Swine wastewater treatment by combined process of iron carbon microelectrolysis-physical adsorption-microalgae cultivation

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ABSTRACT

Combined treatments were designed based on iron-carbon micro-electrolysis treatment (ICME), physical adsorption (PA) with zeolite (Z) or vermiculite (V) and microalgae cultivation (MC, \textit{C. vulgaris}) for removing pollutants from swine wastewater (SW), herein are ICME + MC (IM), ICME + Z + MC (IZM) and ICME + V + MC (IVM). Results showed that the minimum total nitrogen (TN) of 43.66 mg \textsuperscript{L}\textsuperscript{-1}, NH\textsubscript{4}\textsuperscript{+}-N of 1.33 mg \textsuperscript{L}\textsuperscript{-1} and total phosphorus (TP) of 0.14 mg \textsuperscript{L}\textsuperscript{-1} were obtained by IVM, while the minimum chemical oxygen demand (COD) was 105 mg \textsuperscript{L}\textsuperscript{-1} via IM. During the process of combined treatments, ICME contributed most to the removal of TN (84.52\% by IZM), TP (97.78\% by IVM and IZM) and COD (62.44\% by IVM), and maximum NH\textsubscript{4}\textsuperscript{+}-N removal (55.64\%) was obtained by MC procedure in IM process. Vermiculite performed better than zeolite during all the combined treatments. Besides, the maximum cell dry weight (CDW, 0.74 g \textsuperscript{L}\textsuperscript{-1}) of \textit{C. vulgaris} was obtained by IM on day 13. The results provide an efficient integrated method for swine wastewater treatment.

Key words: adsorption, microalgae, micro-electrolysis, wastewater

HIGHLIGHTS

- Process combing with iron-carbon microelectrolysis, vermiculite adsorption and microalgae cultivation was the most efficient for treatment on TN, NH\textsubscript{4}\textsuperscript{+}-N, TP removal.
- Iron carbon micro-electrolysis combined with microalgae cultivation was the most efficient treatment for COD removal.
- The maximum biomass harvest was observed on day 13 via combined iron-carbon microelectrolysis and microalgae cultivation.

INTRODUCTION

Large amount of swine wastewater (SW) is generated from concentrated animal feeding operations (CAFOs), leading to increasing concern for environmental safety. It was estimated that approximately 0.16 billion tons of SW is produced per year in China (Yu \textit{et al.} 2020), which contains high concentrations of nutrients (nitrogen and phosphorus) and organic matter. If discharged untreated, SW would lead to environmental pollution such as contamination of soil, surface water, and ground water and the fresh water algal blooms. Furthermore, it increases the risks of human exposure to harmful pathogens and fish kill incidents (Cole \textit{et al.} 2000; Smith & Schindler 2009; Damodara Kannan & Parameswaran 2021). Therefore, feasible and effective treatments are necessary before its discharge into the environment. Technologies such as biotreatment (anaerobic and aerobic), natural process (soil, lagoon, and wetland), physical treatment (adsorption and filtration) and chemical precipitation have been extensively applied worldwide for SW treatment (Szögi & Dept. Of Agriculture 2000; Chung \textit{et al.} 2004; Huang \textit{et al.} 2015). Different methods of treating swine wastewater and their removal efficiency are shown in Table 1. However, kinds of disadvantages such as high operational costs or occupation of too much land sources have been found associated with each single process during practical utilization. Thus, it was speculated that the combined process capable of adopting the advantages of individual methods that could supplement each other, may be a more effective way to treat the aquaculture wastewater.

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Iron–carbon microelectrolysis (ICME) is a useful method for removal of wastewater pollutants. When a mixture of iron scraps and granular activated carbon (GAC) is in contact with SW (electrolyte solution), numerous microscopic galvanic cells are formed between the particles of iron (anode) and carbon (cathode) (Ying et al. 2012). The electrode reactions can be represented in terms of Equations (1) and (2) (Ju & Hu 2011) as follows:

Anode (oxidation): \( 2\text{Fe} (s) \rightarrow 2\text{Fe}^{2+} + 4e^- \)

Cathode (reduction): Acidic \( 4\text{H}^+ (aq) + 4e^- \rightarrow 2\text{H}_2(g) \)

Under aerating conditions in the ICME reactor, the oxygen competes as the electron acceptor and \( \text{H}_2\text{O}_2 \) is generated \textit{in situ} via the reaction below (3):

\[
2e_{aq} + \text{O}_2 + 2\text{H}^+ \rightarrow \text{H}_2\text{O}_2
\]

The as-generated \( \text{H}_2\text{O}_2 \) generated subsequently combines with ferrous ions, released by dissolution of iron scraps, to form Fenton’s reagent. It is a strong oxidizing agent that generates of hydroxyl radical \( \cdot\text{OH} \) through the well-known Fenton (Yao et al. 2020). Therefore, it is also referred to as the IME-Fenton reaction (Ying et al. 2012). The organic pollutants can be oxidized by radicals and can also be removed through adsorption, coprecipitation and enmeshment in the ferrous and ferric hydroxide floc (Cheng et al. 2007). The ICME method features with low cost, simple operation, high efficiency, and low consumption since it can remove refractory pollutants and improve the biodegradability of waste-water by changing the structure of some organic matters (Wang et al. 2016). It was reported that the ICME offers advantages in purifying wastewater containing with high-concentration organic matter, and a chemical oxygen demand (COD) removal of 55% was achieved from an acrylonitrile–butadiene–styrene resin manufacturing process (Lai et al. 2012). Although there are many advantages of ICME for wastewater treatment, single treatment procedure of ICME cannot meet the relevant need of nutrient removal. For example, the removal of ammonia nitrogen by ICME was reported 15.5% (Liu et al. 2012). Therefore, further processing is undeniable required.

Moreover, it was found that ICME also exhibited poor per performance toward total nitrogen (TN) removal (Zhang et al. 2014), which may can be expected to be covered by physical adsorption (PA). Zeolite (Z) has been widely used to remove ammonia and organic substances from wastewater owing to its low cost, abundance, simple operation, recyclability, and high adsorption capacity through ion exchange (Wang et al. 2011). Vermiculite (V) is well known for adsorption of ammonium and other impurities (Huo et al. 2012). The previous study revealed zeolite, pumice, and sand can remove 13.3–75.2, 12.3–73.9, and 79–99% of total phosphorus (TP), respectively (Uzun et al. 2021). Huang et al. (2010) reported the Chinese zeolite can remove 92.6% of chemical oxygen demand (COD) and 80% of \( \text{NH}_4^-\text{N} \). Stefanakis & Tsihrintzis (2012) conducted experiment and point out bauxite can remove 54% of TP, 65% of COD, 35% of TN, and 38% of \( \text{NH}_4^-\text{N} \).

| Methods | TP removal | COD removal | TN removal | \( \text{NH}_4^-\text{N} \) Removal | Refs. |
|---------|------------|-------------|------------|-------------------------------|------|
| Electrocoagulation | 93% | / | / | / | Mores et al. (2016) |
| Microalgae cultivation of \( \text{Tribonema} \) sp. And \( \text{Synechocystis} \) sp. | 71.4–72.7% | 55.60% | / | 75.8–89.9% | Cheng et al. (2020a, 2020b) |
| Microalgae cultivation of \( \text{Coelastrella} \) sp. | 74–78% | / | / | 90–100% | Luo et al. (2016) |
| Chlorella vulgaris | / | 60–70% | / | 40–90% | Wang et al. (2015) |
| Biofiltration | 59% | 90% | 89% | / | Kim et al. (2016) |
| Acrobic | / | 34.20% | / | / | Inaba et al. (2018) |
| Photosynthesis | / | 83% | / | / | Wen et al. (2016) |
| Anoxic/aerobic process | 75–86% | 89–97% | 80–94% | / | Yang et al. (2016) |
| Anaerobic digestion | / | 65.8% | / | / | Lourinho et al. (2020) |
The utilization of wastewater as a nutrient source for microalgae cultivation (MC) is one of the best biological treatments considering the significant reduction of nutrients (Luo et al. 2016) and valuable by products of MC system (Cheng et al. 2020a, 2020b). SW is a great nutrient source for MC because of its nutrient composition comprising both the major nutrients and micronutrients (Zhang et al. 2016). However, the wastewater requires pretreatment before microalgae inoculation to reach the tolerance concentration of nutrients of microalgae (Cheng et al. 2020a, 2020b). Employing the ICME or ICME + PA as pretreatment, combined process of ICME + PA + microalgae cultivation (MC) could be a promising method for SW treatment.

In this study, a novel integrated treatment including ICME combined with PA and MC was proposed to investigate the efficiency of pollutants removal and biomass synthesis. The objective of this study was to figure out: (i) the efficiency of combined treatments on pollutants removal under the best optimized operating conditions; (ii) growth and biomass harvest of microalgae in SW.

**METHODS**

**Swine wastewater and preliminary treatment**

The swine wastewater (SW) was collected from the experimental farm of Sichuan Agricultural University (SAU, Ya’an, Sichuan, China). Large non-soluble particulates in the SW were removed by sedimentation and filtration with gauze. Iron scraps were obtained from an agricultural machinery plant of Ya’an in China. Activated carbon, natural zeolite and vermiculite were purchased from the Qingyuan activated carbon factory of He’nan in China. The microalgae strain *Chlorella vulgaris* (*C. vulgaris*, FACHB-24) was purchased from Freshwater Algae Culture Collection (FACC) at the Institute of Hydrobiology (IH) of Wuhan, in China.

Before experiment, the iron scraps were soaked in 10% sodium hydroxide solution for 2 h to remove oil from the surface. Activated carbon particle was soaked in SW for 48 hours and dried at 105 °C to eliminate the adsorbent effect. The zeolite (Z) and vermiculite (V) were washed three times with distilled water and then dried at 105 °C. The *C. vulgaris* strain was pre-cultivated in Blue-Green (BG11) medium until reaching the exponential growth phase for inoculation. Before *C. vulgaris* cultivation, the pH value of SW was adjusted to around 7.1.

**Determination of optimized operating conditions**

For ICME procedure, the iron scraps and activated carbon were mixed in the mass ratio of 1:1 and experiments were conducted at different dosages (50, 100, 150, 200, 250, 300, 400, and 450 g L$^{-1}$), at different pH values (2.0, 3.0, 4.0, 5.0, 6.0, 7.0) for different times (0, 30, 60, 90, 120, 150, 180, 210, and 240 min) to optimize the operating conditions, and air were allowed to flow through the bottom of reaction devices (3 L min$^{-1}$). Taking samples for water quality measurements, and the best conditions for ICME treatment were determined as follows: mass of 200 g L$^{-1}$, pH of 3, and 150 min in glass beakers with 200 mL SW.

For PA procedure, zeolite or vermiculite was added in wastewater (100 mL), which were then vibrated on a thermostat shaker at 150 rpm, respectively. The optimized operating conditions including mass of 350 g L$^{-1}$ and 5 h were confirmed by pretreating at different dosages of zeolite or vermiculite (0, 50, 100, 150, 200, 250, 300, 350, 400 and g L$^{-1}$) and adsorption times (0, 1, 2, 3, 4, 5, 6, 7, and 8 h).

**Combined experiments**

Three combined treatments were conducted to purify SW as shown in Figure 1 shows. IM indicates that SW was treated by iron-carbon micro-electrolysis (ICME) and *C. vulgaris* cultivation, successively. IZM indicates that SW was treated by ICME, physical adsorption using zeolite and MC, successively. IVM indicates that SW was treated by ICME, PA using vermiculite, MC, successively. For MC, *C. vulgaris* with a proportion of 20% (v/v) was inoculated in a 5 L flask. The cultivation condition was conducted at 25 ± 1 °C and under a continuous cool-white fluorescent light with an illumination of 3000–4000 Lx (12 L:12D). The air was allowed to flow through the bottom of the culture devices to agitate the algal broth as well as to supply carbon dioxide (1.5 L min$^{-1}$ for first 6 days and 3 L min$^{-1}$ for the remaining days for each flask). Blue–Green (BG11) medium was used as control for measurement of microalgae growth. All experiments were performed in triplicate.
Measurement of water quality parameters

The water samples were collected at the completion of ICME and PA procedures. The samples subjected to MC procedure were collected daily from each flask, then centrifuged at 8,000 rpm for 15 min, and the supernatant was taken for water quality measurement. The supernatant of each procedure was appropriately diluted for analyses of ammonium (NH$_4^+$-N), total nitrogen (TN), and TP by ultraviolet spectrophotometry (Mapada UV-1200, Shanghai). The COD was measured using a Lovibond ET99722 multi-parameter water quality analyzer Manual (Lovibond, Germany). Nutrient removal rate (%) was calculated according to the method proposed by Folino et al. (2020) by using the following equation:

$$R_i = \frac{(S_{io} - S_{it})}{S_{io}} \times 100\%$$

where $R_i$ is the removal efficiency (%) of substrate $i$ (NH$_4^+$-N, TN, TP or COD); and $S_{io}$ and $S_{it}$ are the initial and final concentrations of $i$ during the treatment, respectively.

Microalgae growth measurement

For microalgae growth analysis, samples (10 mL) were collected daily from each flask and dried in an oven at 105 °C (until constant weight) to evaluate microalgae cell dry weight (CDW) using a balance.

Data analysis

The experimental results were analyzed by using EXCEL (Microsoft Office Enterprise, 2010) and SPSS software (SPSS, v11.5). Statistically significant differences among means were determined by one-way analysis of variance (ANOVA) followed by Dunnett’s test with significance at $p < 0.05$ for all tests.

RESULTS AND DISCUSSION

Removal of total nitrogen from swine wastewater

Figure 2(a) exhibits that the TN decreases from 104 to 43.66–50.01 mg L$^{-1}$ after combined treatments, and the maximum reduction was observed via IVM. Specifically, the ICME removed TN from 104 mg L$^{-1}$ to 58.47 mg L$^{-1}$, with the reduction rate of 43.67% and contribution rate of 75.37–84.52% by the three combined treatments. Lower TN removal rate (about 35%) of ICME was reported in a literature study (Lv et al. 2011) when Fe/C was utilized in the ratio of 1:1. This could be explained by the difference of the carbon particle size and initial pH between the two studies. The ICME procedure contributed most to the TN removal during combined treatments, because the iron ion, active hydrogen, and OH radicals generated in SW
destabilized the colloidal pollutants, lead to the occurrence of redox reaction, induced floc formation and dissolved compounds adsorption and finally the pollutants were removed by flocculation and precipitation (Han et al. 2020).

Besides, the PA removed TN from 58.47 to 45.37 mg L\(^{-1}\) (IZM) and 30.52 mg L\(^{-1}\) (IVM), with the reduction rate of 12.62\% and 26.92\%, respectively. More contribution rate of PA was observed in IVM (46.48\%) than in IZM (24.43\%) (Figure 2(b)). This result was attributed to stronger adsorption ability of vermiculite than zeolite toward TN removal. The effluent of TN includes total inorganic nitrogen (NH\(_4^+\)-N + NO\(_3^−\)-N + NO\(_2^-\)-N) and total organic nitrogen (TON) (Czerwionka et al. 2012). The zeolites have no affinity for anions because of the presence of permanent negative charge on their surface (Haron et al. 2008), which leads to a lower removal of TN for zeolite.

The MC procedure contributed 16.6\% (IM), –8.95\% (IZM), and –21.85\% (IVM) toward TN removal during combined treatments (Figure 2(b)). Negative contribution was obtained due to the microalgae inoculation. The TN was increased by C. vulgaris solution from 58.47 to 80.44 mg L\(^{-1}\) via IM, from 45.37 to 73.14 mg L\(^{-1}\) via IZM, and from 30.52 to 56.77 mg L\(^{-1}\) via IM as shown in Figure 2(a). The microalgae were inoculated into SW along with the medium solution (BG11), which contained organic nitrogen, leading to the increase of TN. At the completion of MC process, the removal rates of TN in IM, IZM, and IVM were 38.53, 31.39, and 22.85\%, respectively (Figure 2(c)). The nitrogenous compound in SW supplied nutrients to microalgae for their growth and proliferation (Stawin\’ski et al. 2018), leading to the decrease of TN. TN of IM, IZM and IVM was 49.45, 50.17, 43.66 mg L\(^{-1}\), respectively. IVM was significant higher than IM and IZM (\(P<0.05\)) and there was no significant difference between IM and IZM (\(P>0.05\)).

Removal of NH\(_4^+\)-N in swine wastewater

The Figure 3(a) demonstrate that concentration of NH\(_4^+\)-N decreases from 56.73 to 1.33–3.42 mg L\(^{-1}\) after combined treatments, and the maximum reduction was observed by IVM. Specifically, the ICME procedure reduced NH\(_4^+\)-N from 56.73 to 33.08 mg L\(^{-1}\), with the reduction rate of 41.69\% and contribution rate of 42.69–44.36\% in three combined treatments (Figure 3(a) and 3(b)). It was reported that the removal of ammonia nitrogen by ICME is a comprehensive process in which both physical and chemical adsorption are involved (Stawin\’ski et al. 2018). Besides, the pH values of SW increased from 7.46 to 8.89 after ICME treatment (Table 2). This can be explained by the electrode reactions of ICME, where the hydrogen ions in cathode (carbon) were reduced and converted to hydrogen gas, causing the pH of the solution to increase (Yang et al. 2009) and thus indicating the increase in the amount of hydroxyl groups increased during ICME.
The PA led to the decrease in the amount of NH$_4^+$-N from 33.08 to 21.58 mg L$^{-1}$ and 5.6 mg L$^{-1}$ via IZM and IVM, with the reduction rate of 20.27 and 48.44%, respectively (Figure 3(a)). More contribution rate of PA was observed by IVM (49.6%) than that by IZM (22.09%) (Figure 3(b)). According to literature (Fan et al. 2021), the removal of ammonia nitrogen by zeolite reached up to 66.97%, and that was much higher than the removal rate of 20.27% obtained in this study. It was mainly due to the alkaline initial pH value of PA procedure (8.89, Table 2) and the alkalinity of solution was adverse for ammonia removal. The previous study revealed that weak acidic condition was favorable for ammonia nitrogen removal and the ammonia nitrogen reduction by zeolite began to decline when pH of wastewater was above 6.18 (Fan et al. 2021), and it decreased sharply when pH of solution was above 8 (Huang et al. 2010). Thus, according to the previous studies, the NH$_4^+$-N removal rate of vermiculite increased slightly when pH was in the range of 6–10 and could reach up to 73% when pH was 9 (Huang et al. 2010). Therefore, NH$_4^+$-N adsorption ability of vermiculite was stronger than that of zeolite under such an alkaline condition of this experiment.

The MC procedure contributed 55.64%, 33.63% and 7.71% in IM, IZM, and IVM, respectively (Figure 3(b)) to NH$_4^+$-N removal during combined treatments. According to previous study, ammonia nitrogen could be assimilated into algal cells in the liquid as well as a portion of ammonia was trapped in the liquid due to its solubility (Kang & Wen 2015). As a result, the concentration of NH$_4^+$-N in three groups declined and the difference of removal rate among the three treatments was due to the different initial NH$_4^+$-N content during MC. The concentration of effluent NH$_4^+$-N of IM, IZM and IVM was 3.42, 3.32 and 1.33 mg L$^{-1}$, respectively.

![Figure 3](image)

**Figure 3** | (a) The NH$_4^+$-N of IM, IZM, and IVM. (b) Contribution rate of NH$_4^+$-N removal in IM, IZM, and IVM.

| Procedure                                      | pH       |
|-----------------------------------------------|----------|
| Raw                                           | 7.46 ± 0.05 |
| After ICME                                    | 8.89 ± 0.12 |
| After adsorption with zeolite                 | 7.70 ± 0.47 |
| After adsorption with vermiculite             | 7.89 ± 0.58 |

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|-----------------------------------------------|----------|
| Raw                                           | 7.46 ± 0.05 |
| After ICME                                    | 8.89 ± 0.12 |
| After adsorption with zeolite                 | 7.70 ± 0.47 |
| After adsorption with vermiculite             | 7.89 ± 0.58 |

The PA led to the decrease in the amount of NH$_4^+$-N from 33.08 to 21.58 mg L$^{-1}$ and 5.6 mg L$^{-1}$ via IZM and IVM, with the reduction rate of 20.27 and 48.44%, respectively (Figure 3(a)). More contribution rate of PA was observed by IVM (49.6%) than that by IZM (22.09%) (Figure 3(b)). According to literature (Fan et al. 2021), the removal of ammonia nitrogen by zeolite reached up to 66.97%, and that was much higher than the removal rate of 20.27% obtained in this study. It was mainly due to the alkaline initial pH value of PA procedure (8.89, Table 2) and the alkalinity of solution was adverse for ammonia removal. The previous study revealed that weak acidic condition was favorable for ammonia nitrogen removal and the ammonia nitrogen reduction by zeolite began to decline when pH of wastewater was above 6.18 (Fan et al. 2021), and it decreased sharply when pH of solution was above 8 (Huang et al. 2010). Thus, according to the previous studies, the NH$_4^+$-N removal rate of vermiculite increased slightly when pH was in the range of 6–10 and could reach up to 73% when pH was 9 (Huang et al. 2010). Therefore, NH$_4^+$-N adsorption ability of vermiculite was stronger than that of zeolite under such an alkaline condition of this experiment.

The MC procedure contributed 55.64%, 33.63% and 7.71% in IM, IZM and IVM, respectively (Figure 3(b)) to NH$_4^+$-N removal during combined treatments. According to previous study, ammonia nitrogen could be assimilated into algal cells in the liquid as well as a portion of ammonia was trapped in the liquid due to its solubility (Kang & Wen 2015). As a result, the concentration of NH$_4^+$-N in three groups declined and the difference of removal rate among the three treatments was due to the different initial NH$_4^+$-N content during MC. The concentration of effluent NH$_4^+$-N of IM, IZM and IVM was 3.42, 3.32 and 1.33 mg L$^{-1}$, respectively.

**Removal of total phosphorus in swine wastewater**

The TP decreased from 48.29 to 0.14–0.15 mg L$^{-1}$ after combined treatments and the maximum reduction was obtained via IVM (Figure 4(a)). Specifically, the ICME reduced TP from 48.29 to 1.22 mg L$^{-1}$, with the reduction rate of 97.47% and contribution rate of 97.76–97.78% in three combined treatments (Figure 4(b)). Similar study was also reported by Shen et al. (2019), who found that ICME removed 93.63% of TP in constructed wetland system. It was revealed that ICME involved the generation of hydrogen, ferrous ions, ferric ions and hydroxide (Yang et al. 2009; Zhang et al. 2018). The hydrogen gas contributed to floatation of the flocculated particles out of wastewater (Braun et al. 2019). The iron scrap of ICME was the main reason of TP removal, because phosphorus ions have strong affinity for Fe$^{3+}$. Oxides of Fe in aqueous medium consist of surface OH groups, which can cause surfaces adsorption of P by complexation (Stawiński et al. 2018).
The PA removed TP from 1.22 to 0.54 mg L\(^{-1}\) and 0.59 mg L\(^{-1}\) with the reduction of rate of 1.41% and 1.3% by IZM and IVM, respectively (Figure 4(a)). The TP decreased in IVM owing to the adsorption by vermiculite (Zhao et al. 2016), and in IZM owing to both adsorption and precipitation of phosphate by zeolite (Karapınar 2009). Therefore, the reduction of TP by zeolite was slightly higher than that of vermiculite.

The MC procedure contributed to TP removal of 2.22% (IM), 0.81% (IZM) and 0.93% (IVM) during combined treatments (Figure 4(b)). Phosphorus is an essential element for the microalgae growth and proliferation and it can be assimilated and accumulated into the microalgae biomass (Karpagam et al. 2015). The effluent TP contents in IM, IZM, and IVM were 0.15, 0.15, and 0.14 mg L\(^{-1}\).

**Removal of chemical oxygen demand in swine wastewater**

The COD decreased from 1,880 mg/L to 105–116.67 mg L\(^{-1}\) after combined treatments and no significant difference (\(P\geq0.05\)) was observed among IM, IZM, and IVM (Figure 5(a)). Specifically, the ICME procedure reduced COD from 1,880 to 779 mg L\(^{-1}\), with the reduction rate of 58.56% and contribution rate of 62.03–62.44% (Figure 5(b)) in three combined treatments. Higher COD reduction by ICME was found in this study compared with the literature report (Ma et al. 2019) report (removal rate of 27.61%). This result may be attributed to higher dosage of iron–carbon system utilized in this experiment. The proportion of iron and carbon utilized in this experiment was 0.2, while the counterpart was 0.06 in the literature study (Ma et al. 2019).

![Figure 4](image1.png) | (a) The TP of IM, IZM, and IVM. (b) Contribution rate of TP removal in IM, IZM, and IVM.

![Figure 5](image2.png) | (a) The COD of IM, IZM, and IVM. (b) Contribution rate of COD removal in IM, IZM, and IVM.
The COD removal rate by PA procedure was inefficient. It contributed -0.04% and -1.27% via IZM and IVM, respectively (Figure 5(b)). According to literature, substance with hydrophobic surfaces is more suitable for adsorption of organic substance (Yang et al. 2020). The surface of zeolite is hydrophilic (Halim et al. 2010), the hydrophilic nature of vermiculite also hindered its adsorption capacity toward the removal of organic contaminants (Mujtaba et al. 2018). This can explain the poor adsorption characterization of zeolite and vermiculite to organic material.

The effluent COD after MC procedure was 105, 110.33, and 116.67 mg L\(^{-1}\) in IM, IZM, and IVM, respectively (Figure 5(a)). IVM was significant higher than IM \((P<0.05)\) and there was no significant difference between IVM and IZM \((P>0.05)\) and no significant between IZM and IM \((P>0.05)\). No significant difference \((P>0.05)\) on removal rate of COD was found among IM (35.85%), IZM (35.60%), and IVM (36.41%), and contribution rates of IM (37.97%), IZM (37.79%), and IVM (37.56%) (Figure 5(b)). The higher COD removal rate (72.6%) was found by Wang et al. (2015)'s research, where the initial ammonia nitrogen was 80 mg L\(^{-1}\). However, initial ammonia nitrogen of MC was 33.08 mg L\(^{-1}\) (IM), 21.58 mg L\(^{-1}\) (IZM), and 5.6 mg L\(^{-1}\) (IVM) in this study. Lack of nitrogen sources can trigger the secretion of extracellular organic matters (EOM), leading to the increase of COD (Wang et al. 2015). Moreover, the higher contents of initial nitrogen and phosphorus will promote the growth of microalgae, leading to a higher COD removal (Scarponi et al. 2021).

**Growth of C. vulgaris**

The biomass of *C. vulgaris* was analyzed in four groups (Figure 6). The declined CDWs on day 2 were related to the flocculation and precipitation caused by suspended impurities along with cells in wastewater. The CDWs in IM showed an increasing trend while that in IVM and IZM showed a decreasing trend. The result indicated there were no sufficient nutrients (TN, TP, and NH\(_4^+\)-N) in IVM and IZM to support growth of *C. vulgaris*, which led to the limited growth of microalgae (Vargas-Estrada et al. 2021). Minimal nutritional requirements can be estimated by the approximate molecular formula (CO\(_{0.48}\)H\(_{1.83}\)N\(_{0.11}\)P\(_{0.01}\)) of the microalgal biomass (Chisti 2007). Although accounting for only 1%, phosphorous is often one of the most important growth-limiting factors (Ferreira et al. 2021). It can be adsorbed into cells by microalgae and then participate in the process of energy synthesis through a variety of phosphorylation process such as substrate level phosphorylation, oxidative phosphorylation, and photosynthetic phosphorylation (Wang et al. 2021). In this study, 3 mg L\(^{-1}\) of phosphorus was required for microalgae (CDW of 0.3 g L\(^{-1}\)) of IM on day 2. However, the content of TP was 0.54 and 0.59 mg L\(^{-1}\) in IZM and IVM, respectively, after the PA process, which led to the decline of CDWs of algae. After 13-day MC, the CDW of IM started to decline and CDW of IM dropped to 0.42 g L\(^{-1}\) on day 16. The maximal CDW (0.74 g L\(^{-1}\)) was observed in IM on day 13 and it was much higher than those in other treatments including control. This may be attributed to the effective electrode transfer after ICME stimulated microbial growth and metabolic enzyme activity, and further promoted to biodegradation ability of microorganism (Zhang et al. 2012). Besides, on day 13, the TP, NH\(_4^+\)-N and COD in IM were 0.15, 6.7, and 116 mg L\(^{-1}\)

**Figure 6** | CDW of *C. vulgaris* during the culture in swine wastewater.
and there was no significant difference ($P > 0.05$) between day 13 and day 16. Therefore, for both purification of SW and harvest of biomass, ICME procedure integrated with MC for 13 days was the best option among three combined treatments.

**Reusability and cost of adsorbents**

According to the previous studies (Halim *et al.* 2010), the reusability of regenerated zeolite was better than that of the fresh one toward ammoniacal nitrogen. Moreover, the ammoniacal nitrogen and COD removal by regenerated zeolite increased by 7.3% and decreased by 2.6%, respectively (Halim *et al.* 2010). Fan *et al.* (2021) reported the effect of the zeolites, which remained stable after reuse for three times. In generally, 21 g of ammonium nitrogen can be adsorbed by 21 kg of vermiculite within 2 h; however, the removal efficiency of vermiculite dropped to 52% after 3 h and 38% after 5 h, respectively (Rama *et al.* 2019). Zhang *et al.* (2021) conducted experiment and pointed out that removal efficiency of biochar drops to 71.3% after three regeneration cycles. It can be concluded from above findings that the biochar, zeolite and vermiculite can be reused for three or two times at least for ammonia nitrogen removal.

The market unit price of natural zeolite, vermiculite and biochar were $50–300 (Szerement *et al.* 2021), $850 (Brião *et al.* 2021) and $2062–2512 (Campbell *et al.* 2018) respectively. The corresponding cost for removing 1,000 g ammonia nitrogen was investigated and the smallest cost was $13 for zeolite (Table 3).

**CONCLUSIONS**

All the combined treatments IM, IZM, and IVM can remove pollutants in swine wastewater. The minimum effluent TN, NH$_4^+$-N, and TP was obtained via IVM, and the minimum effluent COD was observed in IM. During the combined treatments, ICME contributed the most to the TN (84.52% in IZM), TP (97.78% in IVM and IZM), and COD (62.44% in IVM), while MC contributed the most to the NH$_4^+$-N removal (55.64%). Vermiculite performed better than zeolite during all the combined treatments. Moreover, the maximum biomass harvest was obtained via IM. The results provide useful information for developing efficient and economical methods to remove pollutants from swine wastewater. Undeniably, a lot more systematic explorations are still demanded to investigate the removal of more pollutants such as heavy metals, antibiotic and hormones should be explored by combined treatment that will be pursued in the near future.

**ACKNOWLEDGEMENTS**

This research was financially supported by the National Natural Science Foundation of China (31702156), and the Sichuan Swine Innovation Team Construction Project of National Modern Agricultural Industry Technology System of China (sccxtd-2021-08), and Chongqing & Rongchang Agriculture and Animal Husbandry High-tech Industry Research and Development Special Project (cstc2019ngzx0004).

**DATA AVAILABILITY STATEMENT**

All relevant data are included in the paper or its Supplementary Information.

**REFERENCES**

Braun, J. C. A., Borba, C. E., Godinho, M., Perondi, D., Schontag, J. M. & Wenzel, B. M. 2019 Phosphorus adsorption in Fe-loaded activated carbon: two-site monolayer equilibrium model and phenomenological kinetic description. *Chemical Engineering Journal* 361, 751–763.

Brião, G. D. V., Da Silva, M. G. & Vieira, M. G. A. 2021 Expanded vermiculite as an alternative adsorbent for the dysprosium recovery. *Journal of the Taiwan Institute of Chemical Engineers* 127, 228–235.
Campbell, R. M., Anderson, N. M., Daugaard, D. E. & Naughton, H. T. 2018 Financial viability of biofuel and biochar production from forest biomass in the face of market price volatility and uncertainty. *Applied Energy* **230**, 330–343.

Cheng, H., Xu, W., Liu, J., Wang, H., He, Y. & Chen, G. 2007 Pretreatment of wastewater from triazine manufacturing by coagulation, electrolysis, and internal microelectrolysis. *Journal of Hazardous Materials* **146**, 385–392.

Cheng, H., Narindri, B., Chu, H. & Whang, L. 2020a Recent advancement on biological technologies and strategies for resource recovery from swine wastewater. *Bioresource Technology* **303**, 122861.

Cheng, P., Chen, D., Liu, W., Cobb, K., Zhou, N., Liu, Y., Liu, H., Wang, Q., Chen, P., Zhou, C. & Ruan, R. 2020b Auto-flocculation microalgae species Tribonema sp. and Synechocystis sp. with T-IPL pretreatment to improve swine wastewater nutrient removal. *Science of The Total Environment* **725**, 138263.

Chisti, Y. 2007 Biodiesel from microalgae. *Biotechnology Advances* **25**, 294–306.

Chung, Y. J., Choi, H. N., Cho, J. B. & Park, S. K. 2004 Treatment of swine wastewater using MLE process and membrane bio-reactor. *Water Science and Technology* **49**, 445–450.

Cole, D., Todd, L. & Wing, S. 2000 Concentrated swine feeding operations and public health: a review of occupational and community health effects. *Environ Health Perspect* **108**, 685–699.

Czerwionka, K., Makinia, J., Pagilla, K. R. & Stensel, H. D. 2012 Characteristics and fate of organic nitrogen in municipal biological nutrient removal wastewater treatment plants. *Water Research* **46**, 2057–2066.

Damodara Kannan, A. & Parameswaran, P. 2021 Ammonia adsorption and recovery from swine wastewater permeate using naturally occurring clinoptilolite. *Journal of Water Process Engineering* **43**.

Fan, J., Wu, H., Liu, R., Meng, L., Fang, Z., Liu, F. & Xu, Y. 2021 Non-thermal plasma combined with zeolites to remove ammonia nitrogen from wastewater. *Journal of Hazardous Materials* **401**, 126272.

Ferreira, G. F., Ríos Pinto, L. F., Maciel Filho, R. & Fregelente, L. V. 2021 Effects of cultivation conditions on Chlorella vulgaris and Desmodesmus sp. grown in sugarcane agro-industry residues. *Bioresource Technology* **342**, 125949.

Halim, A. A., Aziz, H. A., Johari, M. A. M., Ariffin, K. S. & Adlan, M. N. 2010 Ammoniacal nitrogen and COD removal from semi-aerobic landfill leachate using a composite adsorbent: fixed bed column adsorption performance. *Journal of Hazardous Materials* **175**, 960–964.

Han, Y., Wu, C., Su, Z., Fu, X. & Xu, Y. 2020 Micro-electrolysis biological fluidized bed process for coking wastewater treatment. *Journal of Water Process Engineering* **38**, 101624.

Haron, M. J., Ab Rahim, F., Abdullah, A. H., Hussein, M. Z. & Kassim, A. 2008 Sorption removal of arsenic by cerium-exchanged zeolite P. *Materials Science and Engineering: B* **149**, 204–208.

Huang, H., Xiao, X., Yan, B. & Yang, L. 2010 Ammonium removal from aqueous solutions by using natural Chinese (Chende) zeolite as adsorbent. *Journal of Hazardous Materials* **175**, 247–252.

Huang, H., Xiao, D., Liu, J., Hou, L. & Ding, L. 2015 Recovery and removal of nutrients from swine wastewater by using a novel integrated reactor for struvite decomposition and recycling. *Scientific Reports* **5**.

Huo, X., Wu, L., Liao, L., Xia, Z. & Wang, L. 2012 The effect of interlayer cations on the expansion of vermiculite. *Powder Technology* **224**, 241–246.

Inaba, T., Hori, T., Navarro, R. R., Ogata, A., Hanajima, D. & Habe, H. 2018 Revealing sludge and biomass structural dynamics during anaerobic digestion of swine wastewater. *Journal of Environmental Science and Technology (Tehran)* **17**, 4917–4938.

Luo, L., He, H., Yang, C., Wen, S., Zeng, G., Wu, M., Zhou, Z. & Lou, W. 2016 Nutrient removal and lipid production by Coelastrella sp. in anaerobically and aerobically treated swine wastewater. *Bioresource Technology* **216**, 135–141.

Lv, Y., Wang, Y., Shan, M., Shen, X. & Su, Y. 2011 Denitrification of coking wastewater with micro-electrolysis. *Journal of Environmental Sciences* **23**, S128–S131.

Ma, W., Han, Y., Xu, C., Han, H., Zhong, D., Zhu, H. & Li, K. 2019 The mechanism of synergistic effect between iron-carbon microelectrolysis and biodegradation for strengthening phenols removal in coal gasification wastewater treatment. *Bioresource Technology* **271**, 84–90.
Mores, R., Treichel, H., Zakrzewski, C. A., Kunz, A., Steffens, J. & Dallago, R. M. 2016 Remove of phosphorous and turbidity of swine wastewater using electrocoagulation under continuous flow. *Separation and Purification Technology* 171, 112–117.

Mujtaba, G., Rizwan, M., Kim, G. & Lee, K. 2018 Removal of nutrients and COD through co-culturing activated sludge and immobilized Chlorella vulgaris. *Chemical Engineering Journal* 343, 155–162.

Rama, M., Laiho, T., Eklund, O. & Wärnä, J. 2019 An evaluation of the capability of nanomodified vermiculite to in situ ammonium removal from landfill leachate. *Environmental Technology & Innovation* 14, 100340.

Scarpone, P., Volpi Ghirardini, A. M., Bravi, M. & Cavinato, C. 2021 Evaluation of Chlorella vulgaris and Scenedesmus obliquus growth on pretreated organic solid waste digestate. *Waste Management* 119, 235–241.

Smith, V. H. & Schindler, D. W. 2009 Eutrophication science: where do we go from here? *Trends in Ecology & Evolution* 24, 201–207.

Stawinski, W., Węgryzn, A., Mordarski, G., Skiba, M., Freitas, O. & Figueiredo, S. 2018 Sustainable adsorbents formed from by-product of acid activation of vermiculite and leached-vermiculite-LDH hybrids for removal of industrial dyes and metal cations. *Applied Clay Science* 161, 6–14.

Stefanakis, A. I. & Tsihrintzis, V. A. 2012 Use of zeolite and bauxite as filter media treating the effluent of Vertical Flow Constructed Wetlands. *Microporous and Mesoporous Materials* 155, 106–116.

Szeremeta, J., Szatanik-Kloc, A., Jarosz, R., Bajda, T. & Mierzwia-Hersztek, M. 2021 Contemporary applications of natural and synthetic zeolites from fly ash in agriculture and environmental protection. *Journal of Cleaner Production* 311, 127461.

Szügi, A. A. & Dept. Of Agriculture, F.S.U. 2000 Treatment of swine wastewater using a saturated-soil-culture soybean and *Algal Research* 222, 327–335.

Uzun, O., Gokalp, Z., Irik, H. A., Varol, I. S. & Kanarya, F. O. 2021 Zeolite and pumice-amended mixtures to improve phosphorus removal efficiency of substrate materials from wastewaters. *Journal of Cleaner Production* 317, 128444.

Vargas-Estrada, L., Longoria, A., Okoye, P. U. & Sebastian, P. J. 2021 Energy and nutrients recovery from wastewater cultivated microalgae: assessment of the impact of wastewater dilution on biogas yield. *Bioresource Technology* 341, 125755.

Wang, Q., Yang, Y., Yu, C., Huang, H., Kim, M., Feng, C. & Zhang, Z. 2011 Study on a fixed zeolite bioreactor for anaerobic digestion of ammonium-rich swine wastes. *Bioresource Technology* 102, 7064–7068.

Wang, Y., Guo, W., Yen, H., Ho, S., Lo, Y., Cheng, C., Ren, N. & Chang, J. 2015 Cultivation of Chlorella vulgaris JSC-6 with swine wastewater for simultaneous nutrient/COD removal and carbohydrate production. *Bioresource Technology* 198, 619–625.

Wang, L., Yang, Q., Wang, D., Li, X., Zeng, G., Li, Z., Deng, Y., Liu, J. & Yi, K. 2016 Advanced landfill leachate treatment using iron-carbon microelectrolysis- Fenton process: process optimization and column experiments. *Journal of Hazardous Materials* 318, 460–467.

Wang, J., Song, A., Huang, Y., Liao, Q., Xia, A., Zhu, X. & Zhu, X. 2021 Domesticating Chlorella vulgaris with gradually increased the concentration of digested piggy wastewater to bio-remove ammonia nitrogen. *Algal Research* 60, 102526.

Wen, S., Liu, H., He, H., Luo, L., Li, X., Zeng, G., Zhou, Z., Lou, W. & Yang, C. 2016 Treatment of anaerobically digested swine wastewater by Rhodobacter capsulatus. *Bioresource Technology* 222, 33–38.

Yang, X., Xue, Y. & Wang, W. 2009 Mechanism, kinetics and application studies on enhanced activated sludge by interior microelectrolysis. *Bioresource Technology* 100, 649–653.

Yang, D., Deng, L., Zheng, D., Wang, L. & Liu, Y. 2016 Separation of swine wastewater into different concentration fractions and its contribution to combined anaerobic-aerobic process. *Journal of Environmental Management* 168, 87–93.

Yang, S., Huang, Z., Li, C., Li, W., Yang, L. & Wu, P. 2020 Individual and simultaneous adsorption of tetracycline and cadmium by dodecyl dimethyl betaine modified vermiculite. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* 602, 125171.

Yao, Q., Huang, C., Wang, M., Xiong, L. & Chen, X. 2020 Treatment of water hyacinth anaerobic fermentation wastewater by combining Fe-C micro-electrolysis with Fenton reaction. *Journal of Environmental Chemical Engineering* 8, 104157.

Ying, D., Peng, J., Xu, X., Li, K., Wang, Y. & Jia, J. 2012 Treatment of mature landfill leachate by internal micro-electrolysis integrated with coagulation: a comparative study on a novel sequencing batch reactor based on zero valent iron. *Journal of Hazardous Materials* 229–230, 426–433.

Yu, J., Hu, H., Wu, X., Zhou, T., Liu, Y., Ruan, R. & Zheng, H. 2020 Coupling of biochar-mediated adsorption and algal-bacterial system to enhance nutrients recovery from swine wastewater. *Science of The Total Environment* 701, 134935.

Zhang, J., Zhang, Y. & Quan, X. 2012 Electricity assisted anaerobic treatment of salinity wastewater and its effects on microbial communities. *Water Research* 46, 3535–3543.

Zhang, J., Chen, S., Zhang, Y., Quan, X., Zhao, H. & Zhang, Y. 2014 Reduction of acute toxicity and genotoxicity of dye effluent using Fenton-coagulation process. *Journal of Hazardous Materials* 274, 198–204.

Zhang, B., Han, H., Fu, S., Yang, P., Gu, Z., Zhou, Q. & Cao, Z. 2016 Dehydrogenase inhibits gastric cancer cell growth and tumorigenesis by selectively inducing tumor-suppressive endoplasmic reticulum stress and a moderate apoptosis. *Biochemical Pharmacology* 104, 8–18.

Zhang, X., Lin, H. & Hu, B. 2018 The effects of electrocoagulation on phosphorus removal and particle settling capability in swine manure. *Separation and Purification Technology* 200, 112–119.

Zhao, J., Zhao, Y., Xu, Z., Doherty, L. & Liu, R. 2016 Highway runoff treatment by hybrid adsorptive media-baffled subsurface flow constructed wetland. *Ecological Engineering* 91, 231–239.

First received 25 June 2021; accepted in revised form 29 November 2021. Available online 13 December 2021