Photosynthetic bacteria pond system with infra-red transmitting filter for the treatment and recovery of organic carbon from industrial wastewater

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Abstract A photosynthetic bacteria pond system was applied to the treatment of food industrial wastewater and recovery of carbon in the form of purple non-sulphur bacterial (PnSB) cell. The effect of infra-red transmitting filter on the selection of microbial groups in the system was investigated. It was found that more than 90% of organic removal could be achieved when the system was operated at HRT of 3 to 10 days, even though some fluctuations were observed at lower HRT. Infra-red transmitting filter could suppress the growth of microalgae in the system and allow the purple non-sulphur to grow in the system. Nevertheless, they could be outgrown by sulphate-reducing bacteria at higher organic loading rates. The growth of purple sulphur bacteria associated with sulphate reducing bacteria was also observed. ORP is a crucial operating factor to control the system under micro-anaerobic conditions which is preferred to the growth of purple non-sulphur bacteria.

Keywords Carbon recovery; infra-red transmitting filter; photosynthetic bacteria pond; purple non-sulfur bacteria

Introduction

The application of purple non-sulphur bacteria to wastewater treatment is an attractive method since the microorganisms are widely available in nature and capable of removing organic substances even under high organic load (Hiraishi *et al.*, 1989; Sasikala and Ramana, 1995) especially in sunlight intensive area like tropical countries. Consequently, the treatment will require minimum additional energy apart from natural sunlight and the treatment cost can be substantially reduced. They are applicable to organic compounds (Madigan *et al.*, 2000) and wastewater from various industries (Noparatnaraporn *et al.*, 1986). By introducing this system, it might be possible to develop a high-efficiency treatment pond without odorous gas production (Figure 1). The proposed system consisted of two ponds connected in series. In the first pond of this process, it is expected that acidogenic bacteria consume organic matter in wastewater and purple non-sulphur bacteria grow with their metabolite. Thereafter, the following aerobic fishpond is expected to have a high fish yield. Moreover, the by-product of the treatment, the purple non-sulphur bacteria cells, has high nutrition such as protein and vitamin (Kobayashi *et al.*, 1971; Kobayashi and Tchan, 1973; Getha *et al.*, 1998; Banjaree *et al.*, 2000) which is a good alternative for fish feed.

This research focused on the application of naturally cultivated purple non-sulphur photosynthetic bacterial system to the treatment of sulphur containing food industrial wastewater. Influencing factors and appropriate operating conditions in the system were studied. The predominating microbial group and composition of bacterial community in the system was determined under natural operating conditions.

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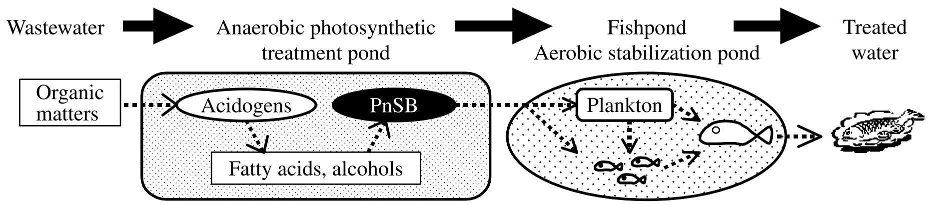

Figure 1 Process flow of photosynthetic bacteria pond treatment system

Materials and methods

Two laboratory-scale reactors simulating photosynthetic bacteria pond were used. Each reactor was made of glass vessels with 3 L working volume. The vessel dimension was 16 cm in diameter and 30 cm in height. The reactor was illuminated for 12 hours per day by two 60 W incandescent lamps from both side. Observed light intensity at the surface of the reactor was 45 W/m^2. Temperature was kept at room temperature and the heat emitted from incandescent lamps was dispersed by electrical fans. The system was operated as a single pass complete mixed reactor by providing magnetic stirrer mixing. Feeding of food industrial (noodle processing) wastewater and withdraw of reactor effluent was carried out once a day. The oxidation-reduction potential (ORP) and temperature of mixed liquor in the reactor were monitored. pH was monitored and controlled between 7 and 8 with the addition of either 0.1 M NaOH or 0.1 M HCl. The schematic of laboratory-scale experimental system is shown in Figure 2.

Enriched purple non-sulphur bacteria (wild strains) were used as seeding in the reactor. The hydraulic retention time (HRT) of 10, 6 and 3 days and the presence of infra-red transmitting filter were examined. The organic loading to the system was 0.16, 0.27 and 0.53 kgBOD/m^3.d, respectively. The performance of the system was evaluated in terms of effluent qualities at different hydraulic retention times (and organic loading rates). The analytical parameters for the influent and effluent from reactors include pH, soluble BOD (sBOD), soluble COD (sCOD), suspended solids (SS), sulphite (SO_3^-), sulphate (SO_4^{2-}) and hydrogen sulphide (H_2S).

The microbial population in the system was studied by determining bacterio-chlorophyll-a (Bchl.a) for photosynthetic bacteria and chlorophyll-a for microalgae. Purple non-sulfur bacteria (PnSB) were also quantified by fluorescent *in-situ* hybridization (FISH) technique. The populations of sulfate reducing bacteria (SRB) and purple sulfur bacteria

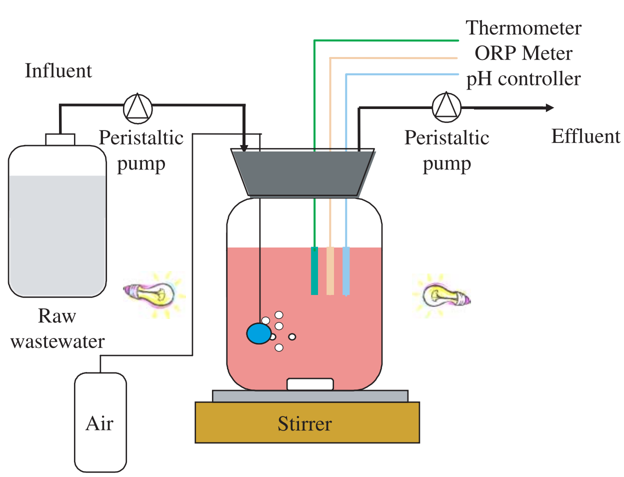

Figure 2 Schematic of laboratory scale experimental system

(PSB) were quantified by conventional plate count methods. Crude protein content of sludge withdrawn from the reactor was also analyzed.

Results and discussion

Effect of HRT and infra-red transmitting filter on the treatment performance

Two reactors (with and without infra-red transmitting filter) were operated at different HRTs of 10, 6 and 3 days corresponding to the average organic (sCOD) loading of 0.16, 0.15 and 0.24 kg/m^3.d. During the experiments, intermittent aeration was supplied to prevent a permanent drop in oxidation-reduction potential (ORP). Table 1 shows the water qualities obtained from both reactors. It was found that a high degree of organic (BOD and COD) removal was obtained at HRT of 10 and 6 days, yielding consistent effluent qualities. However, when the HRT was decreased to 3 days, organic removal rate fluctuated. The removal of organic substance in the reactor is mainly accomplished by PnSB which utilised organic substrates in the presence of light from wastewater as photosynthetic electron donor and carbon sources. In addition, sulphate reduction can also help eliminate some fractions of organic substances.

It was found that a high SCOD removal rate of more than 90% was achieved under the organic (sCOD) loading rate of 0.1 to 0.3 kg/m^3.d. High sCOD removal was obtained when the HRT in the system operated between 3 and 10 days, even though occasional fluctuations on the removal efficiency were observed at low HRT (Figure 3). The difference in treatment performance on the system with and without infra-red transmitting filter was not significant, showing that the infra-red transmitting filter did not have a significant effect on the organic removal in the system.

The reduction of HRT not only increased organic substance, but also raised sulphur compound loading to the system. As a result, they produce sulphide which is an inhibitory compound for PnSB. The sulphite and sulphate concentration in the influent varied between 53–241 mg/L and 10–183 mg/L. The sulphite concentrations in effluent were kept below 20 mg/L while sulphate concentrations in effluent increased. These increases in sulphate concentrations were mainly due to the oxidation of incoming sodium metabisulphite which was used for bleaching and antioxidant purposes. Nevertheless, the sulphate concentration was found to gradually decline towards the end of the experiment due to the development of sulphate reduction in the system. The accumulation of hydrogen sulphide and sulphite concentrations was not significant in the system. A similar trend was also observed in the reactor without infra-red transmitting filter.

Figure 4 shows the variation of ORP in a feeding cycle of wastewater. It can be seen that the organic loading rate and the presence of an infra-red transmitting filter affected the variation of ORP. The ORP dropped significantly after the feeding of wastewater to the system and gradually increased afterwards. In the reactor without infra-red transmitting filter, ORP increased much higher than those in the reactor with infra-red transmitting filter after feeding because oxygen was produced from the photosynthesis of microalgae in the presence of light. A higher organic loading (at HRT of 3 days) and the use of an infra-red transmitting filter could control the ORP, mostly in the negative range. Microanaerobic conditions are preferable to the growth of PnSB as compared to microalgae but too low a level of ORP might also promote the SRB activities.

Dynamic of microbial community in the system

In the reactor with infra-red transmitting filter, there were three major bacterial groups, i.e. PnSB, PSB and SRB. Figure 5 show the variation of microbial population in the reactor with an infra-red transmitting filter at different HRT. There was a decreasing trend of Bchl.a at HRT of 10 and 3 days. At HRT of 6 days, the Bchl.a content increased at the beginning

Table 1 Water qualities in the system with and without infra-red transmitting filter at different HRT

Parameters		HRT 10 days			HRT 6 days			HRT 3 days		
		Inf.	Eff.(a)	Eff. (b)	Inf.	Eff.(a)	Eff. (b)	Inf.	Eff.(a)	Eff. (b)
pH	range	5.6–6.0	7.1–7.9	7.0–8.0	5.2–6.4	7.0–7.6	7.0–7.5	5.3–6.5	7.0–7.9	7.0–7.7
	avg.	5.7	7.4	7.5	5.8	7.4	7.3	5.9	7.5	7.4
ORP	max.	21	297	188	47	191	226	119	270	228
(mV)	min.	244	280	265	174	148	171	245	260	250
	avg.	77	0	80	110	19	30	156	111	27
SBOD	max	723	26	28	425	40	21	400	16	18
(mg/L)	min	1490	123	113	785	56	55	780	137	157
	avg.	1151	58	52	638	38	39	618	52	52
SCOD	max	972	44	52	241	61	61	360	43	54
(mg/L)	min	2380	304	336	1214	110	133	1558	845	1056
	avg.	1579	119	117	899	94	104	791	216	231
SS	max	124	168	180	88	250	296	24	104	210
(mg/L)	min	544	653	770	936	568	870	1140	568	644
	avg.	298	437	529	361	430	541	252	343	406
Sulpide	max	0.11	0.01	<0.01	0.01	0.01	0.01	0.01	0.01	0.01
(mgS/L)	min	0.27	0.11	ND	0.35	0.35	0.08	2.46	2.46	0.43
	avg.	0.24	0.02	<0.01	0.06	0.06	0.02	0.51	0.51	0.27
Sulphite	max	97	10	13	86	2	2	53	2	2
(mgS/L)	min	241	20	15	241	13	13	239	16	16
	avg.	188	14	13	133	11	11	108	11	12
Sulphate	max	10	42	51	20	12	20	14	20	49
(mgS/L)	min	182	207	266	183	327	383	67	253	271
	avg.	69	116	130	52	178	188	45	135	140

(a)With infra-red transmitting filter (b)without infra-red transmitting filter

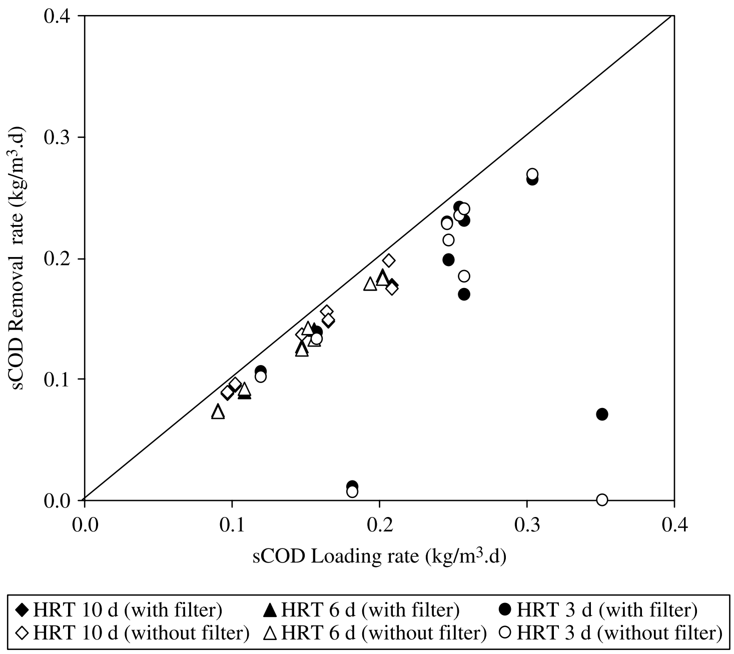

Figure 3 Relationship between sCOD loading and removal rate in the system

and started dropping after the operation went beyond 5 HRT. Nevertheless, the growth of SRB was found to be significant after 4 HRT in both cases. SRB in the reactor at HRT of 3 days was maintained relatively constant. This was due to the fact that a higher concentration of SRB was maintained under this operation from the beginning. However, SRB could increase in all conditions provided that the sulphur concentration of incoming wastewater was sufficiently high. The opposite trend of PnSB and SRB suggested that there was a competitive relationship among them. When sulphate reduction took place in the system, the produced sulphide inhibited the growth of PnSB (Madigan *et al.*, 2000). The growth of PSB associated with SRB was also observed at high organic loading rates as PSB could utilise sulphide produced from SRB activities for their growth.

Chl.a was found abundant in the reactor without an infra-red transmitting filter. There was an increasing trend of Chl.a after 40, 12 and 6 days at HRT of 10, 6 and 3 days, respectively. Since the visible wavelength could be easily absorbed by microalgae during photosynthesis, microalgae became predominant in the reactor without infra-red

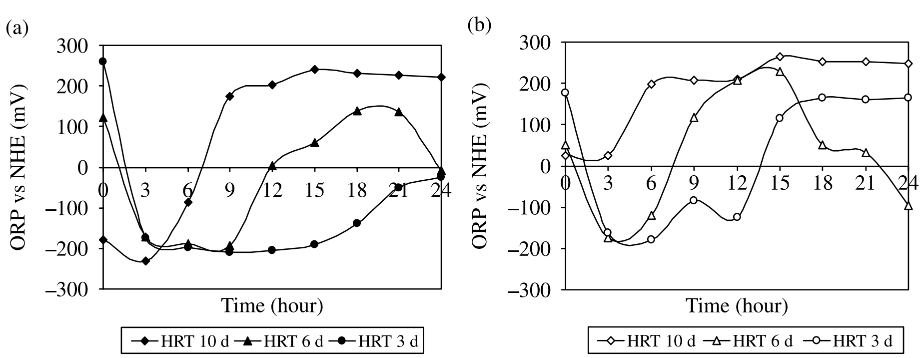

Figure 4 Variation of ORP within a feeding cycle (24 hours) in the reactor (a) with infra-red transmitting filter and (b) without infra-red transmitting filter

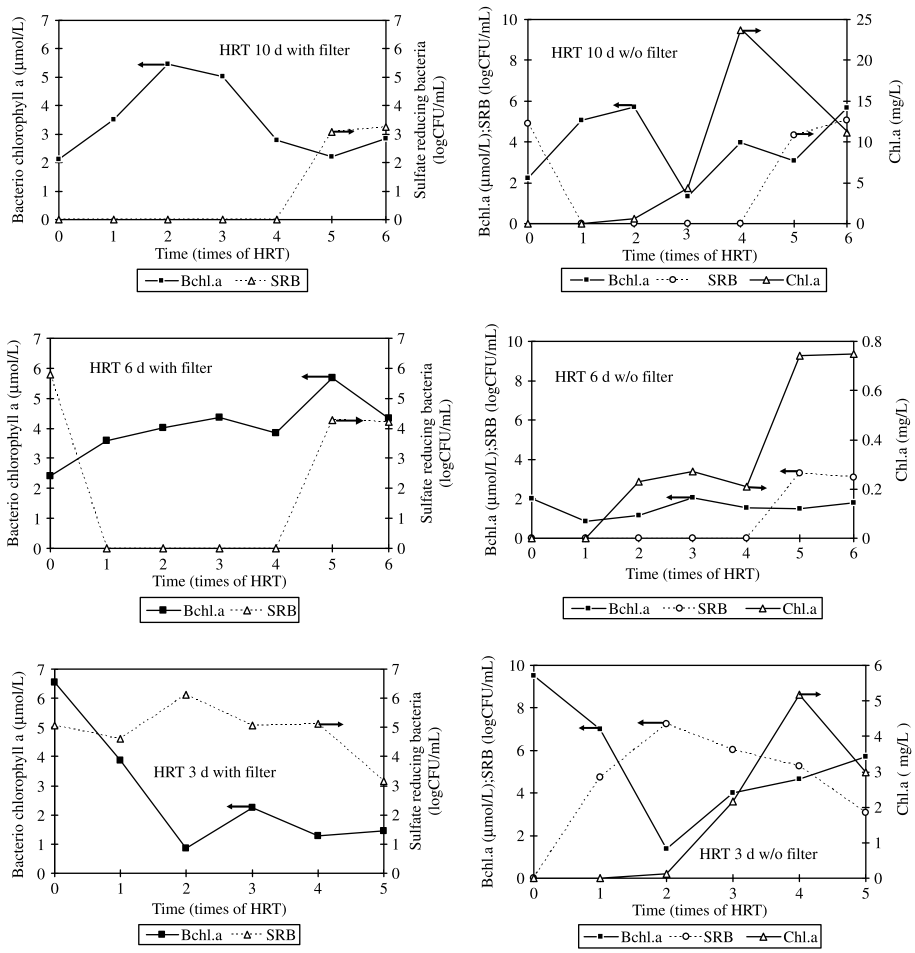

Figure 5 Dynamic of bacterial population within in the system at different HRT

transmitting filter. However, there was a significant reduction in the amount of Chl.a towards the end of the experiment as they were outgrown by SRB. These results clearly suggested that an infra-red transmitting filter could be used to suppress microalgae growth and successful microalgae control was accomplished.

Quantification of PnSB by fluorescent *in situ* hybridization

PnSB were detected and quantified using fluorescent *in situ* hybridization (FISH) with six oligonucleotide probes (Rpal686 for *Rps. Palustris*, Rbla1372 for *Rba. Blasticus*, Rbsp084 for *Rba. sphaeroides* and *Rba. Azotoformans*, Rbmr151 for *Rhb. marinum* and *Rps. Julia*, Blsv1030 for *Bla. sulfoviridis* and *Bla. viridis*; and Rvsl139 for *Rv. Sulfidophilum*). All bacteria groups were firstly simultaneously stained with DAPI (4″,6-diamidino-2-phenylindole). Then, the target bacteria were quantified as a ratio against total bacteria. *In situ* hybridization revealed that photosynthetic bacterium cultivated from natural mixed culture in existing wastewater treatment system from the industry consisted of at least two species of PnSB; *Rps. palustis* and *Rba. blasticus.* Figure 6 shows the variation of *Rps. palustis* and *Rba. blasticus* ratio along the experimental period in the reactor with an infra-red transmitting filter at different HRT. At HRT of 10 days, the average ratio of *Rps. palustis* and *Rba. blasticus* were initially 0.29 and 0.06 and reduced drastically to 0.02 at the end of the experiment. A similar trend was observed at HRT of 6 days.

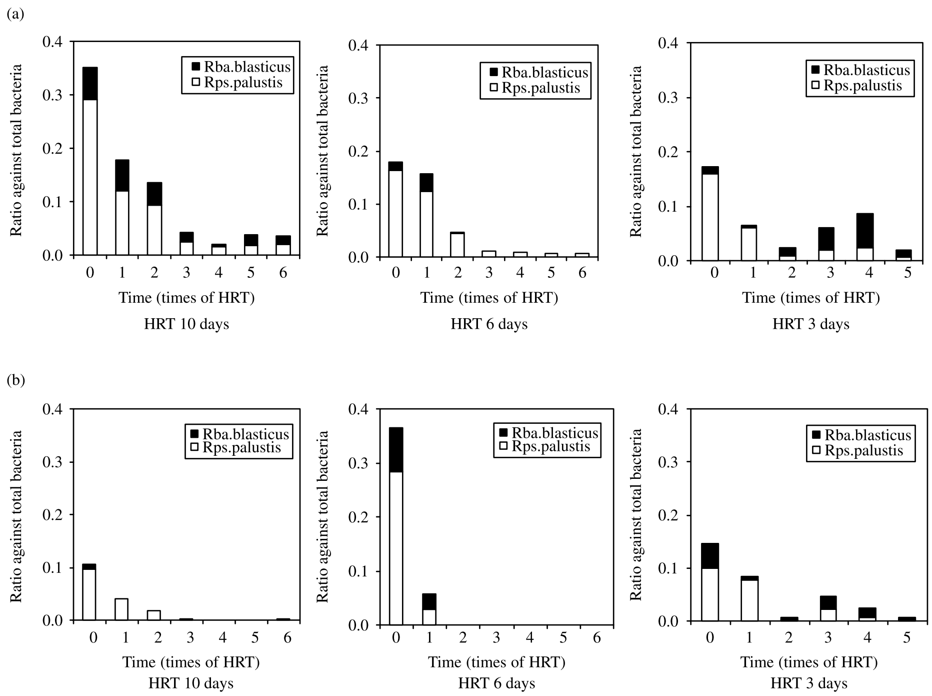

Figure 6 Ratio of specific PnSB against total bacteria (DAPI) in the system at different HRT (a) with infra-red transmitting filter (b) without infra-red transmitting filter

In the reactor without an infra-red filter, the initial *Rps. palustis* and *Rba. blasticus* ratios were 0.28 and 0.11 and they disappeared after 2 HRT. This was due to excessive microalgae growth and a reduction in PnSB population. At HRT of 3 days, the ratio of *Rps. palustis* and *Rba. blasticus* varied significantly. Initially, the reactor was operated under intermittent aeration of 1 min every 1 h but it was found that PnSB disappeared shortly after the operation began. Therefore, the aeration supply was increased to 1 min every 30 min. It was probably caused by the fluctuation of sulphite and sulphate levels in the influent which created toxic conditions to PnSB.

It was found that the reactor with an infra-red transmitting filter at HRT of 6 and 10 days had good treatment performance and microalgae growth could be suppressed in the system. Nevertheless, the population of PnSB could not be maintained and disappeared as the sulphate reduction took place and PSB became the predominant photosynthetic organisms in the reactor.

Single cell protein (SCP) production

It was found that the crude protein content in the sludge obtained from the reactor with the infra-red transmitting filter at all HRT was more than 50% (Table 2). The protein content of sludge is comparatively high enough to be utilised as SCP. At HRT of 10 and 6 days, the crude protein content in the reactor with an infra-red transmitting filter were found to be higher than without an infra-red transmitting filter as the photosynthetic bacteria cell contains higher protein than microalgae. However, there was no significant difference among them at HRT of 3 days because high organic loading created anaerobic conditions and there was neither the growth of PnSB nor microalgae but PSB in both reactors.

Table 2 Production of SCP from photosynthetic bacteria at different HRT

Run	HRT (d)	Light	Organic-N (mg/L)	Crude protein (mg/L)	SS (mg/L)	Crude protein content (g/g-dry weight)
A-1	10	with filter	38	234	437	0.54
A-2		w/o filter	33	208	529	0.39
B-1	6	with filter	46	287	430	0.67
B-2		w/o filter	52	326	541	0.60
C-1	3	with filter	35	181	343	0.53
C-2		w/o filter	41	254	458	0.55

Conclusions

A high organic removal of more than 90% was achieved in the photosynthetic bacteria pond operated at HRT of 3 to10 days. An infra-red transmitting filter was effective in suppressing the growth of microalgae and allowed purple non-sulphur to grow in the system. Two species of purple non-sulphur bacteria were identified in the system (*Rps. Palustis* and *Rba. Blasticus*). However, they could be outgrown by sulphate reducing bacteria at higher organic loading rates. ORP needed to be carefully controlled to suppress sulphate reduction and the growth of sulphate reducing bacteria. The growth of purple sulphur bacteria associated with sulphate reducing bacteria was observed. The sludge withdrawn from the reactor with the infra-red transmitting filter contained a high protein content of more than 50%, comparatively sufficient to be utilised as single cell protein.

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