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Evaluating integrated strategies for robust treatment of high saline piggery wastewater

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A B S T R A C T

In this study, we integrated physicochemical and biological strategies for the robust treatment of piggery effluent in which high levels of organic constituents, inorganic nutrients, color, and salts remained. Piggery effluent that was stabilized in an anaerobic digester was sequentially coagulated, micro-filtered, and air-stripped prior to biological treatment with mixotrophic algal species that showed tolerance to high salinity (up to 4.8% as Cl^-). The algae treatment was conducted with continuous O_2 supplementation instead of using the combination of high lighting and CO_2 injection. The microalga *Scenedesmus quadricauda* employed as a bio-agent was capable of assimilating both nitrogen (222 mg N g cell^{-1} d^{-1}) and phosphorus (9.3 mg P g cell^{-1} d^{-1}) and utilizing dissolved organics (2053 mg COD g cell^{-1} d^{-1}) as a carbon source in a single treatment process under the heterotrophic growth conditions. The heterotrophic growth of *S. quadricauda* proceeded rapidly by directly incorporating organic substrate in the oxidative assimilation process, which coincided with the high productivity of algal biomass, accounting for 2.4 g cell L^{-1} d^{-1}. The algae-treated wastewater was subsequently ozonated to comply with discharge permits that limit color in the effluent, which also resulted in improved biodegradability of residual organics. The integrated treatment scheme proposed in this study also achieved 89% removal of COD, 88% removal of TN, and 60% removal of TP. The advantage of using the hybrid configuration suggests that this would be a promising strategy in full-scale treatment facilities for piggery effluent.

1. Introduction

Anaerobic digestion (AD) is a useful technology to degrade livestock manures, producing biogas and digestate. The digestate still contains high concentrations of inorganic nutrients and biodegradable organics, and thus requires post remediation strategies. Microfiltration (MF) and ultrafiltration (UF) are being more frequently used for solid–liquid separation when treating digested livestock manures (Ledda et al., 2015), while reverse osmosis membranes are essential for many advanced treatment scenarios (Masse et al., 2007; Ledda et al., 2013). Both capital and operating costs of membrane processes are affected by membrane fouling, which decreases permeate flux and in extreme cases can result in membrane failure (Kim and Dempsey, 2013). The use of membrane filtration for manure treatment is technically feasible with proper pretreatment (Masse et al., 2007). Coagulation/sedimentation are commonly combined with the post-filtration with granular media or membranes (Nieuwenhuijzen and Graaf, 2011). Coagulation with metallic salts has been used to remove dissolved organics as well as suspended solids from a wide range of water sources (Duan and Gregory, 2003). However, the use of coagulants for the removal of suspended solids to low levels can lead to several problems: (1) the addition of high coagulant doses increases the volume and mass of solid residuals; (2) the addition of coagulants increases the total dissolved salt in the effluent; and (3) the addition of high doses of coagulants can result in increased irreversible-fouling of membranes (Choi and Dempsey, 2004; Galbelich et al., 2006). In the context of the effectiveness of post-treatment with microorganisms, the use of high doses of metallic coagulants can also result in the depletion of soluble phosphorus that is a necessary element for

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the growth of bio-agents. In this study, we therefore employed an organic polymer as a primary coagulant for the removal of suspended solids from anaerobically digested piggery effluent. It is often found that quaternary polymers such as polydiallyldimethylammonium chloride (pDADMAC) are quite effective flocculants (Bolto and Gregory, 2007). These additives are sometimes known as coagulants that have been frequently employed for treating surface water and wastewater (Bolto and Gregory, 2007; Kim et al., 2014c).

Biological processing is one of the most common methods for removing organic matter and nutrients from a wide range of wastewaters. These processes are made up of microbial activities that result in decomposition and removal of targeted components from a solution. Salinity is considered to be one of the major constraints on microbial viability and species diversity in wastewater treatment systems (Pronk et al., 2014). Piggery effluents commonly contain high concentrations of carbon, nitrogen, and phosphorous; along with a high salinity, these effluents can adversely affect biological growth and thus deteriorate wastewater treatability. It has been reported that saline wastewater with more than 2% salt results in low removals of organic and nutrients and poor settleability of microbial cells in conventional biological processes (Pronk et al., 2014). Thus, the application of salt-tolerant microorganisms in the biological treatment of saline wastewater has been attempted using a wide variety of bio-agents (Xiao and Roberts, 2010). In particular, algae differ in their adaptability to salinity based on their tolerance. Use of salt-tolerant algal strains for the remediation of piggery effluent can also result in operational simplicity in the simultaneous removal of nitrogen and phosphorus in a single treatment process, in contrast to conventional biological nutrient removal (BNR) processes that are comprised of nitrification/denitrification and luxury P uptake under strictly controlled operating conditions (Metcalf and Eddy, 1991). BNR processes typically require an amount of external carbon source for denitrification under anoxic condition. Along with the removal of inorganic nutrients using algae treatment, the removal of carbonaceous organics can also be achieved using specific algal strains, unless the organic carbon source for 'heterotrophically-growable' bio-agents is a limiting factor. We previously determined that *Scenedesmus quadricauda* is very effective in removing inorganic nutrients. In this previous study, we noticed that *S. quadricauda* can perform heterotrophic growth, besides the common autotrophic growth when using CO_2 as the sole carbon source. *S. quadricauda* is a microalga that can be photoautotrophically or heterotrophically grown under different culture conditions. The heterotrophy may be used as a supplementary strategy when autotrophic energy is not enough (e.g., when light intensity is low), which can result in the disappearance of chlorophyll in cells (Miao and Wu, 2006). The heterotrophic growth of microalga can proceed more rapidly by directly incorporating an organic substrate in the oxidative assimilation process for storage material production (Wang et al., 2010). Therefore, this mode of culture eliminates the requirement for light and offers the possibility of greatly increased cell density and productivity (Liang et al., 2009). Biomass productivity is strongly associated with assimilating substrates and thus heterotrophic growth is more desirable for the better removal of contaminants compared to photoautotrophic growth that typically requires an external CO_2 supplement under continuous illumination.

Local facilities for treating piggery effluent are currently facing more stringent regulations on their discharges of process wastewaters. In many cases, these limits are based on acceptable color levels, biodegradability, and biotoxicity, not simply on organic concentrations. In particular, acceptable color levels are generally location-dependent and are affected by the treatment capacity of the receiving post-treatment facilities and the volume of effluent discharged. Although the effect of light scattering causing apparent color can be eliminated by membrane filtration, chemical treatment processes are more applicable for removing true color which implies the presence of dissolved substances that absorb light of wavelength 400–800 nm (Langlais et al., 1991). In this work, we have tested the feasibility of using ozone to comply with discharge permits that limit color in the effluent, especially by replacing conventional bleaching processes to reduce the use of chlorine. Even though chlorination and Fenton oxidation can lead to satisfactory color abatement, ozonation is the treatment most effective in oxidative color removal with no residue or sludge formation (Langlais et al., 1991). Activated carbon adsorption is also one of the most commonly used methods for color removal, but regeneration or re-use results in a steep reduction in performance (Robinson et al., 2001). Many researchers have evaluated the possibility of ozonation in the context of advanced treatment for potable and wastewater, such as advanced oxidation of natural organic matter (NOM) from conventionally treated surface water (Kim et al., 2006), disinfection of swine wastewater (Macauley et al., 2006), removal of antibiotic-resistant bacteria from piggery effluent (Lee et al., 2011), and degradation of pharmaceutical contaminants from urban wastewater (Espejo et al., 2014). Biological treatment following ozonation is a well-established strategy to effectively remove dissolved organic matter and micro-pollutants by converting refractory organic compounds into easily-biodegradable fractions. Ozonation increases the polarity and hydrophilicity of NOM in the aqueous phase and only small portions of the organics can be completely mineralized (Kim and Yu, 2005). This implies the increase of low-molecular and thus easily-assimilative fractions in bulk organics.

The goal of this study was to investigate an integrated physicochemical and biological approach to achieve the following objectives during the treatment of piggery effluent at high salinity: (1) effective removal of total suspended solids (TSS), total chemical oxygen demand (TCOD), total nitrogen (TN), total phosphorus (TP), and color; (2) substantial decrease in refractory organic fractions in the treated wastewater to alleviate the burden of post-treatment facilities (usually domestic wastewater treatment plants) and further receiving water bodies; (3) a better understanding of how saline tolerant algal species work on wastewater remediation under heterotrophic growth conditions (i.e., without strong lighting and CO_2 injection); and (4) operational simplicity in the simultaneous removal of TN and TP in a single biological process. Physicochemical and biological strategies were integrated in this study for the robust treatment of piggery effluent in which high levels of organic constituents, inorganic nutrients, and salts remained. The performance was evaluated with respect to the removal of TSS, TCOD, TN, TP, and color using the hybrid configuration designed to be employed in a full-scale treatment facility for piggery effluent. The piggery effluent has been treated with the following processes (1) coagulation/sedimentation, (2) algae treatment, and (3) ozonation.

2. Materials and methods

2.1. Pretreatment of piggery effluent prior to algae treatment

Anaerobically digested piggery effluent was collected from commercial treatment facilities (ENR Solution Co., Ltd., South Korea). All facilities operate at a flow rate of 23 $m^3\ d^{-1}$ and use the upflow sludge blanket reactor process for the mesophilic anaerobic digestion of organic substrates. Table 1 shows the average characteristics of the AD effluent samples collected during this study. Fig. 1 shows a flow diagram of the wastewater treatment strategy proposed in this study. The concentrate from microfiltration process should be fed to a settling tank with added coagulant to further

Table 1
Characteristics of anaerobically digested piggery effluent.

Parameter	Value (mean ± SD)
pH	8.7 ± 0.1
Zeta potential (mV)	−35.4 ± 5.5
Chloride (mg L^{-1})	47,955 ± 141
Color (mg Pt–Co L^{-1})	17,369 ± 224
TCOD (mg L^{-1})	21,600 ± 283
SCOD (mg L^{-1})	18,450 ± 71
DOC (mg L^{-1})	4486 ± 31
UV254 (cm^{-1})	65 ± 0
TSS (mg L^{-1})	9100 ± 141
Turbidity (NTU)	3005 ± 7
TN (mg L^{-1})	4380 ± 60
SN (mg L^{-1})	3648 ± 30
NH_4–N (mg L^{-1})	2945 ± 49
TP (mg L^{-1})	142 ± 3
SP (mg L^{-1})	108 ± 0

remove the remaining solids that are retained and concentrated in submerged membrane reactors. Similarly, air stripping often requires a post-treatment polishing step using a solid or liquid sorbent to remove ammonia stripped off. Further research is thus required to complete a full scale application for robust treatment of piggery effluent.

The effluent was then coagulated with pDADMAC (P/N: 409022, Sigma–Aldrich) followed by sequential solid/liquid separations to remove the microbials and suspended solids from the mixed liquor. pDADMAC was diluted with deionized water to 1% (v/v) before its use. Jar tests were performed to determine the effect of coagulant concentrations on contaminant removals after sedimentation. The tests were conducted using a 6 stirrer with conventional blades and cylindrical 1 L beakers. pDADMAC was added to the beakers at seven different doses ranging from 0 to 10,000 ppm (μL L^{-1}), which corresponded to 12.4 meq-cationic charge L^{-1}. Rapid mixing was conducted at a velocity gradient of 230 s^{-1} for 1 min and the mixture was then left to flocculate for 30 min at 25 s^{-1} followed by quiescent settling for 1 h. The supernatant was collected from each beaker after settling to measure the removal of contaminants such as turbidity, TSS, TCOD, TN, NH_4–N, and TP. The supernatant collected was also filtered manually with 0.2 μm cellulose acetate (CA) membranes (C020A047A, Advantec, Japan) to determine the residual dissolved organic carbon (DOC), the ultraviolet (UV) absorbance at 254 nm (UV254), and color.

Based on the color removal estimated for AD effluent, the best polymer dose was selected for further experiments. The collected supernatant was subsequently filtered by CA membranes with a nominal pore size of 0.2 μm (Advantec Toyo Roshi Kaisha, Ltd., Japan) using vacuum pressure to remove microbials and suspended solids. The CA filters were selected to avoid not only carbon bleeding from the filters but also the undesirable adsorption of organic components onto the filters.

Passage of wastewater through a fixed-bed column of carriers was investigated with the objective of selecting a standard stripping protocol (see Fig. 2). The filtered piggery wastewater was stripped with air using an acrylic column (ID 5 cm × H 100 cm) in which polyethylene carriers (AnoxKaldnes, Sweden) were filled up to 80 cm. Four different pH conditions (pH 8.5, 10, 11, and 12) were examined for the wastewater circulating from the top to the bottom of the column at a flow rate of 40 mL min^{-1}, while injecting air (60 L min^{-1}) to create a counter flow. Residual NH_4–N was determined for all stripping conditions examined. A carrier packing height of 80 cm resulted in good removal of ammonia from piggery wastewater at pH 10. The wastewater was recirculated three times and the final effluent was neutralized (pH 7.5) before further biological treatment. Ammonia-rich vapor was vented to an exhaust pipe.

2.2. Biological treatment with *S. quadricauda*

S. quadricauda AG 10003 was obtained from the Korea Collection for Type Culture at the Korea Research Institute of Bioscience and Biotechnology. The algal stain was inoculated into 1000 mL tubular photo-bioreactors containing 800 mL BG-11 medium (Rippka et al., 1979). The photo-bioreactors were incubated under white fluorescent light illumination at 75 μmol photon m^{-2} s^{-1} at 25 °C for two weeks while continuously supplementing with 5% CO_2 (v/v) which was placed into the photo-bioreactors.

Batch experiments were conducted to determine the growth rate of *S. quadricauda* using a tubular borosilicate reactor (ID 3.4 cm × H 52 cm). Algal biomass from a two-week-old culture was harvested by centrifugation (3000 rpm for 10 min at room temperature) and was inoculated at 500 mg L^{-1} (as dry cell weight) into the bioreactor containing 400 mL pretreated piggery wastewater. The bioreactor was incubated at 25 °C for 138 h while aerating through an inlet port at the conical shaped bottom of the reactor. The aeration was carried out at a flow rate of 80 mL min^{-1} to supply O_2, resulting in the culture well mixed in the reactor. No fluorescent light illumination or CO_2 supplement was applied for algal growth in this study. During the incubation, a 10 mL of mixed liquor was collected several times from the bioreactor for measurement of dry cell weight. TCOD, TN, NH_4–N, and TP were also measured after filtration with 0.2 μm membranes. After algae cultivation for a given period of time, the mixed liquor was immediately placed in a cylindrical 1 L beaker to allow quiescent settling for 1 h. Supernatant collected was used for further ozonation tests described in Section 2.3.

The cell growth was determined by measuring the dry cell weight concentration using APHA Standard Method 2540 D (1998). The growth coefficient (μ, d^{-1}) and the specific nutrient consumption rate (q, mg-N or -P g-cell d^{-1}) for a specific period were calculated using Equations (1) and (2), respectively.

$$\mu = ln(AB_2/AB_1)/(t_2 - t_1) \tag{1}$$

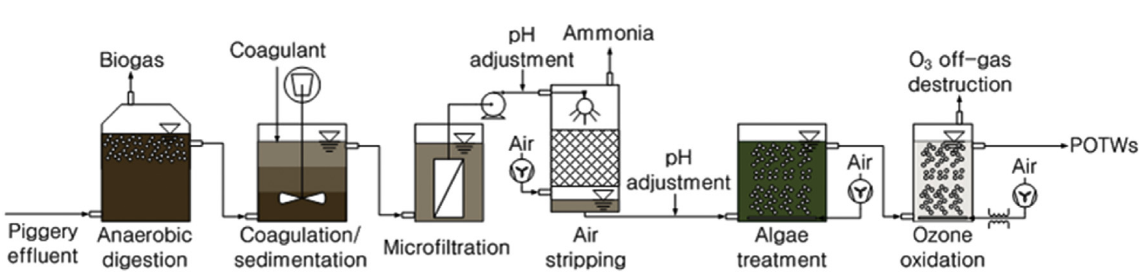

Fig. 1. Schematic of integrated strategies for robust treatment of high saline piggery wastewater.

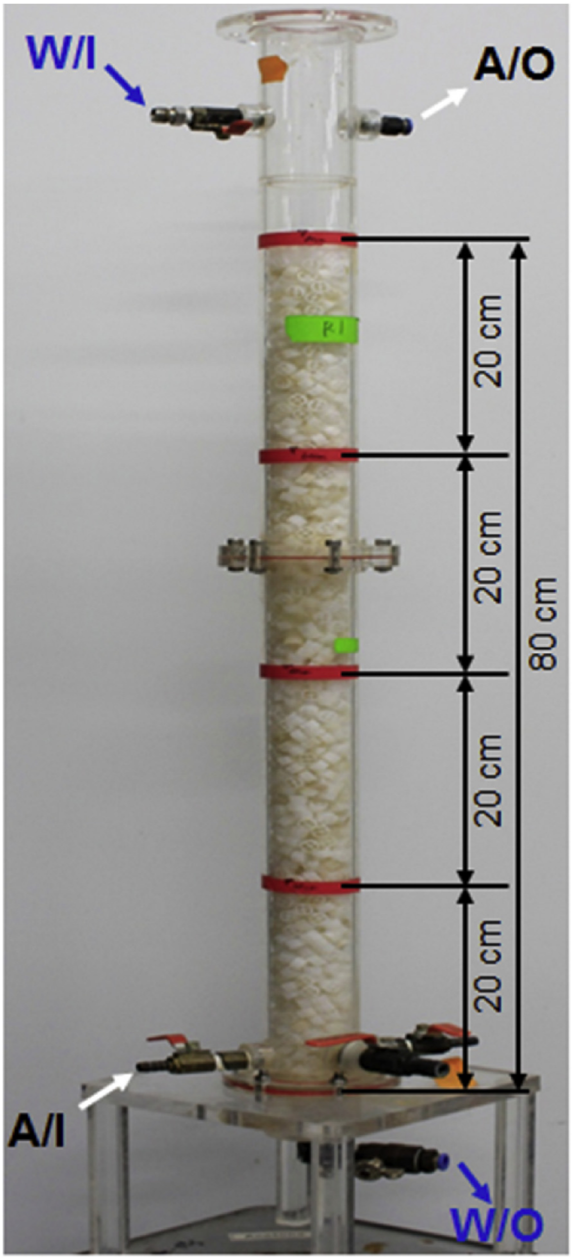

Fig. 2. Stripping column packed with polyethylene carriers. Four ports were installed on the column for water inlet (W/I), water outlet (W/O), air inlet (A/I), and air outlet (A/O).

$$q = (S_1 - S_2)/[AB' \times (t_2 - t_1)] \quad (2)$$

Here, AB_2 and AB_1 are the algal biomass concentrations as dry cell weight (g L^{-1}) at times t_2 and t_1, respectively. S_1 and S_2 are the assimilable nutrient concentrations (mg-N or -P L^{-1}) at times t_1 and t_2, respectively. AB' is the average algal biomass concentration between moment time t_1 and t_2. Therefore, the maximum value of the growth coefficient (μ_{max}) and the maximum specific nutrient consumption rate (q_{max}) were obtained between the closest two data points of the maximum slope on the dry cell weight concentrations and the assimilated nutrient concentrations plotted against time, respectively.

2.3. Ozonation of algae-treated wastewater

Algae-treated piggery wastewater was ozonated at a dose of 87 mg L^{-1} for 60 min to identify the minimum O_3 dose for compliance with color goals. The ozonation apparatus consists of a glass ozone contactor (ID 8 cm × H 33 cm), an ozone generator (Lab2B, Ozonia, Korea), and an oxygen tank. Ozonation was performed in the batch contactor equipped with a water jacket to maintain 15 °C while injecting ozone at a flow rate of 1 L min^{-1}. During the ozonation, a 5 mL liquid sample was collected from the contactor at 10, 20, 30, and 60 min to determine the soluble COD (SCOD), DOC, and UV254. Gaseous ozone exhausted from the contactor was detected and quantified using an ozone analyzer (Model H1, IN USA Inc., USA).

2.4. Characterization of dissolved organic matter

Advanced characterization techniques were used to determine the effects of ozonation and algae cultivation on the compositional and functional properties of organic components and to differentiate organics originating from algal cell growth. The molecular weight (MW) distribution of dissolved organic matter was determined using liquid chromatography-organic carbon detection (LC-OCD) (DOC-Labor, Germany), in which size-exclusion chromatography was coupled with on-line equipment for real-time measurement of both UV254 and organic carbon concentration of effluent passing through the exclusion column. More detailed information of the LC-OCD system can be found in an earlier study (Huber et al., 2011).

Fluorescence spectra were collected using a Perkin–Elmer LS-50B luminescence spectrometer, which uses a 450 W xenon lamp source. All liquid samples were diluted with carbon-free electrolyte solution under ambient pH conditions. Spectroscopic analysis was carried out at a concentration of 1 mg C L^{-1}. The acquisition interval and integration time were maintained at 1 nm and 0.1 s, respectively. Right-angle geometry was used for liquid samples in a 10 mm fused-quartz cuvette. Three-dimensional spectra were obtained by repeatedly measuring the emission spectra within the range from 200 to 600 nm, with excitation wavelengths from 200 to 400 nm, spaced at 5 nm intervals in the excitation domain. Spectra were then concatenated into an excitation-emission matrix (EEM).

2.5. Analytical methods

The chemical oxygen demand (COD) in the water samples was measured by the dichromate method using a DR/5000 spectrophotometer (Hach, USA). The TP was measured by the acid persulfate digestion method and the NH_4–N was measured by the salicylate method using the identical spectrophotometer (Kim et al., 2014a). Total organic carbon (TOC) (TOC-V CPN) and TN (TNM-1) were also determined (Shimadzu, Japan) using whole samples. The Hach DR/5000 spectrophotometer was used to measure chromaticity and UV254 after filtration with 0.2 µm membrane filters. The specific UV absorbance (SUVA) value is calculated from the UV254 divided by the DOC of the water sample, where the UV254 is mainly generated by electron-rich sites such as aromatic functional groups and double-bonded carbon groups in an organic molecule (Kim and Lee, 2006; Lamsal et al., 2011). Turbidity was measured using a Hach 2100P turbidimeter. Light intensity was determined using LI-250A (LI-COR, USA). The pH was measured using a pH meter (Orion 3 STAR, Thermo Scientific, USA). Metals were analyzed using an iCE 3500Z atomic absorbance spectrometer (Thermo Scientific, USA). Each measurement was carried out in triplicate and average values were reported.

3. Results and discussion

3.1. Overall fate of contaminants through piggery wastewater treatment

Table 2 shows the results of batch experiments with piggery effluent stabilized in an anaerobic digester in terms of mass balances to each component. The coagulation/sedimentation facilitated partial removal of TSS (89%), TCOD (44%), TN (30%), TP (32%), and color (64%). Subsequent microfiltration of the settled wastewater resulted in the complete removal of TSS, while negligible changes were found in the concentrations of other components. The chromaticity of piggery effluent which was subjected to algae treatment was 6175 ± 26 mg Pt–Co L^{-1}, which absorbed almost all of the light illumination within a path length of 1 cm. Under heterotrophic growth conditions the algae treatment achieved >51% removal of TCOD (11,100 ± 141 mg L^{-1}) remaining after coagulation/microfiltration. The level of TN decreased to 981 ± 1 mg L^{-1} by the stripping of free ammonia fraction, after which the level further decreased to 536 ± 9 mg L^{-1} with algae treatment. Likewise, TP was assimilated by the algal cells, which accounted for 20 mg L^{-1} of TP. The algae-treated wastewater was finally ozonated to minimize refractory organic fractions in the treated wastewater to alleviate the burden of post-treatment processes. A significant decrease in TCOD and the associated color removal were achieved by ozonation at 87 mg O_3 L^{-1} for 10 min. The integrated treatment scheme which consists of coagulation/microfiltration, stripping, algae treatment, and ozonation achieved 100% removal of TSS, 96% removal of color, 89% removal of TCOD, 88% removal of TN, and 60% removal of TP prior to conveying the piggery effluent to neighboring domestic wastewater treatment facilities. Further details of each treatment strategy are described and discussed below.

Sedimentation is often used in wastewater treatment applications where space and equipment mobility are not limiting factors. Good removals of TSS and turbidity indicate good removal of flocculated particles by sedimentation. The pDADMAC polymer was effective in removing contaminants after sedimentation and a 1.2 meq L^{-1} dose achieved 70% removal of color and 40% removal of SCOD (see Supplementary information, SI-1). Sufficient pDADMAC (>6.0 meq L^{-1}) was added to achieve complete removals of TSS and turbidity within 1 h of settling, which resulted in decreased removals of color and SCOD compared to when using a 1.2 meq L^{-1} dose. This is based on the result showing that high doses of the pDADMAC polymer resulted in charge re-stabilization for dissolved organics, but not for particles and colloids. This suggests charge neutralization as well as polymer bridging as the dominant mechanisms for pDADMAC.

A steep decrease of color was achieved with a 1.2 meq L^{-1} of pDADMAC. The residual color increased significantly, but did not exceed the initial level of color in the wastewater as the polymer dose was increased from 1.2 meq L^{-1} to 6.2 meq L^{-1}. A further increase of the polymer dose up to 12.4 meq L^{-1} resulted in a negligible change in the residual color. It is well known that the anionic charge carried by color-causing materials (e.g., humus-like substances) plays a major part in their interactions with metal ions and other cationic species and thus optimum coagulation occurs at a polymer dosage very close to that required for charge neutralization (Bolto and Gregory, 2007). The optimum dose of polymer for TSS (or turbidity) removal is not always the same as those for the removal of dissolved organics in wastewater. The polymer dose optimal for particle removal caused overdosing for COD removal (see Supplementary information, SI-1). Our result shows that the residual color can be used to control coagulant dosing for removing dissolved organics in piggery wastewaters. This provides a simple and robust method compared to measuring the zeta potential, as used to judge the stability of particles in coagulation processes.

It is worth noting that the polymer dose (~1.2 meq L^{-1}) did not remove a significant amount of TP a crucial element for algal growth, in contrast to conventional chemical precipitation that can result in the nearly complete depletion of TP via the precipitation of metallic hydroxide phosphates. When used as primary coagulants, cationic polymers do not produce large floc volumes because organic coagulants can be effective at much lower coagulant dosages than inorganic coagulants (Bolto and Gregory, 2007). This paper mainly focuses on the growth of mixotrophic microalga in high saline wastewater and the associated removal of residual contaminants under heterotrophic conditions; thus, we did not expand the research scope to consider the effects of coagulation conditions (e.g., chemical doses and mixing intensity and duration) on the performance of downstream microfiltration. Many studies have employed the concept of under-dosing for achieving a better performance of membrane filtration (Choi and Dempsey, 2004; Kim et al., 2014c). The under-dosing refers to coagulant additions that are lower than the charge neutralization dose, resulting in residual negative charge on the coagulated flocs. Similarly, in this study, we selected a 1.2 meq L^{-1} dose for coagulation of piggery effluent prior to microfiltration.

The level of free ammonia is considered to be one of the most crucial factors impacting on algal growth. The formation of free ammonia depends on both the ammonium level and aqueous pH of the growth media. Prior to algae treatment, we used air stripping to minimize the concentration of non-ionized ammonia (i.e., free ammonia) that could adversely affect the growth of microalgae in the pretreated piggery wastewater. Stripping was conducted at pH 10 using a standard stripping protocol which was selected through preliminary tests (details described in Section 2.1). The result showed that the NH_4–N concentration decreased to 644 ± 11 mg L^{-1}, corresponding to 14.1 mg L^{-1} free ammonia (see Supplementary information, SI-2).

3.2. Algae treatment of pretreated piggery effluent

The chromaticity of pretreated piggery effluent was 6175 ± 26 mg Pt–Co L^{-1}, which absorbed 85% of the light illumination within a path length of 1 cm and thus resulted in a nearly complete inhibition of photosynthesis. Instead of using the

Table 2
Percentages of TSS, color, TCOD, TN, and TP remaining after sequential treatments of AD effluent. Values in the parentheses show the initial concentration of each component in the AD effluent.

Treatment	TSS (9100 ± 141 mg L^{-1})	Color (17,369 ± 224 mg L^{-1})	TCOD (21,600 ± 283 mg L^{-1})	TN (4380 ± 60 mg L^{-1})	TP (142 ± 3 mg L^{-1})
Coagulation/Sedimentation	11.0 ± 0.2	36.1 ± 1.3	56.2 ± 1.3	70.4 ± 0.6	68.3 ± 0.6
Microfiltration	None	36.0 ± 0.2	51.9 ± 0.1	67.0 ± 0.2	55.6 ± 0.2
Stripping	None	35.6 ± 0.4	51.4 ± 0.1	22.4 ± 0.4	57.0 ± 0.4
Algae treatment	None	39.0 ± 3.1	24.9 ± 0.3	12.3 ± 0.1	40.9 ± 0.1
Ozonation[a]	None	4.1 ± 0.1	10.9 ± 0.5	11.8 ± 0.1	41.5 ± 0.1

[a] Ozonation was 60 min at a dose of 87 mg O_3 L^{-1}.

combination of high lighting and CO_2 injection that is typically used in auto- and mixo-trophic algal growth, algae treatment was conducted with continuous O_2 supplementation to assimilate carbonaceous organics from piggery wastewater. The results of the batch experiment with piggery wastewater using a microalga *S. quadricauda* are shown in Fig. 3a, in which the concentration of dry cell weight is plotted against time. The stagnant growth of the algal strain was found to be a result of being acclimated to the significantly different growth conditions compared to when grown in a synthetic medium under autotrophic conditions. During the transition from the lag phase to the stationary phase, substantial removals of SCOD and inorganic nutrients were accomplished by algal cells. Almost no change was found in the SCOD concentration after 40 h of cultivation, but the growth of microalgae continued even up to the 90 h cultivation. This observation demonstrates that microalgae produce storage materials (e.g., polysaccharides and polyphosphates) as reserve materials when resources are abundant and draw upon them in periods of starvation. SP was decreased by less than 70% of the initial concentration by algal cells during the first 40 h of cultivation, after which it gradually increased to more than 80% of the initial SP.

Based on the results shown in Fig. 3, the algal strain employed as bio-agents was capable of assimilating both nitrogen (222 mg N g cell^{-1} d^{-1}) and phosphorus (9.3 mg P g cell^{-1} d^{-1}) in a single treatment process. The bio-agents also decreased SCOD (2053 mg O_2 g cell^{-1} d^{-1}) by utilizing dissolved organics as a carbon source under the heterotrophic growth conditions, and the removal of SCOD was associated with high productivities of algal biomass, accounting for 2400 mg cell L^{-1} d^{-1}. This was much higher than that reported in earlier work, in which identical algal strain was grown on diluted piggery wastewater supplemented with CO_2 (Kim et al., 2014b). The maximum algal growth coefficient (μ_{max}) for *S. quadricauda* under the mixotrophic conditions was 0.37 d^{-1} (Kim et al., 2014b), which was much smaller than that observed in this study under heterotrophic conditions (1.06 d^{-1}). Likewise, the maximum specific nutrient consumption rate (q_{max}) was also higher for heterotrophic conditions than that observed for mixotrophic conditions. This result indicates that the heterotrophic growth of *S. quadricauda* can proceed more rapidly by directly incorporating organic substrate in the oxidative assimilation process for storage material production, which coincided with a previously reported work (Kim et al., 2014b).

The TN removal was governed by the extent of the reduction in NH_4–N, which was the most dominant fraction among inorganic nitrogen species. *S. quadricauda* converted NH_4–N to their biomass through metabolic pathways to a much greater extent than achieved under mixotrophic growth conditions (Kim et al., 2014a). The NH_4–N concentration can also be decreased via the stripping of free ammonia fraction during aeration, but such ammonia gasification during oxygen transfer in the algae bio-reactor resulted in an insignificant decrease of NH_4–N from 2812 ± 3 mg L^{-1} to 2729 ± 1 mg L^{-1} for the course of these experiments.

3.3. Ozonation for compliance with the permit effluent limits

Biological treatment processes in domestic wastewater treatment plants are generally efficient for meeting the limits of BOD and TSS removal, but are largely ineffective for removing color from the wastewaters. For a full-scale treatment system, highly-colored wastewater should be properly treated before it is conveyed to the post-treatment facilities. In this study, we referred to operating permit effluent limits imposed by a public sanitary authority that is responsible for managing and operating the receiving post-treatment plants and the discharge of treated water into surface

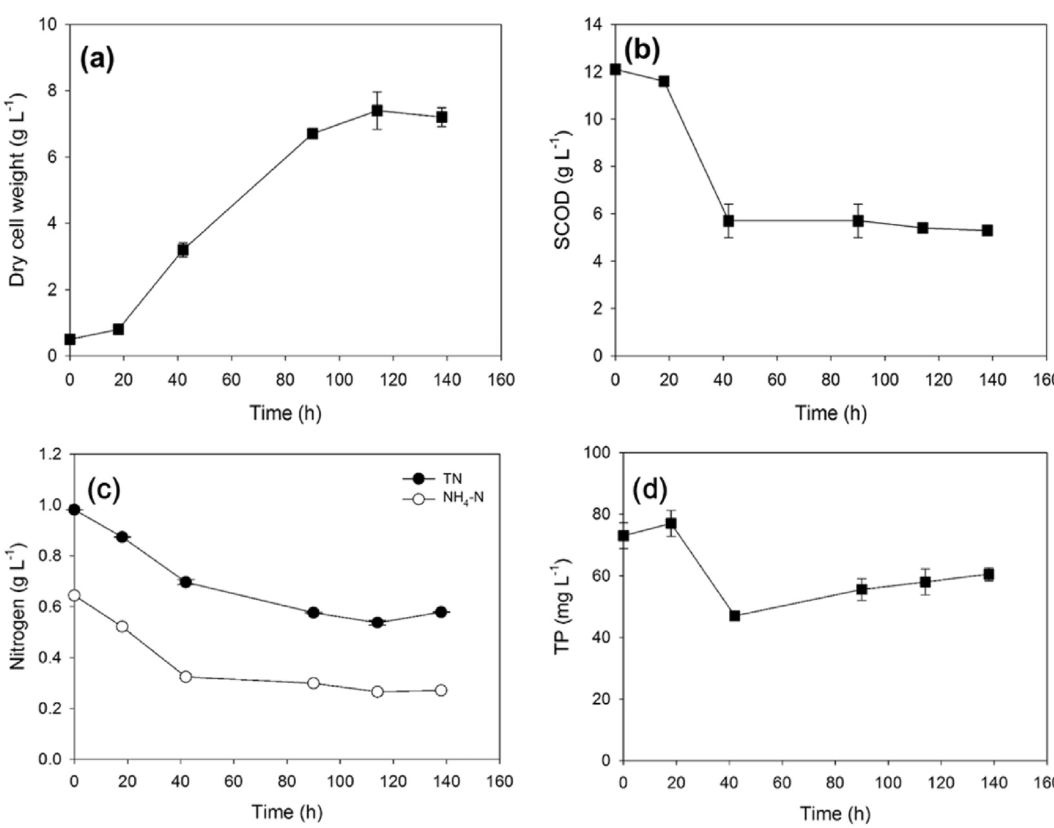

Fig. 3. Growth of *S. quadricauda* and the associated removal of contaminants. The residual concentrations of COD, TN, NH_4–N, and TP were determined after filtration (0.2 μm).

waters. Our examination revealed that ozonation can achieve lower discharge concentrations and greater reliability for compliance with color goals.

A substantial decrease of chromaticity was achieved as ozonation continued for 20 min, which complied with discharge permits that limit color to 1000 mg L^{-1} and COD to 3000 mg L^{-1} in the effluent (see Fig. 4). The chromaticity was further reduced at higher ozone doses (which were equivalent to longer reaction times), but only to a small extent. SCOD decreased from 5.4 ± 0.1 to 1.0 ± 0.1 g L^{-1} when the concentration of ozone injected into algae-treated piggery wastewater was increased. Similarly, a significant decrease of DOC was found as the ozone dose was increased up to 2.9 mg-O_3 mg-C^{-1}, corresponding to 60 min of ozonation. A substantial decrease in SUVA was observed upon ozonation for 10 min, but an insignificant decrease was achieved with further increase of ozonation of up to 60 min. Based on the formula $CH_2O + 2O_2 = 2CO_2 + H_2O$, which gives a 32/12 ratio for COD/TOC, the SCOD/DOC ratio was very close to the theoretical conversion ratio of 2.67 for algae-treated wastewater with non-ozonation, whereas the ratio decreased for the effluent that was ozonated for 10 min or longer. This was likely attributed to the much slower DOC reduction than COD reduction. The relative oxidation state of the byproducts with more oxygenated functionalities (e.g., hydroxyl, carboxyl, etc.) was much higher than for the parent organic molecules, as evidenced by a decrease in the SCOD/DOC mass ratio upon ozonation. The result also suggests that pretreatment with proper levels of ozone substantially improves the bioavailability of organic matter for heterotrophs in the bioremediation of piggery wastewater, which concurred with previously reported work (Kim et al., 2014a).

In contrast to the removal of dissolved organics upon ozonation, almost no change was found in the total mass of nitrogen. The level of residual NH_4—N was gradually decreased from 271 ± 7 to 188 ± 4 mg L^{-1} with increasing ozonation duration. No significant change was observed in the level of residual TN upon ozonation for up to 60 min, and thus the ammonia gasification that could occur when bubbling the ozone through wastewater was negligible. This result indicates that ammonium was oxidized to nitrite/nitrate ions by ozone molecules at alkaline pH (Langlais et al., 1991).

In the context of mixotrophic growth for algae treatment of piggery wastewater, ozonation can be used to increase light transmittance in the photosynthesis of microalgae. The ozonation pretreatment could result in the robust growth of microalgae and improved removal of inorganic nutrients during the subsequent cultivation of microalgae in the ozonated wastewater. However, this combination of pre-ozonation and algae treatment might only be applicable if the organic carbon source for heterotrophs is a limiting factor. The biomass productivity that is strongly associated with contaminant removals is lower for the mixotrophic growth than for heterotrophic growth. The external CO_2 supplement under continuous illumination is typically required for mixotrophic growth of microalgae, resulting in increased process costs for algae treatment with relatively low cell density (Chen et al., 2011; Pleissner et al., 2013). The amount of newly produced algal biomass should be properly eliminated from the bioreactor due to the self-shading effect occurring in the photo-bioreactors. Likewise, repetition of the algal harvesting results in an accumulation of non- or less-biodegradable organic constituents in the culture, which may also require post-treatment.

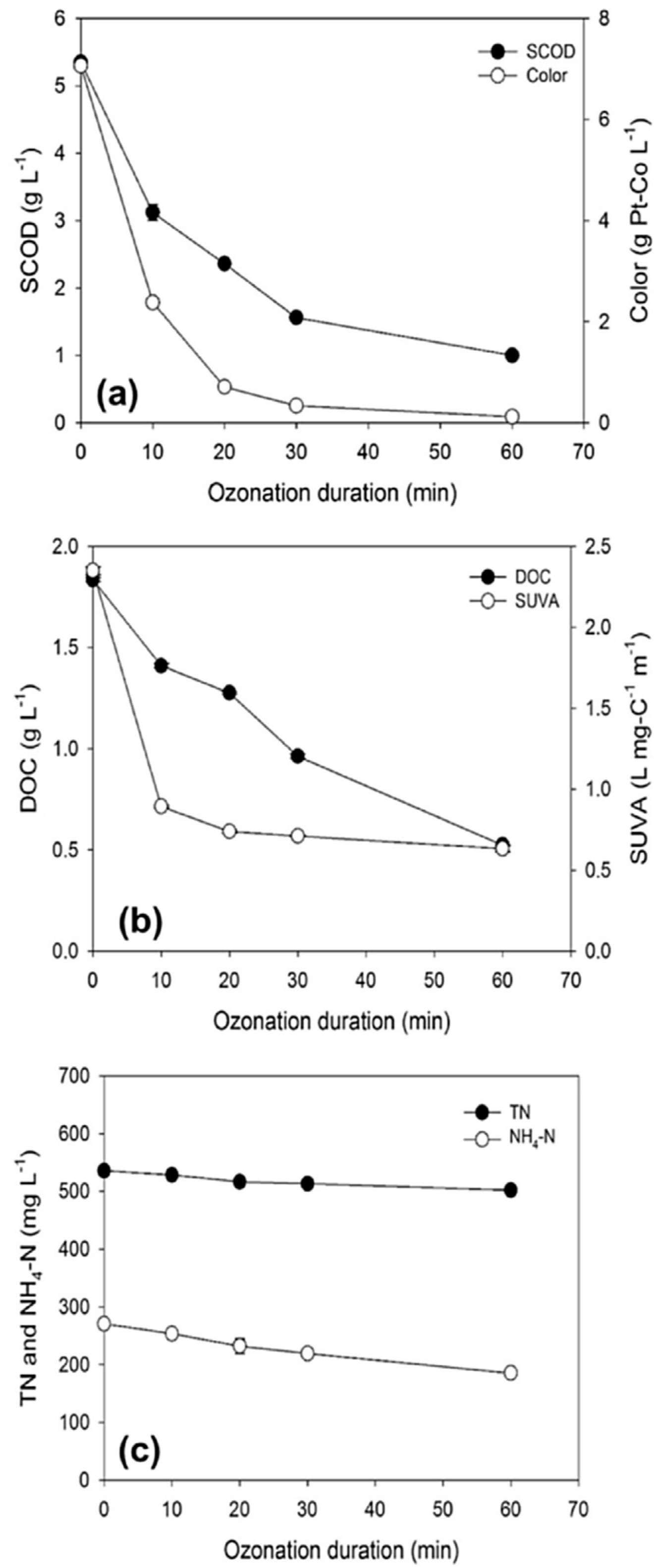

Fig. 4. Impact of ozonation on the characteristics of piggery wastewater. The ozonation of piggery wastewater continued up to 60 min at a dose of 87 mg O_3 L^{-1}.

3.4. Characterization of organic constituents in piggery effluent

Using advanced characterization techniques, the analysis revealed that the integrated treatment scheme substantially changed the compositional and functional properties of organic compounds in piggery effluent. Bulk organics can be classified as illustrated in Fig. 5a based on the fact that the retention time of organic components in the exclusion column of LC-OCD depends on their MW. In this study, LC-OCD chromatograms were divided

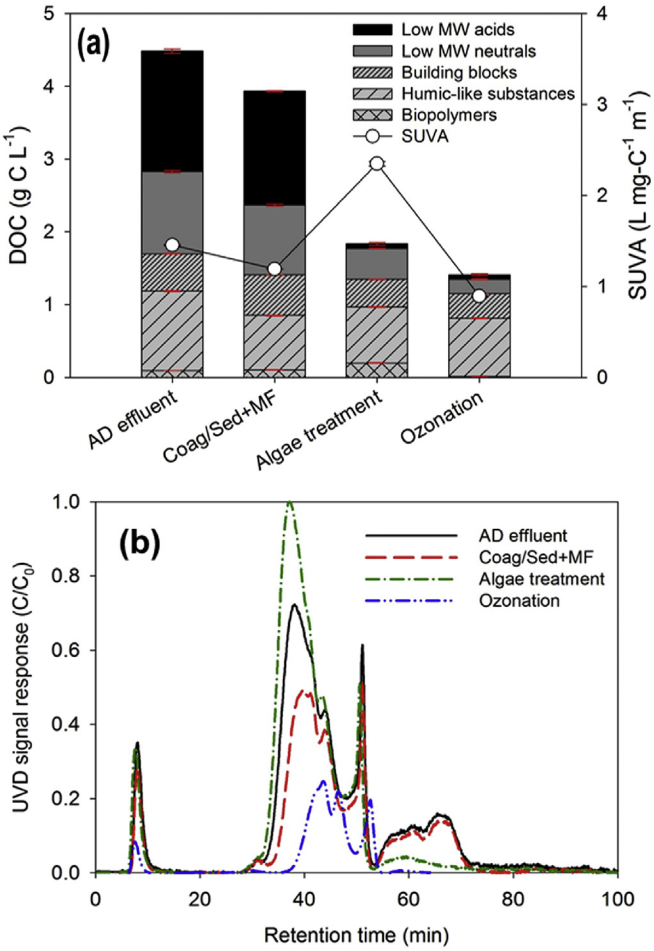

Fig. 5. (a) Effects of sequential treatments on the distributions of five different organic fractions in piggery effluent. (b) LC-UVD spectra for organic materials in the piggery wastewater samples. Ozonation was 10 min at a dose of 87 mg O_3 L^{-1}.

into five different fractions: biopolymers (>20,000 Da), humic-like substances (1200–500 Da), building blocks (500-350 Da), low MW acids (<350 Da), and low MW neutrals (<350 Da) (Huber et al., 2011). The organic materials in wastewater collected after anaerobic digestion of piggery effluent were dominantly low MW acids (37% of DOC) and neutrals (25% of DOC). The piggery organic matter also had a high percentage of humic-like substances (24% of DOC) and the percentage decreased with coagulation/filtration. Coagulation with metallic salts and organic polymers is commonly used to remove dissolved organics as well as colloidal materials from a wide variety of water sources for the purposes of both potable and non-potable uses; in particular, hydrophobic organics are preferentially removed compared to hydrophilics which have a lower molecular weight and higher repulsion against flocculants formed as a result of coagulation (Kim and Lee, 2006). In this study, microfiltration following coagulation with pDADMAC decreased both DOC and SUVA, which was mainly associated with the removal of humic-like substances that are relatively stable (i.e., slowly biodegradable and/or non-biodegradable). SUVA has been used as an indicator of the humic content in water environmental systems (USEPA, 1999; Edzwald and John, 1999; Ratpukdi et al., 2010). Subsequent cultivation of microalgae on the wastewater that was air-stripped for free ammonia removal resulted in a significant increase of SUVA, which was attributed to the removal of hydrophilic fraction in bulk organics. A relative increase in UV254 upon algal cultivation indicates that the hydrophilic organic components in the wastewater were more preferably assimilated by algal cells during the heterotrophic growth of microalgae (see Fig. 5b). Algae treatment removed nearly all of the low MW acids and >55% of the low MW neutrals, while it could not effectively remove other fractions and rather doubled the level of biopolymers that were associated with extracellular polymeric substances formed during microbial metabolism and biomass decay.

Fig. 5a also shows that a 10 min ozonation achieved almost complete removal of large organic molecules designated as biopolymers in the piggery wastewater. The level of humic-like substances was slightly increased by ozonation for 10 min, after which it gradually decreased with increasing ozonation duration due to both mineralization and low-molecularization (see Supplementary Information, SI-3). For low MW acids, the amount of organic carbon converted from larger organic components had an increasing tendency with the increasing ozonation duration. Unlike low MW acids, some fractions in low MW neutrals were removed by ozonation for 10 min, probably due to their electrophilic or nucleophilic characteristics that can very quickly react with molecular ozone.

Fluorophores typically contain several combined aromatic groups, or plane or cyclic molecules with some π bonds (Shimizu and Hiyama, 2010). Fulvic acid-like substances (peak region D) dominated among the fluorescent organic constituents in the raw piggery effluent which was collected after anaerobic digestion of piggery effluent (see Supplementary information, SI-4). The fluorescence EEM spectra of organic materials in wastewater samples were normalized for further interpretation based on the fluorescence intensities at the highest peak observed for raw piggery effluent (Table 3). The results indicated that algae treatment was believed to remove protein-like substances (peak regions A and B) to a much greater extent than humic-like substances (peak regions C and D). On the other hand, the residual humic-like substances were dramatically removed by ozonation at a minimum of 10 min. These trends indicated that organic fractions with a higher degree of aromaticity were accumulated as a result of microalgae cultivation and that ozonation post-treatment can be adopted to remove the fluorescent moieties in the residual hydrophobic organics.

3.5. Advantages of using the integrated strategies for livestock manure treatment

The use of microalga *S. quadricauda* for treatment of digested piggery effluent provides an operational simplicity in the simultaneous removal of inorganic nutrients (N/P) and COD in comparison with a conventional hybrid system of coagulation and membrane bioreactor (MBR). MBR is credited as a promising alternative for treating organic-rich wastewater and is increasingly combined with chemical coagulation/flocculation for industrial wastewater treatment such as dairy (Chen and Liu, 2012) or piggery (Kornboonraksa et al., 2009) effluents. A substantial decrease in effluent TP is associated with the use of metallic coagulants (e.g., ferric chloride and alum), whereas sequential nitrification/denitrification process is commonly integrated with MBR for biological nitrogen removal under strictly controlled operating conditions. However, the long sludge retention time in the MBR is considered essential for complete nitrification with slow-growing microorganisms. The consistent nitrification may also be feasible with proper pretreatment like we used to decrease ammonia in our study, since the activity of ammonia-oxidizing bacteria can be significantly inhibited in systems that have high ammonia concentrations (Anthonisen et al., 1976). Moreover, the denitrification typically requires an amount of external carbon source. Although coagulation followed by microfiltration were similarly used in this study for the removal of suspended solids from digested piggery effluent, the use of metallic coagulants that can lead to several

Table 3
Effects of sequential treatments on fluorescent properties of dissolved organic matter in piggery effluent. Fluorescence intensities collected from four different regions were normalized based on the highest value (3.8×10^5) among the peaks observed for anaerobically-digested piggery effluent.

Treatment	Peak A (Tryptophan protein-like)	Peak B (aromatic protein-like)	Peak C (humic acid-like)	Peak D (Fulvic acid-like)
AD effluent	0.45	0.96	0.63	1.00
Coag/Sed + MF	0.39	0.80	0.56	0.92
Algae treatment	0.03	0.07	0.41	0.50
Ozonation (min)				
10	0.02	0.10	0.13	0.09
20	0.01	0.09	0.07	0.06
30	0.04	0.09	0.03	0.04
60	0.05	0.10	0.01	0.04

problems was eliminated. While composting solid wastes from the coagulation of digestion residues is a promising strategy, the high content of metals in the solid wastes may adversely affect the composting bacteria and further plant growth, soil organisms, and water quality in the land application of the final compost. The cost of using organic polymers could be partially offset by the increased loading for membrane facilities and decreased costs for the separate disposal of solid wastes produced when using metallic salts. In addition, there are a variety of potential uses for the algal biomass from livestock manure treatment that could defray treatment costs (Pizarro et al., 2006). More work will need to be conducted to quantitatively assess the capital and operational costs for the integrated treatment strategies based on practical experiences and optimization work through pilot tests.

4. Conclusions

Physicochemical and biological strategies were integrated for the robust treatment of piggery effluent in which high levels of organic constituents, inorganic nutrients, and salts remained. The performance was evaluated using the hybrid configuration to be employed in the full-scale treatment facility for piggery effluent. The multi-strategic approach was successful in treating piggery wastewater and resulted in the following benefits: (1) effective biological treatment with *S. quadricauda* even at the high salinity typical of piggery wastewaters; (2) lower discharge concentrations and greater reliability for compliance with color goals; (3) substantially increased particle removal over a broad range of coagulant doses in comparison with dissolved organic removal; and (4) large increases in the treatability of residual organics prior to conveying piggery effluent to neighboring domestic wastewater treatment facilities.

Very few studies have dealt with the concurrent removal of organic and inorganic components from high saline piggery wastewater by means of 'heterotrophically-growable' microalgae. The simultaneous removal of inorganic nutrients and carbonaceous organics using saline tolerant algal species was achieved under the heterotrophic growth conditions, resulting in high bio-assimilability relative to photoautotrophic and mixotrophic conditions using light illumination and CO_2 supplement. In addition, a process combining ozonation post-treatment with algae cultivation provided a valuable solution to the recalcitrant livestock effluent, especially for improving contaminant removal and thus alleviating the burden of the receiving water bodies.

Liquid chromatographic and spectroscopic techniques were successfully adopted to investigate the effects of physicochemical and biological treatment strategies on the compositional and functional properties of residual organic components. Ozonation provided the means to low-molecularize and even remove the high MW organic fractions that were increased by algae treatment. The low MW organics can be again consumed by microalgae which in turn increase the high MW ones, provided that the nutrients are available in adequate amounts relative to each other. This system may be considered as a semi-closed loop recirculating ozonated wastewater to the algae bioreactors until the desired effects of ozone are judged to be negligible. The use of recirculating water systems is worth further investigation for minimizing effluent concentrations of inorganic nutrients and refractory organic matter.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.watres.2015.11.054.

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