Analysis on influencing factors of detecting chemical oxygen demand in water by three-dimensional spectroscopy*

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This paper focuses on the standard chemical oxygen demand (*COD*) liquid and studies the impact of pH, nitrite nitrogen, nitrate nitrogen, heavy metals, salinity, and other factors on fluorescence intensity and fluorescence peak positions during the detection of *COD* in water using fluorescence spectrometry. The influence mechanisms of different environmental factors on fluorescence spectra are also analyzed. Results indicate that pH value affects the fluorescence emission wavelength (*Em*), resulting in a red shift from 1.5 to 7.2, and a blue shift from 7.2 to 12.3. Nitrate nitrogen can react with organic matter in water to form nitro compounds, leading to a decrease in fluorescence intensity. Salinity has a negligible effect on T1 peak but a relatively large effect on T2 peak. Heavy metal ion concentration has a significant impact on T2 peak, while T1 peak position shifts with an increase in heavy metal ions. This study aims to explore the factors that can impact the detection of *COD* in water using three-dimensional fluorescence spectrometry, providing references to improve accuracy and practicability for *COD* detection based on three-dimensional fluorescence spectrometry.

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Spectroscopic techniques have widespread applications in various fields, including food[1,2], agriculture[3,4] and environmental ecology[5,6], medical diagnosis and analysis[7,8], public safety[9,10], materials science[11], and water quality parameter monitoring[12,13]. Chemical oxygen demand (*COD*) is a crucial indicator of water quality pollution, reflecting the extent of water pollution[14]. Three-dimensional fluorescence spectroscopy method for detecting *COD* in water offers the advantages of rapid, accurate, and non-hazardous assessment, without the usage of toxic and harmful chemical reagents[15]. However, water environment factors can affect three-dimensional fluorescence spectra, leading to measurement inaccuracy and reduction in precision. To further advance and enhance the utilization of this technology in the domain of water quality testing, an exploration of the principal factors driving the detection of *COD* through three-dimensional fluorescence spectroscopy is imperative. Thus, this study aims to investigate the influencing factors of three-dimensional fluorescence spectroscopy for sensing water quality *COD*, thereby providing a useful reference for improving the accuracy and practicality of *COD* detection.

The fluorescence spectra and intensity of aromatic compounds with acidic or alkaline groups change due to the ionization of acidic groups or the protonation of alkaline groups. Therefore, pH control is crucial in fluorescence analysis. This study investigates the influence of pH on the fluorescence peak center positions and fluorescence intensities of T1 and T2 in water samples with varying acidity and alkalinity.

The relationship between fluorescence intensity and pH variation of actual water samples is illustrated in Fig.1. As shown in Fig.1(a), a river water sample with *COD* concentration of 4.0 mg/L displays distinct pH variation patterns. Likewise, Fig.1(b) shows the pH variation patterns of a river water sample with *COD* concentration of 14.2 mg/L. The differences in pH variation patterns between the water samples are attributed to the varying types and structures of organic matter present.

Our findings highlight the importance of pH control in fluorescence analysis, especially when dealing with water

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samples with varying acidity and alkalinity. This work provides valuable insights for the development of more effective and accurate methods for the detection of water quality *COD*.

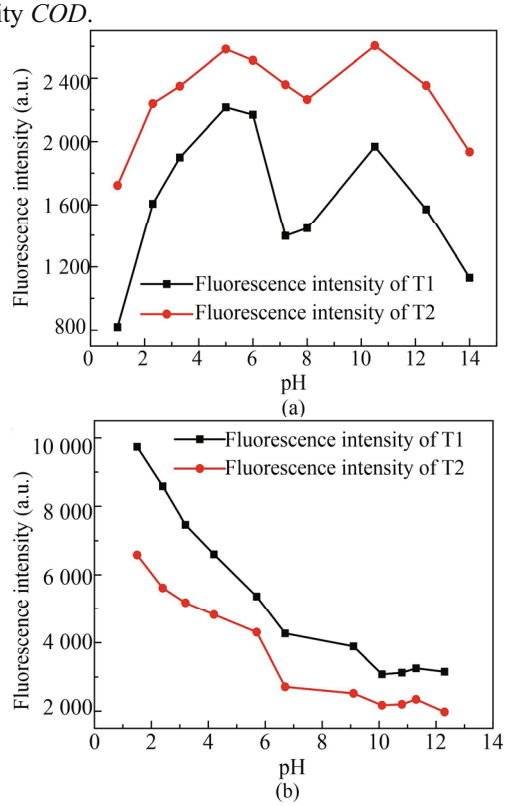


Fig.1 Influence of pH on the fluorescence peaks intensity of river water samples: (a) *COD*=4.0 mg/L; (b) *COD*=14.2 mg/L

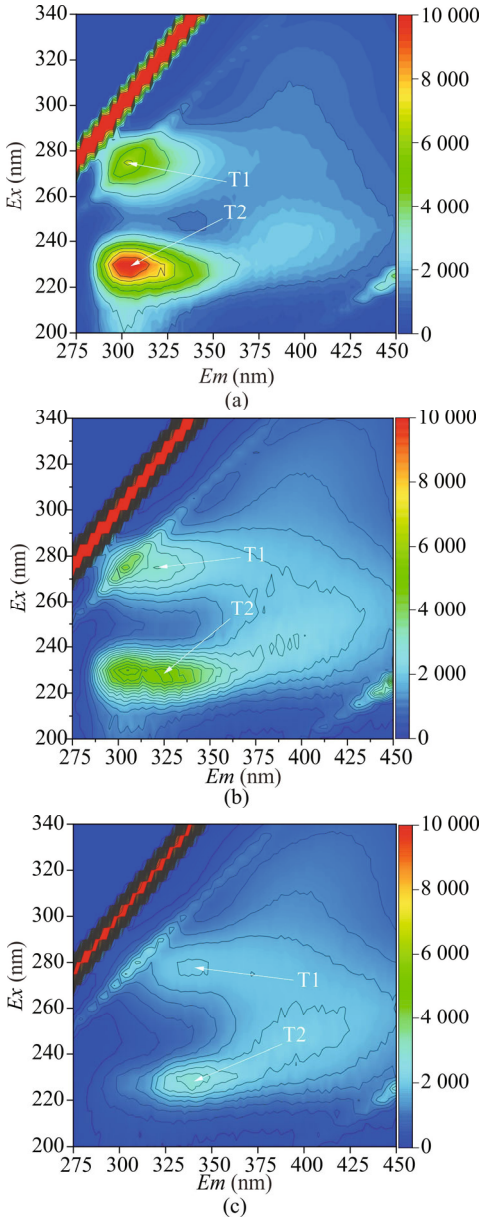
Analysis of Fig.1(a) reveals that the fluorescence intensities of T1 and T2 peaks exhibit a bimodal relationship with pH, with the two peaks appearing at pH=5 and pH=10.5, respectively. This behavior arises due to the pH-dependent dissociation of carboxyl and phenolic hydroxyl groups, with pKa values ranging from 3 to 6 and 9 to 11, respectively, in the organic macromolecular structure present in water. As the pH drops from 6 to 3, the dissociation of carboxyl groups leads to the stretching of macromolecular configurations in dissolved organic matter (DOM), resulting in the exposure of fluorescent groups in the solution, with the maximum fluorescence peak occurring at pH=5. On the other hand, as pH increases from 9 to 11, dissociation of phenolic hydroxyl groups and repulsion of anions cause the stretching of DOM molecules, exposing a large number of fluorescent groups in the solution, which increases fluorescence intensity. The maximum fluorescence intensity is attained at pH=10.5.

Analysis of Fig.1(b) shows that within the pH range of 1.5—12.3, the fluorescence intensity of the sample gradually decreases with increasing pH, indicating an increase in the degree of aggregation of some fluorescent groups in the DOM of the sample with increasing pH. Some fluorescent groups are more prone to stretch in an acidic environment, resulting in more exposed fluorescent groups in the solution and enhanced fluorescence intensity.

Fig.2 shows the contour maps of three-dimensional fluorescence spectra of a river water sample with *COD* concentration of 14.2 mg/L at different pH conditions, with pH values of 1.5, 5.7, 7.2 and 12.3, respectively.

As shown in Fig.2, the center positions of T1 and T2 fluorescence peaks change with increasing pH. The specific positions of T1 and T2 fluorescence peaks under different pH conditions are presented in Tab.1.

According to Tab.1, as pH increases, the excitation wavelength (*Ex*) changes minimally, while the emission wavelength (*Em*) changes significantly. The trends in changes for T1 and T2 peaks are the same. As pH increases from 1.5 to 7.2, *Em* wavelength increases, indicating a red shift in fluorescence spectra. As pH further increases from 7.2 to 12.3, *Em* wavelength decreases, indicating a blue shift in fluorescence spectra. The underlying reason lies in the fact that both dissociation



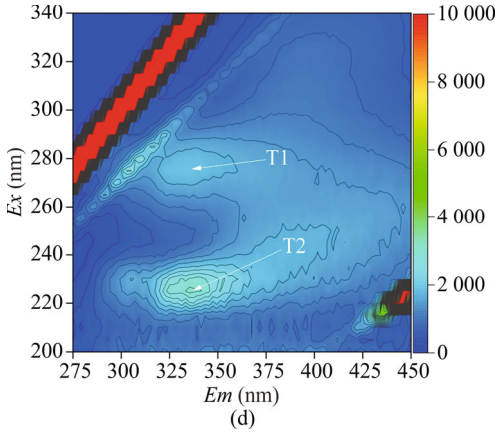


Fig.2 Influence of pH on the fluorescence peak positions of a river water sample: (a) pH=1.5; (b) pH=5.7; (c) pH=7.2; (d) pH=12.3

Tab.1 Influence of pH on the T1 and T2 peak positions

pH	T1 *Ex/Em* (nm)	T2 *Ex/Em* (nm)
1.5	275/303	230/302
5.7	275/320	230/323
7.2	275/338	230/340
12.3	275/333	225/335

and protonation can cause changes in the energy gap between the ground state and excited state of the molecule, resulting in a shift in fluorescence spectra. If the solution is initially alkaline and H_2SO_4 is added, the pH of the solution decreases, and the concentration of H^+ ions increases, leading to an enhancement of protonation effect on electron-withdrawing groups such as carboxyl groups (-COOH), which in turn causes a red shift in fluorescence spectra. Conversely, if the solution is initially acidic and NaOH is added, the pH of the solution increases, causing an enhancement of ionization effect on organic molecules in the solution. This leads to an increase in dissociation effect on electron-withdrawing groups, such as carboxyl groups, resulting in a blue shift in fluorescence spectra.

Nitrate nitrogen and nitrite nitrogen exist in aqueous solution in the forms of nitrate ion and nitrite ion, respectively. Fig.3 shows the fluorescence intensity variations of T1 peak of a 20 mg/L standard solution under different NO_2^- and NO_3^- ion concentration conditions, where the concentration range is 0—30 mg/L.

As shown in Fig.3, within the ion concentration range of 0—30 mg/L, the fluorescence intensity at T1 peak is not significantly affected by NO_3^- ion concentration but shows a decreasing trend with increasing NO_2^- concentration. This is because NO_2^- can react with organic matter in water to generate nitro compounds. With the increase of ion concentration, more nitro compounds are formed, forming a fluorescence quencher. The molecules of the fluorescence substance interact with those of the quencher to generate coordination compounds that do not emit light, resulting in weakened fluorescence intensity. In fact, both NO_2^- and NO_3^- can react with organic matter in water to generate nitro compounds and reduce fluorescence intensity, but 30 mg/L of NO_3^- is not enough to generate sufficient nitro compounds for fluorescence quenching.

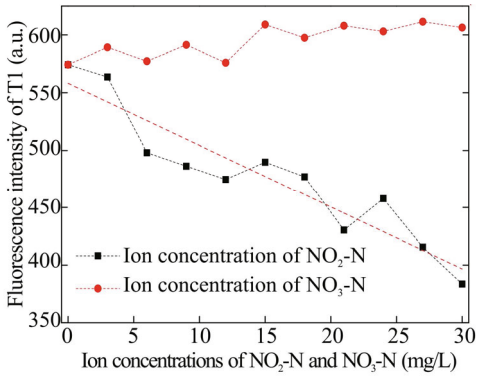


Fig.3 Influence of NO_2^- and NO_3^- on the T1 intensity of standard solution

This study provides insights into the effects of nitrate and nitrite ions on the detection of *COD* in water using fluorescence analysis.

Three-dimensional fluorescence spectra of NaCl solutions with mass fractions of 5‰, 15‰, 25‰, 35‰ and 45‰ were collected, with distilled water as a blank background at a mass fraction of 0‰. The fluorescence intensities of T1 and T2 peaks were recorded using a method of taking the average of three measurements, as shown in Fig.4.

It can be observed from Fig.4 that the fluorescence intensity of the T1 peak remains essentially constant with increasing NaCl concentration, while the fluorescence intensity of the T2 peak decreases and tends to level off. This is because the emitting species of the T2 peak, which is low-excitation wavelength tryptophan, has an unstable structure. The chloride ions in the NaCl solution can inhibit the ionization of the functional groups of the fluorescence species, and the degree of molecular conformational curling of the fluorescence species gradually increases with the concentration of Cl^- ions, leading to a decrease in fluorescence intensity. However, because the chloride ions have a relatively small inhibitory effect on ionization, the fluorescence intensity of the T2 peak slowly decreases with increasing NaCl concentration.

Therefore, when using the fluorescence intensity at the T2 peak to establish the relationship between water quality *COD* and fluorescence spectra for water samples with different salinities, the influence of salinity on fluorescence intensity must be considered to avoid reducing the predictive ability of the model.

Natural water bodies contain various metal elements, such as K, Ca, Na, Mg, Al, Fe, Mn, etc. With the advancement of technology and the improvement of people's living standards, various untreated sewage and

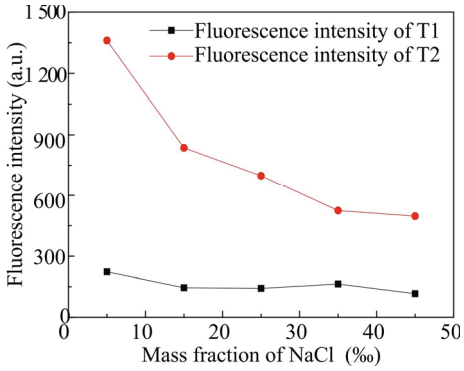


Fig.4 Fluorescence intensity of characteristic peaks of NaCl solution

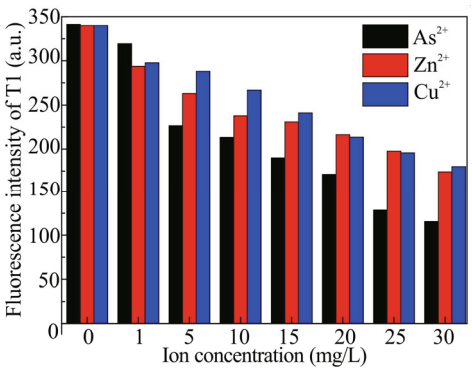


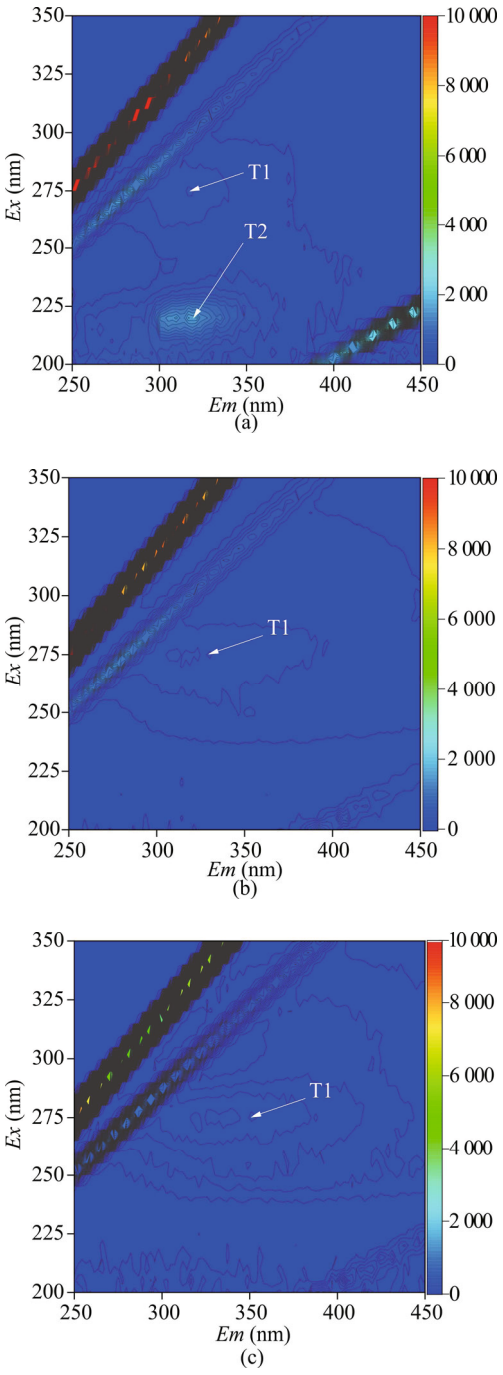
Fig.5 Influence of heavy metal ion on the fluorescence intensity of T1 peak

wastewater are discharged into nature, causing natural water bodies to be polluted by various heavy metal elements, such as Hg, Cd, Cr, Zn, As, Cu, Ni, etc. Although the content of heavy metals is trace, they are all toxic elements. In this study, the potassium hydrogen phthalate standard solution is studied, and the impact of As, Zn, Cu heavy metal ion concentrations on the T1 peak fluorescence intensity at different concentrations was analyzed to investigate the influence of these three heavy metal ions on the fluorescence detection of water quality *COD*. To highlight the impact of heavy metal elements on fluorescence intensity, the potassium hydrogen phthalate concentration was 1 mg/L, and the concentrations of As^{2+}, Zn^{2+} and Cu^{2+} ions ranged from 0 to 30 mg/L. The heavy metal ion standard solution with an original concentration of 1 000 mg/L was proportionally diluted with distilled water. The impact of the three heavy metal ions on the fluorescence intensity at the T1 peak of the water sample is shown in Fig.5.

As shown in Fig.5, with the increase of ion concentration, the fluorescence intensity at the T1 peak gradually decreases. This is because most inorganic salt metal ions do not produce fluorescence, and some fluorescent substances in the DOM of the water body combined with heavy metal ions to undergo quenching reactions, leading to weakened DOM fluorescence. The vast majority of metal ions in solution can only undergo non-radiative transitions and cannot produce fluorescence. Although there are some organic compounds with conjugated double bonds in actual water bodies that can form chelates with heavy metal ions, they do not produce fluorescence due to their non-rigid structure and non-coplanarity of the molecules, resulting in reduced in fluorescence intensity.

The variation of the center positions of T1 and T2 fluorescence peaks in water samples with different concentrations of heavy metal ions is shown in Fig.6.

Fig.6(a) shows the three-dimensional fluorescence contour spectrum of the standard solution without heavy metal ions. Fig.6(b) and (c) depict the change in the T1 peak of the standard solution with increasing Zn^{2+}



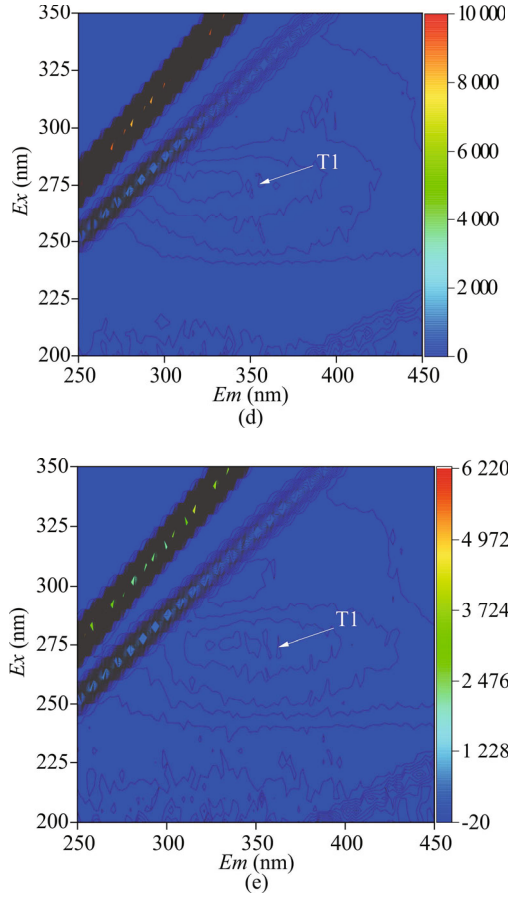


Fig.6 Influence of heavy metal ion concentration on the positions of fluorescence peaks: (a) Standard solution 1 mg/L; (b) Zn^{2+} concentration is 15 mg/L; (c) Zn^{2+} concentration is 30 mg/L; (d) As^{2+} concentration is 15 mg/L; (e) As^{2+} concentration is 30 mg/L

concentration, while Fig.6(d) and (e) show the change in the T2 peak of the standard solution with increasing As^{2+} concentration. It can be observed that the T2 peak disappears in the presence of heavy metal ions, indicating its high sensitivity to these ions, and the T1 peak shifts in position. Tab.2 displays the specific positions of the T1 peak at different concentrations of heavy metal ions.

Tab.2 Influence of heavy metal ion on the fluorescence position of T1 peak

Heavy metal ion	Ion concentration (mg/L)	T1 *Ex/Em* (nm)
Zn^{2+}	0	275/333
	15	275/327
	30	275/350
As^{2+}	0	275/333
	15	275/355
	30	275/363

From Tab.2, it is evident that the change in heavy metal ion concentration does not affect the *Ex*, but causes a shift in the *Em*. When the Zn^{2+} ion concentration varies in the range of 0—15 mg/L, *Em* shifts towards the blue region, while a red shift is observed when the Zn^{2+} ion concentration varies in the range of 15—30 mg/L. In the case of As^{2+} ions, *Em* shifts towards longer wavelength with increasing ion concentration, resulting in a red shift. This is because the coordination of fluorescent ligands with metal ions can be considered as an acid-base reaction. Therefore, the spectral changes of the ligand-electron spectrum resulting from metal ion coordination are similar to the effects of solution pH on the ligand spectrum.

In fact, the coordination of aromatic ligands with non-transition metal ions (such as Zn^{2+}) produces a polarization effect on the ligands at the coordination site, similar to the spectral shift caused by protonation of the ligands at the coordination site. However, the coordination of many transition metal ions with aromatic ligands often leads to static quenching of the ligand fluorescence.

External environmental changes can also affect three-dimensional fluorescence spectrum. Tab.3 shows that pH, NO_2-N, NO_3-N, and salinity have little effect on the T1 fluorescence peak of the standard solution's three-dimensional fluorescence spectrum. Therefore, when analyzing the influence of external environmental factors on the T1 peak, the effects of pH and salt substances in the water sample can be ignored, and the main influencing factors for the T1 peak are the turbidity and temperature of the water sample.

Tab.3 Influence of environmental factors on spectral characteristics of standard solutions

Influencing factor	Fluorescence intensity
pH	In the range of 4—12.3, the fluorescence intensity of T1 and T2 changes little.
NO_2-N (NO_2^-)	The fluorescence intensity at T1 decreases gradually with the increase of ion concentration, but the intensity changes little.
NO_3-N (NO_3^-)	Ion concentration has little effect on fluorescence intensity at T1 peak.
Salinity (NaCl)	The fluorescence intensity at T1 has little change, while that at T2 decreases with increasing salinity.
Heavy metal ion	The fluorescence intensity at T1 decreases gradually with the increase of ion concentration, while the fluorescence intensity at T2 drops sharply.

The study presents an analysis and investigation of the impact of various factors (namely, pH, nitrite nitrogen, nitrate nitrogen, heavy metals, and salinity) on fluorescence intensity and fluorescence peak position during three-dimensional fluorescence spectrum detection of water-quality *COD*. In this paper, we perform some simple mechanism analyses of the impact of relevant factors on the three-dimensional fluorescence spectrum. However,

these analyses are not sufficient to fully explain the influence of these factors on the spectrum. Thus, further investigation is needed to better understand their influence. The study aims to identify key factors that affect the accuracy of *COD* detection results and to provide technical support and basis for the improvement of this detection method. The results of this study have significant reference value and guidance significance for the further application and promotion of water quality parameter detection based on three-dimensional fluorescence spectrum.

Ethics declarations

Conflicts of interest

The authors declare no conflict of interest.

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