

The enhanced effect of activated sludge attached to the roots of *Pistia stratiotes* on nutrient removal for secondary effluent

Jian-Zhen Zhou, Xian-Qiu Zhang, Wen-Ming Xie, Shun-Qing Yang, Ming-Xia Du, Ke-Wei Dai and Xue Han

ABSTRACT

Aquatic plants are widely used for treating wastewater treatment plant secondary effluent. During this process, some residual activated sludge in the secondary effluent is intercepted and attaches to the plant roots. However, the effect of the attached activated sludge on nutrient removal in secondary effluent has up to now been unknown. Aiming at this problem, this investigation was conducted to compare the nutrient removal rates in secondary effluent by washed *Pistia stratiotes* (washed batch) and *Pistia stratiotes* with activated sludge attached to the roots (study batch). Extracellular polymeric substances (EPS) from the activated sludge attached to the roots were extracted and characterized by three-dimensional excitation emission matrix (3D-EEM) fluorescence spectroscopy. The results showed that the nutrient removal rates in the study batch were better than that in the washed batch. The 3D-EEM results showed that the protein content of EPS increased during the experiment, indicating the growth of microorganisms in the attached activated sludge. Our work demonstrated the enhanced effect of activated sludge attached to the roots of *Pistia stratiotes* on the removal of pollutants in secondary effluent, which is useful to guide the practical engineering of secondary effluent treatment.

Key words | activated sludge, nutrient removal, *Pistia stratiotes*, secondary effluent

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INTRODUCTION

Although activated sludge technology can remove most of the pollutants in municipal wastewater, the residual nitrogen, phosphorus and chemical oxygen demand (COD) in secondary effluent can still be significant, which deteriorates the receiving water quality (Giraldo & Garzon 2002; Wang *et al.* 2013). Other effective methods should be adopted to further remove these pollutants. An aquatic plant treatment system (APTS) is one of the most commonly used technologies for secondary effluent purification for the advantages of good purification efficiency, environmental friendliness, easy management and low cost (Sun *et al.* 2009). In recent decades, APTS has been used to remove nutrients and heavy metals from wastewater. Studies have shown that APTS can effectively remove pollutants from industrial wastewater (Yapoga *et al.* 2013), livestock and poultry wastewater (Sooknah & Wilkie 2004; Akinbile & Yusoff 2012; Chen *et al.* 2014), domestic sewage (Vaillant *et al.* 2003) and landfill

leachate (El-Gendy *et al.* 2006). It has also been used for treating the eutrophication in Lake Massaciuccoli in Italy and Lake Dianchi in China (Ciurli *et al.* 2009; Wang *et al.* 2012).

Past studies have shown that aquatic plants are highly efficient in purifying urban sewage tail water (Kim & Kim 2000; Kumari & Tripathi 2014). However, the effluent from the secondary settler usually contains suspended solids, namely residual activated sludge, which can be intercepted and attaches to the plant roots. The effect of the attached activated sludge on nutrient removal has up to now been unknown.

In this study, the effects of the attached activated sludge on nitrogen, phosphorous and COD removal in the secondary effluent were investigated by batch experiments. The first batch was a control with effluent alone to evaluate the effect of microorganisms in raw water on the pollutant removal. The second batch was effluent with washed *Pistia stratiotes*

(washed batch) and the third batch was effluent with *Pistia stratiotes* with activated sludge attached to the roots (study batch). The plant height, root length and nutrient removal were measured and analyzed. The mechanism for the activated sludge attached to the roots of *Pistia stratiotes* to affect nutrient removal was explored with three-dimensional excitation emission matrix (3D-EEM) fluorescence spectroscopy. Hopefully the results of this study will be useful for the design of secondary effluent polishing.

METHODS

Test plants and water samples

Pistia stratiotes, a perennial floating herbaceous plant, belongs to the Araceae family. The plants were cultured in sewage water with 3 g L⁻¹ activated sludge for 2 weeks for acclimation and activated sludge attachment. The plants with similar size and root length were selected for batch experiments. The activated sludge and sewage used in the experiment were both collected from the local wastewater treatment plant (WWTP) in Nanjing, China. The influent to this WWTP is a mix of about 80% domestic wastewater and 20% industrial wastewater. The secondary effluent characteristics are shown in Table 1.

Batch experiments

The experiment was divided into three batches and each batch was performed twice at the same time. Batch A, the control batch, contained 6 L secondary effluent without plants to evaluate the effect of microorganisms in raw water on the pollutant removal. Batch B, the washed batch, contained 6 L secondary effluent and two *Pistia stratiotes*, whose roots had been washed using deionized water to remove the activated sludge on the roots. Batch C, the study batch, contained 6 L secondary effluent and two *Pistia stratiotes* plants with activated sludge attached to the roots. The dry weight of the activated sludge in batch C was about 1.2 g, according to the dry weight of the washed activated sludge from the other two plants with similar size and root length. The wet weight of plants

in batch B and batch C was about 50 g (25 g per plant). The experiment was conducted under natural light to ensure that the plants grew normally and the water temperature was maintained within 20–22 °C.

The plant height, root length, COD, NH₄⁺-N, NO₃⁻-N, total nitrogen (TN) and total phosphorus (TP) were measured every other day. Extracellular polymeric substances (EPS) were extracted from the activated sludge in batch C at the beginning and the end of the experiment to analyze the three-dimensional fluorescence.

Analysis

The COD, NH₄⁺-N, NO₃⁻-N, TN and TP were measured following *Standard Methods* (APHA 1998). The plant height and root length were measured with a ruler. The sludge EPS were extracted using a thermal extraction process. The activated sludge was firstly centrifuged for 10 minutes at 4,000 rpm, then washed with 0.9% NaCl solution and centrifuged for 10 minutes again at 4,000 rpm to remove the supernatant (repeated twice). Secondly, the activated sludge was re-suspended with 0.9% NaCl solution and heated for 1 h at 80 °C, then centrifuged for 10 minutes at 5,000 rpm. The supernatant was filtered as the test solution of EPS (Morgan et al. 1990). EPS extraction was characterized by EEM with a fluorescence spectrophotometer (Lumina, Thermo, USA). The scanning emission wavelength (Em) ranged from 280 to 550 nm at 1 nm increment by varying the excitation wavelength (Ex) from 200 to 400 nm at 5 nm increments. The scanning speed was set at 2,400 nm min⁻¹ for all measurement. The spectrum of the deionized water was recorded as the blank (Sheng & Yu 2006). Raman scattering was removed by subtracting the EEM spectrum of the Milli-Q water blank, and Rayleigh scattering was removed using interpolation.

RESULTS AND DISCUSSION

Batch experiments were repeated twice at the same time. The results were reported as the mean and standard deviation of the two repetitions.

The growth of aquatic plants

The changes of plant height and root length in batches B and C are presented in Figure 1. The results showed that the plant height and root length in batch B and batch C both increased without significant difference between the two

Table 1 | The characteristics of secondary settler effluent (mg L⁻¹, except pH)

TP	TN	NH ₄ ⁺ -N	NO ₃ ⁻ -N	COD	pH
0.48	12.83	1.33	8.24	48.08	7.95

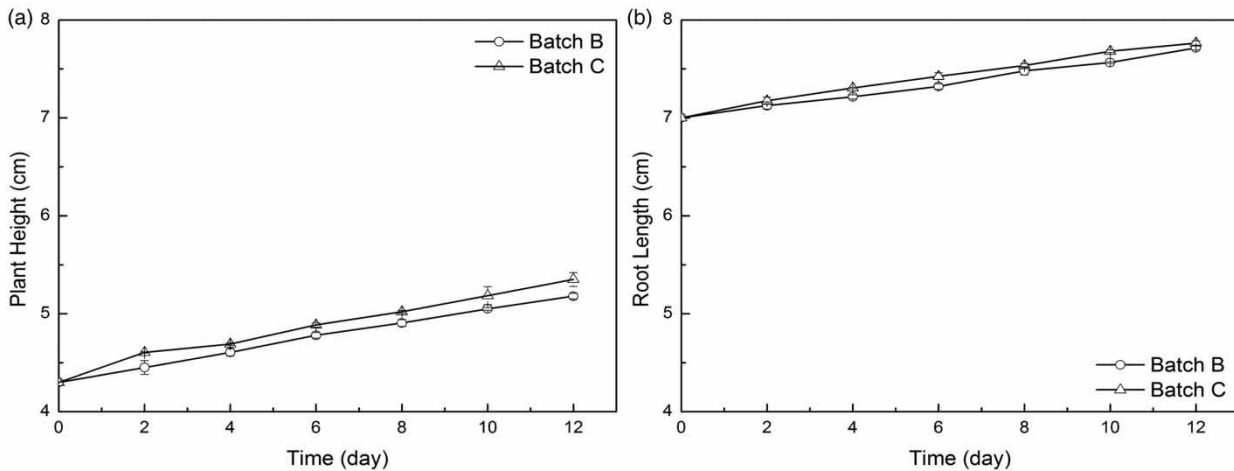


Figure 1 | The changes of plant height (a) and root length (b) in batches B and C.

batches, suggesting that the attached activated sludge had no remarkable effect on plant growth.

The changes of nutrient removal

The changes of COD are shown in Figure 2. In batch A, COD slightly decreased, indicating the degradation of organic pollutants in the secondary effluent caused by microbial activity. Compared with batch A, both batch B and batch C showed a significant decrease in COD. However, the COD removal in batch C was more considerable than that in batch B, demonstrating that the removal rate of organic pollutants was enhanced by the attached activated sludge.

As shown in Figures 3–5, the concentrations of $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$ and TN in batch A showed a very slight decrease,

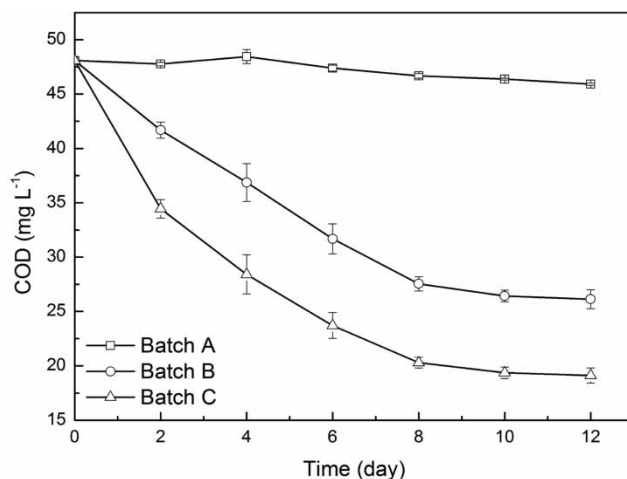


Figure 2 | The changes of COD in batches A, B and C.

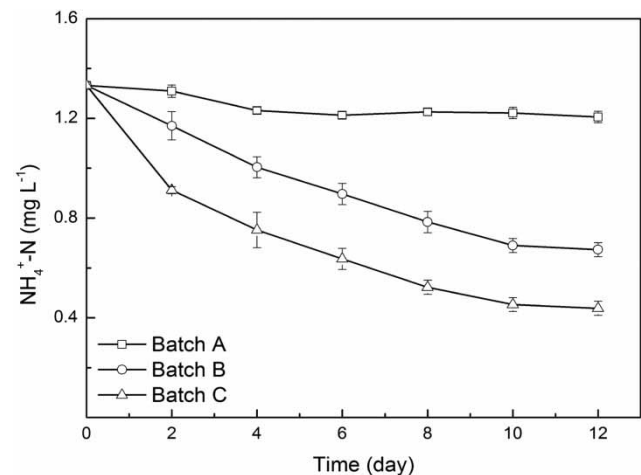


Figure 3 | The changes of $\text{NH}_4^+\text{-N}$ in batches A, B and C.

similar to the change in COD. The nutrient concentration in batch B and batch C both decreased significantly. However, batch C removed nutrients more rapidly and in greater amounts than batch B.

In our systems, the nitrogen could be mainly removed by two mechanisms, one is the absorption of *Pistia stratiotes*, and the other is the degradation of microorganisms in the attached activated sludge. *Pistia stratiotes* could effectively absorb ammonia nitrogen and nitrate nitrogen. This is consistent with the results of Awuah et al. (2004) and Akinbile & Yusoff (2012). At the same time, the role of the microorganisms in attached activated sludge in nitrogen removal cannot be ignored (Jayaweera & Kasturiarachchi 2004). In our experiment, the nitrogen removal rate in batch C was higher than that in batch B. This accelerated removal in batch C can be ascribed to the nitrification and

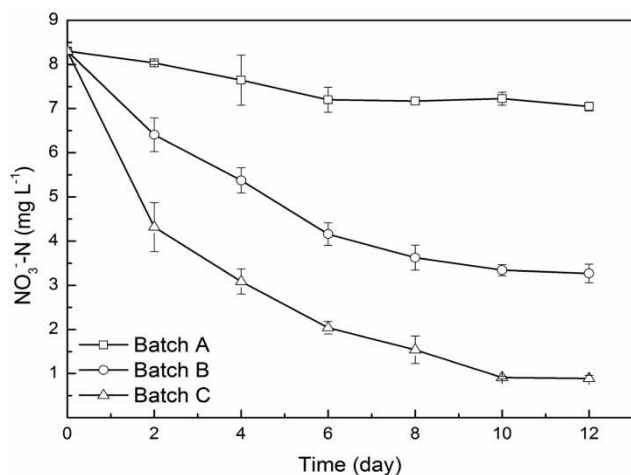


Figure 4 | The changes of NO₃⁻-N in batches A, B and C.

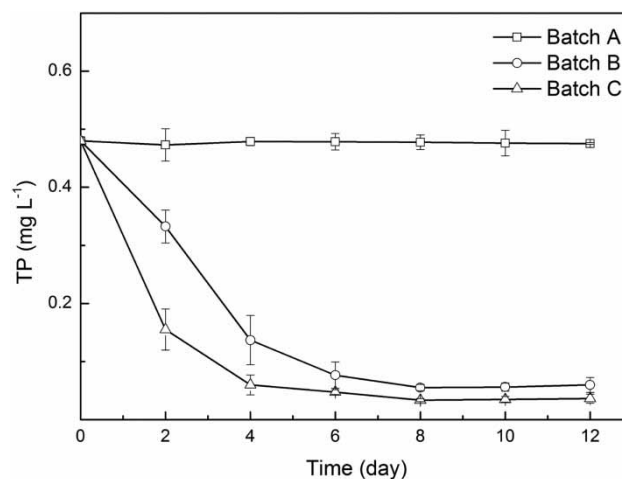


Figure 6 | The changes of TP in batches A, B and C.

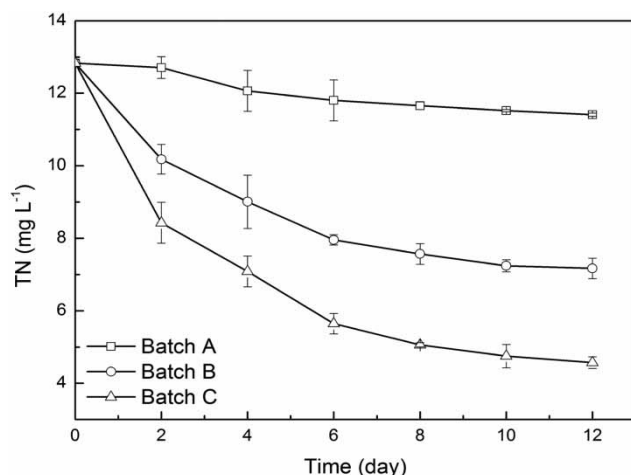


Figure 5 | The changes of TN in batches A, B and C.

denitrification by microorganisms in the attached activated sludge. In batch C, the dissolved oxygen concentration was 2–3 mg L⁻¹ during the daytime but it was lower than 1 mg L⁻¹ at night, which would make nitrification and denitrification possible. In short, it was the nitrification and denitrification of microorganisms in the attached activated sludge that promoted nitrogen removal.

The changes of TP in batch experiments are shown in Figure 6. The result showed that the TP in batch B and batch C decreased significantly without obvious difference between the two batches at the end of the experiment. It was demonstrated that *Pistia stratiotes* itself had good TP removal ability and could reduce TP to very low concentrations by continuously absorbing phosphorus from the water over a number of days. This is consistent with the results of Lu *et al.* (2010) and Gupta *et al.* (2012). However,

in the early stage of the experiment, batch C removed TP more rapidly than batch B. The adsorption by the attached activated sludge could be responsible for this difference (Zhang *et al.* 2013).

Nutrient removal rates at the end of the experiment

The average nutrient removal rates at the end of the experiment are shown in Table 2. It is clearly shown that the order of removal rates was batch C > batch B > batch A, suggesting the enhanced removal of pollutants by the activated sludge attached to the roots of *Pistia stratiotes* in the secondary effluent.

EEM results of extracted activated sludge EPS

EPS are generally considered to be the microbial extracellular macromolecules, which are mainly secreted by microorganisms. When microorganism are under sufficient substrate conditions, enhanced secretion of EPS occurs with microorganism growth (Higgins & Novak 1997; Wilen *et al.* 2003). The 3D-EEM spectroscopy was proven to be an effective method to characterize the EPS (Sheng & Yu 2006; Ni *et al.* 2009), and the fluorescence peak at

Table 2 | The average removal rates of COD, NH₄⁺-N, NO₃⁻-N, TN and TP at the end of the experiment

Batches	COD	NH ₄ ⁺ -N	NO ₃ ⁻ -N	TN	TP
Batch A	4.5%	9.5%	15.1%	11.1%	2.0%
Batch B	45.7%	49.5%	60.6%	44.1%	87.6%
Batch C	60.2%	67.1%	89.3%	64.4%	92.5%

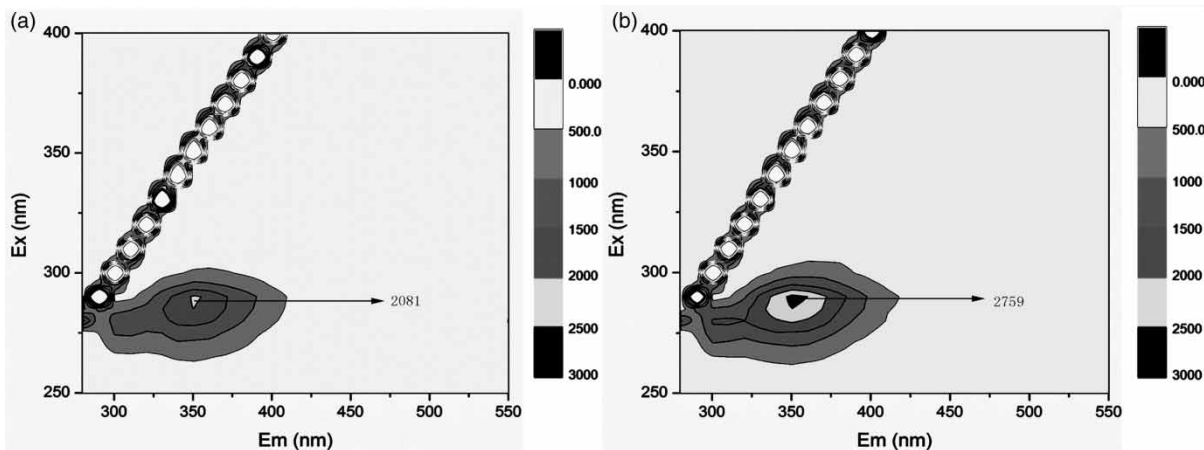


Figure 7 | The changes of EPS-EEM of batch C at the beginning (day 0) and the end (day 12) of the experiment.

the excitation/emission wavelengths of 290/350 nm is assigned to proteins (Wei et al. 2014). At the beginning and the end of batch experiments, the EPS were extracted from the activated sludge and characterized by EEM, and the result is shown in Figure 7. The fluorescence peak intensity at the excitation/emission wavelengths of 290/350 nm increased at the end of the experiment, indicating the growth of microorganisms in the attached activated sludge. Therefore, it was the microorganisms in the attached sludge that improved the removal of nutrient in batch C.

CONCLUSION

In this study, batch experiments were conducted to compare nutrient removal in a secondary effluent by washed *Pistia stratiotes* (washed batch) and *Pistia stratiotes* with activated sludge attached to the roots (study batch). The results showed that the nutrient removal rates in the study batch were better than those in the washed batch. The 3D-EEM results showed that the better nutrient removal in the study batch was due to the microorganisms in the attached activated sludge. The synergistic effect of *Pistia stratiotes* and attached activated sludge can enhance pollutant removal in secondary effluent.

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