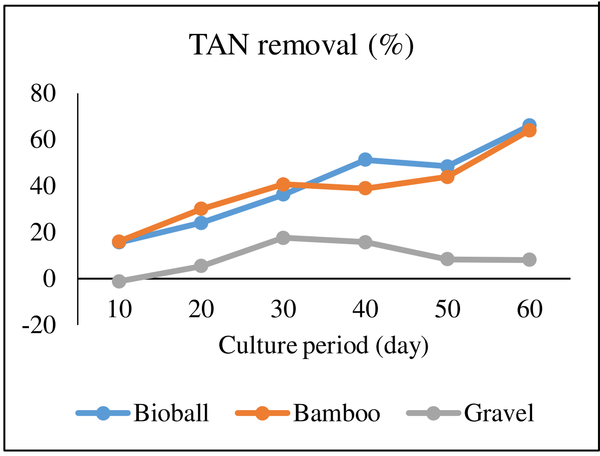
Remarks: the values followed the same letter are not significantly different (P>0.05)

Table 2. The range of water quality of common carp during cultured period at different substrate of trickling filter

Parameters	Substrates			Optimal condition
	Bio ball	Bamboo	Gravel	
Temperature (°C)	26.2-28.3	26.8-27.9	26.6-28.4	25-30[17)]
pH	6.0-7.5	6.0-7.5	6.0-7.5	6-9[16,17)]
DO (mg/l)	3.33-5.88	3.9-5.99	3.29-4.91	≥ 3[16,17)]
TAN (mg/l)	0.16-0.31	0.19-0.47	0.15-0.80	<1.00[16)]
Nitrite (NO_2-N) (mg/l)	0.0015-0.0076	0.0019-0.0061	0.0071-0.023	<0.01[16)]
Nitrate (NO_3-N) (mg/l	1.532-2.227	1.742-2.239	1.715-3.496	50[16)]
Phosphate (PO_4-P) (mg/l)	0.20-0.33	0.24-0.45	0.22-0.53	10[17)]
Alkalinity (mg/l)	97.63-115.34	85.43-124.14	103.73-134.24	50-500[16)]

The percentage average of removal efficiency of TAN, nitrite, nitrate, and phosphate using bioball was the highest than that of bamboo, and gravel (Table 1). Statistical analysis revealed that the percentage of removal efficiency using bio ball and bamboo was better than that of gravel (P<0.05). Water quality parameters observed during the common carp cultured such as temperature, pH, DO, TAN, nitrate, phosphate, and alkalinity were within the optimal range, except the nitrite concentration at gravel substrate was beyond the optimal range (Table 2).

3.3.*The pattern of removal efficiency of TAN, nitrite, nitrate, and phosphate*
The patter of removal efficiency of TAN, nitrite, nitrate, and phosphate during the cultured period of common carp at different substrate of trickling filter is shown at Figure 4a-d.



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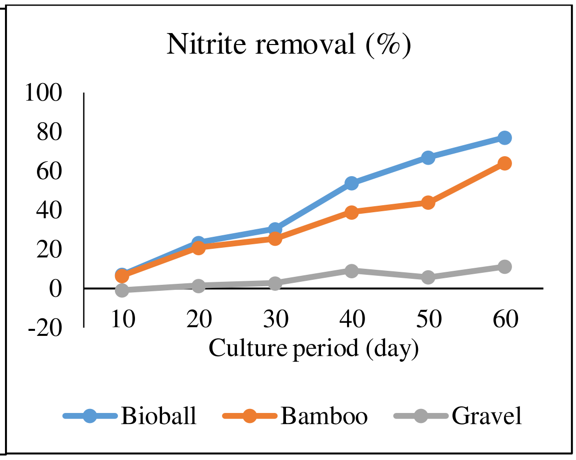

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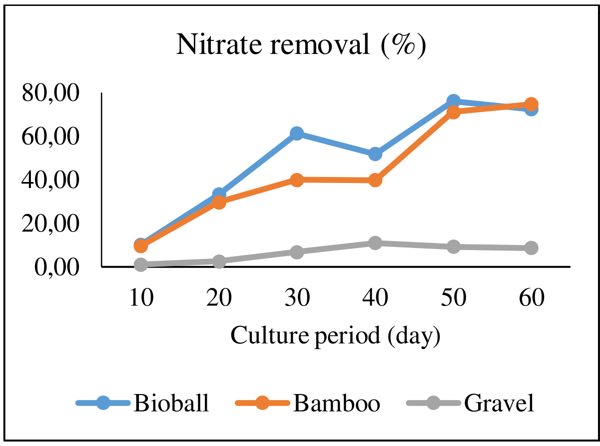

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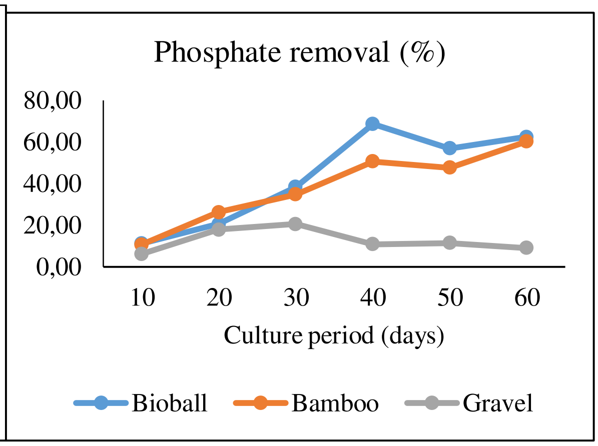

d

Figure 4. The pattern of removal efficiency of common carp cultured at different substrate of trickling filter. a) Removal efficiency of TAN; b) Removal efficiency of nitrite; c) Removal efficiency of nitrate; and d) Removal efficiency of phosphate.

The pattern of removal efficiency of TAN, nitrite, nitrate, and phosphate showed that bioball and bamboo substrates were the same pattern which is increased with increasing culture period while gravel substrate showed the low in term of removal and almost relative constant (Figure 4a-d). This indicated that bamboo and bioball is very effective in term of reducing such pollutant where gravel substrate showed ineffective.

4. Discussion

4.1. Survival, specific growth, and biomass of common carp

Fish growth, and survival are influenced by some of factors such as food availability, stocking density, and water quality [18]. The present experiments indicated that trickling filter with bioball and bamboo substrates are better than that of gravel substrate for survival, growth, and biomass (Figure 3a-d). This condition may be correlated with water quality which affects such biological performance. Water quality (Table 2) shows that the nitrite concentration has over the limit for common carp culture. Nitrite is an intermediate and important product in bacterial nitrification and denitrification processes in the nitrogen cycle. Nitrite has multiple physiological effects, one critical consequence of nitrite accumulation is the oxidation of hemoglobin to methemoglobin, thus, the amount of methemoglobin affect fish growth and survival [19].The present experiment shows that nitrite concentration at trickling filter using gravel stone substrate is higher than that of bio ball and bamboo substrates. Therefore, the common carp cultured using gravel stone as a substrate have produced low in survival, growth, and biomass (Figure 3 a-d). High nitrite concentration exposure will reduce growth and food intake [20].

4.2.Removal efficiency and water quality

Trickling filter have been used for biological wastewater treatment since 1890s. A trickling filter is a fixed-film reactor with no submerged medium over which wastewater is distributed. Treatment of wastewater occurs when wastewater passes through the biofilm attached to the medium. Trickling filters are used for organic matter removal, simultaneous organic matter removal, and nitrification as well as tertiary nitrification. Their advantages over the activated sludge process are less energy requirement, less need for equipment maintenance, and simple operation [21]. Components of recirculation aquaculture system such as a bio filter is very important in order to removal or reduce the ammonia concentration. The selective if substrates that will use as a media in the biological filter is main to allow nitrifying bacteria attach on the surface area of the substrate [22].The removal efficiency of trickling filter using stonewall are ranged from 50-100% TSS, 50% BOD, COD, 10-60% ammonia, and 0-13% nitrate [20]. Limestone and bio ball has also effective in removal of ammonium with is 15.12% and 11.94%, respectively[23]. The present experiment indicate that bamboo substrate of trickling filter is the same effective like bio ball in removal of TAN, nitrite, nitrate, and phosphate. Thus, bamboo have a potentially to be used as a substrate media for trickling filter.

Water quality is the most important factor in aquaculture. The present experiment shows that overall data of water quality parameters are within in the optimal range, except for nitrite concentration is slightly out of the limit. Nitrite concentration is 0.02 mg/L for long period affect fish growth, survival and low fish produce [24].

5. Conclusion

Bamboo have an effective in removal of TAN, nitrite, nitrate, and phosphate, thus, it can be used for substrate medium of trickling filter.

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