



International  
Standard

**ISO 24758-1**

**Fine bubble technology —  
Evaluation method for determining  
the reactive oxygen species in  
ultrafine bubble dispersions —**

**Part 1:**

**Probe based kinetic model**

*Technologie des fines bulles — Méthode d'évaluation pour  
déterminer les espèces réactives de l'oxygène dans les dispersions  
de bulles ultrafines —*

*Partie 1: Modèle cinétique basé sur les sondes*

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## Foreword

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This document was prepared by Technical Committee ISO/TC 281, *Fine bubble technology*.

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## Introduction

Fine-bubble (FB) technology holds considerable promise for application in water- and wastewater-treatment processes. Over the past several decades, numerous studies have demonstrated that microbubble (MB) and ultrafine bubble (UFB) dispersions can accelerate various advanced oxidation processes (AOPs), such as those using ozone, plasma, ultraviolet (UV), and electrochemical processes, thereby enhancing the efficiency of pollutant treatment. Although the treatment effects of fine-bubble processes are promising, their underlying mechanisms have not been fully understood. Understanding the generation mechanism of reactive species is imperative. Consequently, it is essential to clarify the role of reactive oxygen species (ROS) in pollutant abatement via MB and UFB treatments.

There are many techniques for detecting ROS in water. Electron paramagnetic resonance (EPR) spectroscopy stands out as a proficient tool for the direct detection of ROS, specifically those possessing unpaired electrons, even at concentrations as low as 1  $\mu\text{M}$ . However, its application is limited as EPR falls short in detecting ROS, such as ozone and hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), which are devoid of unpaired electrons. Moreover, challenges persist in the detection of the superoxide anion radical ( $\text{O}_2^{\cdot-}$ ) in water. The  $\text{O}_2^{\cdot-}$  radical can stably exist in alcohols, such as methanol, where they are readily trapped by 5,5-dimethyl-1-pyrroline N-oxide (DMPO). In aqueous solutions, however, the addition rate of  $\text{O}_2^{\cdot-}$  to DMPO is comparatively low, whereas the decomposition of its DMPO-adduct into DMPO-OH occurs at an exceedingly high rate, rendering effective detection impracticable. Additionally, the EPR technique is predominantly employed for the qualitative or semi-quantitative analysis of ROS. Determining the cumulative and real-time concentrations of ROS with precision remains a complex task.

Probe-based kinetic models are useful for optimizing water-treatment processes for efficient pollutant abatement and elucidating the ROS reaction mechanism driven by fine-bubble technology. By employing low concentrations ( $\mu\text{M}$  scales) of ROS probes, which will not significantly affect the reaction system, we can more realistically elucidate the reaction mechanisms occurring in water- and wastewater-treatment processes. With probe-based kinetic models, it is possible to measure the accumulated concentration of these ROS over time and their real-time concentrations at each treatment interval. However, caution is advised in the utilization of this method to measure the concentration of  $\text{H}_2\text{O}_2$  within systems, as the relatively low oxidizing capacity of  $\text{H}_2\text{O}_2$  compared to other ROS may lead to inaccuracies due to significant differences in reaction rate constants, potentially introducing significant errors in the analytical evaluations.

Fluorescence spectroscopy, in which suitable probes (e.g., 3'-(p-aminophenyl) fluorescein (APF)) are employed, is an excellent technique for detecting ROS because of its high sensitivity, simplicity in data collection, and high spatial imaging resolution. The fluorescent response of the APF probe to different ROS varies significantly. Notably, fluorescence spectroscopy is not a direct measurement method. When multiple ROS are present in a system simultaneously, it becomes challenging to distinguish them.

This document specifies detection methods for ROS in MB and UFB dispersions. In ISO 24758-1, the application of probe-based kinetic models for measuring the cumulative concentrations of various ROS over time, as well as their real-time concentrations at each treatment interval, is described. In ISO 24758-2, the application of APF to complement the probe kinetic model, which can detect  $\text{H}_2\text{O}_2$  in MB and UFB dispersions, is described. The establishment of this document will provide substantial value for various industries. Manufacturers of MB and UFB equipment for environmental remediation can adopt this document to assess their equipment and wastewater-treatment processes. Companies with wastewater-treatment needs can use this document as a basis for selecting appropriate processes and instrumentation. This document will play a proactive role in evaluating the application and research of MBs in fields including wastewater treatment and disinfection etc.

