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# Combined ozonation and aquatic macrophyte (*Vallisneria natans*) treatment of piggery effluent: Water matrix and antioxidant responses

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ABSTRACT

The efficiency of the combined process of ozonation with *Vallisneria natans* to improve the quality of piggery effluent was investigated in this study, and the effects of ozonated water on the antioxidant responses of *V. natans* were explored. The effluent was treated with ozone at 10, 30 and 50 mg $L^{-1}$ for 30 min in combination with *V. natans* for 28 d. Combined treatment was successful at removing inorganic nutrients and organic compounds from piggery effluent. Low ozone concentration treatment of water was optimal for the growth of *V. natans*, while exposure to high levels for long periods of time increased reactive oxygen species (ROS) and damaged their antioxidant systems. The results demonstrate that combined ozonation and submerged macrophytes treatment, can remove inorganic nutrients and organic compounds from piggery effluent, reducing the pollution into aquatic ecosystem.



## 1. Introduction

China's rapidly developing economy has led to intensive livestock and poultry breeding, resulting in the production of massive wastes and serious environmental pollution. It is estimated that a total annual discharge of 6.0 billion tons (*t*) is generated from piggery waste in China (Wang et al., 2012), most of which is not disposed properly. Piggery wastewater contains a high concentration of organic components and inorganic nutrients, and many studies have focused on how to improve conventional biological treatments to reduce these pollutants (Harrington and Scholz, 2010; Rajagopal et al., 2011). The results of these investigations have revealed that piggery effluent from these methods still contains nutrient-rich and relatively high levels of biodegradable organic matter, which poses a significant threat to surface and groundwater and requires subsequent treatment (Suzuki et al., 2010). Growing concerns about the requirements of modern piggeries and the environment have resulted in development of new technology and equipment that is friendly and cost effective to reduce problems associated with wastewater (Tripathi and Tripathi, 2011).

Ozone is a powerful oxidizing agent that induces the degradation of organic and inorganic substances either directly or via the formation of hydroxyl and other radicals (Tay and Madehi, 2015). Ozone also efficiently inactivates a wide range of microorganisms (Liltved et al., 2006). Ozonation has been one of the most commonly applied chemical oxidation methods for water treatment in developed countries (Zimmermann et al., 2011; Wadhawan et al., 2014; Altmann et al., 2012), and has been demonstrated to be efficient in removing of pharmaceuticals and other micropollutants from drinking water and wastewater (Westerhoff et al., 2005; Hollender et al., 2009). Indeed, ozonation is now one of the most promising methods for wastewater treatment (Monarca et al., 2000). Though ozonation technology is attractive, total mineralization is usually not achieved (Dodd et al., 2009) A potential disadvantage of ozonation process is the formation of unknown by-products due to partial oxidation and reaction with matrix components (von Gunten, 2003), such as bromate, formaldehyde or carboxyl compounds etc (Hollender et al., 2009), which may sometimes be even more toxic than parent components (Li et al., 2008). These oxidation by-products are usually more easily biodegradable and can be partially removed during biological process (Stalter et al., 2010).

Natural aquatic ecosystems have gained a great deal of attention because of their potential for use in wastewater treatment and resource-saving without the need for additional funds. Aquatic macrophytes are an indispensable part of the aquatic ecosystem

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that plays an important role in the treatment process (Chen et al., 2014). Specifically, aquatic macrophytes may help redistribute nutrients, which are beneficial for bioremediation of eutrophic systems (Zhang et al., 2013). Aquatic macrophytes also have high potential to collect heavy metals (Mishra and Tripathi, 2009), as well as to take up and accumulate microcystins (Jiang et al., 2011). *V. natans* (Lour.) Hara, a perennial submerged macrophyte widely distributed in different freshwater habitats in China (Wang et al., 2008), is a stress-tolerant plant that can withstand large perturbations of the environment (Soana et al., 2012). It has been suggested that this genus can provide positive feedback for aquatic ecosystem restoration (Pinardi et al., 2009). We believed that *V. natans* could be considered a valid option for producing high quality water from ozonated piggery effluent. Combined biofiltration with ozonation has been shown to reduce organic and inorganic content (Tripathi and Tripathi, 2011). However, a number of studies have shown that ozonated wastewater affects growth of organisms, such as *Chlorella pyrenoidosa* and fish (Gan et al., 2014; Stalter et al., 2010). Accordingly, combined treatment of piggery wastewater with *V. natans* and ozonation may have synergistic effects on plants.

Environmental and developmental changes stimulate the production of reactive oxygen species (ROS) through various organelles and enzymes (Van Breusegem and Dat, 2006). Transient increases in ROS may serve as signaling intermediates to promote cross talk (Zhang et al., 2001), which can amplify or diminish the ensuing response through the regulation of antioxidant systems (Pasqualini et al., 2003). Sustained ROS production provokes the accumulation of phytotoxic levels of ROS, leading to visible necrosis or induced programmed cell death (Van Breusegem and Dat, 2006). Therefore, understanding the characteristics of ROS metabolism could help to improve the effects of ozonation or an ability of *V. natans* to withstand the environmental changes.

Combined treatment with ozonation and aquatic macrophytes is one of the most promising technologies among advanced wastewater treatment methods. However, ozonation of wastewater can have both positive and negative effects on aquatic macrophytes. Therefore, the present study was conducted to investigate the degradation of pollutants by combining ozonation with *V. natans* for the treatment of piggery effluent, as well as the ROS metabolism responses of *V. natans* to ozonated piggery effluent.

## 2. Materials and methods

### 2.1. Study site and piggery effluent

The present study was conducted at Zhuanghang experimental station (121°23′E, 30°53′N), Shanghai, China. Biologically treated piggery effluent was collected from a local pig farm that used a treatment method that consisted of an oxidation pond (about 150 $m^2$, 2 m deep), subsurface flow constructed wetland (about 25 $m^2$, 1 m deep) and surface flow constructed wetland (about 2000 $m^2$). The daily wastewater output is about 5 t, and the discharge interval is 7 d. Samples were collected in a plastic container from the effluent pond, transferred to the experimental station, and treated with ozone immediately.

### 2.2. Ozonation of piggery effluent

Ozone was generated by an oxygen-supplied corona discharge-type ozone generator (WGS10, China), and ozone flow was regulated at 2.5 L $min^{-1}$. Ozone concentrations were continuously monitored by ozone analyzers (IDEAL-2000, USA). For every treatment, the reactor was filled with the same volume of the piggery effluent (140 L). The effluent was ozonated with three different doses of ozone, up to 10 mg $L^{-1}$ (AO1, after ozonation), 30 mg $L^{-1}$ (AO2) and 50 mg $L^{-1}$ (AO3). Part of the effluent served as an untreated control (BO, before ozonation). The ozone gas was allowed to react with the sample for 30 min, and the aqueous temperature of the reaction was 20 °C. Subsequently, samples were stored for 24 h at ambient temperature with aeration to dissipate any residual ozone.

### 2.3. Vallisneria natans *treatment*

The aquatic macrophyte species, *V. natans* (Lour.) Hara, which is an evergreen variety, was collected from Shanghai Ocean University, China. *V. natans* was cultured in the ordinary pond at the experimental station. Plants with healthy and uniform growth ($40 \pm 5$ cm) were randomly selected for planting in containers (35 cm in diameter, 55 cm in height) with mud (15 cm) at the bottom. The same plant density was maintained and three replicates were performed in each treatment. Untreated and ozone treated water was gently poured into the containers. The physiochemical characteristics of the effluent and plant tissue analysis were measured at 7 d intervals, last 28 d. A constant volume of water and evaporated water was maintained in the container by addition of the corresponding treatment water after sampling.

### 2.4. Analytical methods

Water samples were analyzed for total nitrogen (TN) and total phosphorus (TP) according to the Chinese standard methods. Briefly, TN was measured by the alkaline potassium persulfate digestion-UV spectrophotometric method. TP was measured using the ammonium molybdate spectrophotometric method, where samples were first subjected to digestion using potassium persulfate as the reducing agent for TP analysis. The concentrations of $NH_4^+$, $NO_3^-$ and $PO_4^{3-}$ were measured using a 930 Compact IC Flex ion chromatograph (Metrohm, Switzerland). Dissolved organic carbon (DOC) was analyzed using an Apollo 9000 total organic analyzer™ (Teledyne Tekmar, USA) after polytetrafluoroethylene filters (pore size 0.45 μm). Ultraviolet (UV) spectrophotometers (DR 5000, Hach, USA) at wavelengths of 254 nm ($UV_{254}$) and 436 nm ($UV_{436}$) were utilized to evaluate the degradation and decolourization rate of humic substances, respectively.

Plant samples were analyzed for $O_2^-$ production utilizing a modified version of the method described by Wang and Lou (1990). The key to the method is the detection of $O_2^-$ by oxidation of hydroxylamine, which yields nitrite. The $H_2O_2$ concentration was colorimetrically measured as described by Zou (2000). The intensity of yellow color of the supernatant was measured at 415 nm against a reagent blank. The activity of superoxide dismutase (SOD, EC 1.15.1.1) was assayed by measuring its ability to inhibit the photochemical reduction of NBT using the method described by Beauchamp and Fridovich (1971). The volume of extract corresponding to 50% inhibition of the reaction was considered to be one enzyme unit. Catalase (CAT, EC 1.11.1.6) activity was evaluated as described by Li et al. (2004). One unit of CAT activity was defined as a change in absorbance of 1 unit per min. Peroxidase (POD, EC 1.11.1.7) activity was measured by monitoring the increase in absorbance at 470 nm (Castillo et al., 1984). One unit of POD activity was defined as a change in absorbance of 1 unit/min. Ascorbate peroxidase (APX, EC 1.11.1.11) activity was determined spectrophotometrically by assessing the decrease in absorbance at 290 nm (extinction coefficient of 2.8 m $M^{-1}$ $cm^{-1}$) (Nakano and Asada, 1987). Ascorbic acid (AsA) content was assayed using the method described by Zou (2000). Glutathione (GSH) concentrations were measured using a 5,5-dithio-bis-2-nitrobenzoic acid (DTNB) assay as described by Anderson (1985).