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## Short Communication

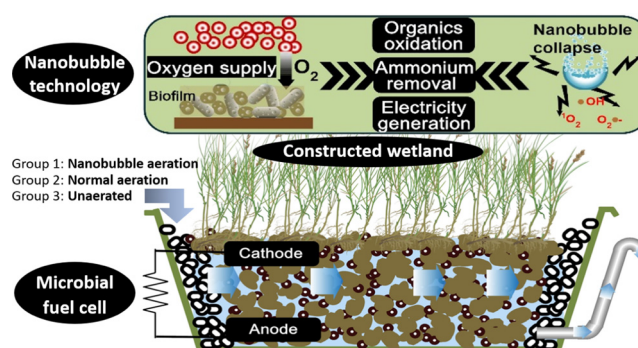
## Nanobubble aeration enhanced wastewater treatment and bioenergy generation in constructed wetlands coupled with microbial fuel cells

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## HIGHLIGHTS

- Nanobubble (NB) aeration is suggested to upgrade conventional artificial aeration.
- NB aeration enhanced the organics and ammonia removal in constructed wetlands (CWs).
- NB technology improved the bioenergy generation in CW-microbial fuel cell systems.
- NB technology has the potential to trigger CWs innovation for wastewater treatment.

## GRAPHICAL ABSTRACT



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## ABSTRACT

Artificial aeration is a widely used approach in wastewater treatment to enhance the removal of pollutants, however, traditional aeration techniques have been challenging due to the low oxygen transfer rate (OTR). Nanobubble aeration has emerged as a promising technology that utilise nano-scale bubbles to achieve higher OTRs owing to their large surface area and unique properties such as longevity and reactive oxygen species generation. This study, for the first time, investigated the feasibility of coupling nanobubble technology with constructed wetlands (CWs) for treating livestock wastewater. The results demonstrated that nanobubble-aerated CWs achieved significantly higher removal efficiencies of total organic carbon (TOC) and ammonia (NH<sub>4</sub><sup>+</sup>-N), at 49 % and 65 %, respectively, compared to traditional aeration treatment (36 % and 48 %) and the control group (27 % and 22 %). The enhanced performance of the nanobubble-aerated CWs can be attributed to the nearly three times higher amount of nanobubbles ( $\varnothing < 1 \mu\text{m}$ ) generated from the nanobubble pump ( $3.68 \times 10^8$  particles/mL) compared to the normal aeration pump. Moreover, the microbial fuel cells (MFCs) embedded in the nanobubble-aerated CWs harvested 5.5 times higher electricity energy (29 mW/m<sup>2</sup>) compared to the other groups. The results suggested that nanobubble technology has the potential to trigger the innovation of CWs by enhancing their capacity for water treatment and energy recovery. Further research needs are proposed to optimise the generation of nanobubbles, allowing them to be effectively coupled with different technologies for engineering implementation.

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## 1. Introduction

Constructed wetlands (CWs), as a nature-based solution, have been widely used for various wastewater treatment applications and offer additional benefits, such as carbon sequestration and biodiversity services (Oral et al., 2020; Wu et al., 2023). In CWs, oxygen plays a crucial role in pollutant remediation, including the chemical oxygen demand (COD) and ammonia ( $\text{NH}_4^+$ -N) removal by nitrification (Vymazal, 2020; Wu et al., 2018). Although wetland plants can supply  $\text{O}_2$  to support microbial biodegradation (Brix, 1997), seasonal variations in plant vitality can limit their contributions (Kadlec, 2016). To overcome the limitation of  $\text{O}_2$  availability, the use of artificial aeration has become a popular approach in CWs, with approximately 500 aerated wetland systems operating worldwide, resulting in approximately 33 % improvement in COD and  $\text{NH}_4^+$ -N removal performances (Nivala et al., 2020). However, traditional aeration techniques have been challenging due to their low oxygen transfer efficiency (6–10 %). Smaller bubbles have a larger surface area and therefore a better gas transfer coefficient, which may benefit biodegradation processes (Fan et al., 2023). Recent studies have incorporated micro-scale bubble aeration in CWs, resulting in significantly improved wastewater treatment performance (Gong et al., 2017; Nascimento et al., 2017).

Nanobubble technology has been recognised as being able to provide revolutionary potential in the area of environmental engineering, and especially for wastewater treatment (Lyu et al., 2019). Nanobubbles are defined as bubbles with diameters  $<1 \mu\text{m}$  (ISO/TC, 2017) and possess unique properties such as longevity, low buoyancy, and high gas solubility. Our previous study demonstrated that a small amount of oxygen-filled nanobubbles could supply  $\text{O}_2$  continuously for over 4 months and reverse hypoxia to aerobic conditions (Zhang et al., 2018). We hypothesize that nanobubble technology could offer effective  $\text{O}_2$  delivery in CWs, thereby enhancing pollutant removal. Additionally, nanobubbles generate numerous reactive oxygen species (ROSs), including superoxide anion radicals ( $\text{O}_2^{\cdot -}$ ) and singlet oxygen ( $^1\text{O}_2$ ), during the process of bubble collapse (Fan et al., 2021b; Tang et al., 2021). These ROSs have strong oxidative properties, which can further facilitate pollutant degradation (Wu et al., 2022). Although excessive DO and the elevated generation of ROS can induce stress and trigger plant senescence (Wang et al., 2020), it has been widely agreed that the addition of nanobubbles can improve the growth of various plants, such as rice, tomato, and aquatic macrophytes (Ahmed et al., 2018; Ebina et al., 2013; Y. Wang et al., 2021). As a result, enhanced plant growth may further benefit nutrient removal through plant uptake in CWs. Microbial fuel cell-coupled CWs (CW-MFCs) have been explored for simultaneous wastewater treatment and energy production (Doherty et al., 2015), but power densities achieved are still too low for practical applications. As cathodic aeration is a common strategy for improving the energetics of CW-MFCs (Gupta et al., 2023), nanobubbles could be expected to provide substantial oxygen as electron acceptors at the cathode and improve the generation of bioelectricity.

The study aimed to investigate the potential of nanobubble technology to upgrade CWs in terms of wastewater treatment and simultaneous energy recovery. The pollutant removal efficiency and electricity generation capacity of CWs equipped with nanobubble and conventional pump aerations were compared. Moreover, the size and distribution of nano-scale bubbles generated by the pumps were determined to provide insight into the underlying mechanisms. Finally, future research needs and potential engineering implementations of the proposed concept were discussed.

## 2. Materials and methods

### 2.1. System setup and operation

Nine vertical flow saturated CWs were set up with identical dimensions of 20 cm inner diameter and 60 cm height, as shown in Fig. 1a. Each CW was filled with quartz sand ( $\text{Ø}$  0.2–0.6 cm) and a layer of gravel ( $\text{Ø}$  1–3 cm) 5 cm thick at the bottom and top. Inlet pipes were placed at the top of each CW, and effluents were collected at the bottom of each system,

passing through an external pipe with a height of 50 cm. All CWs were planted with 3–4 *Iris pseudacorus* stalks and equipped with microbial fuel cells (MFCs). Graphite cathode (upper) and anode (lower) electrodes with a diameter of 15 cm and thickness of 0.5 cm were buried in the middle portion of each CW with a spacing of 10 cm (Fig. 1b). Each electrode ( $176.6 \text{ cm}^2$ ) consisted of a porous plate to allow wastewater to flow through. A layer of glass wool with a thickness of 2 cm was placed between electrodes to provide a ‘sharp’ redox profile (Doherty et al., 2015). The cathodes and anodes were connected by insulated titanium wire through an external circuit with an external resistance of 950  $\Omega$ .

The experiments were conducted in the glasshouse at Nottingham Trent University, UK, where the CWs were exposed to natural light exposure. The influent was collected from a local chicken farm, and after pre-treatment by settlement, the supernatant was placed in a 50 L tank for each CW group. Three treatment groups with triplicates for each, including control (no aeration), normal pump aerated, and nanobubble aerated groups, were operated in this study. The two aeration approaches were conducted in the influent tanks with one aerated by a normal pump (20 W) with a ceramic diffuser for 100 min per day, and the other aerated for 10 min per day by a commercial nanobubble generator (200 W; KTM, Nikuni Co., Ltd., Kanagawa, Japan). Influent flows were controlled by multichannel peristaltic pumps with the average influent TOC,  $\text{NH}_4^+$ -N,  $\text{NO}_3^-$ -N, and  $\text{NO}_2^-$ -N of all systems were approximately 163, 43, 2.6, and 0.5 mg/L, respectively, for the duration of the experimentation period. The dissolved oxygen (DO), oxidation-reduction potential (ORP) and pH of the initial livestock wastewater (without aeration) were around 0.5 mg/L,  $-120 \text{ mV}$  and 7.6, respectively. All systems were operated under a flow regime providing a hydraulic retention time (HRT) of 1 day. The first four months (May–August) were operated as the stabilisation period before the weekly sampling campaign for the next three months.

### 2.2. Sampling and analysis

The sizes and distributions of nanobubbles ( $<1 \mu\text{m}$ ) in both traditional aerated water and nanobubble-aerated water were determined by nanoparticle tracking analysis using a ZetaView PMX 120 (Particle Metrix, Meerbusch, Germany). Since current techniques, such as dynamic light scattering (DLS) and nanoparticle tracking analysis (NTA), are unable to differentiate bulk nanobubbles from nanoparticles (Paknahad et al., 2021), the samples were prepared by aerating tap water instead of using real wastewater. Both pumps were operated over periods of 5–100 min, after which aliquots of the aerated waters sampled and the analyses carried out at room temperature.

After the stabilisation period, influent and effluent samples (triplicates for each) were collected weekly from all CWs. DO, pH and concentrations of TOC,  $\text{NH}_4^+$ -N,  $\text{NO}_3^-$ -N, and  $\text{NO}_2^-$ -N were determined. The pH and DO were measured using a portable Orion 5-Star meter with a pH electrode (9172BNWP; Thermo Scientific, USA) and a DO electrode (086030MD; Thermo Scientific, USA). TOC was measured by a TOC-V analyser equipped with a TNM-1 unit (Shimadzu Corp., Japan).  $\text{NH}_4^+$ -N,  $\text{NO}_3^-$ -N, and  $\text{NO}_2^-$ -N were analysed by a continuous flow colorimetric technique (SEAL AutoAnalyzer 3, UK). The electrical performance of all CWs was determined by the hourly recording of voltage (V) across the external resistor (R) for two weeks using a data-acquisition and multimeter system (Keithley DAQ6510, UK).

### 2.3. Calculation and statistical analysis

Total inorganic nitrogen (TIN) was calculated using the sum of  $\text{NH}_4^+$ -N,  $\text{NO}_3^-$ -N, and  $\text{NO}_2^-$ -N measured in the water samples. Current ( $I = V/R$ ) and power ( $P = VI$ ) of the MFCs were calculated based on the recorded voltage and the value of the external resistance (950  $\Omega$ ). Then, the power density ( $\text{mW}/\text{m}^2$ ) was then calculated by dividing the power by the surface area ( $\text{m}^2$ ) of the anode. The Kruskal-Wallis tests ( $p < 0.05$ ) were conducted to compare the removal performances of pollutants between different systems.

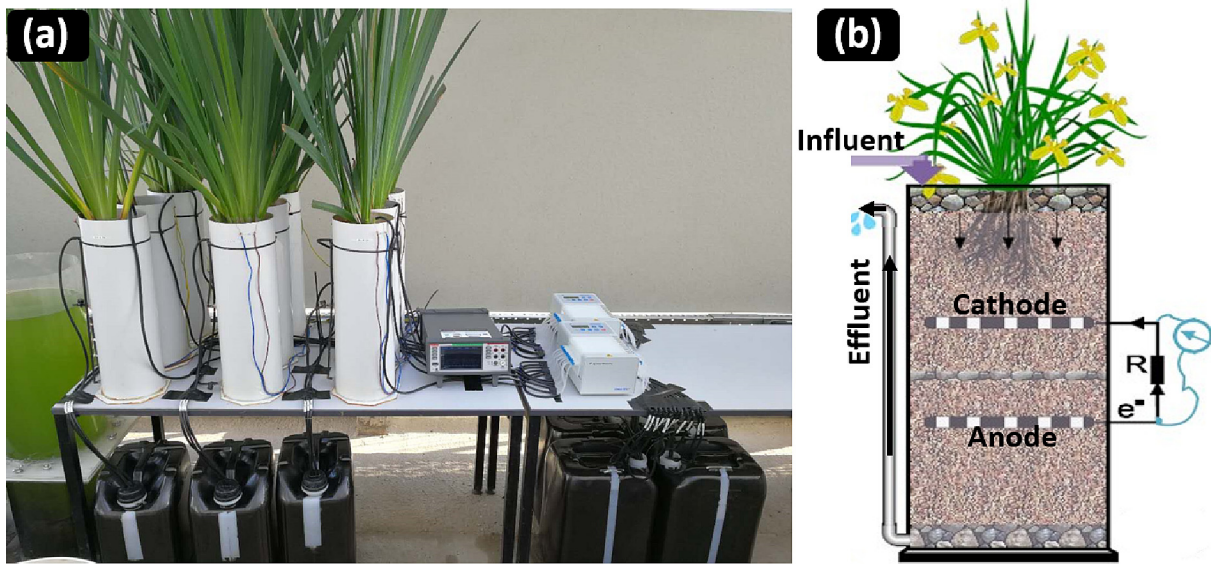


Fig. 1. The photo (a) and schematic (b) of the experimental constructed wetland systems. Both cathode and anode electrodes in (b) are made of graphite.

### 3. Results and discussion

#### 3.1. Nanobubbles generation in the waters

Various methods have been developed to produce nanobubbles, including ethanol–water exchange, direct immersion, temperature change, electrochemical reaction, and hydraulic cavitation (Wu et al., 2022). Among them, the hydraulic cavitation method is deemed the most effective

approach (Zhou et al., 2022), in terms of nanobubble generation speed and stability, which was used in this study. Regardless of the dimensions, all bubbles in water undergo dynamic processes, such as coalescence and breakup (Fig. 2a).

Notably, current Nanoparticle Tracking Analysis (NTA) technology is unable to differentiate nanobubbles from other solid nanoparticles in water samples, which makes it unfeasible to detect them in CWs effluent samples owing to the presence of substrate particles and plant debris.

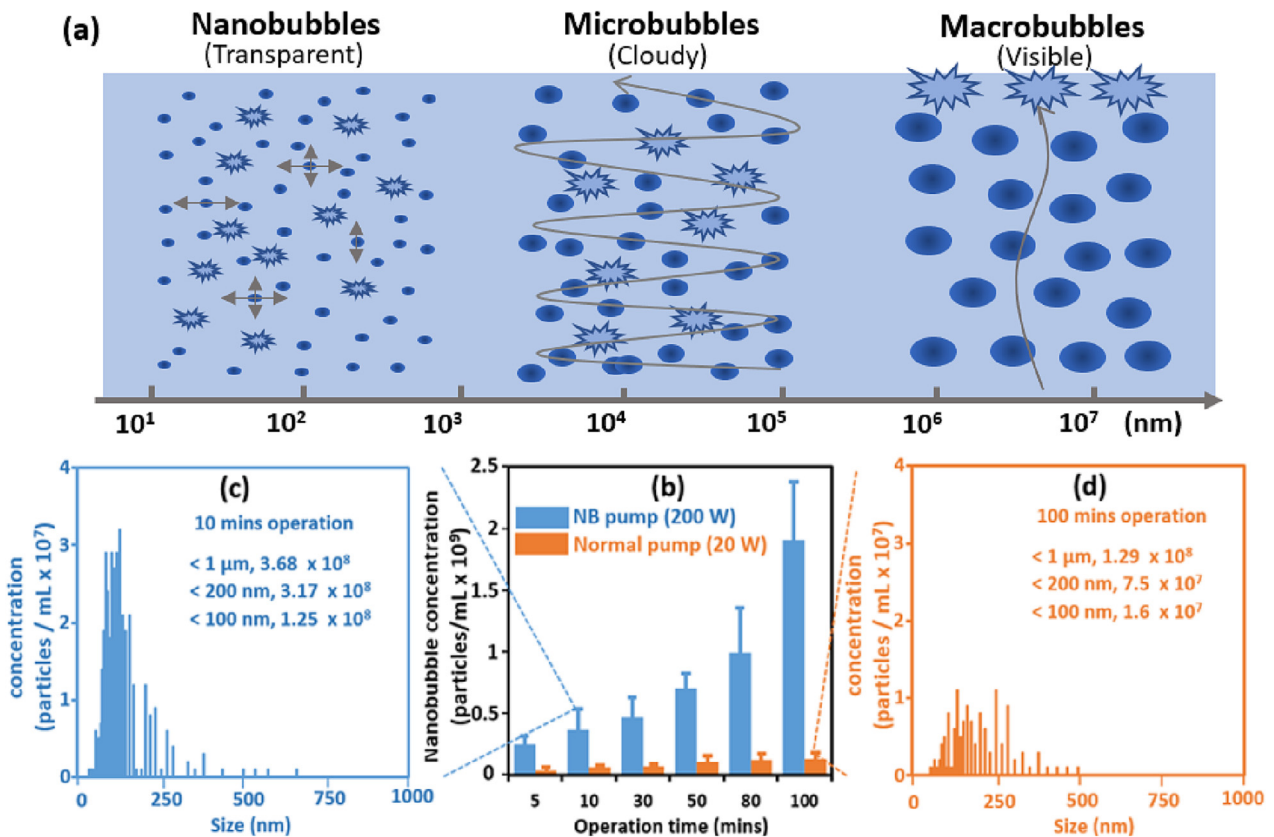


Fig. 2. The characterisations of different sizes of bubbles (a) and the concentrations of nanobubble from the water samples aerated by traditional and nanobubble aeration pumps along with the time (b). The bubble size and distribution status under the selected modes of operation for nanobubble- (c) and traditional- (d) aerated CWs.

Thus, the size and distributions of the nanobubbles from influent could be considered as the current best strategy to link with the relevant removal of pollutants in CWs. Fig. 2b shows that the nanobubble generator could produce 1–2 magnitude higher concentrations of nanobubbles than those from the normal pump over the same operating time from 5 to 100 min. In this study, the nanobubble pump (200 W) and normal pump (20 W) worked for 10 and 100 min/day, respectively, to achieve the same energy consumption (0.033 kWh per day) for CWs aeration. Under such comparable conditions, the nanobubble concentration was  $3.68 \times 10^8$  particles/mL in the nanobubble pump aerated water (Fig. 2c), however, three times lower concentration ( $1.29 \times 10^8$  particles/mL) was observed in the normal pump aerated water (Fig. 2d). Over 86 % of the nanobubbles ( $3.17 \times 10^8$  particles/mL) were <200 nm in the water aerated by the nanobubble generator. A concentration of only  $7.5 \times 10^7$  particles/mL was found in the water aerated with the normal pump. Moreover, unaerated samples from the tap water samples consistently contained  $<10^4$  particles/mL nanobubbles.

The DO concentrations in the water samples aerated with normal and nanobubble pumps were found to be 9 and 20 mg/L (oversaturated), respectively, which were significantly higher than the control group (0.5 mg/L). The size of the bubbles plays a significant role in determining the rate of oxygen transfer in water during aeration. Previous studies have shown that smaller bubbles with a larger surface area facilitate a better oxygen transfer rate (Fan et al., 2023). Nanobubbles have a higher capacity to diffuse gases into the water before floating to the surface compared to macrobubbles (Fig. 2a), based on classical bubble diffusion theory. Theoretically, the high Laplace pressure within the nanobubbles due to their small curvature radius should drive bubble dissolution in the liquid almost instantly ( $\sim 1 \mu\text{s}$ ) (Lyu et al., 2019). Numerous studies have observed that nanobubbles can remain stable for hours to days. Atomic force microscopy (AFM) has detected heterogeneous pressures inside nanobubbles, and subsequent molecular dynamics simulations have demonstrated a high-density gas state, rather than a high-pressure state, inside the nanobubbles (S. Wang et al., 2021). Hence, the gas diffusion process from nanobubbles is reasonably slow and can last for a prolonged period, which is beneficial for pollutant biodegradation in CWs.

### 3.2. Better performance for wastewater treatment

Both aerated CWs exhibited significantly higher efficiencies for TOC and  $\text{NH}_4^+\text{-N}$  removal (Fig. 3a and b) than those with unaerated CWs (27 % and 22 % removal efficiencies, respectively). These observations can be explained by the improved aerobic conditions in the CW beds, which acted to stimulate bacterial oxidation and nitrification for organics and ammonium degradation (Nivala et al., 2020). Moreover, the nanobubble aerated systems also showed significantly higher removal of TOC (49 %) and  $\text{NH}_4^+\text{-N}$  (65 %) than those CWs aerated by traditional pumping systems (36 % and 48 %, respectively). Notably, the organic form of nitrogen in the chicken farm wastewater was not monitored in this study. However, it can be anticipated that mass removal of  $\text{NH}_4^+\text{-N}$

would be much higher due to the conversion of organic nitrogen into  $\text{NH}_4^+\text{-N}$ . Overall, these results illustrate that nanobubble aeration could provide higher oxygenation capability for the more effective removal of pollutants. The findings are in line with previous studies that have demonstrated the ability of nanobubble aeration to stimulate biofilm growth and shift microbial community functions towards enhanced removal of COD and ammonia in biological wastewater treatment processes (Xiao et al., 2021; Xiao and Xu, 2020).

This removal of organic pollutants could be attributed either to biodegradation by the stimulated microbial communities or to the direct breakdown of organic structures by reaction with the ROSs (Zhou et al., 2022) provided by disintegrating nanobubbles. In a hydroponic study with simulated clean lake water, S. Wang et al. (2021) reported that the nanobubble concentration threshold for the switch from growth promotion to growth inhibition in *Iris pseudacorus* was found to be  $3.45 \times 10^7$  particles/mL. However, despite the inlet water in this study containing a higher nanobubble concentration ( $10^8$  particles/mL), there were no significant differences observed in the plant morphology among the three groups. It is hypothesized that the wetland media and adsorbed substances may act as a barrier, mitigating the direct impact of nanobubble-induced ROS on the plant roots. Furthermore, this study was specifically conducted for wastewater treatment, which contains high levels of pollutants that can compete with the consumption of the generated ROS. Nevertheless, the detection of ROSs and the microbial community response should be studied in future to fully illustrate the mechanisms of operation.

In contrast to the decreased concentrations of  $\text{NH}_4^+\text{-N}$  observed after treatment, the average concentrations of  $\text{NO}_3^-\text{-N}$  increased from 2.6 mg/L in the influent to 24.4, 15.9 and 9.3 mg/L in the effluent for the nanobubble aerated, normal aerated and control groups, respectively. Additionally, the effluent concentration of  $\text{NO}_2^-\text{-N}$  ranged from 0.5 to 1.2 mg/L in all three groups without showing significant differences. Therefore, the removal of TIN (the sum of  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_3^-\text{-N}$ , and  $\text{NO}_2^-\text{-N}$ ) from nanobubble-aerated systems was found to be only slightly more effective (21 %) than that from the normal pump-aerated systems (18 %), which may be due to aerobic conditions that could only enhance nitrification rather than denitrification processes. However, further optimisation of the system configuration, such as implementation of part-aeration or of hybrid CWs, could be investigated to increase the removal of TIN.

### 3.3. Higher energy recovery from CW-MFCs

The non-aerated CWs did not exhibit a considerable ability to produce electricity (Fig. 3c), with a maximum voltage and calculated power density of 0.17 V and 1.7 mW/m<sup>2</sup>, respectively. The higher maximum voltage and power density of 0.29 V and 5.2 mW/m<sup>2</sup>, respectively, were observed in the normal pump aerated systems. The higher electricity generation may be attributable to the better aerobic conditions in the volume around the cathode, owing to the higher oxygen content of the influent flow, which would better facilitate the transfer of electrons to the cathode from the anode (Zhao et al., 2013). This arrangement would also contribute to the significantly higher

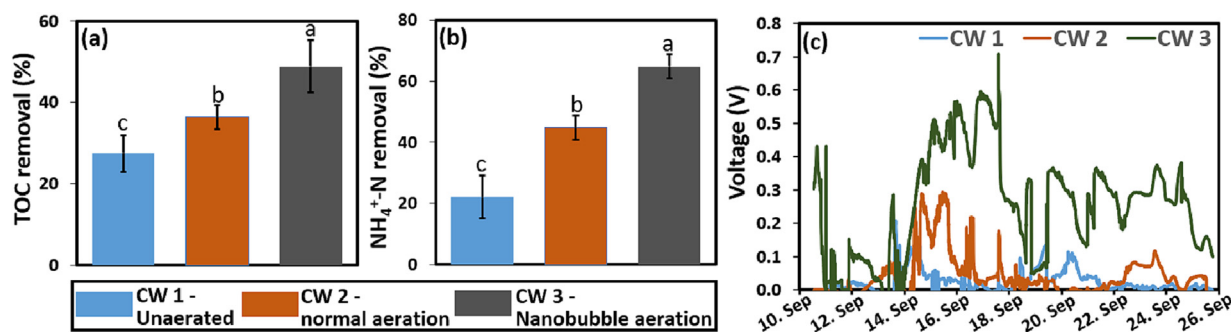


Fig. 3. Removal efficiencies of the TOC (a) and ammonium (b,  $\text{NH}_4^+\text{-N}$ ), and the electricity generation (c) from the three CWs equipped with MFCs while operating under the external resistance of 950  $\Omega$ .

maximum voltage (0.71 V) and power density (29 mW/m<sup>2</sup>) obtained in the nanobubble aerated systems.

The measured average power densities (1.7–29 mW/m<sup>2</sup>) observed from all three CWs were comparable to those (3.37–15.73 mW/m<sup>2</sup>) obtained from previous studies (Oon et al., 2015; Yadav et al., 2012; Zhao et al., 2013). A previous study has demonstrated that the special operation on cathode aeration significantly enhances the peak power density from 2.5 to 112 mW/m<sup>2</sup> in a CW-MFC when treating wastewater from anaerobic digestion processes (Wu et al., 2017). This current study did not focus on optimisation of the configuration of the CWs, and the specific electrode materials employed, but it illustrated that nanobubble aeration could improve the electricity generation under the basic vertical flow CWs regime by a factor of around 5.5. The result may be due to the superior oxygen transfer efficiency and presence of oxidising species, for instance free radicals, which could facilitate the acceptance of the cathode for electrons. Further studies will undoubtedly be needed to study the optimisation of the coupling between other types of CWs and MFCs for the recovery of stable and high levels of bioelectrical energy.

### 3.4. Insights for engineering implications and future research

Building upon knowledge, the development of new, innovative, and sustainable solutions to water treatment processes, is crucial, especially at a time when new, and more demanding, water regulations are being established under the UN's Sustainable Development Goals initiative. Artificial aeration has long been considered a technology entailing major energy consumption in wastewater treatment. For example, the conventional mechanical aerators in wastewater treatment plants account for 75 % of their total energy costs (Rosso et al., 2008). Similarly, to develop and deploy an efficient aeration approach is of paramount importance to maintain the cost-effective nature of CWs for wastewater treatment. Thus, the use of nanobubble technology for aeration paves a sustainable way by which to improve the efficiency of oxygenation for wastewater treatment.

Nanobubbles are gas-filled 'nanoparticles', which is a different concept to the traditional understanding of the nanoparticle being a pollutant (e.g. silver nanoparticles). They can provide an effective and chemical-free way for gas transfer and stimulate relevant biochemical reactions for the sustainable removal of pollutants. Nanobubbles filled with different gases could, in addition, function in different ways, e.g. N<sub>2</sub> filled nanobubbles could improve methane production in anaerobic digestion (Chuenchart et al., 2021) and O<sub>3</sub> filled nanobubble could enhance photodegradation of micropollutants (Fan et al., 2021a). However, several technical and scientific thresholds still need to be addressed to help the understanding and implementation of the underlying mechanisms (Zhou et al., 2022). As noted in this current research, aeration in the influent was conducted differently from the conventional approach, which typically involves a pump and a diffuser to directly introduce air into the CWs. It was mainly determined by the currently available nanobubble generation methods (X. Wang et al., 2021). The ceramic membrane filtration approach seems to be the closest to conventional diffuser-based aeration, but it is less efficient in stabilising the generated nanobubbles compared to other methods (Ahmed et al., 2018). Other effective methods rely on hydraulic cavitation in liquid/gas mixture, including the use of a venturi tube (Li et al., 2021) and the gas-water circulation approach currently utilised, which can only be applied in the influent. Additionally, it is expected that solid particles in the solution may impact the cavitation process, affecting cavitation nucleus formation and the flow patterns of liquid and gas (Lyu et al., 2019). Therefore, we urge that further studies should focus, as a priority, on the optimisation of nanobubble generation, to ensure that the special characteristics of nanobubbles can be effectively utilised, when coupled with different wastewater treatment technologies.

## 4. Conclusion

This study, for the first time, demonstrated the feasibility of integrating nanobubble technology with constructed wetlands for efficient wastewater

treatment. Compared to traditional aerated treatment and control groups, this innovative method achieved significantly higher removal efficiencies of organics and NH<sub>4</sub><sup>+</sup>-N. Additionally, the embedded microbial fuel cell systems exhibited a remarkable 5.5 times higher electricity energy recovery rate in nanobubble-aerated CWs, suggesting the potential of nanobubble technology to enhance the capacity of CWs for water treatment and energy recovery. However, further research is required to investigate the microbial community response and develop suitable nanobubble generation methods for the practical application.

## CRedit authorship contribution statement

**Tao Lyu:** Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Writing – original draft. **Yuncheng Wu:** Investigation. **Yang Zhang:** Writing – review & editing. **Wei Fan:** Writing – review & editing. **Shubiao Wu:** Data curation, Validation, Writing – review & editing. **Robert J.G. Mortimer:** Writing – review & editing. **Gang Pan:** Writing – review & editing, Project administration, Funding acquisition.

## Data availability

Data will be made available on request.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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