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Enhancing Swine Wastewater Treatment: A Sustainable and Systematic Approach through Optimized Chemical Oxygen Demand/Sulfate Mass Ratio in Attached-Growth Anaerobic Bioreactor

Mehdi Lamssali [1], Shobha Mantripragada [2], Dongyang Deng [1,*] and Lifeng Zhang [2,*]

[1] Department of Built Environment, College of Science and Technology, North Carolina A&T State University, Greensboro, NC 27411, USA
[2] Department of Nanoengineering, Joint School of Nanoscience and Nanoengineering, North Carolina A&T State University, Greensboro, NC 27401, USA
* Correspondence: ddeng@ncat.edu (D.D.); lzhang@ncat.edu (L.Z.); Tel.: +1-336-285-3139 (D.D.); +1-336-285-2875 (L.Z.)

Abstract: The swine industry generates millions of gallons (thousands of cubic meters) of wastewater every day, posing significant environmental risk due to high concentrations of organics and nutrients. This study aims to investigate the effectiveness of attached-growth anaerobic bioreactors for treating swine wastewater by utilizing sulfate-reducing bacteria, focusing on the impact of chemical oxygen demand (COD)/sulfate mass ratios on organics degradation. A series of lab-scale anaerobic bioreactors were employed to treat swine wastewater for a 14-day period. The study evaluated changes in pH, acidity, alkalinity, COD, sulfate, and various nutrients along with total suspended solids (TSS) and volatile suspended solids (VSS) before and after treatment. At a COD/sulfate mass ratio of 2:1, the bioreactors achieved optimum removal efficiencies of 80% for TSS, 83% for VSS, 86–88% for COD, 82–87% for sulfate, 73% for sulfide, and 73% for sulfite. The nutrient removal efficiency was 67% for nitrate and 72% for nitrite. The acidity and alkalinity were effectively controlled, with alkalinity values reaching up to 2161 $\pm$ 92.5 mg/L and pH within the range of 7–7.24. The findings demonstrated that anaerobic bioreactor at a COD/sulfate mass ratio of 2:1 significantly enhanced the degradation of organic matter coupling with sulfate reduction in swine wastewater, providing an efficient and sustainable treatment method.

Keywords: swine wastewater; wastewater treatment; anaerobic bioreactor; sulfate-reducing bacteria; COD/sulfate mass ratio

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1. Introduction

The swine industry, a vital component of agricultural output and economy, has experienced significant growth in recent years. Its expansion has led to a substantial increase in swine wastewater production, which has become a major environmental concern. In the United States, the swine industry generates about 245 million gallons (more than 925,000 cubic meters) of manure daily, containing high concentrations of organic matters and nutrients [1–3]. The release of untreated swine wastewater into water bodies can lead to oxygen depletion, eutrophication, and high turbidity, which causes fish kill and other ecological disturbances. Thus, it is crucial to effectively manage swine wastewater to mitigate its adverse effects on water quality and aquatic environment [4,5].

Swine wastewater is a complex mixture of organic matters, nutrients, microbes, solids, heavy metals, and antibiotics. These constituents can pose significant risks to both the environment and human health [6,7]. One effective technology for swine wastewater treatment is constructed wetlands, which utilize natural processes involving wetland vegetation, soils, and their associated microbial assemblages to treat wastewater [8]. These systems are cost-effective and require low maintenance. Advanced oxidation processes (AOPs) and membrane bioreactors (MBRs) are also employed to enhance the removal of pollutants and pathogens from swine wastewater [9,10]. AOPs use chemical oxidants to break down contaminants, while MBRs combine biological treatment with membrane filtration, offering high-quality effluent suitable for reuse. Integrating these technologies can significantly improve the efficiency of swine wastewater management, reduce greenhouse gas emissions, and promote resource recovery.

Among various technologies for wastewater treatment, anaerobic digestion stands out as a prominent method due to its effectiveness in reducing organic load and generating biogas as a renewable energy source. This process involves the microbial decomposition of organic matter in the absence of oxygen, leading to the production of methane-rich biogas and digestate, which can be used as a fertilizer [11]. A common feature of the anaerobic digestion method is the exploitation of sulfate-reducing bacteria (SRB) to render sulfate reduction to sulfide, alkalinity generation, and subsequent metal sulfide precipitation, and convert organics to bicarbonate ions instead of CO_2 and methane as opposed to traditional anerobic digestion, which is dominated by methanogens [12–14]. In anaerobic digestion system, both methanogens and SRB play critical roles in the degradation of organic matter. Methanogens produce methane as a byproduct of their metabolic processes, while SRB reduce sulfate to sulfide, utilizing organic compounds or hydrogen as electron donors. These two groups of microorganisms often compete for the same substrates. The chemical oxygen demand (COD)/sulfate mass ratio is a crucial parameter that determines which microbial community will dominate. When the COD/sulfate ratio is low, SRB tend to outcompete methanogens because they have a higher affinity for hydrogen and acetate, allowing them to effectively outcompete methanogens on these substrates [15–17]. Conversely, when the COD/sulfate ratio is high, methanogens are more likely to dominate, as the availability of organic substrates exceeds the sulfate reduction capacity.

Studies have demonstrated that SRB can effectively reduce the levels of organic pollutants, sulfates, and heavy metals in wastewater [18,19]. Along with COD/sulfate mass ratio, several factors influence the activity and efficiency of SRB in treating wastewater, including pH, temperature, and the availability of electron donors. Optimal pH levels for SRB activity typically range between 6.5 and 7.5, while temperatures between 25 °C and 37 °C are considered ideal [20,21]. Additionally, the presence of readily available organic substrates such as lactate, acetate, and ethanol can enhance the metabolic activity of SRB, leading to more efficient sulfate reduction [22]. There is an optimum COD/sulfate mass ratio for SRB in wastewater treatment to maximize treatment efficiency by ensuring sufficient organic substrate availability for SRB while maintaining an effective sulfate reduction and preventing inhibitory conditions. The optimum COD/sulfate ratio typically varies, depending on operational conditions and wastewater composition [23–25]. For example, sulfate removal efficiencies exceeding 90% were achieved in synthetic wastewater with sulfate concentrations up to 1965 mg/L and COD/sulfate ratios higher than 1.7 [24]. Despite their potential, the application of SRB in wastewater treatment also has challenges. The production of hydrogen sulfide, a toxic and odorous gas, necessitates careful management and control to prevent environmental and health hazards [26]. Deng and Lin have utilized ferrous chloride to take out sulfide generated from the sulfate reduction process and form ferrous sulfide precipitates to reduce the sulfide toxicity [27].

In spite of a number of reports in literature exploring SRB and COD/sulfate ratio for wastewater treatment, there is limited research in this regard for swine wastewater. Given the sulfate prevalence and the large amount of readily available organics in swine wastewater [28], a similar SRB mechanism may be effectively applied to swine wastewater treatment. The coupling of sulfate with organic degradation could reduce organics to bicarbonate, thus adding alkalinity to the system instead of producing greenhouse gases [28–30]. For the first time, this study aimed to investigate the effectiveness of treating swine wastewater using SRB in the anaerobic attached-growth bioreactor and explore the impact of COD/sulfate mass ratio on organics degradation and sulfate reduction. A series of lab-scale anaerobic bioreactors were employed and monitored to evaluate pH change, alkalinity production, and COD, sulfate, and nutrient reduction in swine wastewater after treatment. Our results revealed that at an optimum COD/sulfate ratio of 2:1, the bioreactors achieved significant contaminant and nutrient removal efficiencies for swine wastewater. This research demonstrated the great potential of optimizing COD/sulfate mass ratio in the attached-growth anaerobic bioreactors for swine wastewater treatment. Not only can such a treatment be environmentally friendly but also energy efficient because it does not require aeration for the microbial oxidation of organics in the swine wastewater. Additional benefits may include reduced CO_2 and methane production and emission, the removal of selected heavy metals (e.g., Cd, Cu, Hg, Pb and Zn) (if present) due to their low solubility with sulfide, and low biomass production. The findings from this study provided a holistic approach (as illustrated in Figure 1) to treat swine wastewater and contributed to the knowledge of treating high-strength agricultural wastewaters, where efficient reductions in organics and nutrients are required to meet environmental discharge standards.

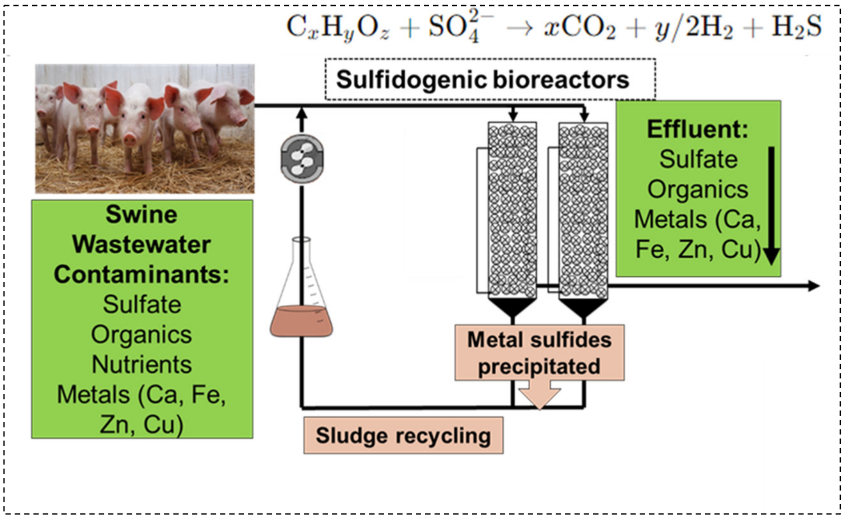

Figure 1. Schematic diagram of anaerobic attached-growth bioreactor system for swine wastewater treatment utilizing sulfate-reducing bacteria (SRB).

2. Material and Methods

2.1. Anaerobic Bioreactors Setup

Four lab-scale anaerobic bioreactors were established to investigate the removal of chemical oxygen demand (COD), sulfate, and nutrients from swine wastewater. The control bioreactor contained 2000 mL of sterile swine wastewater, collected from the swine farm operation facility at North Carolina Agricultural and Technical State University. In contrast, each of the three experimental bioreactors consisted of 1950 mL of swine wastewater and 50 mL of anaerobic digester sludge, sourced from a municipal wastewater treatment plant as the bacteria inoculum. Throughout the 14-day experimental period, all bioreactors were continuously stirred and anaerobic conditions were maintained via N_2 sparging.

Potassium hydrogen phthalate ($C_8H_5KO_4$) and sodium sulfate (Na_2SO_4), sourced from Fisher Scientific, were used to manipulate the COD/sulfate mass ratios in the bioreactors when necessary. The bioreactors were kept at a consistent temperature of 28 $\pm$ 1 °C [31,32]. To maintain anaerobic conditions, the airtight bioreactors were periodically sparged with syringe-filtered N_2 gas before and during operation.

Each bioreactor was filled with 50 pieces of Kaldnes K1 plastic media (Evolution Aqua Ltd., Wigan, UK) to support the growth of attached anaerobic microorganisms. The unique design of the K1 media provides an extensive surface area (approximately 500 m^2/m^3) for improving microbial colonization, enhancing biomass retention, increasing resistance to operation condition changes, and higher contact surface between the substrate and microorganisms [32,33].

2.2. Operation

To evaluate the effect of the COD/sulfate mass ratios on swine wastewater treatment, swine wastewater samples were collected from three separate wastewater collection trips, and the three bioreactors were individually operated based on the water collected from each trip. The results from each reactor represent distinct experimental runs (reactor 1 refers to trip 1, reactor 2 refers to trip 2, and reactor 3 refers to trip 3). The bioreactors were operated in batch mode at four different COD/sulfate mass ratios: 6:1, 4:1, 2:1, and 1:1. Each ratio was maintained to ensure consistent interactions between the microorganisms and the swine wastewater.

Operational conditions, such as temperature, were regularly monitored to maintain consistency. A daily 50 mL sample was collected from each bioreactor for analysis purposes. This sample volume was selected to ensure comprehensive data collection throughout the study while minimizing disturbance to the reactor environment. The samples were rigorously tested to measure pH, acidity, alkalinity, COD, sulfate, nutrient, and solid levels, providing insights into the performance of the bioreactors at each COD/sulfate mass ratio.

2.3. Measurement

Acidity and alkalinity were measured using an Auto titrator (Thermo Scientific Orion 950, Thermo Fisher Scientific, Waltham, MA, USA) following Standard Methods for the Examination of Water and Wastewater 2320 B and 2310 B, respectively [34]. pH was determined with a pre-calibrated pH meter (Mettler Toledo EasyPlus Easy pH Acid/Base Titrator). Total solids (TS), total suspended solids (TSS), total dissolved solids (TDS), and volatile suspended solids (VSS) were measured using Standard Methods for the Examination of Water and Wastewater [34]. COD concentrations were assessed using a closed reflux, colorimetric method with a Hach DR-3900 spectrophotometer [35]. Nutrient levels, including phosphorus (PO_4^{3-}), nitrogen-ammonia (NH_3-N), nitrite (NO_{2^-}), nitrate (NO_{3^-}), and various sulfur species including sulfate (SO_4^{2-}), sulfide (S^{2-}), and sulfite (SO_3^{2-}), were determined using a Hach (Loveland, CO, USA) DR-3900 spectrophotometer in accordance with Hach methods 8180, 10205, 10019, 8192, 8051, 8131, and 10308, respectively.

A scanning electron microscope (SEM, Auriga, Carl Zeiss, White Plains, NY, USA) was employed to examine microorganism morphology and accumulation on the K1 plastic media. For this purpose, one piece of K1 media was removed from each bioreactor at the beginning (day 0) and the end (day 14) of the experiment. The samples were then dried and prepared for SEM analysis to compare the surface morphology over time. To reduce surface charging during SEM, all samples were coated with gold–palladium [36].

3. Results and Discussion

3.1. Water Quality of Initial Swine Wastewater

The initial values of pH, acidity, alkalinity, nutrients, sulfate, and COD along with solids concentration of the collected swine wastewater are presented in Table 1.

Table 1. The initial water quality data of swine wastewater.

Parameter	Average ± SD
pH	7.1 ± 0.04
Alkalinity (mg/L)	1620.3 ± 9.4
Acidity (mg/L)	245 ± 6.2
Nitrite NO_2^-–N (mg/L)	0.14 ± 0.005
Nitrate NO_3^-–N (mg/L)	1.3 ± 0.10
Phosphorus PO_4^{3-} (mg/L)	284.6 ± 7.3
Nitrogen, Ammonia NH_3–N (mg/L)	255.4 ± 1.5
Sulfide S^{2-} (μg/L)	137 ± 2.7
Sulfite SO_3^{2-} (mg/L)	1.0 ± 0.004
Sulfate SO_4^{2-} (mg/L)	133.1 ± 1.9

Table 1. *Cont.*

Parameter	Average ± SD
COD (mg/L)	953.9 ± 0.7
TSS (mg/L)	1195 ± 8.5
TDS (mg/L)	2011.8 ± 5.1
VSS (mg/L)	804.5 ± 3.3

3.2. Swine Wastewater Treatment

3.2.1. Solids Concentration (TSS, VSS, and TDS)

The efficacy of anaerobic bioreactor treatment is strongly influenced by the COD/sulfate mass ratios, as evidenced by the variable removal rates of TSS and VSS in this study (Figure 2). At a COD/sulfate ratio of 6:1, the average TSS and VSS removal efficiencies were 29% and 25%, respectively. The TSS and VSS removal efficiencies increased to 48% and 37%, respectively, at a COD/sulfate ratio of 4:1. The removal efficiencies further improved to 80% and 83%, respectively, achieving their highest levels at a COD-to-sulfate ratio of 2:1, indicating an optimum mass ratio for solids management. TSS, encompassing both organic and inorganic particles like metal hydroxides, decreased significantly as the majority of organic matter was degraded and metals were precipitated with hydroxide ions that were generated from the hydrolysis of produced bicarbonate ions. Consequently, this reduction in organics (including biomass) also led to a decrease in VSS. There was a noticeable decline in the average TSS and VSS removal efficiencies to 65% and 48%, respectively, at a COD/sulfate ratio of 1:1 compared to 2:1. The decreased solids removal efficiencies at ratios other than 2:1 can be attributed to an imbalance between sulfate and COD. An excess of sulfate relative to COD or an excess of organics can hinder the optimal growth of SRB, resulting in reduced organic degradation and metal precipitation [37,38].

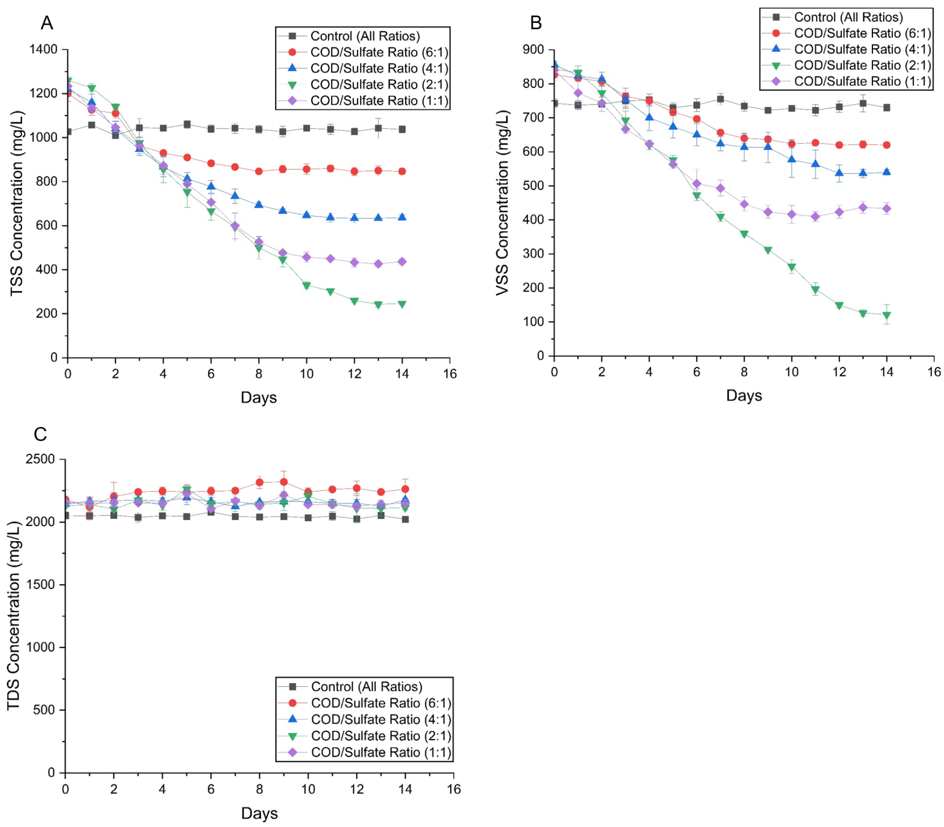

Figure 2. Profiles of total suspended solids (TSS) (**A**), volatile suspended solids (VSS) (**B**), and total dissolved solids (TDS) (**C**) in the bioreactors at various COD/sulfate ratios across the treatment period of 14 days.

While the removal efficiencies of TSS and VSS showed significant variation upon the change in COD/sulfate ratios, TDS remained relatively constant across all the bioreactors with negligible removal efficiencies observed (Figure 2). This observation suggests that TDS components, likely comprising inorganic salts, minerals and other small particles, are less amenable to the biological treatment processes presented in this study. The observed stability of TDS concentrations across different COD/sulfate ratios is consistent with the principle that anaerobic processes predominantly degrade biodegradable organic and inorganic matter, exerting minimal influence on dissolved inorganic substances [39].

Furthermore, the control reactor showed no substantial degradation of TSS, TDS, or VSS, irrespective of the COD/sulfate ratios. This indicated the observed removal of solids in the bioreactors was primarily a result of biological degradation, rather than physical or chemical pathways. The lack of degradation within the control reactor reinforced the importance of biological activity to achieve a reduction in solids in swine wastewater and the necessity of maintaining an appropriate balance of bacteria, sulfate, nutrients, and electron acceptors to stimulate the microbial consortia responsible for the anerobic bioreactor treatment process [40].

3.2.2. pH, Acidity, and Alkalinity

In the context of anaerobic bioreactor, pH, acidity, and alkalinity stand as important parameters that can profoundly impact system performance and stability. SRB thrive in slightly acidic to neutral pH environments, typically between pH 6.0 and 8.0. Outside this range, their metabolic activities can be inhibited [16]. The pH, acidity, and alkalinity of all the bioreactors under different COD/sulfate conditions were monitored in this research (Figure 3).

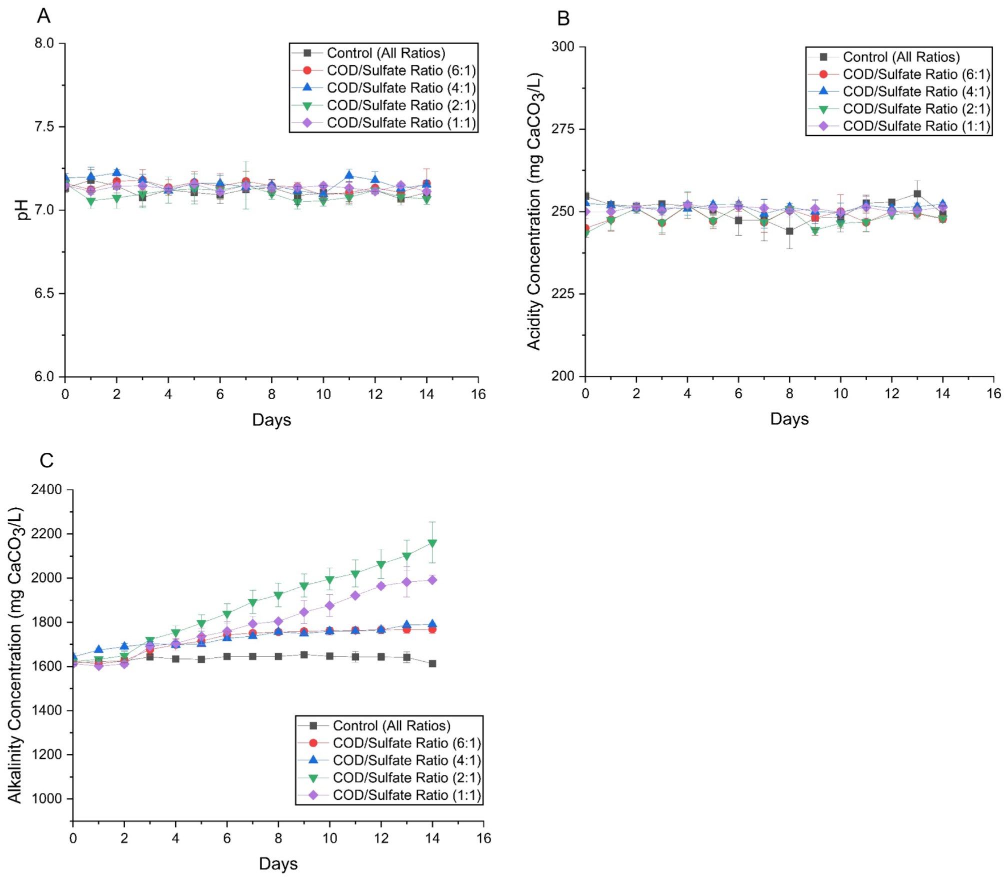

Figure 3. Profiles of pH (**A**), acidity (**B**), and alkalinity (**C**) in the bioreactors at various chemical oxygen demand (COD)/sulfate ratios across the treatment period of 14 days.

Regardless of the COD/sulfate ratios employed, the pH in the bioreactors remained within the range of 7–7.24. It is reported that this pH range supports the stability and activity of the enzymes involved in sulfate reduction, promoting efficient microbial growth and function [41,42]. The pH in our bioreactors indicated that SRB can effectively carry out their metabolic processes, including the reduction of sulfate to sulfide.

The consistent acidity results, irrespective of the COD/sulfate ratios, further validated the robustness of the sulfate reduction, dominating the anaerobic bioreactor environment [43,44]. While the control reactors exhibited mostly stable alkalinity profiles, bioreactors R1–R3 showed consistent increases in alkalinity, particularly at COD/sulfate ratios of 2:1 and 1:1 (Figure 3). These rises in alkalinity peaked at 2161.1 ± 92.5 mg/L and 2349.1 ± 17 mg/L for the respective ratios, which can be attributed to the SRB's metabolic processes. When SRB reduce sulfate (SO_4^{2-}) to sulfide (S^{2-}), they consume hydrogen ions (H^+) in the process. This consumption of H^+ ions increases the pH and contributes to alkalinity. Additionally, the production of bicarbonate (HCO_3^-) as a byproduct of SRB metabolism also adds to the alkalinity of the environment [13,45].

Overall, the bioreactors exhibited a stable and efficient treatment performance, as indicated by consistent pH and acidity values as well as an alkalinity increase, and reached the optimum level at a COD/sulfate ratio of 2:1.

3.2.3. COD and Sulfate Removal

In this study, the variation in the COD/sulfate mass ratios presented significant changes in the removal efficiencies of COD and sulfate. At a COD/sulfate ratio of 6:1, the removal efficiencies for both COD and sulfate were around 60% in bioreactors R1–R3 (Figure 4). As the ratio increased to 4:1, there was a marked improvement in the bioreactors' removal efficiencies of COD and sulfate with both exceeding 70%. The zenith of bioreactor performance was observed at a 2:1 COD/sulfate ratio. Both COD and sulfate removal efficiencies ranged from 81% to 86% across reactors R1–R3. A shift to the 1:1 ratio showed a slightly lower but still significant COD and sulfate removal efficiency in the mid-to-high range (70%).

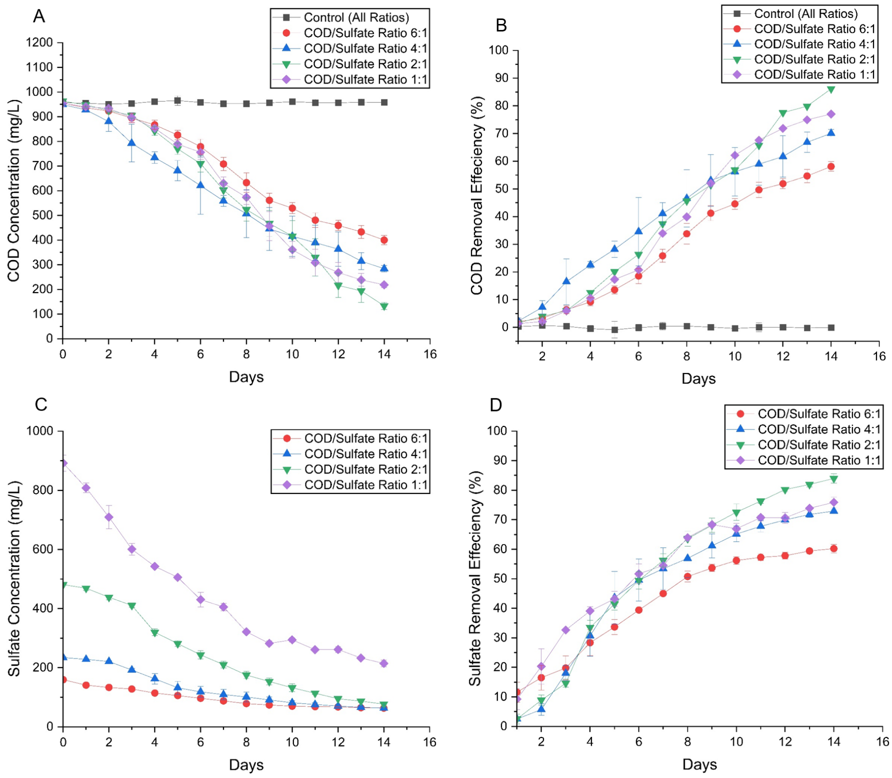

Figure 4. Profiles of COD and sulfate concentrations (**A**,**C**) and their removal efficiencies (**B**,**D**) in the bioreactors at various COD/sulfate ratios across the treatment period of 14 days.

It is reported that the COD/sulfate mass ratio significantly influences the activity of SRB [13]. Omil et al. reported that a COD/sulfate ratio of 2:1 resulted in the highest sulfate reduction efficiency and COD removal rates in anaerobic bioreactors treating industrial wastewater [18]. Lens et al. found that a COD/sulfate ratio between 1.8:1 and 2.2:1 provided optimal conditions for SRB activity, leading to effective sulfate reduction and organic matter degradation [46]. In another study, Stefanie et al. suggested that a COD/sulfate ratio of approximately 1.5:1 to 2:1 supported balanced microbial growth and efficient sulfate reduction in sulfate-reducing bioreactors [47]. Our results are consistent with previous studies showing that when the COD/sulfate ratio was maintained at 2, sulfate reduction processes were maximized, and the production of methane was substantially minimized [48,49]. This ratio represented the balance between available organic matter (COD) and sulfate, which SRB used as an electron acceptor in their metabolic processes. This optimal ratio ensured that SRB had sufficient organic substrate to thrive and effectively reduce sulfate. At this ratio, the sulfate presence was sufficient to serve as the primary electron acceptor, and SRB could efficiently utilize the available organic matter. This reduced the substrates available for methanogens, thereby minimizing methane production. The amount of sulfate that was reduced in our processing also proved the dominance of SRB.

3.2.4. Nutrient Degradation and Reduced Sulfur Species

At the COD/sulfate ratio of 6:1, across the bioreactors R1–R3, the average removal efficiencies for sulfide, sulfite, nitrate, and nitrite reached 39%, 43%, 38%, and 52%, respectively (Figure 5). With the COD/sulfate ratio of 4:1, the bioreactors exhibited enhanced nutrient removal. The observed efficiencies for sulfide, sulfite, nitrate, and nitrite were 46%, 55%, 53%, and 62%, respectively. When the COD/sulfate ratio was adjusted to 2:1, significant removal efficiencies were noted; i.e., removal efficiencies of sulfide and sulfite both reached 73%, while nitrate and nitrite degradation reached 67% and 72%, respectively. Interestingly, at the COD/sulfate of 1:1, while the sulfide and sulfite removal efficiencies remained high at 65% and 66%, nitrate and nitrite removal efficiencies declined to 57% and 46%, respectively.

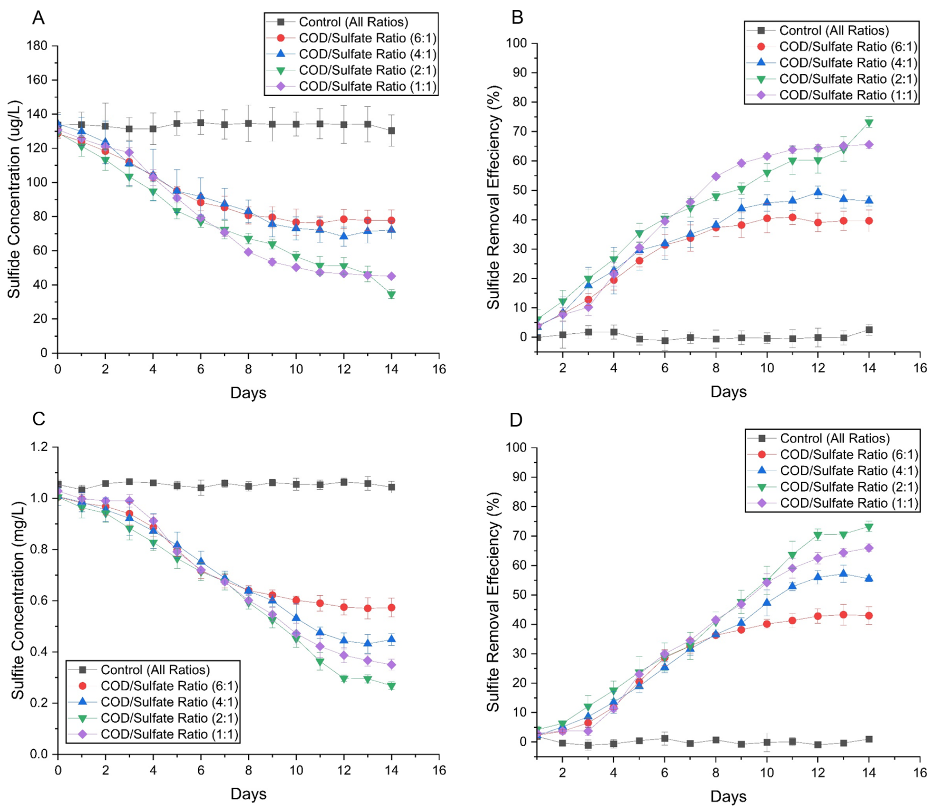

Figure 5. *Cont.*

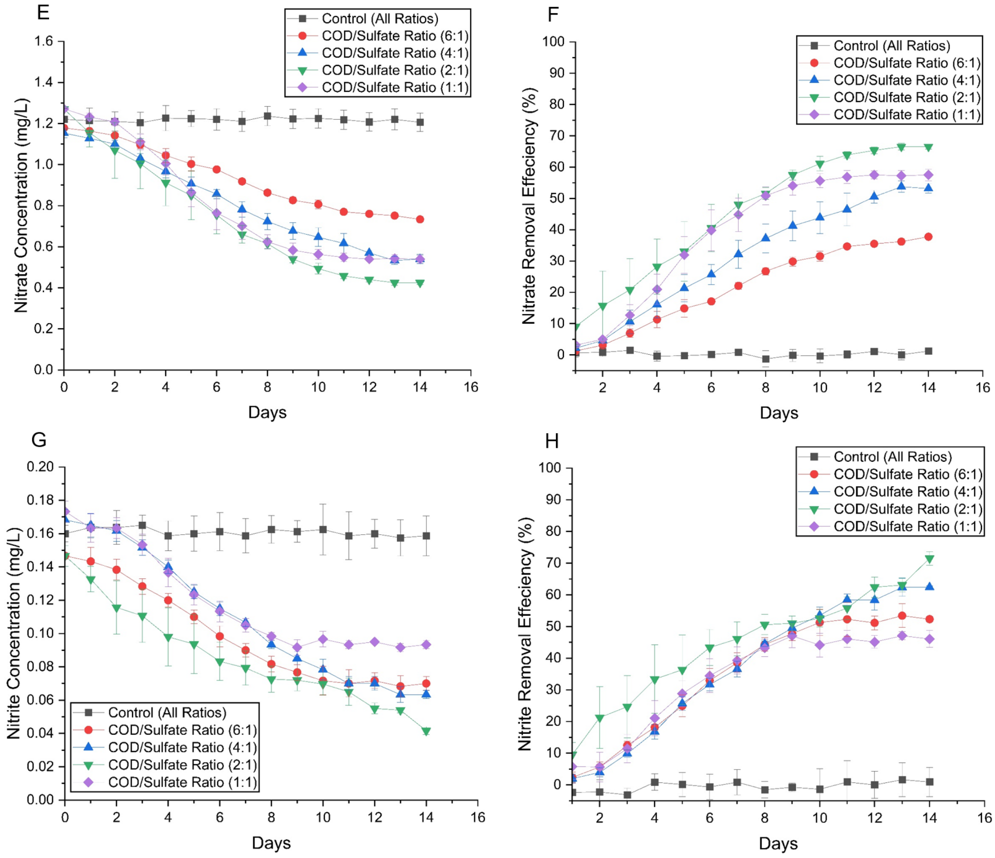

Figure 5. Profiles of sulfide, sulfite, nitrate, nitrite concentrations (**A**,**C**,**E**,**G**) and their removal efficiencies (**B**,**D**,**F**,**H**) in the bioreactors at various COD/sulfate ratios across the treatment period of 14 days.

The reduction of nitrate, nitrite, sulfide, and sulfite in swine wastewater could be attributed to the complex action of the overall microbial community in the bioreactors. It is known that the reduction of nitrate and nitrite in an anaerobic bioreactor with dominant SRB is a complex process, which can be influenced by microbial competition, environmental factors, and the metabolic versatility of the microbial community [16]. Nitrate and nitrite reduction can occur through the activities of denitrifying bacteria, nitrate-reducing SRB, and even SRB under certain conditions [50]. The coexistence of these microbial populations is possible and often observed, contributing to the overall stability and efficiency of anaerobic wastewater treatment systems. Sulfide and sulfite reduction can be a result of sulfate reduction process. In anaerobic digestion reactors, both sulfite and sulfide can be reduced due to the presence of SRB [42]. These bacteria utilize organic compounds as electron donors to reduce sulfate to sulfide, while sulfite, being an intermediate in the sulfate reduction pathway, can also be directly reduced by these bacteria under anaerobic conditions [16]. The simultaneous reduction of sulfite and sulfide occurs as part of the complex biochemical processes within the reactor, contributing to the breakdown of organic matter and the production of hydrogen sulfide (H_2S). Hydrogen sulfide remaining in the treated wastewater can be precipitated through the addition of ferrous chloride ($FeCl_2$) to form ferrous sulfide (FeS) to eliminate H_2S toxicity in the water [27].

Across all COD/sulfate ratios, phosphorus and nitrogen-ammonia showed limited degradation (Figure 6). The phosphorous removal hovered around 3.8–7.7%, and the removal of nitrogen-ammonia was between 5.8 and 10.9%. Despite the evident relationship between COD/sulfate ratio and sulfur-based or nitrogenous compounds, phosphorous and nitrogen-ammonia compounds remained largely unaltered within the studied range of COD/sulfate ratios. Such observations are consistent with previous reports [51,52], in which limited removal efficiencies for phosphorous and nitrogen-ammonia were observed during the wastewater treatment processes. These findings suggested that phosphorous and nitrogen-ammonia degradation depended on different microbial or physicochemical processes, which were not present in our bioreactors, and thus, alternative post-treatment methods may be required for the effective removal of these contaminants [53–55].

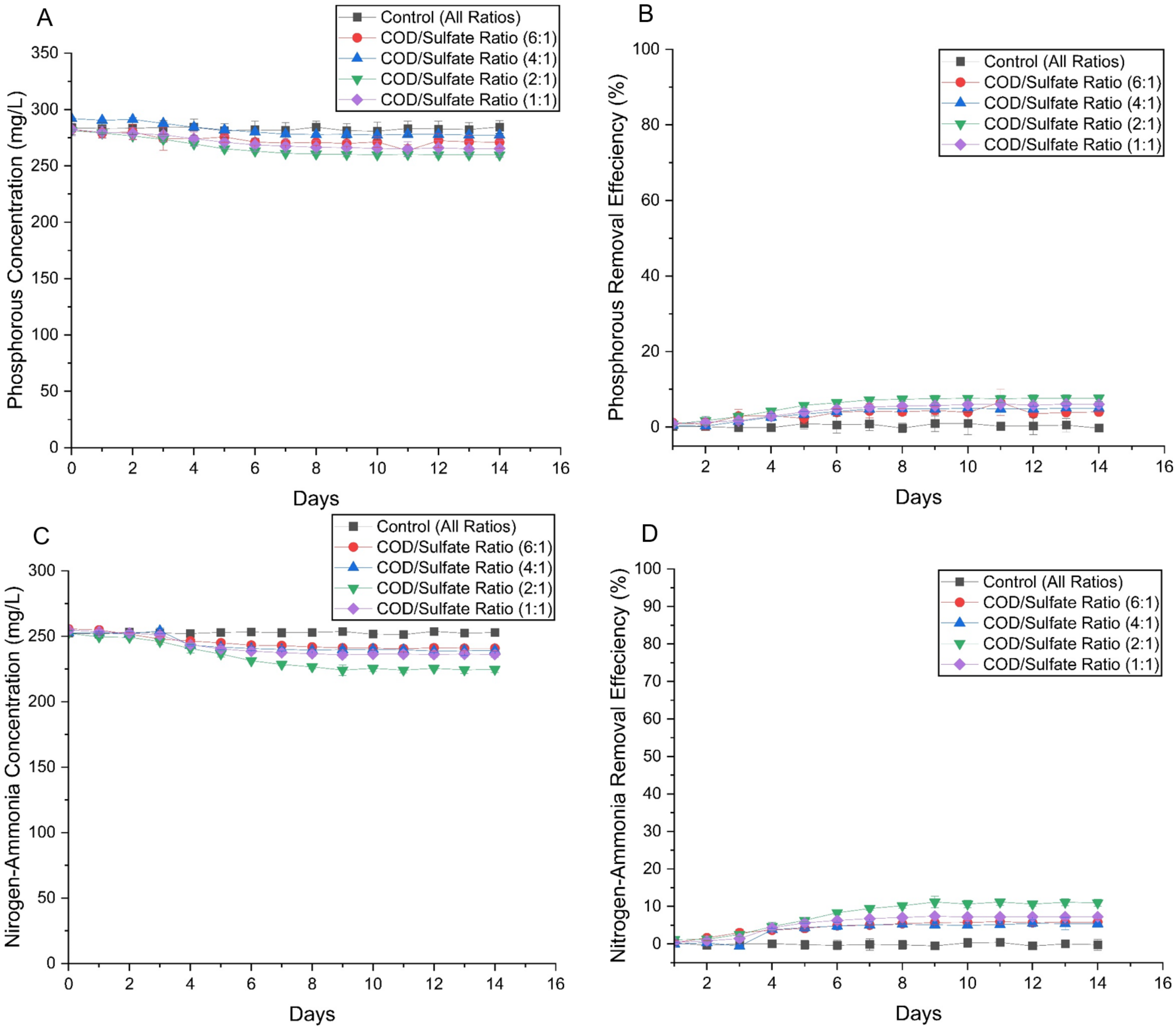

Figure 6. Profiles of phosphorous and N-ammonia concentrations (**A**,**C**) and their removal efficiencies (**B**,**D**) in the bioreactors at various COD/sulfate ratios across the treatment period of 14 days.

3.3. Solid Characterization by Scanning Electron Microscopy (SEM)

SEM was conducted to examine the change in the bioreactor sludge before and after treatment at different COD/sulfate ratios in order to obtain the microbial evolution information for a deeper understanding of the COD/sulfate mass ratios effect (Figure 7).

At a COD/sulfate ratio of 6:1, there was no significant presence of anaerobic bacteria on the sludge. At a COD/sulfate ratio of 4:1 and 1:1, there was only a marginal formation of anaerobic bacteria. This is consistent with the lower removal efficiencies as observed for TSS and VSS at these COD/sulfate ratios. The limited bacterial presence may be indicative of an environment not fully conducive to the proliferation of anaerobic bacterial colonies, particularly those of SRB and acidogenic bacteria, which are crucial for initiating the degradation process in anaerobic systems [56–58].

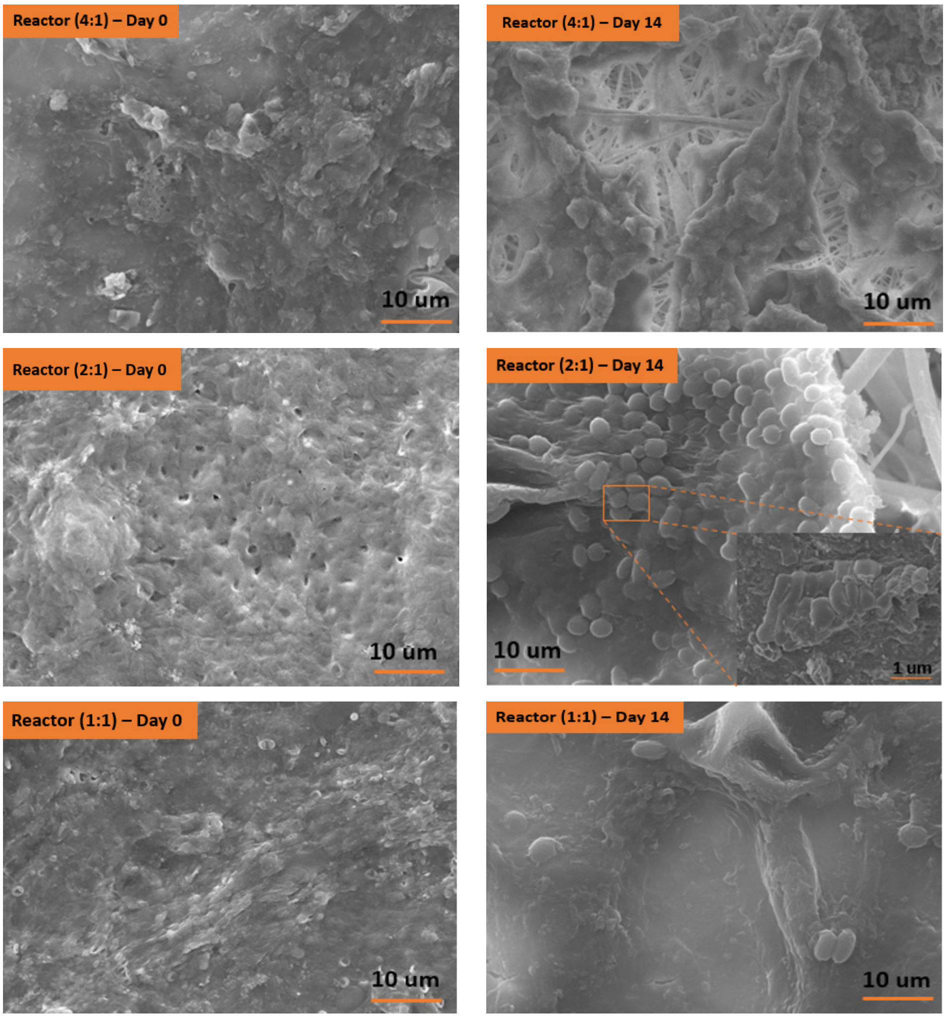

Figure 7. SEM images of sludge surface before (day 0) and after (day 14) bioreactor treatment at different COD/sulfate ratios (showed in bracket).

Conversely, at COD/sulfate ratio of 2:1, the SEM image of the sludge surface demonstrated a robust presence of anaerobic bacteria, a substantial biofilm development (Figure 7: reactor (2:1)–Day 14). Biofilm formation is a hallmark of healthy anaerobic bacterial activity and is known to enhance the degradation of multiple waste components [59–61]. The rod-shaped bacteria observed were consistent with known species such as *Clostridium spp.* and *Desulfovibrio spp.*, which are recognized for their roles in acidogenesis and sulfate reduction, respectively [57,62–65].

These SEM results are consistent with the removal efficiencies of TSS and VSS, suggesting that the COD/sulfate ratio of 2:1 is an optimal environment for the growth of sulfate reducers. The correlation between the microbial presence from the SEM examination and the anaerobic treatment efficiency underscores the need for tailoring the COD/sulfate balance to support the microbial community that is necessary for efficient waste degradation.

4. Conclusions

This study demonstrated the efficacy of treating swine wastewater using anaerobic bioreactors, with emphasis on the crucial role of the chemical oxygen demand (COD)/sulfate mass ratio in the process of treatment. By employing a COD/sulfate ratio of 2:1, the bioreactors achieved significant contaminant removal efficiencies: 86–88% for COD, 82–87% for sulfate, 80% for total suspended solids (TSS), and 83% for volatile suspended solids (VSS). Additionally, the following nutrient removal efficiencies were recorded: 73% for sulfide, 73% for sulfite, 67% for nitrate, and 72% for nitrite. These results highlighted that an optimal COD/sulfate ratio could enhance overall swine wastewater treatment efficiency by promoting a balanced microbial ecosystem, fostering synergistic interactions between sulfate-reducing bacteria (SRB) and methanogens. Scanning electron microscopy (SEM) further validated these findings by showing substantial biofilm development and active anaerobic bacterial presence at the optimal COD/sulfate ratio.

Overall, this research provided valuable insights into optimizing anerobic bioreactor processes for efficient and environmentally friendly treatment of high-strength wastewaters from swine farms, paving the way for more effective and sustainable management of swine wastewater, which is crucial to meet environmental discharge standards and promoting eco-friendly agricultural practices.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/environments11080162/s1.

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