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An MFC-Based Online Monitoring and Alert System for Activated Sludge Process

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In this study, based on a simple, compact and submersible microbial fuel cell (MFC), a novel online monitoring and alert system with self-diagnosis function was established for the activated sludge (AS) process. Such a submersible MFC utilized organic substrates and oxygen in the AS reactor as the electron donor and acceptor respectively, and could provide an evaluation on the status of the AS reactor and thus give a reliable early warning of potential risks. In order to evaluate the reliability and sensitivity of this online monitoring and alert system, a series of tests were conducted to examine the response of this system to various shocks imposed on the AS reactor. The results indicate that this online monitoring and alert system was highly sensitive to the performance variations of the AS reactor. The stability, sensitivity and repeatability of this online system provide feasibility of being incorporated into current control systems of wastewater treatment plants to real-time monitor, diagnose, alert and control the AS process.

Activated sludge (AS) process is one of the most widely used technologies in wastewater treatment[1]. Since AS process is sensitive to the fluctuations of wastewater quantity and environmental instability[2,3], it is crucial to real-time monitor its status in order to achieve better control and performance[4]. A significant number of chemical, physical and electrochemical approaches have been established to monitor the variation of ammonia nitrogen, COD (chemical oxygen demand), DO (dissolved oxygen), pH and temperature as well as other parameters in AS processes[5]. However, each of these methods can only accurately determine the value of one specific parameter and cannot be able to provide sufficient information to monitor the overall status of the AS process, especially under various unstable conditions[6,7].

Microbial fuel cell (MFC) technology has been intensively investigated as it is able to recover energy through treating wastewater[8–10]. In MFCs, microorganisms oxidize organic matter and release electrons at anode, while the terminal electron acceptors (e.g., O_2) are reduced by reacting with the electrons at cathode[11–13]. Two electrodes are connected by a wire containing a load, which results in the generation of electric voltage. Recently MFC-based biosensors have been successfully developed to determine BOD (biological oxygen demand)[6,14], DO[7], volatile fatty acids[15] and toxicity[16] of water samples. Although in some cases a close relationship between the concentration of the measuring object and MFC signal was obtained, previous studies mainly focused on detecting one particular parameter using MFC technology. However, due to the high complexity of AS process and the unclear inter-relations of many involved parameters, such a single "biosensor" technology is not sufficient for on-line monitoring the status of the AS process. Moreover, none of these MFC biosensors had diagnosis and alert functions when suffering potential risks for AS process. In addition, most of previous MFC-type biosensors adopted cation exchange membrane (CEM) as the separator between anodic and cathodic chambers, resulting in a decrease in anolyte pH, which is detrimental to the biological activity at the anode[6].

Therefore, the present study aimed at developing a novel monitoring and alert system for the AS process, which was composed of a single-chamber MFC, a signal acquisition subsystem, and an alert subsystem with self-diagnosis function. The compact single-chamber MFC without CEM, the core of the online system, was assembled and submersed into an AS reactor. The organic substrates and oxygen in the AS reactor were utilized as the electron donor and acceptor respectively by the MFC, which was used to record the variations of the AS reactor. The new alert subsystem with self-diagnosis function was established based on the statistical analysis and logic judgment programs in a Lab View virtual instrument platform. In order to evaluate the reliability and sensitivity of this online monitoring and alert system, various shock tests were imposed to the AS reactor and the corresponding responses of the submersible MFC were examined. In this way, a new online monitoring and alert system for AS process was established.

Results

Stable performance of AS and MFC. After 8-d operation, the AS reactor reached its steady state, and the removal efficiencies of both COD and NH_4^+-N kept at 90.2 ± 4.1% and 96.5 ± 3.2%, respectively (Fig. 1). The concentrations of SS and VSS were 4516 ± 282 and 3657 ± 546 mg/L, respectively, while the DO concentration was within 4.4–5.1 mg/L in the AS reactor. On the other hand, the MFC voltage reached 0.41 ± 0.02 V after 30-d operation and then kept stable (Fig. 1). Furthermore, the MFC-based online monitoring system was operated stably for 6 months without any additional maintenance (Fig. 1).

To further examine the MFC performance, the polarization curves were determined (Fig. S1). The open circuit voltage of the MFC was 0.88 V (Fig. S1A), whereas the maximum power density was 4.7 W/m^3 (Fig. S1B). The power outputs and current of this study were similar with those in the previous studies in which membrane-less MFCs were used[13,17]. The internal resistance of the MFC was calculated as 34.5 ± 2.4 Ω by the slope of polarization curve, while the CE (coulombic efficiency) of the MFC was 6.23%.

Organic overloading shock. In the 5-h tenfold organic overloading (P1 in Fig. 2), the COD concentration in the AS effluent substantially increased from 22 ± 2.1 to 386 ± 15.6 mg/L, while the effluent NH_4^+-N slightly increased from 0.6 ± 0.21 to 5.3 ± 1.2 mg/L (Fig. 2A). On the contrary, the DO concentration decreased from 5.1 ± 0.3 to 0.2 ± 0.1 mg/L in the AS reactor. Simultaneously, the MFC had a quick response to this shock. The variation of the MFC voltage rapidly dropped from −0.005 ± 0.0018 to −0.24 ± 0.0038 V (Fig. 2B), while the anode potential decreased by 0.0749 V (Table S1). As a consequence, a lasting warning signal was provided by the alert system throughout P1. After the overloading shock was terminated, the variation of MFC voltage slowly returned back to 0.0041 ± 0.0021 V after 13 h (P2 in Fig. 2B). During the P3 period, the variation of the MFC voltage approached zero and consequently the warning signal was stopped. This implies that the AS reactor had restored to a new steady state, as evidenced by the low AS reactor effluent COD level.

Ammonia overloading shock. Since nitrifying bacteria in AS grow slowly and are highly sensitive, it is important to monitor the ammonia nitrogen removal performance of AS systems. In general, the production of MFC voltage is not directly linked with the ammonium concentration. However, a high ammonia concentration could increase DO consumption and consequently decrease DO concentration in the AS reactor. Since the MFC adopted O_2 as terminal electron acceptor in the online system, the variation of MFC voltage was able to have a quick response to the ammonium overloading shock.

In the 5-h NH_4^+-N overloading shock, the variation trend of MFC voltage was highly consistent in two repeated experiments (Fig. 3). The MFC voltage variation decreased from 0.0012 ± 0.0031 V to a minimum value of −0.1172 ± 0.0152 V, while the anode potential kept almost constant (Table S1), resulting in a warning signal by the alert system. Simultaneously, the effluent NH_4^+-N concentration of the AS reactor significantly increased from 0.25 ± 0.18 to 156 ± 5.9 mg/L, while the effluent COD concentration slightly increased from 22.8 ± 3.6 to 27.9 ± 2.3 mg/L (P1 in Fig. 3A). In addition, the DO concentration in the AS reactor decreased from 4.9 ± 0.2 to 3.6 ± 0.3 mg/L. After the termination of the ammonia overloading shock, the variation of the MFC voltage was close to zero through 5-h operation (P2 in Fig. 3B). Meanwhile, the removal efficiencies of both NH_4^+-N and COD were constant in the AS reactor, indicating that the AS reactor was restored from the shock and reached a new steady state (P3 in Fig. 3).

On the other hand, the nitrate could be formed in the AS reactor due to nitrification, especially at a high ammonia concentration. Therefore, the nitrate effect on the accuracy of MFC-based online monitoring system was further investigated in this study. As shown in Fig. S2, the variation of MFC voltage was slight when 700 mg/L nitrate was introduced into the AS reactor, suggesting an insignificant effect of nitrate on the accuracy of the MFC-based online monitoring system.

Temperature shock. The variation of the MFC voltage during the temperature shock period is shown in Fig. 4. When the temperature was decreased from 25.2°C to 10.3°C and 5°C at Hour 3 and Hour 5, the AS effluent NH_4^+-N concentration increased from 0.9 ± 0.3 to 8.3 ± 1.5 and 25.5 ± 3.2 mg/L respectively, and the effluent COD level increased from 23.6 ± 1.5 to 38.8 ± 4.5 and 85.5 ± 5.4 mg/L (Fig. 4A) respectively. Meanwhile, a substantial variation of the MFC voltage was observed with a minimum value of −0.2767 ± 0.0067 V after 5-h temperature shock (P1 in Fig. 4B), and the anode potential increased by 0.0884 V (Table S1). As a consequence, an early warning signal was given by the alert system at Hour 1.5 and

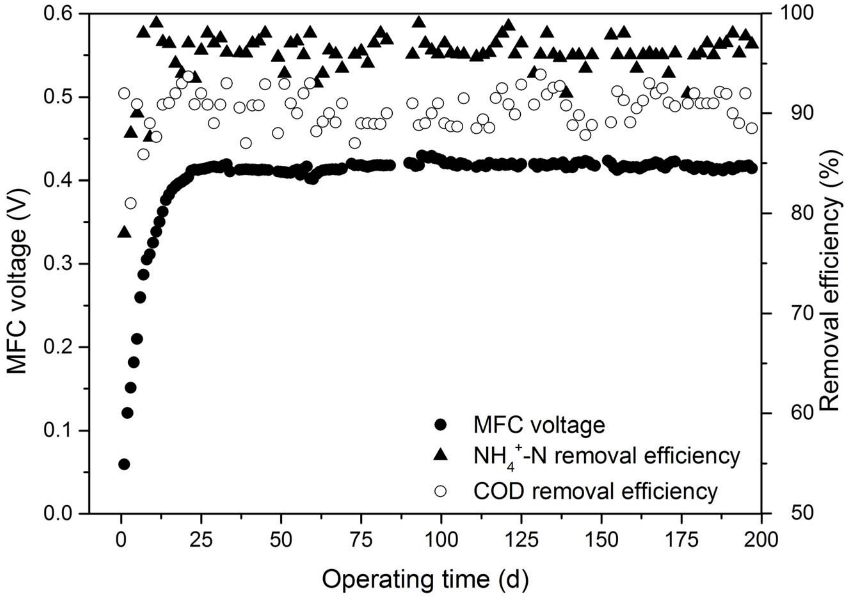

Figure 1 | **Stability of the MFC-based online monitoring and alert system and the AS reactor without any shocks.**

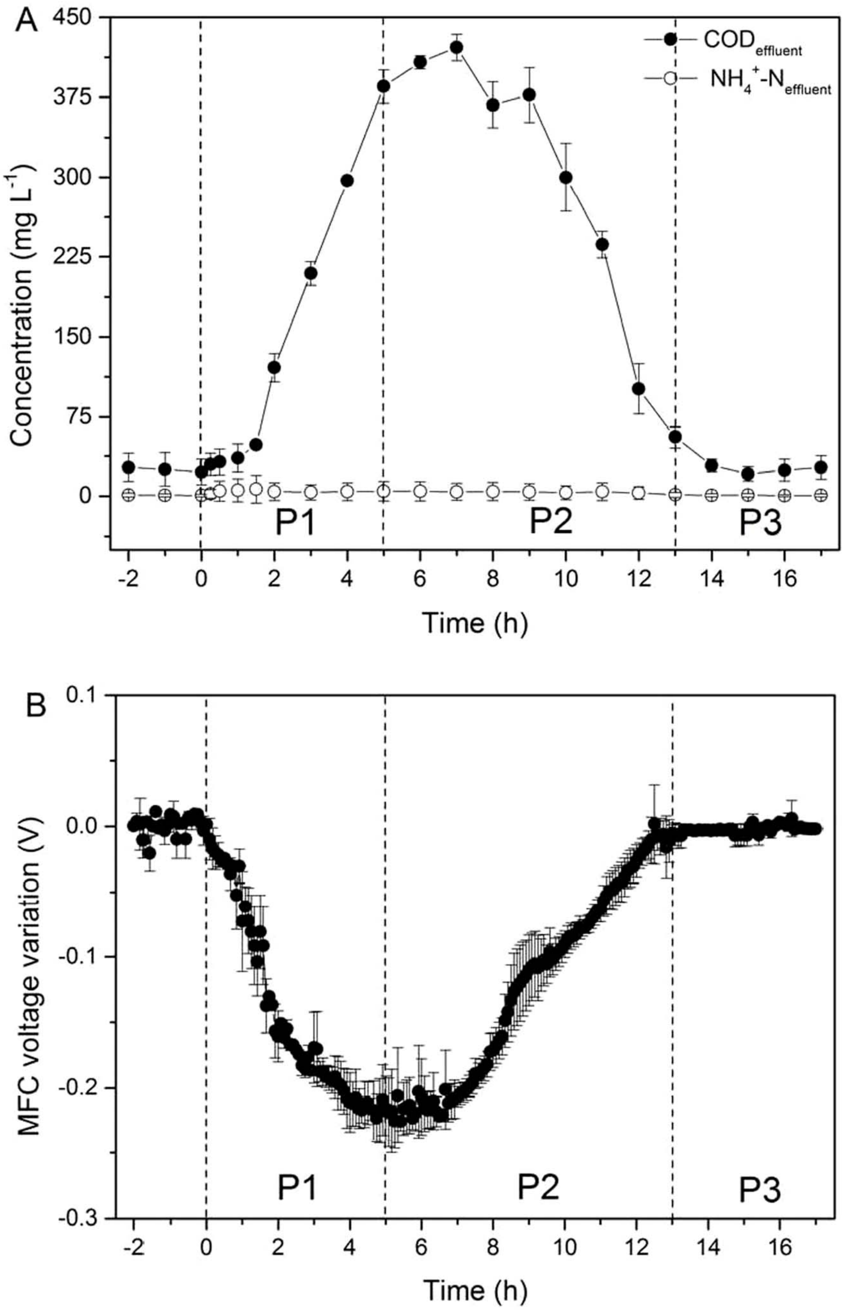

Figure 2 | Performance of MFC-based online monitoring and alert system under 10-fold organic overloading shock: (A) COD$_{effluent}$ and NH$_4^+$-N$_{effluent}$ of the AS reactor; and (B) MFC voltage variations.

lasted throughout P1. Within the temperature shock, the NH$_4^+$-N removal was completely inhibited in Hours 0–20, and the MFC voltage was below 0.2 V. At the end of temperature shock (P2), the variation of the MFC voltage drastically increased from -0.28 ± 0.018 to 0.0014 ± 0.0008 V, and a slow increase in the COD and NH$_4^+$-N removal efficiencies was observed. This result suggests the performance recovery of the AS reactor. As a result, the alert signal was stopped.

Toxicant loading shock. During the toxicant loading shock period (P1 in Fig. S3), the AS effluent COD concentration increased from 21.5 ± 1.5 to 115.8 ± 6.5 mg/L with a corresponding decrease in removal efficiency from 92.8 ± 0.5% to 61.4 ± 2.2%. At the same time, the effluent NH$_4^+$-N concentration increased from 0.8 ± 0.12 to 21.2 ± 2.5 mg/L with a corresponding removal efficiency decline from 97.3 ± 0.4% to 29.3 ± 8.3% (Fig. S3A). These results indicate that cadmium chloride had a severe inhibition on the AS process, especially for COD and NH$_4^+$ removals. On the other hand, the MFC had a rapid response to this toxicant shock and its voltage drastically reduced from 0.41 ± 0.024 to 0.06 ± 0.002 V within 5 h (P1 in Fig. S3B). Meanwhile, the anode potential increased by 0.1425 V (Table S1). In this case, the variation of the MFC voltage was higher than the preset threshold value after the shock, a lasting alert signal was generated by the alert system. The sharp decrease in the MFC voltage indicates a severe collapse of the AS reactor in this toxicant shock. The AS reactor needed one week to recover after the termination of the toxicant shock.

Discussion

MFC-based "biosensor" technology has been recently developed to determine various individual parameters of water samples, while this study focused on the establishment of a novel MFC-based monitor-

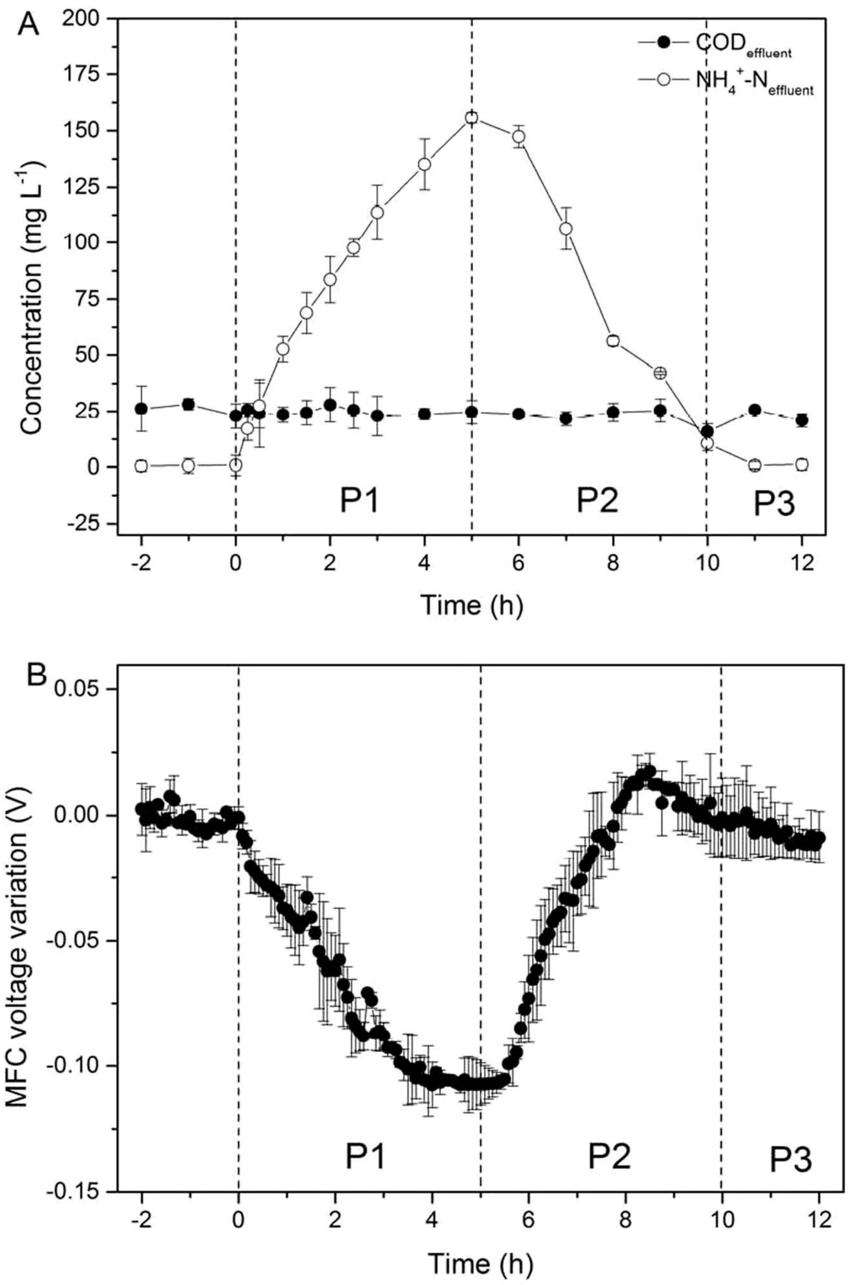

Figure 3 | Performance of MFC-based online monitoring and alert system under 10-fold ammonia overloading shock: (A) $COD_{effluent}$ and NH_4^+-$N_{effluent}$ of the AS reactor; and (B) MFC voltage variations.

ing and alert system with self-diagnosis function rather than a biosensor for the AS process. The main differences between our MFC-based diagnosis monitoring and alert system with the MFC sensors reported in literature are summarized in Table S2.

The submerged single-chamber MFC was constructed with a novel design in this study, as shown in Fig. S4. The anodic microorganisms in the MFC could sustain themselves using substrate from the AS influent, while plenty of oxygen in the AS reactor could be adopted as the electron acceptor for the cathode of the submerged MFC. Thus, Such MFC design could not only provide evaluation on the status of the AS reactor, but also significantly reduce the costs of the online monitoring system, especially for a long-term operation. In addition, a new diagnosis and alert program was also developed to rapidly evaluate the AS reactor status based on the signals of MFC voltages, anode potential and operational temperature.

As an online monitoring and alert system, its signal should be stable when the AS reactor is operated at steady state. Otherwise, it would result in an incorrect conclusion. The signal of our developed system, i.e., the MFC voltage variation, was relatively stable under usual conditions and fluctuated only in a 5% range (±0.02 V) over six months without any maintenance (Fig. 1). On the other hand, the MFC-based monitoring system demonstrated a high sensitivity to different shocks. For example, in the organic overloading shock tests, the decrease in the MFC voltage variation had a correlation with the increase in the AS effluent COD level (Fig. 2). The MFC voltage variation had a quick response to such an organic overloading shock.

This online monitoring and alert system also had a capability of early warning to various shocks. Here, we took the temperature shock test as an example. After 1.5-h shock (P1 in Fig. 4), the AS effluent COD and NH_4^+-N concentrations varied slightly from 23.6 ± 1.5 to 34.7 ± 2.5 mg/L, and from 0.9 ± 0.3 to 2.3 ± 0.7 mg/L,

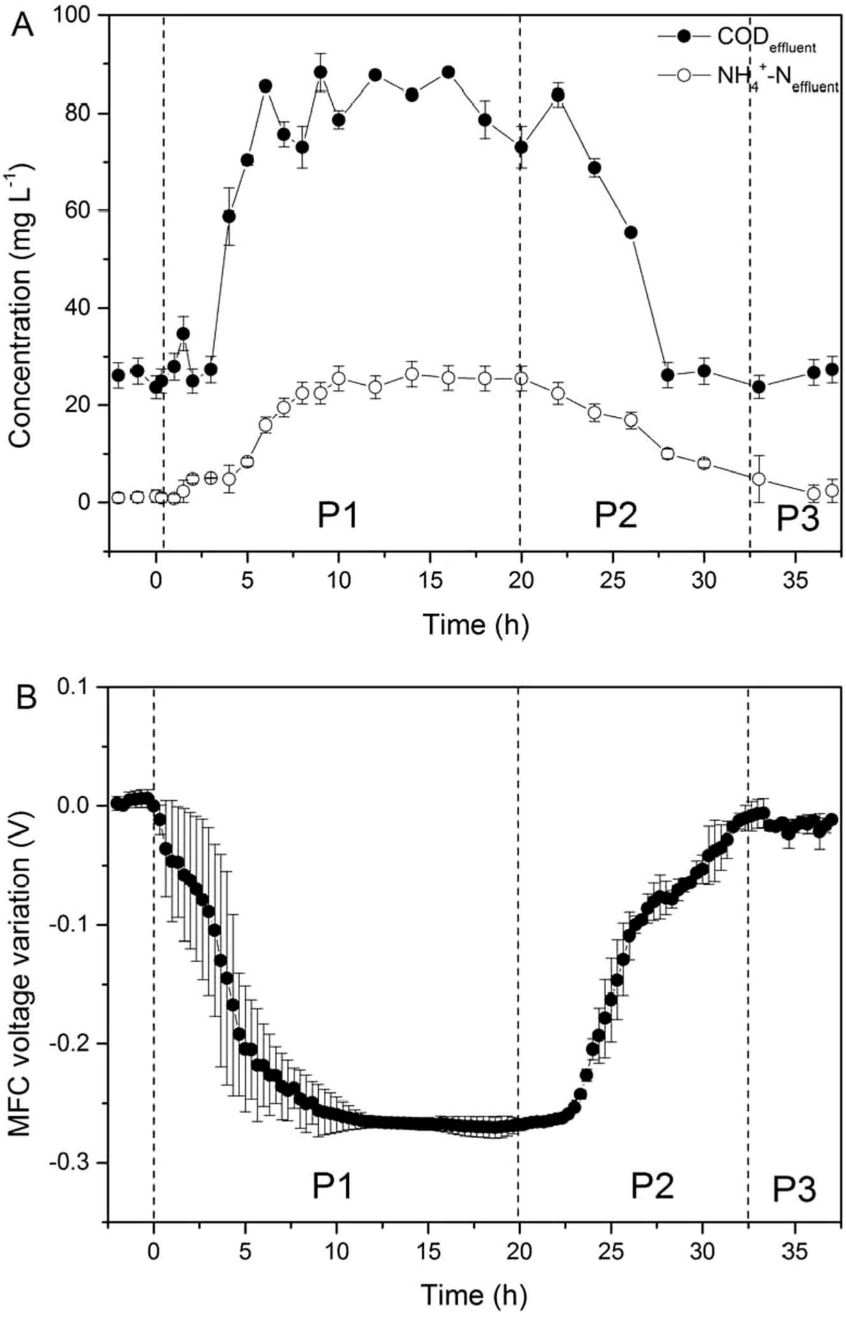

Figure 4 | Performance of MFC-based online monitoring and alert system under 5°C temperature shock: (A) $COD_{effluent}$ and NH_4^+-$N_{effluent}$ of the AS reactor; and (B) MFC voltage variations.

respectively (Fig. 4A). However, the voltage variation changed substantially: the voltage variation exceeded 0.04 V and decreased from 0.0013 ± 0.001 to -0.0644 ± 0.009 V (Fig. 4B). As a result, an early alert warning was given at Hour 1.5 (Table S1). On the other hand, the AS effluent COD and NH_4^+-N concentrations exceeded the China's Discharge Standard (COD $>$ 60 mg/L or NH_4^+-N $>$ 15 mg/L) at Hour 6 (P1, Fig. 4). The warning signal given by the alert program was 4.5 hours earlier than the substantial deterioration of the effluent quality. Therefore, the MFC system could response more rapidly and provide an early alert warning. Additionally, the MFC voltage variations of two repeated tests were consistent, indicating that the MFC-based online system had a high repeatability for different shocks.

Furthermore, on the basis of MFC voltage, anode potential and operational temperature variations, a correlation between these three indexes and AS reactor status was established in order to address differentiation of various types of shocks (Table 1). The variation of MFC voltage alone was insufficient to determine what kind of shock the AS system suffered from, as its value was negative under all four types of shocks. However, the shock type suffered by the AS reactor could be identified through a comprehensive analysis of the variations of MFC voltage, anode potential and operational temperature. As shown in Table 1, a negative variation of both MFC voltage and anode potential means an organic overloading shock, while a negative MFC voltage variation with a positive anode potential variation indicates a toxicant or temperature one. Furthermore, the temperature and toxicant shocks could be distinguished through the variation value of operational temperature. Additionally, a negative variation of the MFC voltage with a constant anode potential implies that the AS reactor might suffer an ammonia overloading shock.

Table 1 | Comprehensive analysis of MFC voltage, anode potential and operational temperature variations to identify shock type

MFC voltage variation	Anode potential variation	Temperature variation	Shock type
negative	negative	zero	organic overloading shock
negative	close to zero	zero	ammonia overloading shock
negative	positive	negative	temperature shock
negative	positive	zero	Toxicant shock

Compared with the conventional online monitoring systems, the new design MFC without CEM in our system could sustain itself using substrates in wastewater and thus reduce the costs of its long-term operation. In addition, the sensitivity of this online monitoring and alert system ensure a reliable early warning of potential risks, and the relative simplicity, stability and adaptability of this system provide high feasibility of being incorporated into current control systems for wastewater treatment plants.

Methods

AS reactor. As shown in Fig. S4, the cylindrical AS reactor had a working volume of 7.1 L with 30-cm height and 11.5-cm diameter. The AS reactor was inoculated with 7.1 L activate sludge from the Wangtang Municipal Wastewater Treatment Plant (Hefei, China), and the concentration of suspended solids (SS) and volatile suspended solids (VSS) was 2752 ± 160 and 2120 ± 127 mg/L, respectively.

MFC-based online monitoring and alert system. The established online monitoring and alert system included three parts: a single-chamber MFC, a signal acquisition subsystem and an alert subsystem with self-diagnosis function (Fig. 5). The schematic of the MFC is shown in Fig. S4. The nonwoven cloth was used as the separator of the cathodic and anodic chambers in the MFC. To prevent leakage, the nonwoven cloth was pretreated by tetrafluoroethylene as described previously[18,19], and was supported with a polyvinyl chloride tube. The total empty volume of the anodic chamber was 636 mL. Granular graphite with a diameter of 3–5 mm (Sanye Carbon Co., China) was used as the electrode material in the anode compartment, reducing the compartment liquid volume to 145 mL. In addition, a graphite rod with 6 mm diameter (Sanye Carbon Co., China) was inserted into the anode compartment for connection. A Ag/AgCl reference electrode was inserted into the anode chamber for monitoring anode potential. A 0.6-cm thickness carbon-graphite felt with 380 cm^2 of surface area (Sanye Carbon Co., China) was used as the cathode material without any pretreatment. The graphite rod and carbon felt were connected by titanium wires across an external resistance (100 Ω). The 50-mL inoculated sludge of the MFC was the concentrated

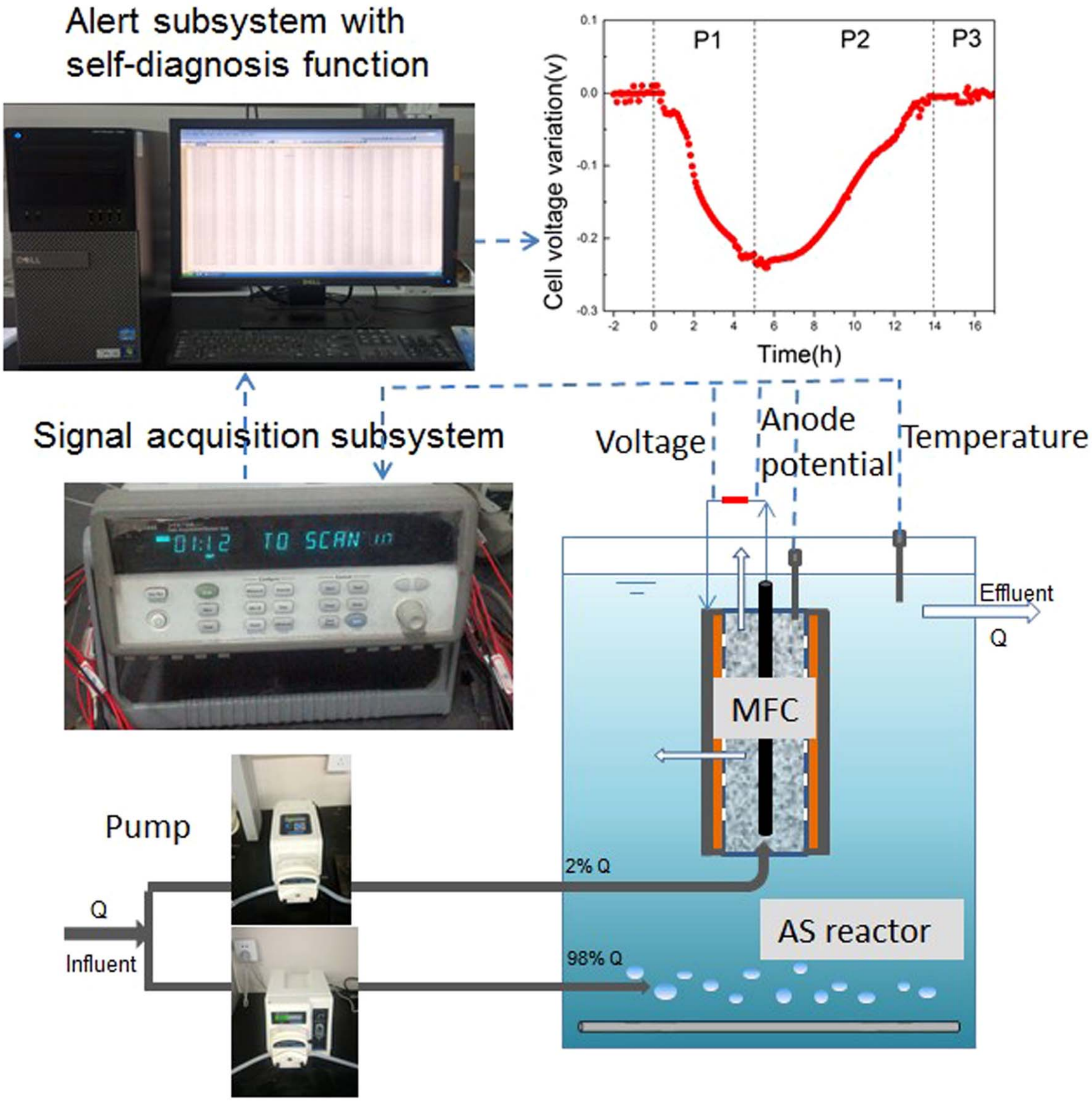

Figure 5 | Schematic of the MFC-based online monitoring and alert system.

Table 2 | Experimental condition for various shock tests

Run	Test	Duration	Shock operating conditions
35–38 d	10-fold organic overloading shock	36 h	influent COD 3000 mg/L; influent NH_4^+-N 30 mg/L; HRT 5 h; temperature 25°C; influent conductivity 4390 ± 527 μs/cm
47–49 d	10-fold ammonia overloading shock	25 h	influent COD 300 mg/L; influent NH_4^+-N 300 mg/L; HRT 5 h; temperature 25°C; influent conductivity 2025 ± 325 μs/cm
85–90 d	5°C temperature shock	80 h	influent COD 300 mg/L; influent NH_4^+-N 30 mg/L; HRT 5 h; temperature of 5°C; influent conductivity 785 ± 185 μs/cm
109–114 d	Toxicant shock	370 h	influent COD 300 mg/L; influent NH_4^+-N 30 mg/L; cadmium chloride 500 mg/L; HRT 5 h; temperature 25°C; influent conductivity 1454 ± 210 μs/cm

anaerobic sludge with an SS concentration of 10 g/L from an upflow anaerobic blanket reactor at our laboratory.

A data acquisition subsystem (34970A, Agilent Co., USA) was used to real-time collect and record MFC voltage, anode potential and operational temperature every 5 min. Based on the statistical analysis and logic judgment programs, the alert system with self-diagnosis function was developed with a LabView virtual instrument platform (National Instruments Co., USA) as described previously[20]. In this alert program, the variation value of MFC voltage was calculated as the voltage value at a certain time minus the average one at steady state. An absolute value of MFC voltage variation lower than the pre-set threshold value of 0.04 V (Page 2–3 in SI) indicated a steady state of the AS process. If the absolute value of MFC voltage variation exceeded the pre-set threshold value of 0.04 V, a warning signal would be given by this alert system to indicate possible risks for the AS reactor. Moreover, a higher absolute value of MFC voltage variation means a higher degree of risk for the AS reactor.

Experimental operation. Under normal conditions, the HRT (hydraulic retention time) of the AS reactor was kept at 5 h. The synthetic wastewater was separately fed into the AS reactor and the anode of the submersible MFC with a flow rate of 1421 and 29 mL/h, respectively. The compositions of the synthetic wastewater were as follows: 0.64 g/L $CH_3COONa\cdot3H_2O$; 0.125 g/L NH_4Cl; 0.1 g/L KH_2PO_4; 12 mg/L $CaCl_2$; 12 mg/L $MgSO_4$; and 10 mL of trace element solution as described previously[19]. The SS concentration of the AS rector was 4516 ± 282 mg/L and the solids retention time (SRT) was kept at 20 d through discharging excess sludge (Fig. S5). DO and pH in the AS reactor were 4.4–5.1 mg/L and 6.7–7.2, respectively. The conductivity of AS reactor was 985 ± 212 μs/cm under normal conditions, and the influent conductivity under different shocks is listed in Table 2.

A series of shock tests were conducted for the AS reactor, as summarized in Table 2. The influent COD concentration was increased by 10 times for the organic overloading shock. The ammonia overloading shock was imposed by switching the feed NH_4^+-N concentration by 10 fold. For the temperature shock, the reactor was put into a freezer at 5°C. Cadmium chloride with 500 mg/L was imposed into the AS reactor for the toxicant shock. Each shock test was repeated twice.

Analysis. The concentrations of SS, VSS, COD, NH_4^+-N, and temperature were measured according to the APHA standard methods[21]. DO and pH value were determined by a portable DO/pH meter (HQ 40d, Hach Co., USA). The conductivity of influent was measured by a conductivity meter (DDSJ-308A, INESA Scientific Instrument Co., China). The polarization curves of MFC were obtained by an electrochemical workstation (CHI660C, Shanghai Chenhua Co., China) at a scan rate of 1 mV/s and a prior open circuit potential period of 12 h. The current density and power density were normalized to the total anodic chamber volume. The CE was calculated as described previously[19].

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Author contributions

G.H.X. carried out the experiments, analyzed the data, and wrote the paper; Y.K.W. and G.P.S. analyzed the data; H.Q.Y. and Y.M. designed the experiments, analyzed the data, and wrote the paper.

Additional information

Supplementary information accompanies this paper at http://www.nature.com/scientificreports

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