The Detection Methods of Hydroxyl Radical: A Review

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Abstract. Hydroxyl radical (·OH) can rapidly react with biological macromolecules, organic and inorganic substances on account of its strong oxidizing property, so it is widely used in sewage treatment, chemiluminescence analysis, biological macromolecule modification and other fields. The strengthening effect of Hydrodynamic cavitation on chemical reactions depends on the number of free radicals initiated by cavitation. These free radicals are highly reactive and react rapidly with pollutants and other species in wastewater. Therefore, the systematic summary of detection methods of hydroxyl radical and the description of its advantages and disadvantages can provide technical support for scientific researchers, and guides the research in the future.

1. Introduction

In recent years, sewage treatment gradually become a hotspot. Hydrodynamic cavitation is an important means to develop intensive and miniaturized wastewater treatment technology. It is a greener and more efficient method to treat industrial wastewater. Occuring with the collapse of bubbles, hydrodynamic cavitation can break the strong chemical along with the high temperature and high pressure[1-3]. Among the free radicals formed during the collapse process, hydroxyl radical (·OH) has very strong oxidation property[4]. Hydroxyl radical is a kind of strong oxidizing radical, which can initiate and strengthen the chemical reaction process. The quantity produced in the cavitation process represents the intensity of cavitation[5-7]. Therefore, it is very important to detect the amount of hydroxyl radicals produced by Hydrodynamic cavitation for explorering the occurrence and strengthening effect of cavitation.

The hydroxyl radical has an unpaired electron, which is highly reactive and has a strong oxidation capacity. In addition, ·OH has a high reaction rate constant and a short life[8-9]. ·OH is a powerful oxidant with high efficiency and no secondary pollution[10]. However, for the detection of ·OH, there is no accurate and effective direct determination method at present. The only way to detect the amount of ·OH is to capture ·OH by agent and then detect the adducts indirectly. Therefore, such detection methods have high requirements on the capture efficiency of the agent, the stability and reactivity of the adduct, but the capture efficiency of the agent for ·OH is low in actual reactions. At present, the main methods include spectrophotometry, fluorescence spectrophotometry (FD), electron spin capture (ESR), high performance liquid chromatography (HPLC), chemiluminescence (CL), electrochemical detection (ECD) and so on.

2. Free radical detection technique

2.1 Spectrophotometric method

The ESR method and HPLC method are expensive and difficult for general laboratories to bear. Therefore, photometric method is commonly used by domestic researchers. Spectrophotometry is efficient, fast and practical, and the instrument is cheap. Through the development of technology in recent years, spectrophotometry has become a mature method for the detection of ·OH. Spectrophotometry uses the strong oxidation of ·OH to change the structure, properties and color of the probe capture agent, so as to change the absorption of the specific spectrum of the capture agent and indirectly determine ·OH.

Li Jiwu et al. [11] used bromophenol blue (BPB) as a liquid radical probe catcher to quantitatively detect the production of ·OH in the discharge process of high voltage corona liquid film. Yan Jun et al. [12] adopted visible spectrophotometry and used salicylic acid as the capture agent of hydroxyl radical ·OH produced by Fenton reagent reaction, making $FeSO_4$, H_2O_2, and salicylic acid-ethanol into a reaction system. They investigated the influencing factors of hydroxyl radical ·OH capture by salicylic acid, and used Fenton's reaction system as antioxidant filter. Yang Chunwei et al. [13] took methylene blue indicator as an example to capture hydroxyl free radicals generated by Fenton reagent reaction. With its high sensitivity and stability, it is suitable to be applied to study the mechanism of advanced oxidation method of environmental aqueous Fenton reagent. Wang Jingang et al.[14] used methylene blue spectrophotometry to detect hydroxyl radical by the change of absorbance of methylene blue before and after oxidation.

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2.2 Fluorescence photometric method

Fluorescence spectrophotometry has higher sensitivity than spectrophotometry and is not easily affected by other ions at specific wavelength. Although the accuracy and sensitivity of this method is less than that of ESR method, fluorescence photometry is simple to operate and easy to implement, so it is the most widely used method at present. The disadvantage of fluorescence photometry is that the adduct product of hydroxyl or the catcher itself needs characteristic fluorescence.

Liu Ting et al.[15] prepared Fe_2O_3-TiO_2-Al_20_3 catalyst and established a three-phase fluidized bed photoassisted heterogeneous Fenton reaction system. In the experiment, coumarin was used as ·OH capture agent, and the ·OH generated in the photoassisted heterogeneous Fenton reaction system was detected by fluorescence spectroscopy. Yang et al.[16] use quantitative DMSO to capture hydroxyl radicals and generate methyl radicals. The methyl radicals react with NitroXide-Linkednaphthalence, resulting in enhanced fluorescence intensity. The fluorescence intensity is proportional to the concentration of hydroxyl radicals and 0.1-2.0 μmol/ L of H_2O_2 can be detected, the lowest detection limit of H_2O_2 is 0.04μmol/L. Ding Haiyang et al.[17] studied the generation of ·OH in the degradation process of Ti/SnO_2 electrode by fluorescence photometry, and discussed the feasibility of the application of this method in the electrocatalytic oxidation process. At the same time, Ti/RuO_2 electrode was used as comparing electrodes, so as to study the different properties and mechanisms of the two electrodes in electrocatalytic oxidation activity.

2.3 Optical fiber spectral system measurement method

It is different from the existing spectrophotometry and fluorescence spectro-photometry. In order to detect radical concentration rapidly and accurately at low cost, Liu Huimin et al.[18] proposed to couple the online detection technology of ·OH radical and ·OH radical concentration optical fiber spectrum by UV-excited hydrogen peroxide production, and set up the online detection system of ·OH radical concentration optical fiber spectrum. The response characteristics of optical fiber spectra to methylene blue, and the effects of light conditions and initial concentration of hydrogen peroxide on the production and detection performance of ·OH radical were investigated. The results show that the concentration of ·OH radical can be accurately measured online by using the optical fiber spectrum system based on MB as ·OH radical catcher.

2.4 Electron spin capture method

Electron spin resonance (ESR), also known as electron paramagnetic resonance, was first discovered in 1944. It uses the characteristics that the material with unpaired electrons absorb the energy of electromagnetic wave under the action of magnetic field to make the electron transition between energy levels to detect and analyze paramagnetic substances. The method of spin capture is to add unsaturated diamagnetic compounds (spin capture reagent) into the reaction system, and combine them with all kinds of high activity and short life free radicals produced in the reaction system to form relatively stable spin adduct, which is suitable for ESR detection. The principle is to combine appropriate spin capture reagent and active short life free radicals to generate relatively stable spin adducts, the amounts of spin adducts can be detected by electron spin spectroscopy, and the amounts of spin adducts can be used to calculate the number of original free radicals. ESR is the most direct and effective technology for studying free radicals, but these free radicals must be relatively stable and reach to a certain concentration before they can be detected and studied by ESR technology. However, most of the free radicals produced in biological systems are unstable, which cannot be detected by conventional ESR spectroscopy. In order to overcome this limitation of ESR technology, spin capture technology has been developed, which is the most widely used and successful method for studying active free radicals in biological and medical systems [19].

Typical spin capture agents are nitrite or nitroxide compounds. When sufficient amounts of spin capture agent are added to the free radical producing system, the spin capture agent will react rapidly with any free radical present, producing stable and detectable nitroxide free radical adducts. The ESR spectrum of the free radical adduct formed has hyperfine cleavage given by the free radical trapping gene, which can identify the free radical adducts formed by the free radical and trapping agent which is very sensitive to the free radical structural changes[20]. There are three main types of spin capture agents, namely nitroso compounds, circular nitrones and linear nitrones.

Ding Qinxue et al. [21] used electron spin capture technology to detect hydroxyl radical (·OH) produced by the antitumor antibiotic boamycin in vitro. 5,5'-dimethylpivrlin-n-oxide was used as hydroxyl radical catcher, and the free radical signal was detected by electron spin resonance (ESR) spectrometer at room temperature. It was found that if nitrogen was added to the solution, no free radicals were produced when boamycin or ferrous sulfate were added to phosphate buffer alone or both. At the same time, if boamycin and ferrous sulfate were added to the buffer and oxygen, the signal appeared, and the strength of the signal was positively related to the concentration of boamycin. Cong Jianbo et al. [22] used liquid nitrogen (770 K) to preserve spin adducts to prolong the life of spin adducts, and the results showed that the preservation of short-lived free radical spin adducts in liquid nitrogen could prolong their life. For the short-lived free radicals that cannot be measured without ESR measurement conditions, this method can be used to select appropriate trapping agent, which can be stored in liquid nitrogen immediately after capture, and then removed and thawed for measurement when necessary.

Using free radical trap agent - the ESR method detecting hydroxyl radicals is simple and effective, but expensive and low cost. Since the spin adducts are unstable, their life is very short from only a few minutes or tens of minutes, must be measured immediately after capturing free radicals, so its quantitative analysis is not very accurate, which limits many experimental studies and industrial applications.

2.5 High performance liquid chromatography

High performance liquid chromatography (HPLC) is a chromatographic analysis technique used to separate mixtures in order to confirm and quantify the proportions of the individual components. It relies on a pump to pressurize the sample through a pressure column filled with an adsorbent, causing the components of the sample to separate. Free radicals should be captured by a trapping agent, which can react with free radicals to form a stable conjugate and is easy to be separated and detected. General ·OH capture agents are benzoic acid/salicylic acid (SA), phthalic acid, dimethyl sulfoxide (DMSO), etc. ·OH reacts with dimethyl sulfoxide to form mesylate, and its content can be determined by HPLC to infer that of ·OH.

Xue Juanqin et al.[23] used high performance liquid chromatoid-ultraviolet detection (HPLC-UV) to determine ·OH generated in the electrochemical oxidation system by using salicylic acid (SA) and 4-hydroxybenzoic acid (4-HBA) as capture agents. The results show that the electrochemical oxidation reactions of SA and 4-HBA with ·OH are in accordance with the first-order reaction kinetics, and the reaction rate constants K are $2.1833\times10^{-4}s^{-1}$ and $1.3500\times10^{-4}s^{-1}$, respectively. After three repeated experiments, it is found that the initial consumption of 4-HBA is 3 times of SA and the capture time is about 2.3 times of SA when the same ·OH capture amount is achieved. Through comparative study, it is found that SA has higher sensitivity and stronger capturing ability under UV detection conditions. Han Yao et al.[24] established a simple and sensitive method for the determination of ·OH produced by Fe(III) -catalyzed photoreaction. ·OH was captured by dimethyl sulfoxide (DMSO) to form formaldehyde, and then reacted with derivative reagent 2, 4-dinitrophenylhydrazine (DNPH) to form corresponding hydrazone (HCHO-DNPH), afterwards it was analyzed by high performance liquid chromatography. The effects of the concentration,pH, temperature and time of the derivatization on the derivatization reaction were studied. The optimal derivatization conditions were determined at 270μmol/L, pH=4, 50℃ and 30 min. Yang Wei et al.[25] established a simple, efficient and rapid method for the determination of hydroxyl radical by using high performance liquid chromatography combined with Coulomb array multi-electrode detector system (HPLC-ECD).

The advantages of HPLC are convenient measurement, high efficiency and high sensitivity, the detection limit up is to 10^{-9}~10^{-11}g. However, it requires expensive equipment and the reaction process is relatively complex, besides, there are many intermediates and secondary products, so it is still unqualified in the accurate quantitative detection of free radicals.

3. Conclusions

Hydroxyl radical has always been a research hotspot. From the methods mentioned above, it can be seen that in recent years, the detection technology of hydroxyl radical is gradually mature, and there are a variety of detection methods at home and abroad. However, a few methods (such as ESR, laser fluorescence spectroscopy and long optical path absorption spectroscopy) can be directly measured, most of the determination methods are indirect. The indirect methods are affected by many factors, such as measuring system and measuring conditions. However, due to the characteristics of high activity, low concentration and short lifetime of ·OH, there are many interfering factors even for direct measurement. Therefore, the detection of ·OH should be combined with its own research characteristics, and try to use and combine different analysis methods to improve the analysis effects.

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