

# Spectrofluorometric Characterization of Adsorption of Different Molecular Size Fractions of Humic Acid onto Anion-Doped TiO<sub>2</sub> Specimens

P. Akan, N. C. Birben and M. Bekbolet

**Abstract**—In this study, excitation-emission matrix (EEM) fluorescence spectral features were evaluated for the elucidation of surface interactions among diverse molecular size fractions of humic acid (0.45 µm filtered fraction, 100 kDa fraction and 30 kDa fraction) and bare as well as anion-doped TiO<sub>2</sub> specimens (Evonik P-25 and Hombikat UV-100). EEM constitute fluorescence spectra at many different excitation wavelengths providing a general point of view related to all features of organic matter within a particular spectral range. The objective of the present research was to compare the similarities and differences of the origin of different molecular size fractions of humic acid in the presence of bare and anion doped TiO<sub>2</sub> (Evonik P-25 and Hombikat UV-100) specimens by excitation-emission matrix (EEM) fluorescence spectral properties.

**Keywords**— adsorption, anion-doped TiO<sub>2</sub> specimens, excitation-emission matrix, humic acid

## I. INTRODUCTION

Natural organic matter (NOM) existing in all soils, surface or ground waters includes humic substances (HS), which are complex macromolecular products of the chemical and biological degradation of plant and animal tissues, containing lignin and carbohydrates as well as proteins [1]. HS can be divided into three components: humic acid (HA), fulvic acid (FA) and humin according to their solubility properties in acidic solutions [2]. HA include both aromatic and aliphatic components with fundamentally three functional groups: carboxylic acids (COOH), phenolic alcohols (OH), and methoxy carbonyls (C=O) [2; 3]. The presence of HA in water resources has been of concerns in water supply owing to some public problems, namely bladder cancer and birth defects [4; 5]. Recently, owing to these problems the removal of HAs, which bring about to generate disinfection by-products (DBPs) by reacting with chlorine in the chlorination process of drinking water treatment plants, has gained much attention.

TiO<sub>2</sub> is an important adsorbent with regard to the investigation of relationships between the surface charging and adsorption [6]. In other words, one of the most ideal adsorbents that have been employing to explore the influence of the

surface functional groups on adsorption is TiO<sub>2</sub> [7]. TiO<sub>2</sub> displays some superior properties because of high chemical stability, excellent functionality, relative chemical and biological inertness, non-toxicity, relatively low price. However, a remarkable obstacle of TiO<sub>2</sub> for its photocatalytic capabilities is its wide and intrinsic band gap which could employ UV light. Many methods have been elucidated to shift the spectral response of TiO<sub>2</sub> to the visible region without a decrease of photocatalytic activity of TiO<sub>2</sub>. Among these techniques, impurity doping with anionic non-metal dopants, such as carbon, nitrogen as well as sulfur, has been widely applied in order to extend the absorption wavelength range of TiO<sub>2</sub> to the visible region [8].

Recently, fluorescence excitation-emission matrix (EEM) spectrophotometry has been extensively employed to comment fluorescence properties of dissolved organic matter (DOM) containing organic molecules with chromophoric (light absorbing) and fluorophoric (light emitting) moieties [9; 10]. EEM constitute fluorescence spectra at many different excitation wavelengths providing a general point of view related to all features of organic matter within a particular spectral range. Humic substances characteristically state fluorescence in the excitation wavelength range of  $\lambda_{exc} \sim 300\text{-}400$  nm and the emission wavelength range  $\lambda_{emis} \sim 400\text{-}500$  nm [11].

The objective of the present research was to compare the similarities and differences of the origin of diverse molecular size fractions of HA in the presence of bare and anion doped TiO<sub>2</sub> (Evonik P-25 and Hombikat UV-100) specimens by EEM fluorescence spectra.

## II. MATERIAL AND METHODS

Commercial HA was purchased from Aldrich (Aldrich Company, USA). Working HA solution with the concentration of 20 mgL<sup>-1</sup> was prepared by dilution of 1000 mgL<sup>-1</sup> stock solution following filtration through 0.45 µm membrane filter. HA solutions were fractionated using a 50 mL Amicon Model 8050 ultrafiltration stirred cells into two different molecular size fractions *i.e.* 100 and 30 kDa. HA was characterized by DOC as well as UV-vis parameters. An incipient wet impregnation method was used to prepare anion doped TiO<sub>2</sub> specimens (C-doped, N-doped, S-doped and N-S co-doped) as the adsorbents. Bare TiO<sub>2</sub> specimens were Hombikat UV-100 from Sachtleben Chemie (crystal structure: 100% anatase, porous, BET surface area: > 250 m<sup>2</sup>g<sup>-1</sup>, average particle size: <10 nm, density: 3.9 gML<sup>-1</sup>) and Evonik P-25 from Evonik Crop. (crystal structure: 80% anatase and 20% rutile,

P. Akan was with Bogazici University, Institute of Environmental Sciences, 34342, ISTANBUL/TURKEY. P.Akan is now with Hacettepe University, Environmental Engineering Department, 06800, ANKARA/TURKEY.

N. C. Birben is with Bogazici University, Institute of Environmental Sciences, 34342, ISTANBUL/TURKEY.

M. Bekbolet is with Bogazici University, Institute of Environmental Sciences, 34342, ISTANBUL/TURKEY.

nonporous, BET surface area:  $55 \pm 15 \text{ m}^2\text{g}^{-1}$ , average particle size: 30 nm, density:  $3.8 \text{ g mL}^{-1}$ ). Concentrations of bare and anion doped  $\text{TiO}_2$  specimens used in batch adsorption experiments were selected in the range of 0.1-1.0  $\text{mg mL}^{-1}$ . Fluorescence spectroscopic measurements were conducted by a Perkin Elmer luminescence spectrometer. To attain fluorescence EEMs, excitation wavelengths ( $\lambda_{\text{exc}}$ ) were incrementally increased from 200 to 500 nm at 10 nm steps, the emission at longer wavelengths ( $\lambda_{\text{emis}}$ ) was detected at 0.5 nm steps. Excitation and emission slit widths were set to 12 nm. Matlab R2012a was used to plot the EEM data [12; 13].

### III. RESULTS AND DISCUSSION

#### A) Evaluation of EEM fluorescence spectral features of diverse molecular size fractions of HA following adsorption onto $\text{TiO}_2$ specimens

Humic substances characteristically state fluorescence in the excitation wavelength range of  $\lambda_{\text{exc}} \sim 300\text{-}400$  and the emission wavelength range of  $\lambda_{\text{emis}} \sim 400\text{-}500$  nm. Fluorophoric centers could be described as humic-like fluorescence (fluorescence excited between  $\lambda_{\text{exc}} = 220$  and 250 nm, and emitted between  $\lambda_{\text{emis}} = 400$  and 460 nm), fulvic-like fluorescence (fluorescence excited between  $\lambda_{\text{exc}} = 300$  and 340 nm, and emitted between  $\lambda_{\text{emis}} = 400$  and 460 nm) and protein-like (tryptophan and tyrosine) fluorescence (fluorescence excited between  $\lambda_{\text{exc}} = 270$  and 280 nm and emitted between  $\lambda_{\text{emis}} = 330$  and 370 nm) [11]. With reference to the presented EEM fluorescence spectral features of diverse molecular size fractions of HA following adsorption onto bare and anion doped  $\text{TiO}_2$  Evonik P-25 specimens, regional speciation depending on the excitation-emission bands were presented in Table 1. Fluorescence intensity strength was indicated by a symbol (+) with reference to the presented color intensity. Besides the aforementioned fulvic-like (Region III) and humic-like fluorophores (Region V), Region I (tyrosine-like) and Region II (tryptophan-like) signified the fluorophores arising from aromatic proteins either free or as protein constituents. Region IV represented fluorophores attributed to the components of the dissolved organic carbon derived from microbial byproducts [13].

TABLE 1. REGIONAL SPECIATION OF THE EEM FLUORESCENCE SPECTRAL FEATURES OF DIVERSE MOLECULAR SIZE FRACTIONS OF HA FOLLOWING ADSORPTION ONTO BARE AND ANION DOPED  $\text{TiO}_2$  P-25 SPECIMENS

HA system and conditions	Region I Aromatic Proteins I $\lambda_{\text{exc}}$ 220-250 $\lambda_{\text{emis}}$ 280-332	Region II Aromatic Proteins II $\lambda_{\text{exc}}$ 220-250 $\lambda_{\text{emis}}$ 332-380	Region III Fulvic-li ke $\lambda_{\text{exc}}$ 220-250 $\lambda_{\text{emis}}$ 380-580	Region IV Microbial byproduct s $\lambda_{\text{exc}}$ 250-470 $\lambda_{\text{emis}}$ 280-380	Region V Humic-li ke $\lambda_{\text{exc}}$ 220-470 $\lambda_{\text{emis}}$ 380-580
0.45 $\mu\text{m}$ filtered fraction					
HA, initial	n.o.	n.o.	+++++	n.o.	+++
N-doped $\text{TiO}_2$	n.o.	+	+++++	n.o.	+++++
S-doped $\text{TiO}_2$	++	+	+++++	+++	+++++
C-doped $\text{TiO}_2$	++	++	+++++	n.o.	+++++
N-S co-doped	+	+	+++++	n.o.	+++++
Bare $\text{TiO}_2$			++		++

$\text{TiO}_2$					
Bare $\text{TiO}_2$	+	+++	+++++	++	+++++
100 kDa fraction					
HA, initial	n.o.	++	+++++	n.o.	+++++
N-doped $\text{TiO}_2$	+	++++	+++++	++++	+++++
S-doped $\text{TiO}_2$	+	++++	+++++	+++	+++++
C-doped $\text{TiO}_2$	++	+++	+++++	+++	+++++
N-S co-doped $\text{TiO}_2$	+	+++	+++++	+++	+++++
Bare $\text{TiO}_2$	++	++++	+++++	++++	+++++
30 kDa fraction					
HA, initial	+	++++	+++++	+++	+++++
N-doped $\text{TiO}_2$	++	+++	+++++	++++	+++++
S-doped $\text{TiO}_2$	++	+++	+++++	+	+++++
C-doped $\text{TiO}_2$	n.o.	++	+++++	n.o.	+++++
N-S co-doped $\text{TiO}_2$	+	++++	+++++	+++	+++++
Bare $\text{TiO}_2$	+	+++	+++++	++	+++++

n.o., not observed

When the regional speciation profile was assessed, it could be easily said that both humic-like and fulvic-like fluorophores were prominent for all of the molecular size fractions of HA following adsorption onto all  $\text{TiO}_2$  specimens (Table 1). Particularly Region IV displayed that fluorophoric groups were not produced during adsorptive interactions.

According to the data obtained from EEM fluorescence spectral features of diverse molecular size fractions of HA following adsorption onto bare and anion doped  $\text{TiO}_2$  specimens, the most significant results were attained for 30 kDa fraction of HA following adsorption onto S-doped  $\text{TiO}_2$ .

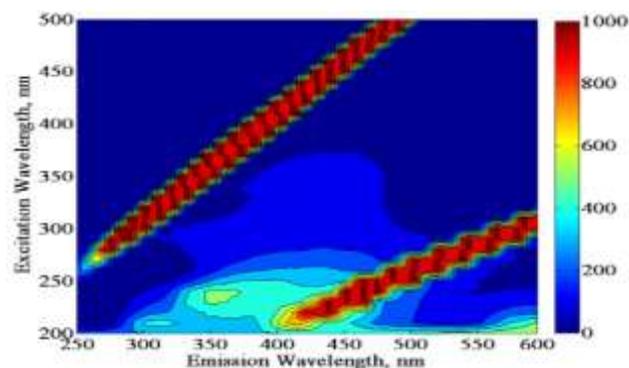


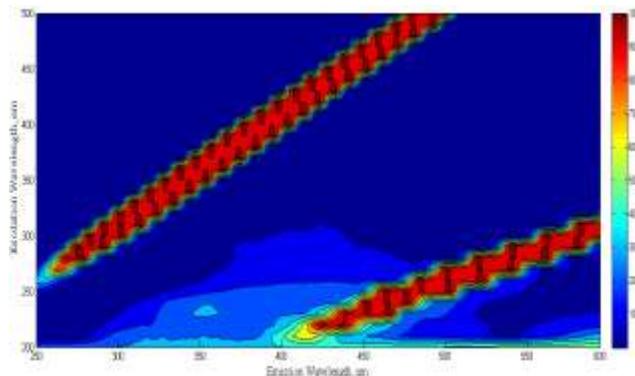
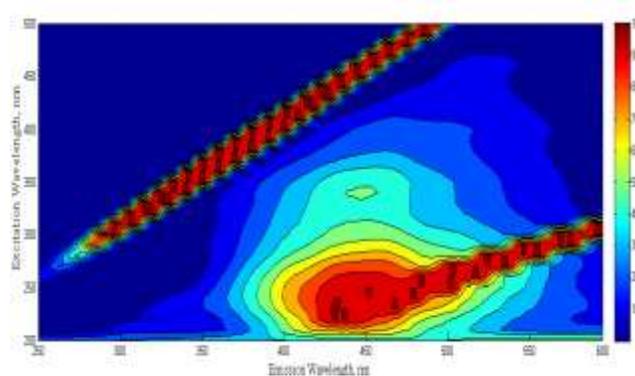
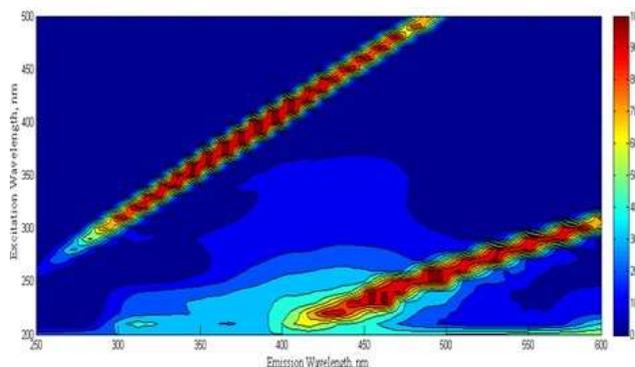
Fig 1. EEM fluorescence spectra recorded under condition: 30 kDa fraction of HA in the presence of S-doped  $\text{TiO}_2$  Degussa P-25.

Regional speciation of diverse molecular size fractions of HA following adsorption onto bare and anion doped  $\text{TiO}_2$  Hombikat UV-100 were displayed in the Table 2.

TABLE 2. REGIONAL SPECIATION OF THE EEM FLUORESCENCE SPECTRAL FEATURES OF DIVERSE MOLECULAR SIZE FRACTIONS OF HA FOLLOWING ADSORPTION ONTO BARE AND ANION DOPED TiO<sub>2</sub> UV-100 SPECIMENS

HA system and conditions	Region I Aromatic Proteins I $\lambda_{exc}$ 220-250 $\lambda_{emis}$ 280-332	Region II Aromatic Proteins II $\lambda_{exc}$ 220-250 $\lambda_{emis}$ 332-380	Region III Fulvic-like $\lambda_{exc}$ 220-250 $\lambda_{emis}$ 380-580	Region IV Microbial byproduct $\lambda_{exc}$ 250-470 $\lambda_{emis}$ 280-380	Region V Humic-like $\lambda_{exc}$ 220-470 $\lambda_{emis}$ 380-580
<b>0.45 <math>\mu</math>m filtered fraction</b>					
HA, initial	n.o	n.o	+++++	n.o.	+++
N-doped TiO <sub>2</sub>	++	++	+++++	+	+++++
S-doped TiO <sub>2</sub>	+++	+++	+++++	+	+++++
C-doped TiO <sub>2</sub>	+	++	+++++	+	+++++
N-S co-doped TiO <sub>2</sub>	+	+++	+++++	+	+++++
Bare TiO <sub>2</sub>	+	++++	+++++	+++	+++++
<b>100 kDa fraction</b>					
HA, initial	n.o.	++	+++++	n.o.	+++++
N-doped TiO <sub>2</sub>	++	+++++	+++++	+++	+++++
S-doped TiO <sub>2</sub>	++	+++++	+++++	++	+++++
C-doped TiO <sub>2</sub>	++	++++	+++++	+++	+++++
N-S co-doped TiO <sub>2</sub>	n.o.	+++++	+++++	+++	+++++
Bare TiO <sub>2</sub>	++	+++++	+++++	+++	+++++
<b>30 kDa fraction</b>					
HA, initial	+	++++	+++++	+++	+++++
N-doped TiO <sub>2</sub>	++	+++++	+++++	++++	+++++
S-doped TiO <sub>2</sub>	+++	+++++	+++++	+++	+++++
C-doped TiO <sub>2</sub>	+	+++	+++++	++	+++++
N-S co-doped TiO <sub>2</sub>	++	+++++	+++++	+	+++++
Bare TiO <sub>2</sub>	+	++	+++++	+	+++++

Following adsorptive interactions between HA and TiO<sub>2</sub> specimens, EEM contour-plots indicated that both humic-like and fulvic-like fluorophores were prominent under all conditions. However, in the presence of anion doped TiO<sub>2</sub> specimens, presence of humic-like fluorophores were found to be superior to fulvic-like fluorophores. It could be deduced that HA fluorophores illustrated the presence of regions III and V expressing fulvic-like and humic-like fluorophores and absence of regions I, II and IV. [14]. The highest intensities related to humic-like and fulvic-like fluorophores were observed in adsorption of all of molecular size fractions of humic acid onto N-S co-doped TiO<sub>2</sub> specimens (Fig.2., Fig 3., and Fig 4.)


 Fig 2. EEM fluorescence spectra recorded under condition: 0.45  $\mu$ m filtered fraction of HA in the presence of N-S-doped TiO<sub>2</sub> Hombikat UV-100.

 Fig 3. EEM fluorescence spectra recorded under condition: 100 kDa fraction of HA in the presence of N-S-doped TiO<sub>2</sub> Hombikat UV-100.

 Fig 4. EEM fluorescence spectra recorded under condition: 30 kDa fraction of HA in the presence of N-S-doped TiO<sub>2</sub> Hombikat UV-100.

In accordance with the regional speciation profile belonging to EEM fluorescence spectra, all of molecular size fractions of humic acid following adsorption onto bare and anion doped (C-doped, N-doped, S-doped and N-S co-doped) TiO<sub>2</sub> specimens (Evonik P-25 and Hombikat UV-100) showed similarities that both humic-like and fulvic-like fluorophores were obviously observed. In the result of this study, it could be absolutely said that humic fluorophores were remarkably evident for binary system (HA and TiO<sub>2</sub> specimens).

#### B) Correlation between fluorescence intensity and specified UV-vis parameters

Fluorescence-derived index described as Fluorescence Intensity (FI) was symbolized by the ratio of the emission intensity at  $\lambda_{emis}$  450 nm to that 500 nm, following excitation at

$\lambda_{exc}$  370 nm. An inverse relationship was analyzed between FI and aromaticity of humic substances. Furthermore,  $FI \leq 1.4$  indicated humic fluorophores of organic matter stemming from terrestrial sources whereas  $FI \geq 1.9$  mainly attributed to microbially derived organic matter. Hence, fluorescence intensity (FI) could well be correlated with the specified UV-vis parameters, *i.e.*  $UV_{254}$  and  $UV_{280}$  [13; 15; 16]. Below presented linear regression equations were derived from the data obtained by the 0.45  $\mu m$  filtered fraction of humic acid following adsorption onto bare  $TiO_2$  Evonik P-25 specimens ( $R^2 > 0.87$ ).

$$FI = -0.0831 \times DOC + 1.37 \quad (1)$$

$$FI = -0.00620 \times UV_{254} + 1.31 \quad (2)$$

$$FI = -0.0294 \times Color_{436} + 1.27 \quad (3)$$

Furthermore, specified UV-vis parameters and DOC data displayed an inverse relationship ( $R^2 > 0.91$ ) with FI for 100 kDa fraction of humic acid following adsorption onto N-S co-doped  $TiO_2$  Evonik P-25 Specimens.

$$FI = -0.171 \times DOC + 1.71 \quad (4)$$

$$FI = -0.0143 \times UV_{254} + 1.53 \quad (5)$$

$$FI = -0.0909 \times Color_{436} + 1.59 \quad (6)$$

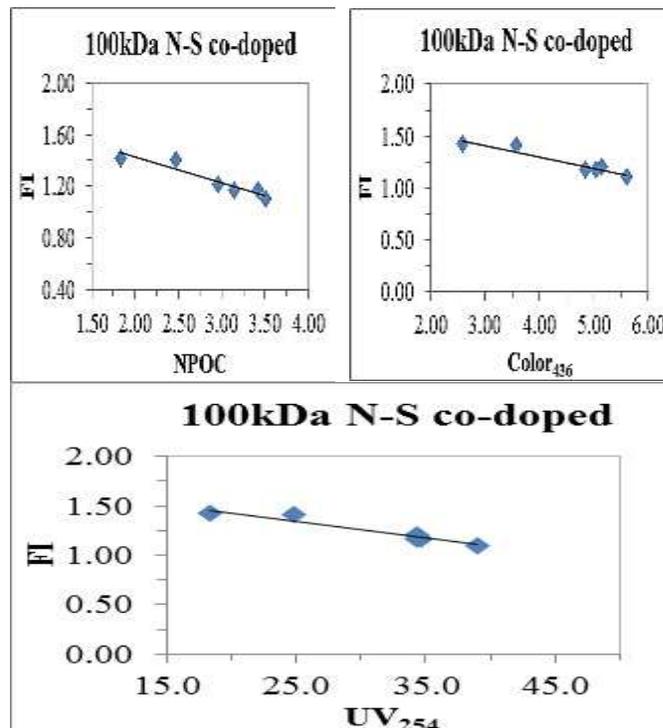


Fig 5. Correlations between fluorescence intensity and specified UV-vis parameters for diverse molecular size fractions of humic acid following adsorption onto  $TiO_2$  specimens.

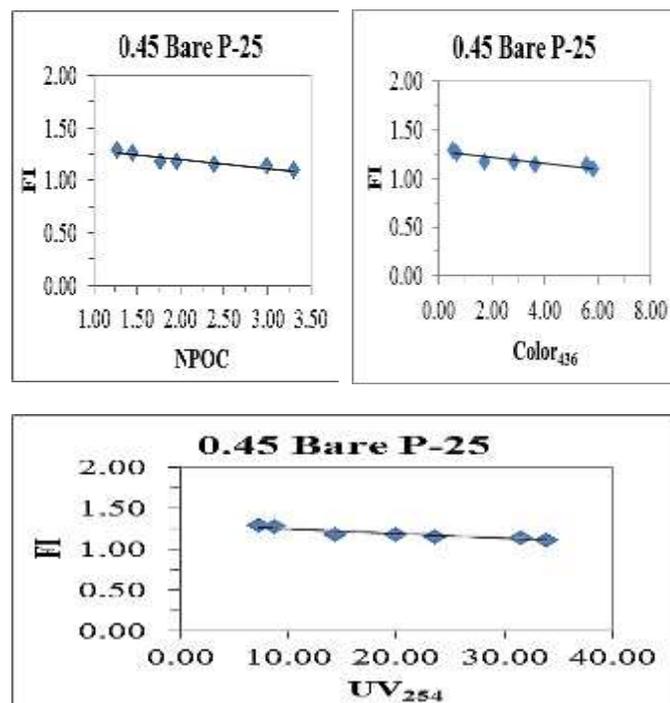
According to the above presented linear equations, the use of the fluorescence methods could present remarkable information about the removal of the specified UV-vis parameters and dissolved organic carbon content [13].

#### IV. CONCLUSIONS

Three-dimensional fluorescence excitation-emission matrix (F-EEM) spectroscopy has been used to distinguish different types and sources of DOM in natural waters [9]. It has been used to characterize DOM and to identify humic-like and protein-like fluorescent signals in water samples from different aquatic environments [11]. In this study, the similarities and differences of the origin of diverse molecular size fractions of humic acid in the presence of bare and anion doped  $TiO_2$  (Evonik P-25 and Hombikat UV-100) specimens were investigated by EEM fluorescence spectral properties. In conclusion, EEM fluorescence spectra of all of molecular size fractions of humic acid following adsorption onto bare and anion doped (C-doped, N-doped, S-doped and N-S co-doped)  $TiO_2$  specimens (Evonik P-25 and Hombikat UV-100) indicated that both humic-like and fulvic-like fluorophores were still prominent under all adsorptive conditions.

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Perihan AKAN: I am a research assistant and also PhD. student at Department of Environmental Engineering, Hacettepe University, Ankara/Turkey. I took my master degree from Bogazici University, Institute of Environmental Sciences, Istanbul/Turkey. I graduated from Environmental Engineering Department, Trakya University, Edirne/Turkey. My research areas are advanced oxidation processes, adsorption as well as air pollution control.

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