**Improving Nutrient and Organic Matter Removal by Novel Integration of a High-Rate Algal Pond and Submerged Macrophyte Pond**

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**Abstract**

This study investigated the remediation effect of a high-rate algal pond (HRAP) and submerged macrophyte pond (SMP) on nutrient and organic matter removal, and explored the effect of SMP on ecological elimination of algae biomass. The results showed that submerged macrophytes could be effective in inhibiting flourishing algae growth. An HRAP-SMP hybrid system exhibited better removal performance than single HRAP and SMP reactor, and 45.6±2.0% TN, 99.0±1.0% TP and 99.0±1.0% DCOD was reduced in the hybrid system. This study demonstrated that the novel integration of HRAP and SMP could be a promising solution for efficient removal of nutrient and organic matter.

**Keywords**: high-rate algal pond, submerged macrophyte pond, algae inhibition, nutrient and organic matter removal

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**Introduction**

Untreated wastewaters are often characterized by their high nutrient and organic matter loads, which consequently cause eutrophication of water bodies [1]. It is increasingly recognized that wastewater should be treated in an ecological and sustainable way for improving water quality. Conventional treatment techniques entail high investment and operational costs [2]. In this regard, algae-based technologies exhibit great promise for wastewater treatment due to their cost-effectiveness and high-quality effluent [3-4]. A high-rate algal pond (HRAP) has been developed for microalgae cultivation in which a large quantity of wastewater can be treated [5]. Algae can utilize nutrients and high levels of organic carbon from wastewater for its flourishing growth, thus contributing to water purification [6].

However, surplus algae in the effluent will pose great threats to people’s lives and production [7]. Therefore, control and elimination of algae biomass has been a significant goal in the aquatic ecosystem. The mechanical removal of algae biomass is impractical while the chemical treatment is undesirable in potable water supplies [8]. The allelopathic inhibition of submerged macrophytes on algae growth is extremely promising due to its low cost and high...
environmental safety. Previous studies have reported that Ceratophyllum demersum, Myriophyllum spicatum and Vallisneria natans could inhibit growth of microalgae by releasing allelochemicals into water [9-11]. Furthermore, submerged macrophytes play an important role in the restoration of lakes suffering from excessive nutrient and organic matter inputs. Submerged macrophytes growing under water provide higher root surface area for microbial growth. They not only absorb high amounts of nutrients but also provide nutrients and oxygen for microbes [12]. Microbial nitrification-denitrification has the most dominant effect on overall nitrogen removal [13]. Oxygen transfer by macrophytes into the root zone plays a significant role in promoting the nitrification process and supporting aerobic bacteria degradation of organics in the wastewater. In summary, submerged macrophytes can contribute to keeping a healthy aquatic ecosystem. Therefore, it is reasonable to use a submerged macrophyte pond (SMP) for deep-purifying HRAP effluents.

In this study, the integration of HRAP and SMP provide a novel and cost-effective treatment system for water purification. However, information on the integration of HRAP and SMP as well as their synergistic effect on nutrient and organic matter removals was unknown. In addition, insufficient knowledge about the operating mode of the hybrid system impeded the optimization of the hybrid system. The study aims to evaluate the variations of algae biomass, nutrient and organic matter in experimental units (single HRAP, single SMP, and hybrid system), evaluate the effect of SMP on optimization of HRAP effluent quality, discuss the effect of hydraulic retention time (HRT) on the performance of hybrid systems, and obtain the optimal operating mode for enhancing nutrient and organic matter removal.

Material and Methods

The present study was conducted in four lab-scale HRAP-SMP hybrid systems located outdoors at Donghua University, Shanghai, China (38°39′27″N, 104°04′58″E). The dimensions (L × W × D) of the HRAP and SMP reactor were identical at 0.96 × 0.60 × 0.60 m, and the total working volume was 300 L. Each HRAP was a single-loop raceway separated by a central baffle, and wastewater in the pond channel was circulated at a velocity of 0.18±0.02 m s⁻¹ by a mechanical stirrer. Algae species Microcystis aeruginosa was obtained from the Institute of Hydrobiology at the Chinese Academy of Sciences, and chlorophyll a (Chl-a) content was determined as an indicator of algae biomass. Synthetic domestic wastewater with tap water was added into experimental units from inlet tanks by peristaltic pumps, and the total inflow was 250±5 L within 1 h. Table 2 shows the composition of the influent wastewater.

The bottom of the SMP reactor was filled with a 10 cm thick layer of fine gravel (Φ = 1.5–2.0 cm), above which was filled with a 5 cm thick layer of washed sand (Φ<0.2 cm) to facilitate macrophyte growth. Perennial submerged macrophytes of Vallisneria natans were collected from Chen Shan Botanical Garden, Shanghai, China (31°04′37.28″N, 121°10′42.76″E), which is widely present in freshwater habitats and eutrophic lakes. Macrophytes with uniform growth (35±5 cm) and similar biomass (30±5 g) were selected. The same plant density (7.5±0.5 g L⁻¹) was maintained.

Experiments were performed in triplicate, and average temperature in the experimental phase (June to October) was 20 to 35ºC. Experiments were carried out in single HRAP (Fig. 1a), single SMP (Fig. 1b) and hybrid systems A and B (Fig. 1c). HRT of four experimental systems were 3d. Of these, single HRAP and SMP were individually operated in an intermittent way with 3d-HRT. Hybrid system A was
operated in an intermittent way with 1d-HRT of HRAP and 2d-HRT of SMP. Hybrid system B was operated in an intermittent way with 2d-HRT of HRAP and 1d-HRT of SMP.

Chl-a content was immediately measured in situ after using a portable water quality multi-probe (Manta 2, EURERA, USA). Total nitrogen (TN, Persulfate Digestion Method), ammonia nitrogen (NH$_4$-N, Nessler’s Reagent Spectrophotometry), nitrate (NO$_3$-N, UV Spectrophotometric Determination), dissolved reactive phosphorus (DRP, Acid Persulfate Reduction Method), total phosphorus (TP, Acid Persulfate Reduction Method) and dissolved chemical oxygen demand (DCOD, Potassium Dichromate Method) were measured using a multi-parameter colorimeter (DR900, HACH, USA) [14]. The samples were filtered through glassfiber filters (0.22 µm) for analysis. All data are presented as mean±SD. Statistical analyses were

### Table 1. Variations of algae concentration with HRT in four experimental systems.

| Systems         | Influent algae content (cells mL$^{-1}$) | Effluent algae content (cells mL$^{-1}$) |
|-----------------|-----------------------------------------|-----------------------------------------|
|                 |                                         | 1d-HRT  | 2d-HRT  | 3d-HRT  |
| Single HRAP     | 580±100                                 | 2652±100 | 13235±200 | 24155±200 |
| Single SMP      | 580±100                                 | 2032±100 | 6787±200  | 8034±200  |
| Hybrid system A | 580±100                                 | 2750±100 | 9212±200  | 12508±200 |
| Hybrid system B | 580±100                                 | 2685±100 | 12952±200 | 19085±200 |

### Table 2. Pollutant removal performance of the four experimental systems.

| Parameters | Influent (mg L$^{-1}$) | System          | Effluent (mg L$^{-1}$) | Removal (%) |
|------------|------------------------|-----------------|------------------------|-------------|
| NH$_4$-N   | 24.0±1.0               | Single HRAP     | 13.7±0.5               | 42.9±2.0    |
|            |                        | Single SMP      | 12.5±0.5               | 47.9±2.0    |
|            |                        | Hybrid system A | 11.4±0.5               | 52.5±2.0    |
|            |                        | Hybrid system B | 12.0±0.5               | 50.0±2.0    |
| NO$_3$-N   | 0.1± 0.1               | Single HRAP     | 0.7± 0.2               | --          |
|            |                        | Single SMP      | 3.0± 0.2               | --          |
|            |                        | Hybrid system A | 2.5± 0.2               | --          |
|            |                        | Hybrid system B | 1.5± 0.2               | --          |
| TN         | 25.2± 1.0              | Single HRAP     | 14.9±0.5               | 40.9±2.0    |
|            |                        | Single SMP      | 15.7±0.5               | 37.7±2.0    |
|            |                        | Hybrid system A | 14.2±0.5               | 43.7±2.0    |
|            |                        | Hybrid system B | 13.7±0.5               | 45.6±2.0    |
| DRP        | 5.40± 0.5              | Single HRAP     | 1.14±0.2               | 78.9±1.0    |
|            |                        | Single SMP      | 0.25±0.2               | 95.4±1.0    |
|            |                        | Hybrid system A | 0.1±0.1                | 99.0±1.0    |
|            |                        | Hybrid system B | 0.1±0.1                | 99.0±1.0    |
| TP         | 5.80± 0.5              | Single HRAP     | 1.30±0.2               | 77.6±1.0    |
|            |                        | Single SMP      | 0.33±0.2               | 94.3±1.0    |
|            |                        | Hybrid system A | 0.1±0.1                | 99.0±1.0    |
|            |                        | Hybrid system B | 0.1±0.1                | 99.0±1.0    |
| DCOD       | 80.0±4.0               | Single HRAP     | 1.0±1.0                | 99.0±1.0    |
|            |                        | Single SMP      | 18.0±3.0               | 77.5±2.0    |
|            |                        | Hybrid system A | 8.0±2.0                | 90.0±2.0    |
|            |                        | Hybrid system B | 1.0±1.0                | 99.0±1.0    |
performed with Origin 8.0 (Origin Lab, MA, USA) statistical software, and significant differences among treatment means \((p<0.01)\) were determined by Student’s \(t\) test.

**Results and Discussion**

During 3 days of treatment, the variations of algae concentration with HRT in four experimental systems are shown in Table 1. Fig. 2 presents the profiles of Chl-a, DCOD, TP and TN in experimental units. As demonstrated by Table 1 and Fig. 2a, algae content in single HRAP increased sharply, whereas it increased gradually in the other three systems followed by a slight fluctuation. The main reason for this phenomenon was the function of submerged macrophytes in SMP. Effluent algae content of single SMP at 3d-HRT \((803±200 \text{ cells mL}^{-1})\) was significantly lower than that \((2415±200 \text{ cells mL}^{-1})\) in single HRAP \((p<0.01)\). The results implied that submerged macrophytes could be effective in inhibiting algae flourishing growth. Hybrid systems A and B were operated with different HRT modes. Effluent algae content of hybrid system A at 3d-HRT \((12508±200 \text{ cells mL}^{-1})\) was significantly lower than that \((19085±200 \text{ cells mL}^{-1})\) in hybrid system B \((p<0.01)\), which was attributed to the longer retention time of wastewater in SMP. In conclusion, it is feasible to use SMP for controlling and decreasing surplus algae from HRAP effluents.

DCOD value gradually declined with HRT in experimental systems (Fig. 2b). Pollutant removal performance of experimental units was presented in Table 2. As presented in Table 2, average DCOD removal in single HRAP and hybrid system B after 3d-HRT was over 99.0%, which was higher than that in single SMP \((77.5±2.0\%)\) and hybrid system A \((90.0±2.0\%)\). The main reason was that dissolved organic matter released from submerged macrophytes led to poor DCOD removal [15]. Hybrid system B exhibited the higher DCOD removal than hybrid System A, which was ascribed to the longer retention time of wastewater in HRAP.

Phosphorus removal mechanisms in the hybrid system include algae assimilation, sedimentation, macrophyte uptake and microbial adsorption [16]. Macrophytes can effectively remove nutrients and play an important part in the restoration of eutrophic waters [17]. As shown in Table 2 and Fig. 2c, reductions in DRP and TP were more significant in hybrid systems A and B \((p<0.01)\) due to the integrated mechanisms for phosphorous removal, where average DRP and TP removal was over 99.0%. Phosphorus removal performance of single SMP was significantly higher than that of single HRAP \((p<0.01)\) due to the high adsorption capacity of *Vallisneria natans*. In summary, besides microbial mechanisms, macrophyte uptake could be an important phosphorus removal pathway.

Algae assimilation and ammonia volatilization are the main N removal mechanism of HRAP, while macrophyte uptake and microbial nitrification-denitrification represents N removal pathway of SMP [18-19]. As demonstrated in Table 2 and Fig. 2d, TN concentration decreased gradually with HRT in experimental units. \(\text{NH}_4^+-\text{N}\) removal performance of single SMP was better than that of single HRAP, whereas single HRAP exhibited higher TN removal than single SMP. \(\text{NH}_4^+-\text{N}\) and TN removal in single SMP were respectively 47.9±2.0% and 37.7±2.0%, and effluent \(\text{NO}_3^--\text{N}\) concentration reached 3.0±0.2 mg L\(^{-1}\). The main reason for this phenomenon was the limitation of microbial denitrification leading to poor TN removal of single SMP. Dissolved oxygen was abundant in single SMP due to photosynthetic macrophytes and algae, which created more favorable conditions for nitrification occurring in oxidized root zone of macrophytes and
inhibited the occurrence of denitrification. Accumulated NO$_3$-N could be partly assimilated by submerged macrophytes, whereas residual NO$_3$-N influenced TN removal. NH$_4$-N and TN reduction in hybrid systems was higher than that in the single HRAP and SMP reactor due to the integrated mechanisms for nitrogen removal. The optimal elimination of NH$_4$-N (52.5±2.0%) and TN (45.6±2.0%) was observed in the hybrid system.

Conclusions

SMP was combined with HRAP for deep purifying HRAP effluents and ecological control of surplus algae. The results demonstrated that SMP is an effective strategy for algae elimination. Better nutrient and organic matter removal performance was obtained in the hybrid system due to the integrated mechanisms for pollutant removal. The novel integration of HRAP and SMP offers more efficient and economical operation process for high-quality effluent. The study may provide an effective option for improving nutrient and organic matter removal from wastewater.

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Conflict of Interest

The authors declare no conflict of interest.

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