Study of oyster shell as a potential substrate for constructed wetlands
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ABSTRACT
We tested the suitability of oyster shell (OS) as a substrate for phosphorus removal in constructed wetlands (CWs) treating swine wastewater. OS is proven to have a significant phosphorus adsorption capacity; significant phosphorus removal was achieved in vertical subsurface flow constructed wetlands (VSSFs) that were filled with OS and used to treat swine wastewater. In the VSSF system, OS adsorption and precipitation played the greatest role in phosphorus removal, and the phosphorus distribution in the substrate layers was attributed to the vertical flow state of wastewater in the system. Ca–P was the predominant form of phosphorus in the system. Overall, the study results showed that OS could be used for phosphorus removal in CWs. OS also allowed for reuse of a waste substance, making the overall system more environmentally friendly.

Key words | constructed wetlands, oyster shell, phosphorus removal, swine wastewater

INTRODUCTION
Recently, China’s Fujian Province has seen a rapid increase in the swine breeding industry, with the development of many large-scale intensive swine farms created to satisfy increasing consumer demand for meat products. These piggeries have resulted in a substantial quantity of feces, urine, and washing water that is discharged into the environment, causing soil pollution and eutrophication of surrounding waters (Deng et al. 2008). Constructed wetlands (CWs) have been widely implemented in the region to address these problems.

CWs are easily operated and maintained, require low input, consume relatively little energy, and are appropriate for various wastewater treatment needs. Generally, CWs perform well in suspended solids (SS) and organic matter (OM) removal, but prove to be inconsistent in nutrient reduction activity and particularly in phosphorus removal (Babatunde et al. 2010).

Many studies have shown that nitrogen and phosphorus are the main culprits of water eutrophication, and phosphorus is of greater significance according to Liebig’s Law of the Minimum. It is thus necessary to strengthen the phosphorus removal capacity of CWs, particularly in light of the fact that swine wastewater contains high concentrations of phosphorus. Phosphorus removal in CWs is a complicated process that is difficult to sustain. It takes place through a combination of several processes: plant uptake, microbial growth, adsorption, precipitation within substrates, etc. Of these processes, adsorption and precipitation within substrates are widely acknowledged to play the largest role in phosphorus reduction (Babatunde et al. 2009). Emphasis should thus be placed on identifying substrates with a high phosphorus removal capacity and suitable physicochemical properties. Substrates should also be inexpensive and locally available to reduce construction costs.

Oyster shell (OS) has a high Ca content (Yoon et al. 2006) and is also a by-product of the mariculture centered on the blue belt zone along the southern coast of Fujian Province. In recent years, oyster production has increased because of market demands and the adoption of hanging oyster techniques in shellfish farms (Ye et al. 2006). Large quantities of OS produced by the farms form a significant source of pollution. If OS were used as a substrate in CWs, OS disposal costs could be diminished, costs of a new CW substrate could be avoided, and a high phosphorus removal rate might be achieved.

Considering the above, we tested OS as a CW phosphate-removing substrate in this study. The objectives of the study were: (1) to measure the physicochemical properties of OS; (2) to conduct phosphorus adsorption
experiments with OS; and (3) to investigate phosphorus removal performances of vertical subsurface flow constructed wetlands (VSSFs) packed with OS.

**MATERIALS AND METHODS**

**OS**

OS was air-dried and ground to pass through a 2-mm mesh sieve, and specific physical and chemical properties were determined as follows.

**Physical properties**

OS porosity was determined using the standard soil science procedure, based on estimations of bulk density and particle density (Yang et al. 2006). Specific surface area (SSA), micropore volume, and micropore size were measured using nitrogen Brunauer Emmett Teller (BET) isotherms on a Micromeritics Gemini 2375 volumetric analyzer.

**Chemical properties**

The pH value was determined with a pH meter (Multi 340i, WTW) after mixing 2 g of milled (<2 mm) material with 20 mL of 0.01 M CaCl2. Electrical conductivity (EC) was measured using nitrogen Brunauer Emmett Teller (BET) isotherms on a Micromeritics Gemini 2375 volumetric analyzer.

**Column experiments**

We filled organic glass columns (i.d.: 20 mm, height: 400 mm) with 100 g of the mixed substrates: sand and OS. The mass fractions of OS in the mixed substrates were 100, 80, 60, 40, 20, and 0%, respectively. Swine wastewater from an anaerobic tank was supplied to the top of each column continuously at a rate of approximately 6.28, 12.56, and 18.84 mL (d·column)⁻¹ [HLR = 0.02, 0.04, and 0.06 m³ (m² d)⁻¹]. The effluent and the volume of each column were measured daily to estimate actual loading, and the flow to each column was continued until the effluent concentration of TP (Cₑ) approached the influent concentration of TP (C₀). A total of 54 columns with three parallels were used for each experimental condition. Two replications were conducted for the column tests.

**Vertical subsurface flow constructed wetlands**

VSSFs were set up under three different HLRs [0.02, 0.04, and 0.06 m³ (m² d)⁻¹]. Each VSSF, made of polyethylene tank with a radius of 0.56 m and depth of 1.30 m, was packed with 100 cm of OS (2 mm) as the substrate layer, and 20 cm of gravel (10–15 mm) as the bottom underdrainage layer. Phragmites australis with an initial height of about 30 cm was planted at a density of 16 plants m⁻² in each system. Swine wastewater from an anaerobic tank (as described under ‘Column experiments’) was pumped continuously into the top of each VSSF throughout the experimental period (January–December 2011), and the effluent flowed out through two drain pipes connected to the bottom of each VSSF. A total of nine VSSFs were constructed, as three parallel VSSFs were operated under each HLR.

**Determination of phosphorus in water samples, plant samples, and substrate samples from VSSFs**

Water samples were collected every 4 d from the inlet and outlet of each VSSF for analysis of TP and SRP. TP and SRP levels were determined using a Hach DR/5000 spectrophotometer according to standard operating procedures (APHA 2005). At the end of all experimental runs, each VSSF was excavated and substrate samples were collected for laboratory experiments. Substrate samples were collected at the depths of 12.5, 37.5, 62.5, and 87.5 cm from each VSSF. TP, inorganic phosphorus, and organic phosphorus of the substrate samples were measured according to standard methods (Yang et al. 2006). The sequential extraction of inorganic phosphorus was conducted according to a method described by Hartikainen (1979). Further, samples of Phragmites australis were rinsed with deionized water and cut into sections. The samples were then dried at 80 °C to constant dry weight and digested with H₂SO₄ and H₂O₂ for TP analysis (Du Laing et al. 2006). Three parallels were used for all measurements.
RESULTS AND DISCUSSION

Physical properties

The physical properties of OS can be seen in Table 1. In this study, OS had a SSA of 9.94 m² g⁻¹. The OS used herein had a comparatively higher SSA than those of other candidate substrates reported in other studies, which included ranges of 2.6–3.9 m² g⁻¹ (Sakadevan & Bavor 1998) and 6.8–31.4 m² g⁻¹ (Drizo et al. 1999). Physical properties indicated that OS had an adequate surface area for biofilm growth and attachment. Table 1 also shows that the mean micropore size of OS was 2.02 μm. Roseth (2000) reported that biofilm bacteria required a growth environment that was typically 1–3 μm, and thus OS provided a suitable environment for biofilm bacterial growth.

Chemical properties

OS EC, which indicates the total soluble metal ion content of OS, was 2,840 μS cm⁻¹ (Table 1). Phosphorus may react with calcium, iron, and aluminum ions released by the substrates and precipitate as insoluble compounds in the interstitial water of CWs, and the adsorption and precipitation within substrates constitute the main phosphorus sink in CWs (Vymazal 2007). OS alkalinity had little influence on microbial growth, and the content of inorganic phosphorus was much higher than that of organic phosphorus in raw OS. Most phosphorus in raw OS was found as Ca–P.

Effect of OS content on the breakthrough curves

We conducted column experiments to estimate the longevity of phosphorus removal, as well as OS phosphorus retention capacity. When the sorption zone moves up and the upper edge of this zone reaches the bottom of the column, the effluent concentration starts to rise rapidly. The point where the effluent concentration of TP reaches 95% (Ct/C0 = 0.95) of its influent value is called the point of column saturation (Suksabye et al. 2008). A total of 100 g of mixed substrates, with 0, 20, 40, 60, 80, or 100% OS content, was mixed into sand, and swine wastewater was loaded continuously at 6.28 mL (d-column)⁻¹ [HLR = 0.02 m³ (m² d)⁻¹]. The breakthrough curve, Ct/C0 versus volume, is shown in Figure 1. At an OS content of 0%, about 0.19 (Ct/C0) was obtained at first, then the Ct/C0 value approached 0.99, and the saturation state was reached where almost no adsorption occurred at the volume of 0.48 L. When OS content was 20, 40, 60, 80, and 100%, respective values of about 0.02, 4.12 x 10⁻³, 1.66 x 10⁻³, 8.04 x 10⁻⁴, and 4.36 x 10⁻⁴ (Ct/C0) were obtained at first. The saturation state, where no adsorption occurred, was reached at respective volumes of 1.42, 2.85,
3.15, 4.09, and 4.40 L. The saturated volumes for 0, 20, 40, 60, 80, and 100% OS were expressed as the following linear regression equation:

\[ Y = 0.0248X - 18.42 \quad (r = 0.9983, \text{significance level} = 0.01) \]

Further, the higher the OS content was, the higher the effluent pH value was (Figure 1).

**Effect of HLR on the breakthrough curves**

We also investigated the effect of HLRs from 0.02 to 0.06 m³/(m²·d) on the breakthrough curves for columns packed with 100 g OS. An increase in HLR at an influent concentration of 83.64 mg L⁻¹ decreased the saturated volume because of a decrease in empty bed contact time (EBCT) (Figure 1). The lower the EBCT was, the less effective the diffusion process became, resulting in a lower phosphorus adsorption capacity (Sarin et al. 2006). The OS saturated volumes under three different HLRs were as follows: 4.40 L under 0.02 m³/(m²·d), 4.24 L under 0.04 m³/(m²·d), and 3.30 L under 0.06 m³/(m²·d). An increase in HLR also decreased the effluent pH value (Figure 1).
Analysis of phosphorus retained in columns

The phosphorus adsorption capacity of each column was examined and is shown in Figure 1. At 0% OS, the phosphorus adsorption capacity of the mixed substrate was about 0.08 mg g\(^{-1}\). As the OS percentage increased, the phosphorus adsorption capacity gradually increased. When the OS content was 20, 40, 60, 80, and 100%, the respective phosphorus adsorption capacity was about 0.65, 1.36, 1.94, 2.58, and 3.17 mg g\(^{-1}\). The phosphorus adsorption capacity for 0, 20, 40, 60, 80, and 100% OS content was expressed as the following linear regression equation:

\[
Y = 32.02X - 2.18 \quad (r = 0.9995, \text{ significance level } = 0.01).
\]

The injected swine wastewater only slightly increased the organic phosphorus content, while inorganic phosphorus increased as the content of OS increased. So, inorganic phosphorus made up most of the TP content in swine wastewater. Inorganic phosphorus content in the substrates gradually increased as the OS content increased (Figure 1).

The results also revealed that the largest amount of inorganic phosphorus was bound to Ca, followed by O–P, loosely bound P (Loosely-P), Al–P, and Fe–P; this is because OS could release a great quantity of Ca\(^{2+}\) in solution, which would react with PO\(_4^{3-}\) and precipitate as insoluble Ca–P compounds. The longevity of CWs vis-à-vis phosphorus saturation could be extended by the addition of OS. As the HLR increased from 0.02 to 0.06 m\(^3\) (m\(^2\) d\(^{-1}\)), a decrease in the OS phosphorus adsorption capacity from 3.17 to 2.00 mg g\(^{-1}\) was observed (Figure 1). This corresponded to a decrease in saturated volume from 4.40 to 3.30 L. Based on the discussion of the HLR effect, it was suggested that lower HLR or longer contact time was required for phosphate removal in these column tests.

Phosphorus removal performance of VSSF

The VSSF phosphorus removal (SRP and TP) performances are shown in Figure 2. The findings showed that 75.53 mg L\(^{-1}\) (90.30% of TP) was in the form of SRP, and the removal of SRP mirrored the pattern of TP removal. The VSSF exhibited stable phosphorus removal rates under three HLRs, whereas phosphorus removal in the VSSF system decreased as HLR was increased. When the HLR was 0.02 m\(^3\) (m\(^2\) d\(^{-1}\)), mean SRP and TP removal rates were 96.80 and 95.88%, respectively, and the effluent concentration of TP was in the range of 3.56 mg L\(^{-1}\). When the HLR was increased to 0.06 m\(^3\) (m\(^2\) d\(^{-1}\)), mean SRP and TP removal rates decreased to 87.76 and 87.96%, respectively. The VSSF also performed better at treating the other swine wastewater pollutants at an HLR of 0.02 m\(^3\) (m\(^2\) d\(^{-1}\)), and the average removal efficiencies were as follows: 95.38% for SS, 94.86% for COD, 97.47% for BOD, 72.43% for TN, 86.62% for NH\(_4^+\)-N, and 88.96% for TKN, respectively. The effluent concentration of each pollutant did not surpass the national discharge standard of livestock wastewater. Therefore, to ensure the pollutant removal efficiencies, an HLR of 0.02 m\(^3\) (m\(^2\) d\(^{-1}\)) was recommended for the VSSF.

Phosphorus removal pathways of VSSF

Following all experimental runs, we determined the phosphorus retained in each VSSF, as well as the phosphorus uptake by plants. Phosphorus uptake levels by plants were 5.68, 5.83, and 5.73 g, respectively (Table 2). These values did not differ significantly from one another when the HLR was increased from 0.02 to 0.06 m\(^3\) (m\(^2\) d\(^{-1}\)). Thus, the effect of plant uptake in VSSFs was not significant enough to improve phosphorus removal overall. Kim & Geary (2001) also reported that the net effect of harvesting plant biomass in a dairy wastewater treatment system under high loading conditions was a removal of less than 5% TP. The present results reflected this: by subtracting plant phosphorus uptake, respective phosphorus amounts that remained in VSSFs were 0.60 kg under 0.02 m\(^3\) (m\(^2\) d\(^{-1}\)), 0.59 kg under 0.04 m\(^3\) (m\(^2\) d\(^{-1}\)), and 0.55 kg under 0.06 m\(^3\) (m\(^2\) d\(^{-1}\)). Therefore, the main VSSF phosphorus removal mechanisms might be microbial immobilization, and adsorption and precipitation within OS. However, Wang & Mitsch (2000) showed that phosphorus microbial immobilization from the water column was low: it represented about 14% of the phosphorus inflow. Further, most assimilated phosphorus was released back to the water column through bottom detritus, and there was no significant correlation found between microorganisms and TP removal rate (Liang et al. 2004). Accordingly, the main removal mechanism for phosphorus in VSSF should be adsorption and precipitation within OS, although the level of removed phosphorus decreased from 0.61 kg under 0.02 m\(^3\) (m\(^2\) d\(^{-1}\)) to 0.56 kg under 0.06 m\(^3\) (m\(^2\) d\(^{-1}\)).

Distribution characteristics of phosphorus retained within substrate layer of VSSF

We analyzed the TP contents within substrate layers at different depths under three different HLRs in VSSFs (Figure 2). TP distribution characteristics were attributed to the vertical flow state of wastewater in each system; the content of TP in the upper substrate layer was much
Figure 2  Phosphorus removal (a-1, a-2) and retention characteristics (b-1, b-2, c-1, d-1, and d-2) of VSSF. (a) Removal efficiency of SRP and TP of the VSSF system under three different HLRs. (b) Distribution characteristics of phosphorus retained within the substrate layer of VSSF when the HLR was 0.02 m³ (m² d⁻¹). (c) Distribution characteristics of phosphorus retained within the substrate layer of VSSF when the HLR was 0.04 m³ (m² d⁻¹). (d) Distribution characteristics of phosphorus retained within the substrate layer of VSSF when the HLR was 0.06 m³ (m² d⁻¹).
higher than that in the lower substrate layer. We also investigated the distribution characteristics of inorganic phosphorus; the high percentage of inorganic phosphorus in TP showed that adsorption and precipitation within OS played the greatest role in phosphorus removal. Once the upper substrate layer was saturated, the content of phosphorus in the lower substrate layer would increase gradually. Furthermore, the contents of organic phosphorus in the substrate layer at different depths were investigated: they were low and were not significantly different from one another. This demonstrated that microbial-immobilized phosphorus typically constituted a small fraction of the TP loaded into the system. We identified phosphorus forms on the substrates by the method of chemical extraction. Specifically, we performed sequential extraction of the samples to investigate the main phosphorus fractions in the substrate layer under the three different HLRs (Figure 2).

In the substrate layer, Ca–P was the predominant form of phosphorus under the three different HLRs. This was because most phosphorus in sewage reacted with calcium ions released by OS and precipitated as insoluble compounds in the interstitial solution of the substrate layer. The distribution characteristics of Ca–P corresponded to the distribution characteristics of TP in the substrate layers: this was further evidence that OS played the greatest role in adsorption and precipitation with phosphorus.

### Table 2 | Phosphorus removal pathways of VSSF

<table>
<thead>
<tr>
<th>HLR/m³ (m² d⁻¹)</th>
<th>Aboveground/underground phosphorus content of plant/mg g⁻¹</th>
<th>Aboveground/underground biomass of plant/kg</th>
<th>Phosphorus uptake of plant/g</th>
<th>Phosphorus removed by microorganism and OS/kg</th>
<th>Phosphorus retained in VSSF/kg</th>
<th>Contribution of plant uptake/%</th>
<th>Contribution of microorganism and OS/%</th>
</tr>
</thead>
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<tr>
<td>0.02</td>
<td>2.19/3.10</td>
<td>1.85/0.52</td>
<td>5.68</td>
<td>0.60</td>
<td>0.61</td>
<td>0.94</td>
<td>99.06</td>
</tr>
<tr>
<td>0.04</td>
<td>2.21/3.14</td>
<td>1.90/0.52</td>
<td>5.83</td>
<td>0.59</td>
<td>0.60</td>
<td>0.98</td>
<td>99.02</td>
</tr>
<tr>
<td>0.06</td>
<td>2.17/3.09</td>
<td>1.87/0.54</td>
<td>5.73</td>
<td>0.55</td>
<td>0.56</td>
<td>1.03</td>
<td>98.97</td>
</tr>
</tbody>
</table>

**CONCLUSIONS**

OS, a byproduct of mariculture in Fujian Province, was shown to be suitable for use as a substrate in CWs. The physicochemical characteristics of OS make it a suitable candidate for a substrate in CWs that will enrich microorganism and plant growth. OS had a significant phosphorus adsorption capacity when treating swine wastewater, ranging from 3.17 to 2.00 mg g⁻¹ as the HLR was increased from 0.02 to 0.06 m³ (m² d⁻¹), 2.55 y under 0.04 m³ (m² d⁻¹), and 1.27 y under 0.06 m³ (m² d⁻¹), respectively. However, the conditions that best mimic the situation in real CWs were difficult to construct in this experiment. Additionally, the VSSF phosphorus removal capability was influenced by water level, plants and biofilm (Liang et al. 2004), so the VSSF phosphorus saturated time is debatable.

### Expected service life of VSSF

The lifetime of a CW can be estimated by multiplying the retention capacities of the substrates by the volume of the substrate layer (Søvik & Klove 2005). This calculation is based on the assumption that the entire CW substrate layer would be saturated with phosphorus at the end of its functional lifetime. In the column experiments of the present study, OS phosphorus removal capacity was estimated to be about 3.17 mg g⁻¹ under 0.02 m³ (m² d⁻¹), 2.67 mg g⁻¹ under 0.04 m³ (m² d⁻¹), and 2.00 mg g⁻¹ under 0.06 m³ (m² d⁻¹), respectively. The saturation time for the phosphorus mass input was 6.05 y under 0.02 m³ (m² d⁻¹), 2.55 y under 0.04 m³ (m² d⁻¹), and 1.27 y under 0.06 m³ (m² d⁻¹), respectively. However, the conditions that best mimic the situation in real CWs were difficult to construct in this experiment. Additionally, the VSSF phosphorus removal capability was influenced by water level, plants and biofilm (Liang et al. 2004), so the VSSF phosphorus saturated time is debatable.
for enhancing wastewater treatment. Such development comes at a time when landfill spaces are decreasing and alternatives to OS disposal are being sought.

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