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COD fractionation and toxicity of pulp and paper mill wastewaters in a tertiary process

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ABSTRACT

The chemical oxygen demand (COD) fractionation, as well as the acute toxicity and UMU genotoxicity of pulp and paper mill wastewater (PPMWW) were investigated to determine the COD compositions of PPMWW and to find the relationship between COD fractionation and toxicity. The fraction comprising non-biodegradable (inert) organic matter (X_I) was the main component in influent PPMWW, accounting for about 80% of the total COD. The acute toxicity of the influent was 0.08–0.16 mg Hg^{2+} L^{-1} and the UMU genotoxicity was 0.6–0.8 μg 4-nitroquinoline-*N*-oxide L^{-1}. Both acute toxicity and genotoxicity were reduced in the biotreatment system. However, coagulation and Fenton oxidation were efficient in eliminating UMU genotoxicity. Total COD, acute toxicity, and genotoxicity showed significant correlation with each other ($p < 0.01$).

Keywords: Pulp and paper mill wastewater (PPMWW); COD fraction; Acute toxicity; Genotoxicity

1. Introduction

Pulp and paper manufacturing is one of the most important industries in the world. It is the fifth largest industry in the US economy [1]. According to the China Paper Association, the total industrial output of Chinese papermaking industry in 2011 was 6,911 billion RMB, generating about 39.37 billion tons of wastewater [2]. Pulp and paper mill wastewater (PPMWW) is difficult to treat for its high chemical oxygen demand (COD), low biochemical oxygen demand (BOD), high toxicity, and dark color. Growing public awareness of the fate of these pollutants and stringent government regulations compel the industry to treat PPMWW before discharging it to the environment. A lot of studies have been conducted to remove these PPMWW pollutants. Lucas et al. [3] reported a novel tertiary treatment on pulp mill wastewater by Fenton (Fe^{2+}/H_2O_2) and solar photo-Fenton ($Fe^{2+}/H_2O_2/UV$) process. The solar photo-Fenton reaction is much more efficient than its corresponding dark reaction under identical experimental conditions. Other studies have reported advanced treatment of PPMWW on optimizing the operation parameters [4–6]. Liu et al. [7] found that pretreatment of PPMWW by magnetization could accelerate the removal of COD_{Cr} and reduce the

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Fe–PA complex dosage by about 7.7%. Dilek and Gokcay [8] reported that utilizing alum as a coagulant enabled 96% removal of COD from effluents released from paper machine processing, 50% removal from pulping effluents, and 20% removal from bleaching effluents. Rohella et al. [9] observed that polyelectrolyte was better than the conventional coagulant, alum, in removing turbidity, COD, and color. Thompson et al. [10] noted that a COD removal efficiency of 80% was consistently achieved using polyelectrolyte coagulants; however, the residual COD was around 800 mg L^{-1}, indicating that additional treatment was necessary.

Various studies have revealed the toxic effects of PPMWW on various fish species [11,12]. But these studies have focused only on COD removal efficiency and PPMWW toxicity to fish. They lack a comparative evaluation of COD and its relationship with toxicity.

Activated Sludge Model 1 was introduced by the International Water Quality Association in 1987, which includes total COD as a water quality parameter. The model divided total COD into several parts: S_S represents soluble, fast biodegradable organic matter; S_I represents soluble, non-biodegradable (inert) organic matter; X_S represents suspended, slowly biodegradable organic matter; X_I represents suspended, non-biodegradable (inert) organic matter; X_A represents autotrophic bacteria; and X_H represents heterotrophic bacteria. X_A and X_H are usually negligible in practice. Due to the consideration of the electron transfer in biochemical reactions, this model has been widely used in recent years [13–15]. Organic matter fractionation [16] has been used innovatively for a number of objectives, such as increasing the regulation of COD in wastewater and delineating the technological limits of a treatment process.

Nevertheless, tests on COD fractions are mainly used for domestic sewage, not industrial wastewater. There are various pollutants in industrial wastewater, and the COD fractions are often closely related to wastewater toxicity [17]. The main objective of this study, therefore, was to investigate the COD fractions of PPMWW and to determine the correlation between COD fractions and toxicity. Luminescent bacteria and vitro SOS/UMU test based on a genetically modified strain of *Salmonella typhimurium* (TA1535/pSK1002) were used to determine the acute and genotoxicity of the PPMWW [18–20]. The results of this study will provide an important reference for COD and toxicity control in PPMWW.

2. Materials and methods

2.1. Wastewater sampling

Wastewater samples were collected from nine sites within a PPMWW treatment plant in northern China. The PPMWW treatment plant has a treatment capacity of 60,000 m^3 d^{-1} and uses a process integrating physicochemical, biochemical, and advanced treatment techniques. The physicochemical process is carried out using a primary sedimentation pool and an oblique net for pulp recovery. The biochemical process consists of anaerobic acidification and low-sludge-load extended aeration. The advanced treatment process consists of two-gradient flocculation deposition and the Fenton oxidation. The Fenton process was conducted by first adjusting the pH with acid. Then, H_2O_2 and Fe^{2+} were added to the reaction chamber. The operating pH of the chamber is between 4 and 5. The amount of H_2O_2 added was 0.05 kg/m^3 with a H_2O_2 to Fe^{2+} ratio of 1:2. The plant has two treatment systems with the same process: system A receives the wastewater from the recycling of waste paper pulp and system B receives the wastewater from wheat straw pulp. After the Fenton oxidation process, the treated water is discharged to a river.

The nine sites in the WWTP were located at (1) inlet A, (2) after primary settling A, (3) after oxic tank A, (4) after secondary settling A, (5) inlet B, (6) after primary settling B, (7) after oxic tank B, (8) after secondary settling B, and (9) after the Fenton oxidation (Fig. 1). The samples were collected in new, clean, high-density polyethylene bottles.

2.2. COD fractionation

COD fractionation experiment was carried out using physicochemical characterization methods. The inert fractions, S_S and X_S, were measured using respirometry batch experiments [21]. The S_I was computed as the soluble COD in the treated effluent. The value of X_I was calculated by subtracting S_I, S_S, and X_S from the influent soluble COD.

A respirometer with three sample chambers was used to measure S_S and X_S. Three replicates were performed for each wastewater sample. Wastewater influent (150 mL) was diluted with 200 mL of distilled water [22]. The resulting solution was added to 350 mL of pre-aerated activated sludge from the WWTP (pre-aeration was carried out for 24 h prior to the procedure). Allyl thiourea (10 mg L^{-1}) was added to inhibit nitrification. Fig. 2 shows oxygen uptake rate (OUR or r_{O_2}) eventually reaching a plateau at 380 min. The values of S_S and X_S were then calculated from the area under the respirogram, using equations (1) and (2). An overall dilution factor (F) of 4.67 was used for the experiments. Additionally, the heterotrophic biomass yield (Y_H) was determined according to the method proposed by Henze [16].

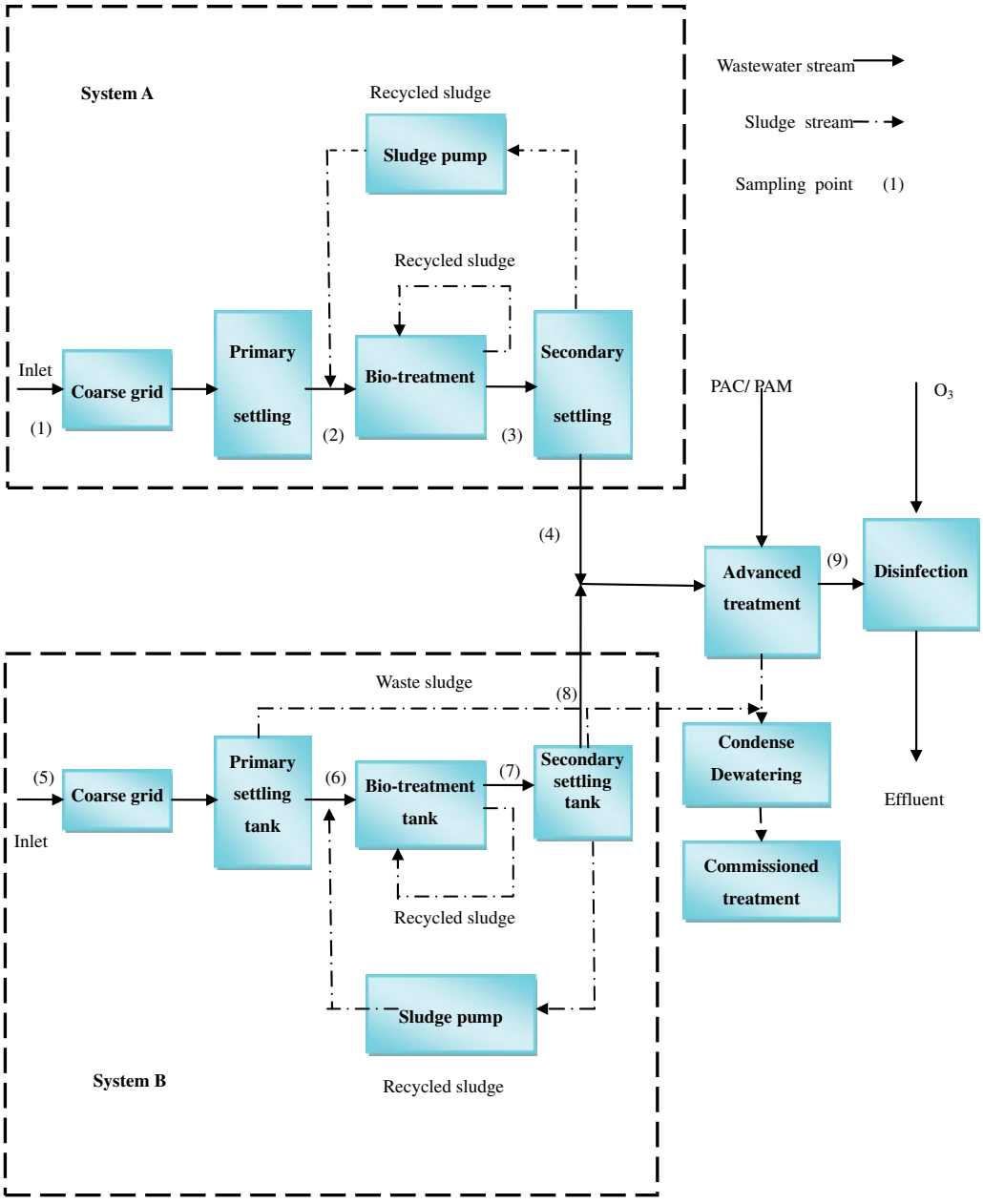

Fig. 1. Flow chart of the wastewater treatment plant displaying sampling locations: (1) inlet A (INA), (2) after primary settling A (PSA), (3) after oxic tank A (OTA), (4) after secondary settling A (SSA), (5) inlet B (INB), (6) after primary settling B (PSB), (7) after oxic tank B (OTB), (8) after secondary settling B (SSB), and (9) after Fenton reaction (FR).

$$S_S = F\frac{1}{1-Y_H}\int_0^{t1}[\mathrm{OUR}(t)-\mathrm{OUR}_{sb}(t)]\cdot \mathrm{d}t \quad (1)$$

$$X_S = F\frac{1}{1-Y_H}\int_{t1}^{t2}[\mathrm{OUR}_{sb}(t)-\mathrm{OU\ Re}]\cdot \mathrm{d}t \quad (2)$$

2.3. Toxicity bioassay

For the toxicity tests, the wastewater samples were taken from the reactors. The toxicity bioassay based on bacterial luminescence was conducted for 15 min by the standard method of Chinese Environmental Protection Agency. The freeze-dried marine bacterium (*Photobacterium phosphoreum*) and the testing instrument (toxicity analyzer model DXY-2) were purchased

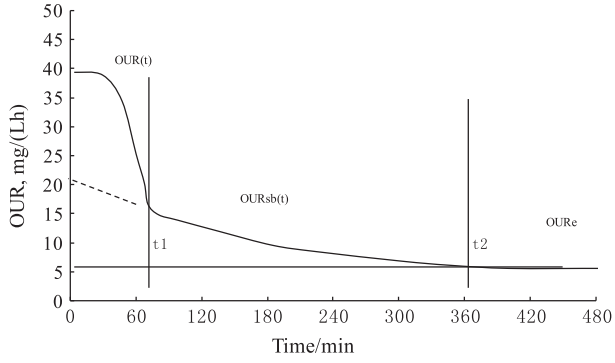

Fig. 2. Intermittent OUR respirometry curve.

from the Institute of Soil Science, Chinese Academy of Sciences. The toxicity analyzer quantifies the decrease in light emission from the bacteria resulting from 15 min exposure to the wastewater. The rate of luminescence inhibition was used as a measure of the toxicity of the wastewater. The acute toxicity of the sample was standardized to an equivalent Hg^{2+} concentration based on the Hg^{2+} dose–response curves for the bacteria.

The wastewater samples were acidified to pH 2 with 2 mol/L H_2SO_4, then passed through cartridges, which had previously been washed with 10 mL methanol, 10 mL acetone, and 20 mL distilled water. The cartridge was loaded with 500 mL of each sample, dried by nitrogen gas, and then eluted with 4 mL of acetone. The eluate was reduced to a small volume by vacuum evaporation, then dried under a stream of nitrogen. The dry residue was dissolved in 2 mL of dimethylsulfoxide (DMSO) and stored in the dark at −20°C before use. The genotoxicity of the concentrated samples were evaluated through the SOS/UMU test using *S. typhimurium* TA1535/pSK1002 without S9 activation. The SOS/UMU test was based on ISO-13829 [23]. In this assay, the galactosidase induced by the genotoxicity chemicals was applied to monitor genotoxicity. 4-nitroquinoline-*N*-oxide (4-NQO) dissolved in DMSO solution was used as the positive control. The genotoxicity of the sample was standardized to an equivalent 4-NQO concentration based on the dose–response curves of 4-NQO.

2.4. Analyses

Mixed liquor suspended solids and COD of wastewater were determined in accordance with the standard methods for the examination of water and wastewater [24].

Calculations were carried out using the hypothesis-testing function in Origin 8.0. Paired *t*-tests were conducted to analyze the results obtained. The hypothesis of significant difference was rejected at a *p*-value of <0.05.

3. Results and discussion

3.1. Wastewater characteristics

The color of the influent PPMWW was uniformly dark brown. The pH of the wastewater varied from 6.2 to 7.3 before the advanced treatment. After the advanced treatment by Fenton oxidation, pH dropped to about 4.0 because of the acid added in the Fenton agents. The total COD of system A was higher than that of system B. This difference indicates the different source of PPMWW samples (one was obtained from recycling of waste paper pulp and the other from processing of wheat straw pulp). COD of the wastewater samples are in accordance with that of typical PPMWW [1].

The five-day BOD/COD of the raw wastewater of systems A and B is 0.30 and 0.26, respectively, which indicates a low proportion of biodegradable organic matter compared with typical values (0.4–0.6 for presettled municipal wastewaters) [25].

Table 1 shows removal efficiencies of 83 and 73% with respect to the incoming total COD during the biotreatment process. Wastewater processed through biotreatment could not meet the limit of discharge standards for COD in China (100 mg L^{-1}). Thus, the advanced process, Fenton oxidation, was the appropriate choice for reducing COD.

Although the COD of the effluent processed through the advanced treatment was about 98 mg L^{-1}, which was complied with the COD standard of emission limit value (100 mg L^{-1}), it did not attain a desirable environmental quality. The effluent remained highly colored and turbid. There is still a need for new legislation to regulate key parameters, such as COD and color.

Table 1
Characteristics of the wastewater*

Sampling point	pH	SS	S_s	X_S	S_I
1#	6.2	1,161	56	117.7	234
2#	6.5	–	85.9	39.5	237.6
3#	6.8	–	62.7	69.1	236.8
4#	7.3	102	65.7	59.5	237.4
5#	6.6	564	55.9	73.2	182
6#	6.5	–	125.7	69.5	186.4
7#	6.8	–	29.8	25.6	187
8#	6.9	106	16.6	11.4	187.4
9#	4.0	64	7.2	4.2	43.2

*Units in mg L^{-1}, except for pH

3.2. COD fractions of PPMWW

The inert soluble COD fraction was estimated by taking a sample of the mixed liquor at the outlet of the plant reactors and re-aerating the sample for 24 h. The purpose of the extended aeration of the mixed liquor was to remove all of the biodegradable substrates. Afterward, S_I was measured by determining the residual final soluble COD. The average S_I value before the advanced treatment was 211 mg L^{-1} COD, which is lower than the treatability results in the effluent of the secondary settling tank (421–460 mg L^{-1} COD). The S_I decreased significantly due to Fenton oxidation.

X_I was calculated by subtracting X_S, S_S, and S_I from the total COD. The X_I fraction (inert particulates) released from the plant is bound in sludge flocs [16]. Although the X_I fraction is inert, it can be removed by biological treatment, physical adsorption, entrapment, and evacuation in the waste activated sludge flow.

The concentration of inert soluble COD remained unchanged in the whole biological treatment process. The COD fractionation pattern thus clearly defines the scope and limitations of the biological treatment used at the plant.

Fast biodegradable organic matter, S_S, accounted for 2.1–14.3% of the total COD in the wastewater and the slowly biodegradable organic matter, X_S, accounted for 2.7–12.9%. X_I in the raw wastewater of system A and system B took up the largest portion, at 80.4 and 85%, respectively. The S_I values in raw wastewater were 234 and 182 mg COD L^{-1}, accounting for 8.6 and 11.4% of the total COD in system A and system B, respectively. The COD in the treated effluent would be higher if only the biotreatment system was used because the biological treatment effluent must contain S_I from raw water. For this reason, it is impossible to achieve an effluent COD of lower than 100 mg L^{-1} with biological treatment alone.

After advanced treatment, the COD removal efficiency of system A and system B were 96.4 and 93.8%, respectively (Table 1). High removal efficiencies for the four COD components were also observed. S_I was mainly removed in the advanced treatment stage.

3.3. Changes in the toxicity of wastewater in the process

3.3.1. Acute toxicity of the wastewater to P. phosphoreum

The acute toxicities of the raw wastewater in system A and system B were 0.16 and 0.08 mg Hg^{2+} L^{-1}, respectively (Fig. 3). The corresponding toxicity grades, therefore, were high and medium, according to the division standard in Table 2. Acute toxicity of the raw wastewater in system A and system B were related to the COD value. The acute toxicity of PPMWW could be reduced by biochemical treatment. It could also be seen in Fig. 3 that COD could be effectively removed by advanced treatment, but advanced treatment was incapable of reducing acute toxicity to *P. phosphoreum*.

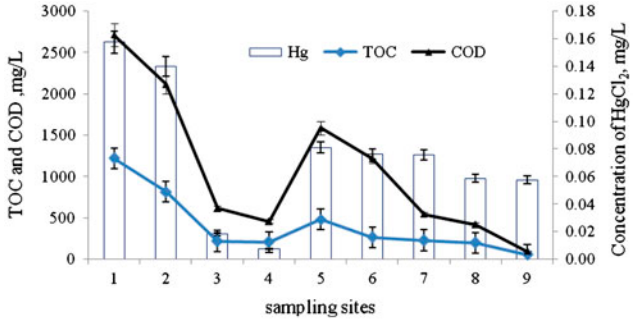

Fig. 3. Acute toxicity, TOC, and COD changes in the paper mill wastewater treatment plant.

Table 2
Classification of acute toxicity of the paper mill wastewater to luminescent bacteria, *P. phosphoreum*

Toxicity grade	Luminescent bacteria reduction rate	$HgCl_2$ concentration (mg L^{-1})
1	<30	<0.07
2	30–50	0.07–0.09
3	50–70	0.09–0.12
4	70–100	0.12–0.16
5	100	>0.16

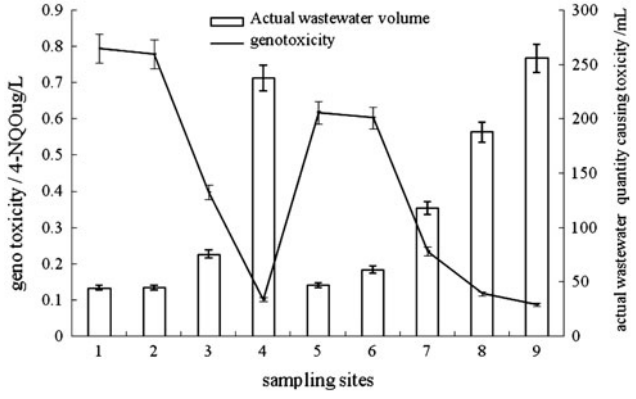

Fig. 4. Variations of the UMU genotoxicity and corresponding volume of wastewater causing genotoxicity in the paper mill wastewater treatment plant.

Table 3
Correlation between COD components and toxicity

	Acute toxicity	Genotoxicity	COD	S_S	X_S	S_I	X_I
Acute toxicity	1						
Genotoxicity	0.775*	1					
COD	0.855**	0.939**	1				
S_s	0.630	0.197	0.464	1			
X_s	0.337	0.694*	0.721*	0.546	1		
S_I	0.142	0.456	0.488	0.521	0.593	1	
X_I	0.892**	0.924**	0.994**	0.395	0.669	0.405	1

*$p < 0.05$.
**$p < 0.01$.

The acute toxicity of PPMWW was far higher than that of municipal wastewater which was given in our previous study [19]. Acute toxicity of PPMWW from system A and system B is also significantly different.

3.3.2. UMU genotoxicity

The genotoxicity of wastewater and the corresponding amount of PPMWW in the various treatment stages that would cause genotoxicity are shown in Fig. 4.

The genotoxicity of raw PPMWW was in the range of 0.6–0.8 μg 4-NQO L^{-1}. This indicates a small amount of organic matter that would cause genotoxicity existing in the raw PPMWW. In addition, the genotoxicity was reduced gradually as the raw water was processed through the wastewater treatment system. Specifically, the genotoxicity was reduced by 87.1 and 80.9% in system A and system B, respectively. Thus, the genotoxicity of the wastewater could also be reduced effectively by the advanced treatment. When IR = 2 (IR is the threshold at which strain mutation might occur), the actual amount of raw PPMWW in system A and system B required to induce genotoxicity was 44.4 and 46.8 mL, respectively, and the effluent volume after the advanced treatment needs to be at least 255.6 mL to induce genotoxicity.

3.4. Correlation between COD fractions and toxicity

The correlation between COD fractions and toxicity was investigated with the purpose of determining the relationship between toxicity, total COD, and COD fractions. The results are shown in Table 3.

In acute toxicity test, toxic substances inhibited bioluminescence in *P. phosphoreum* to cause toxicity, whereas they caused damage to the bacterial DNA and induced the SOS reaction of the bacteria used in the genotoxicity test. However, a correlation ($R^2 = 0.775$) between acute toxicity and genotoxicity was apparent. Although the toxic substances acted on different parts of the organism, there was no significant difference in toxicity among bacterial varieties ($p < 0.05$).

Unlike urban sewage and surface river water, COD of PPMWW showed a significant positive correlation with acute toxicity and genotoxicity at the 0.01 level (R^2 was 0.855 and 0.939, respectively). Thus, the COD of PPMWW had a positive correlation with the toxic effect.

4. Conclusion

This study evaluated the COD fractionation and the toxicity of PPMWW during a tertiary process. The innovative approach used in this study was to investigate the relationship between these variables. The main findings obtained are outlined as followings.

S_S accounted for 2.1–14.3% of the total COD during the paper mill wastewater treatment, whereas X_S accounted for 2.7–12.9%. X_I comprised the largest portion of the total COD in raw wastewater, at 80.4% in system A and 85.0% in system B. The S_I values of the raw wastewater were 234 and 182 mg COD L^{-1}, accounting for 8.6 and 11.4% of the total COD in the first and second stages, respectively. Therefore, effluent COD levels below 100 mg L^{-1} could not be achieved only by biotreatment process.

The acute toxicity and UMU genotoxicity of the wastewater were gradually reduced as the treatment proceeded. The advanced treatment had low efficiency in reducing the acute toxicity, but showed a significant effect in reducing genotoxicity. COD of paper mill wastewater significantly correlated with acute toxicity and genotoxicity.

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