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Algal-Bacterial Symbiosis System Treating High-Load Printing and Dyeing Wastewater in Continuous-Flow Reactors under Natural Light

Chao Lin, Peng Cao *, Xiaolin Xu * and Bangce Ye

Key Laboratory for Green Processing of Chemical Engineering of Xinjiang Bingtuan,
School of Chemistry and Chemical Engineering, Shihezi University, Shihezi 832003, China;
linchaoshzu@163.com (C.L.); bcye@ecust.edu.cn (B.Y.)
* Correspondence: Caop@shzu.edu.cn (P.C.); xuxl@shzu.edu.cn (X.X.);
Tel.: +86-132-0109-1039 (P.C.); +86-135-1993-2599 (X.X.)

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Abstract: This study investigated the symbiotic structure relationship between mixed algae and activated sludge while treating high-load printing and dyeing wastewater under natural light. The effects of hydraulic retention time (HRT) (12 h, 16 h and 20 h) and aeration rate (0.1–0.15, 0.4–0.5 and 0.7–0.8 L/min) on algal–bacterial symbiosis (ABS) and conventional activated sludge (CAS) systems. Experimental results showed that the ABS system exhibited the best removal performance for chemical oxygen demand (COD), ammonia nitrogen (NH_4^+-N) and total phosphorus (TP), which was increased by 12.5%, 23.1% and 10.5%, respectively, and reduced colour 80 times compared with the printing and dyeing wastewater treatment plant. Algae growth could be promoted under lower dissolved oxygen (DO), and the addition of algae could provide more DO to the ABS system. The particle size distribution of sludge in the ABS system was stable, which guaranteed a stable treatment effect. In addition, the COD and colour could be further degraded under the conditions of no external carbon source and longer HRT. It is expected that the present study will provide a foundation for the practical application of the ABS system, and new insights for the treatment of printing and dyeing wastewater.

Keywords: Algal-bacterial symbiosis system; hydraulic retention time; aeration rates; continuous-flow reactors; natural light; high-load printing and dyeing wastewater

1. Introduction

An algal–bacterial symbiosis (ABS) system is known as a classic water self-purification process in natural water [1,2]. Under light conditions, algae can not only remove pollutants through its own degradation ability, such as organic matter (COD), NH_4^+-N, total nitrogen (TN) and total phosphorus (TP), but also generate oxygen through photosynthesis [3,4], which is used by bacteria in activated sludge to degrade pollutants [5]. Therefore, the ABS system not only reduces the amount of aeration, but also better removes contaminants compared to the traditional activated sludge process [6,7]. In addition, such systems are particularly useful in areas with high solar radiation and abundant sunshine [8]. In addition, such systems are especially beneficial in areas with high solar radiation and abundant sunshine [8]. Based on the above advantages, the ABS system has attracted great attention as an emerging technology [9,10].

However, there is still some way to go before the actual application of ABS system. First, most of the research on this topic was still in the stage of processing simulated wastewater, in which pollutants are relatively easy to degrade. For example, Ahmad et al. [4] used 300 mg COD/L (sodium acetate), 10 mg $PO_4^{3-}-P$/L (KH_2PO_4), 100 mg NH_4^+-N/L (NH_4Cl), 10 mg Ca^{2+}/L ($CaCl_2 \cdot 2H_2O$), 5 mg Mg^{2+}/L ($MgSO_4 \cdot 7H_2O$) and 5 mg Fe^{2+}/L ($FeSO_4 \cdot 7H_2O$) instead of actual wastewater to study the stability of algal–bacterial granules. Tang et al. [4] studied the effect of aeration on the performance of ABS system by using 150 mg/L glucose, 150 mg/L starch, 151 mg/L NH_4Cl, 47 mg/L KH_2PO_4, 300 mg/L $NaHCO_3$, 50 mg/L $MgSO_4 \cdot 7H_2O$, 5 mg/L $CaCl_2$ and the trace elements of 2.86 mg/L H_3BO_3, 1.86 mg/L $MnCl_2 \cdot 4H_2O$, 0.22 mg/L $ZnSO_4 \cdot 7H_2O$, 0.39 mg/L $Na_2MoO_4 \cdot 2H_2O$, 0.08 mg/L $CuSO_4 \cdot 5H_2O$, mg/L and 0.05 mg/L $Co(NO_3)_2 \cdot 6H_2O$ to add into synthetic wastewater. Second, the ABS system requires a longer HRT when handling contaminants compared to traditional secondary biochemical treatment, which could lead to a reduction in wastewater treatment capacity. Valigore et al. [11] studied the effect of the food to microorganism (F/M) ratio and HRT on the stability of an ABS system and found that the algal–bacterial system performed better with a longer HRT (4.0 days). Third, most research has presently limited to low-load wastewater for a single ABS system at present. Zhao et al. [12] studied the stability of algal–bacterial granular system using low carbon wastewater of COD (200 mg/L): N(50 mg/L) = 4:1, COD (100 mg/L): N (50 mg/L) = 2:1 and COD (50 mg/L): N (50 mg/L) = 1:1. Fourth, most of these studies were conducted in sequential batch reactors (SBR) reactors [10,13]. This is because from an engineer's viewpoint, continuous-flow reactors are more advantageous than SBRs for pilot or industrial-scale applications due to their lower installation cost and easier operation, control and maintenance [14]. Fifth, algae grows naturally when exposed to natural sunlight [15,16]. However, few researchers currently used an ABS system to treat wastewater under natural light conditions. Markou et al. [17] studied the effect of light intensity on the competition between algae and bacteria in an ABS system and the removal effect of nitrogen. The results showed that algae and bacteria exerted the greatest synergistic effect under the light intensity of 244 μmol/($m^2 \cdot s$), and the removal rate of N reached the highest 8.5 mg N/L·day. Meng et al. [13] studied the effects of light intensity on oxygen distribution, lipid production and biological community of algal–bacterial granules in photo-sequencing batch reactors. Results showed that when the light intensity is $\geq$90 μmol/($m^2 \cdot s$), the algae can provide enough oxygen to enhance the biological activity of the bacteria, thus achieving an efficient algal symbiosis system. Although the increase of light intensity has a better removal effect on pollutants, the addition of light intensity will increase in the operating cost of wastewater treatment. More attention needs to be paid to the effect of wastewater treatment under natural light. Therefore, it is necessary to optimize the shortcomings of the ABS system and apply it to actual wastewater.

On the other hand, an ABS system should present good prospects for application to industrial wastewater [18]. Industrial wastewater has complex composition and deep colour [19]. As emission standards increase, the concentration of pollutants in industrial wastewater needs to be reduced. Traditional activated sludge systems have been unable to meet the treatment requirements of industrial wastewater, especially when dealing with refractory wastewater, such as printing and dyeing wastewater. Khataee et al. [20], Daneshvar et al. [21], Khataee et al. [22] and Kousha et al. [23] reported that algae can degrade dyes well and reduce the colour of wastewater.

In this study, continuous experiments were performed using a multifunctional integrated aerobic bioreactor to evaluate the symbiotic relationship between activated sludge and mixed algae. The optimum treatment performance of an ABS system for printing and dyeing wastewater was explored under different HRTs and aeration rates, and the stability of the ABS system was analysed. The effluent colour and undegraded organic matter were further discussed. This study provides a basis for the application of an ABS system to the cleaning of printing and dyeing wastewater.

2. Materials and Methods

2.1. Algae Species and Sludge

Three algae species that degrade disperse blue 2BLN [24], malachite green and purple crystal were used in this research, which were provided by Xu Xiaolin (Shihezi University, Shihezi, China). The first type of algae was *Chlorella* screened from the Xinjiang Cocoto Sea, which was designated

XJK. The second type of algae was *Filamentous* algae screened from Alcanti Hot Springs, which was designated W6. The third type of algae was *Selenastrum bibraianum* screened from a water sample of the 90th regiment (Xinjiang Autonomous Region Production and Construction Corps), which was designated 902. The algae were cultured in flasks before being added to the system, and the culture conditions were as follows: light intensity 830 μmol/(m^2·s), temperature 25 °C, photoperiod L:D = 24:0 and air flow rate 0.4 m^3/h. The sludge used in the experiment came from the aerobic pool of a printing and dyeing wastewater treatment plant in Shihezi. The properties of the sludge are shown in Table 1.

Table 1. Sludge activity index.

Sludge Activity Index	Mlss (Mixed Liquid Suspended Solids)	SV % (Settling Velocity)	SVI (Switch Virtual Interface)
Content	3000 mg/L	24	80

2.2. Wastewater

The wastewater used in the experiment was collected from a regulating pool of a printing and dyeing wastewater treatment plant in Shihezi, which mainly came from two cotton textile factories. The treatment process in the printing and dyeing wastewater treatment plant is shown in Figure 1. The effluent quality in this experiment will be compared with the wastewater entering the secondary settling pool after anaerobic and aerobic treatment in the printing and dyeing wastewater treatment plant. The quality of the wastewater in the regulating pool and secondary settling pool is shown in Table 2.

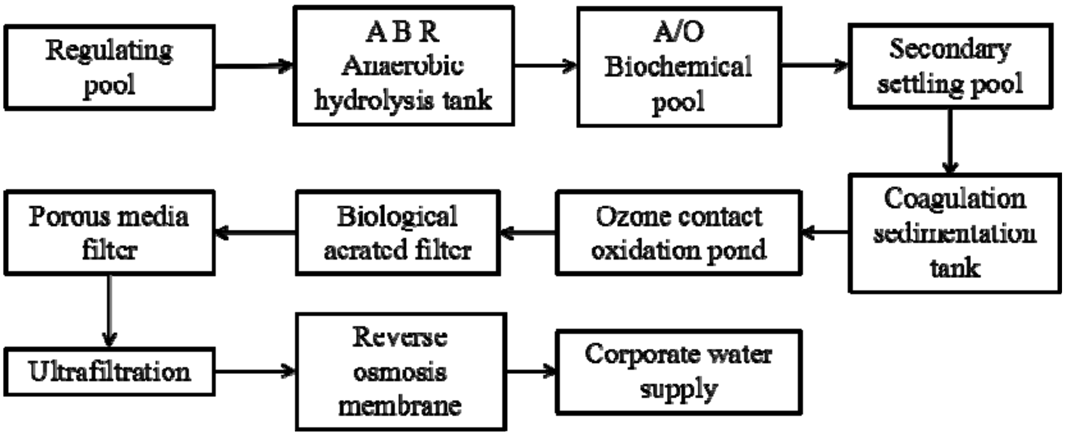

Figure 1. Treatment process in the printing and dyeing wastewater treatment plant.

Table 2. Quality of influent and effluent wastewater from printing and dyeing.

Wastewater Quality	COD (mg/L)	NH_4^+-N (mg/L)	TP (mg/L)	pH	Colour (times)
Regulating pool	650–750	20–30	5.0–6.5	8.5–10.5	500–600
Secondary settling pool	160–220	5–10	4.0–4.5	7.5–8.5	120–160

2.3. Experimental Setup and Operation Conditions

The reactor uses two identical rectangular (30 cm length, 10 cm wide and 22.5 cm high) multifunctional integrated aerobic bioreactors composed of Plexiglass plates, which were used for the printing and dyeing wastewater treatment experiments. The reactor consisted of five parts: an influent zone, a biochemical reaction zone, a three-phase separator, a sedimentation zone and an effluent zone. The effective working volume was 5 L. A structural diagram of the reactor system is shown in Figure 2.

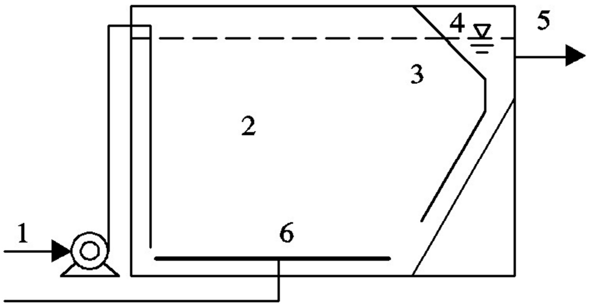

Figure 2. Structure diagram of ABS system and CAS system for printing and dyeing wastewater reactor: (1) influent zone, (2) biochemical reaction zone, (3) three-phase separator, (4) sedimentation zone, (5) effluent zone, (6) Aeration tube.

One reactor was an algal–bacterial symbiosis (ABS) system, operated with mixture of laboratory-grown *Chlorella, Filamentous algae*, and *Selenastrum bibraianum*, mixed with conventional activated sludge, while the other was operated as a conventional activated sludge (CAS) system, in which only the activated sludge was added. Since the operating conditions of the biochemical tank of the printing and dyeing wastewater treatment plant could not be adjusted, a CAS system was established with the same operating conditions in the laboratory. After a period of operation, it was found that the CAS system could reach a treatment level similar to that of the wastewater treatment plant. Therefore, the CAS system was selected as a control to evaluate the pollutant removal ability of the ABS system by changing the same operating parameters.

The experiment was operated at room temperature (25 ± 2 °C) under natural light conditions. The initial mixed liquor suspended solids (MLSS) concentration was 3000 mg/L in each reactor, and the algae-to-sludge ratio of the ABS system was 1:3 (*w*/*w*). The ABS system was obtained using the same cultivation method as described previously [10]. The two reaction systems were run continuously for 95 days, including three phases. In the first phase (1–20 days), the stability of the ABS system and the symbiotic structure were investigated. In the second phase (21–65 days), the removal performance of COD, NH_4^+-N and TP in the two reaction systems under different HRTs were investigated. The optimal HRT was determined. The operating period for each HRT was 15 days, and the sludge residence time was 15 days. The changes of HRT during operation were 12/16/20 h for the two systems. DO conditions were the same: 2.0 ± 0.5 mg/L. In the third phase (66–95 days), under the optimal HRT, the removal performance of COD, NH_4^+-N and TP, and the stability of the two reaction systems were investigated. The operating period for each aeration condition was 10 days. The sludge residence time was 15 days. The amount of aeration during operation and the changes in dissolved oxygen contents in the different systems under these aeration conditions are shown in Table 3. Magnetic stirrers were used to improve O_2 transformation and maintain a homogeneous system.

Table 3. Operating parameters for aeration.

	CAS System			**ABS System**		
Aeration (L/min)	0.15–0.2	0.4–0.5	0.7–0.8	0.15–0.2	0.4–0.5	0.7–0.8
DO (mg/L) ± 0.2	0.45	2.05	3.25	0.85	2.85	3.95

2.4. Analytical Methods

2.4.1. Water Quality Analysis

The effluents from the ABS and CAS systems were sampled and analysed each day. First, a 40 mL water sample was collected from the effluent through a 0.45-micron nitrocellulose membrane filter for water quality testing. The measurements of COD (potassium dichromate method), NH_4^+-N (nessler reagent spectrophotometry) and TP (ammonium molybdate spectrophotometry) in the samples were conducted via the standard methods [25].

2.4.2. Microbial Activity Analysis and Algal Biomass Estimation

In the biochemical reactor of the multifunctional aerobic bioreactor, sludge activity and chlorophyll a, contents were measured in volumes of 100 mL and 40 mL respectively. MLSS and switch virtual interface (SVI) were determined via standard methods [25]. In this study, the chlorophyll a (Chl-a) concentration was used to indicate the biomass of mixed algae [26]. The specific steps for determining the chlorophyll a concentration was as follows: First, the sample was centrifuged for 10 min in a centrifuge rotating at 4000 rpm. The supernatant was removed from the sample after centrifugation. Then, the sample was suspended in 20 mL of a 90% acetone solution with 0.05 g of calcium carbonate. The suspension was sheared with a vortex mixer for 1 minute and refrigerated for 24 h under dark conditions at 4 °C. Finally, the suspension was removed, and centrifuged again at 4000 rpm for 10 min, and the supernatant was used to determine the content of chlorophyll a using the value for a 90% acetone solution as the background, via visible spectrophotometry at four wavelengths: 750 nm, 663 nm, 645 nm and 630 nm to detect chlorophyll a. The concentration of chlorophyll a (c, mg/L) was calculated according to Equation (1), as follows:

$$c = \frac{\lfloor (11.64 \times (OD_{663} - OD_{750}) - 2.16 \times (OD_{645} - OD_{750}) + 0.10 \times (OD_{630} - OD_{750})) \rfloor}{V \cdot \rho} \tag{1}$$

where V is the volume of each sample (L), OD is the absorbency at the corresponding wavelength and ρ is the optical path of the cuvette (cm).

2.4.3. Data Analysis

The removal efficiency was calculated according to Equation (2), as follows:

$$R = \left(1 - \frac{C_i}{C_e}\right) \times 100\% \tag{2}$$

where R is the removal efficiency, and C_i and C_e are the concentrations of the water quality indexes of the effluent and influent, respectively.

The average removal efficiency was calculated according to Equation (3), as follows:

$$\overline{R} = \frac{\sum_1^i (R_1 + R_2 + R_3 + \cdots + R_i)}{d} \tag{3}$$

where $\overline{R}$ is the average removal efficiency, $\sum_1^i (R_1 + R_2 + R_3 + \cdots + R_i)$, i = 1, 2, 3, … , t (sum of removal rates under the same operating conditions), and d is the number of days of operation under the same operating conditions.

The structural state of the ABS system was evaluated using a biological microscope (Axio Observer.A1, Zeiss, Germany). The sludge particle size distribution was determined with a laser particle size analyser (S3500, Microtrac, Largo, FL, USA) using a tri-laser system. Full-wavelength scanning was conducted with a UV-vis spectrophotometer (UV-3200PCS, Mapada, Shanghai, China). Separation of the algae mixture and wastewater was conducted using a high-speed refrigerated centrifuge (HC-3016R, ZONKIA, Anhui, China). The pH and DO were directly measured using a pH instrument (PHS-3C, INESA, Shanghai, China) and a DO instrument (JPSJ-605F, INESA, Shanghai, China).

3. Results and Discussion

3.1. ABS System Startup

The ABS system treats dyeing wastewater in a multi-functional integrated aerobic bioreactor under natural light. It was clear that a relatively stable removal rate of COD was maintained (1–10 days) and the COD removal rate was between 70–75%, as shown in Figure 3. These results proved that the ABS system could run stably in the reactor. The ABS system was observed under a microscope on the second, fourth, sixth and eighth days. It was found that XJK (*Chlorella*), W6 (*Filamentous*) and 902 (*Selenastrum bibraianum*) could be better blended with activated sludge. As shown in Figure 4a, on the second day after the activated sludge and mixed algae were added, it was observed that the *Chlorella* and the *Selenastrum bibraianum* were fused with the activated sludge. As shown in Figure 4b, *Chlorella*, *Selenastrum bibraianum* and the activated sludge were fused with each other and that they were wrapped in *filamentous algae* at the outer edge on the fourth day. The structure observed on the sixth day was basically the same as on the fourth day, as shown in Figure 4c. On the eighth day, it was observed that the amount of *Selenastrum bibraianum* decreased, and there were more *filamentous algae*, but the structure of the ABS system did not change, as shown in Figure 4d. These results indicated that the algae and activated sludge could form a symbiosis in a continuous flow reactor and that this system had a similar structure to the symbiotic system of *Chlorella* and sludge flocs found in a previous study [27]. A structural diagram of the ABS system is conjectured, as shown in Figure 5. Next, we continued to treat high-load printing and dyeing wastewater and measured the MLSS and SVI in the ABS system, as shown in Table 4. It was clear that the COD removal rate was significantly improved compared to the initial stage of the startup, which was between 78–85%, as shown in Figure 1. However, although MLSS remained within a certain range, SVI exhibited a significant increase in the later period, as shown in Table 4. Therefore, when the next continuous flow operation was carried out to study the treatment performance of the ABS system under different hydraulic retention times and aeration volumes, the manual sludge discharge was required (an average of 200–300 mL of mud per day).

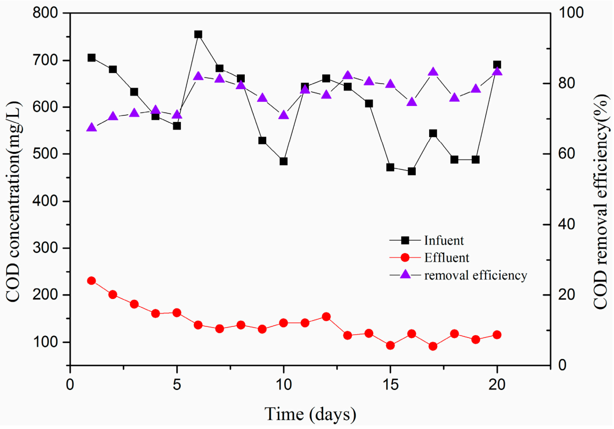

Figure 3. COD influent and effluent concentrations and removal rates under HRT: 16 h, aeration: 0.3–0.4 (L/min), DO: 2.45 ± 0.2 (mg/L).

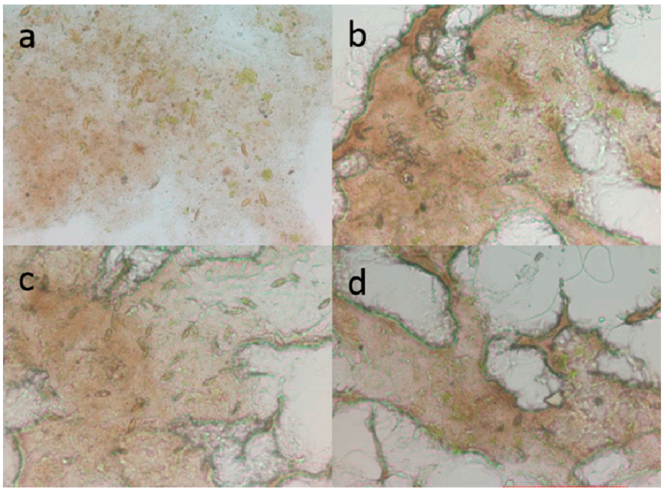

Figure 4. Microscopy examination of the ABS system during a reaction run on the (**a**) second day, (**b**) fourth day, (**c**) sixth day and (**d**) eighth day.

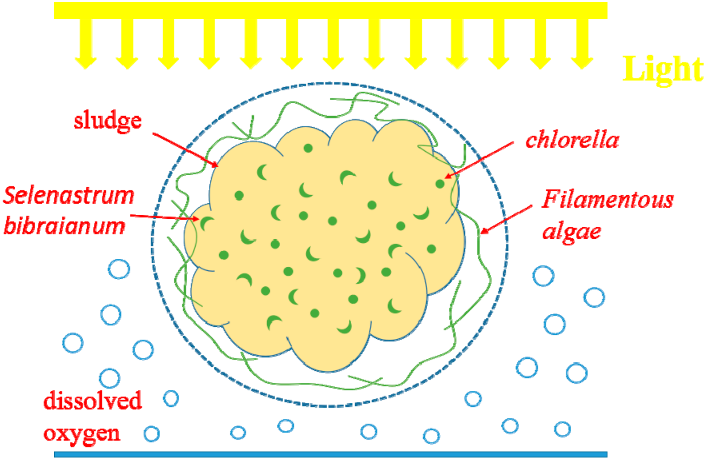

Figure 5. ABS system structure.

Table 4. ABS system activity index.

Activity Index	12th Day	14th Day	16th Day	18th Day	20th Day
MLSS (mg/L)	3100	3200	3300	3500	3400
SVI	100	120	125	135	135

3.2. Performance of the ABS System under Different Hydraulic Retention Times

After the system was stably started, the treatment performance of wastewater under different hydraulic retention times was studied in the CAS and ABS systems. The Figure 6 shows that influent COD, $NH_4{}^+$-N, TP and colour water quality in the two reactors ranged from 586–802 mg/L, 17.2–22.0 mg/L, 4.0–6.1 mg/L and 500–700 times, respectively. In general, the ABS system increased the COD removal rate compared with the CAS system at different HRTs, as show in Figure 6a,b. Under the same HRT, the effluent COD was reduced by 41 mg/L, 35.6 mg/L and 18.6 mg/L, and the average removal rate of COD was increased by 5.5%, 5.3% and 2.8%, respectively, compared to the CAS system. The results showed that the ABS system exhibited a high average COD removal rate, reaching 83%, when the HRT was 16 h. Mclean et al. [28] and Huang et al. [29] reported that as the HRT increases, DO also increases, the photosynthesis of algae is inhibited, and the absorption of nutrients by algae is relatively reduced. When the HRT was 12 h and the reactor ran for 31 days, the COD of the effluent increased due to a change in influent water quality, and the removal rate decreased, after which the reaction system tended to be stable. Compared with the CAS system, the ABS system was a small change in the removal rate of COD and ammonia nitrogen and exhibited high stability to changes in water quality.

The average effluent NH_4^+-N contents were 2.9 mg/L, 2.4 mg/L and 2.3 mg/L, and the average NH_4^+-N removal rates were 84.9%, 88.6% and 89.7% at HRTs, as shown in Figure 6c,d. The removal rate of NH_4^+-N increased obviously with an increase in the HRT of 16 h, which was 3.7% higher than that with an HRT of 12 h, while the removal rate of NH_4^+-N at 20 h was only 1.1% higher than that at 16 h. Compared with the CAS system, the ABS system presented a small improvement in the removal of NH_4^+-N. Peng et al. [30] found that algae can absorb inorganic nutrients in an ABS system and produce oxygen. Oxygen is then used by ammonia-oxidizing bacteria and nitrite-oxidizing bacteria to degrade NH_4^+, NO_3^- and NO_2^- and promote nitrification and denitrification.

The average TP removal rates were 30.2%, 36.6% and 37.7%, as shown in Figure 6e,f. The rate was only 1.1% higher than for an HRT of 16 h when the HRT was 20 h, but the ABS system with an HRT of 16 h showed a more stable TP effluent quality and removal efficiency. The average TP removal rates of the ABS system were 5.8%, 9.0% and 8.9% higher than those of the CAS system under the same hydraulic retention time. Although the removal effect of TP was not ideal, Tang et al. [10] and Tang et al. [31] showed that algae produced oxygen through its own photosynthesis, and polyphosphate bacteria in activated sludge can absorb more phosphorus in the case of more dissolved oxygen, which allows the ABS system to better remove TP compared to a CAS system.

The effluent colour of the ABS system was 60 to 125 times, and the average effluent colour was approximately 90 times, as shown in Figure 6g. The average effluent colour was slightly lower than that of the CAS system. The effluent colour of the ABS system with an HRT of 12 h was significantly better than that of the CAS system. The ABS system exhibited a stable effluent colour at different HRTs. The ABS system achieved the lowest effluent colour of 60 times when the HRT was 16 h, which was superior to the effluent colour of the dyeing wastewater treatment plant of 120–160 times. Although the addition of algae further degraded the colour in the wastewater, it was still far from the ideal processing effect of colour. In Xu Xiaolin's research, it was found that the three algae could achieve a removal rate of more than 90% for the degradation of a single dye in simulated wastewater [24]. The possible reasons included that there were many kinds of dyes in the real wastewater and other easily degradable organic compounds inhibited algae using dyes. Therefore, in the follow-up study, it should pay more attention to the colour degradation of dyeing wastewater.

The chlorophyll a content of the ABS system and the MLSS and SVI of two systems were determined at different HRTs, as shown in Figure 7a,b. Early in the biochemical reaction, the MLSS value of both the CAS system and the ABS system were 3000 mg/L. The chlorophyll a concentration in the ABS system was 1000 μg/L. The MLSS values of the two reaction systems were maintained at 2500–3200 mg/L. When the HRT was 12 h, the chlorophyll a content decreased, and algal growth was inhibited. Valigore et al. [11] found that for autotrophic microbial algae, an increase in biomass requires longer HRT; in the short HRT, the growth of bacteria and algae is inhibited, and the removal of nutrients correspondingly decreased. When the HRT was changed to 16 h, the algal content increased, and the removal rates of COD and NH_4^+-N also increased. However, as the HRT continued to increase, the algal content decreased, which led to a decrease in the COD removal rate. As the hydraulic retention time increased, DO also increased; the photosynthesis of algae was inhibited, and the absorption of nutrients by algae was relatively reduced [28,29]. The SVI of the ABS system increased from an initial value of 76 to 179 as the HRT changed. Through microscope observation, the extension of the HRT caused the *filamentous algae* in the ABS system to grow faster. The mixture of algae into activated sludge improves the sedimentation performance of algae to some extent [32]. However, as the hydraulic retention time increases, excessive filaments or filamentous algae could cause sludge washing and poor sedimentation performance [33]. Therefore, the algal content needs to be controlled within a certain range so that the ABS system will be more stable.

In summary, when the HRT was 16 h, compared with the CAS system, the ABS system showed a better performance, more stable COD, NH_4^+-N and TP contents, and improved effluent quality and removal rates. The COD contents were 75.2 mg/L, 5.1 mg/L and 1.05 mg/L lower than those of the dyeing wastewater treatment plant, and the average COD removal rates were increased by 10.5%, 18.6% and 10.5%.

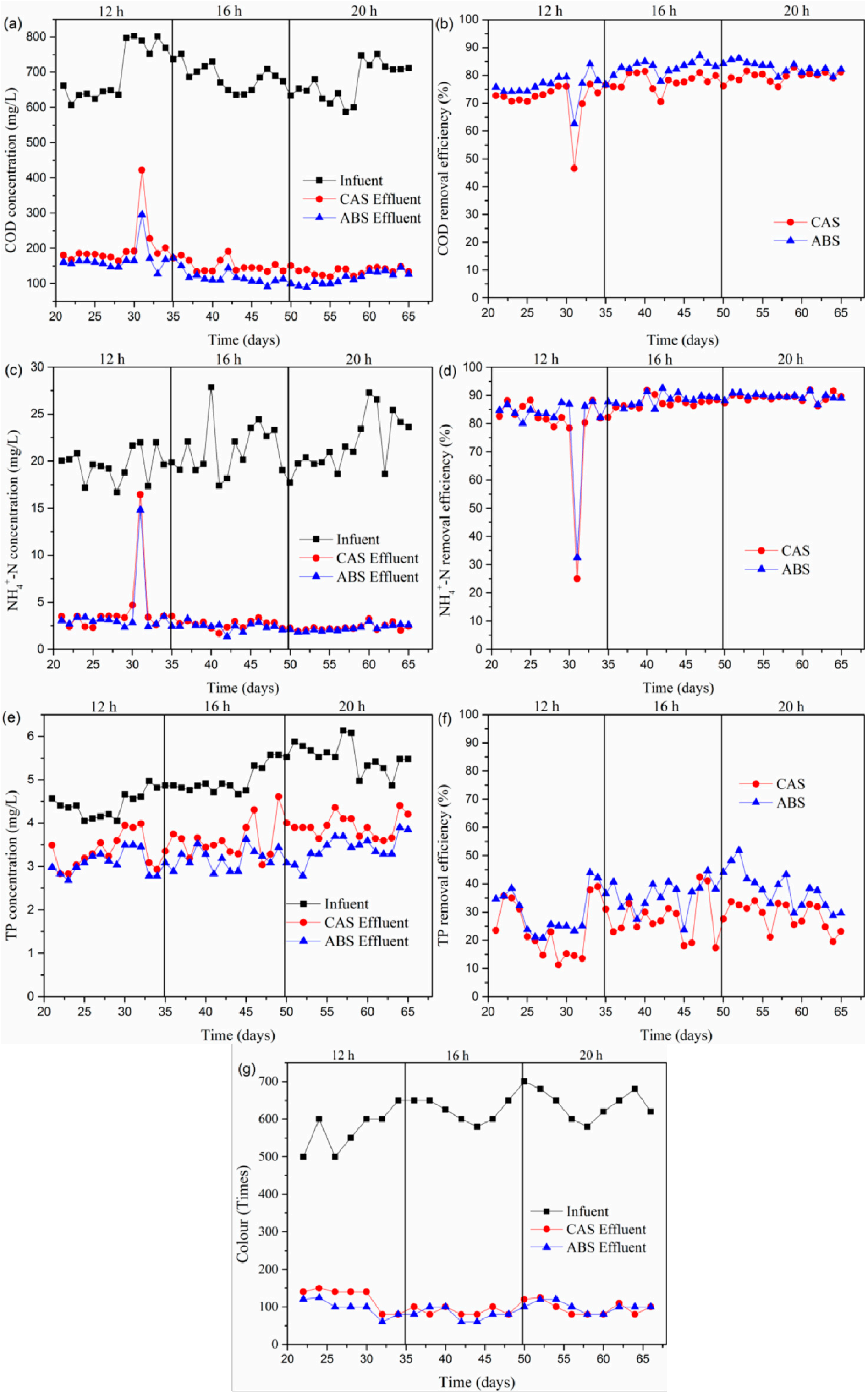

Figure 6. Removal performance of COD (**a**,**b**), NH_4^+-N (**c**,**d**), TP (**e**,**f**) and colour (times) (**g**) in the CAS system and ABS system under different HRTs: 12 h, 16 h and 20 h, and the same aeration: 0.4–0.5 L/min.

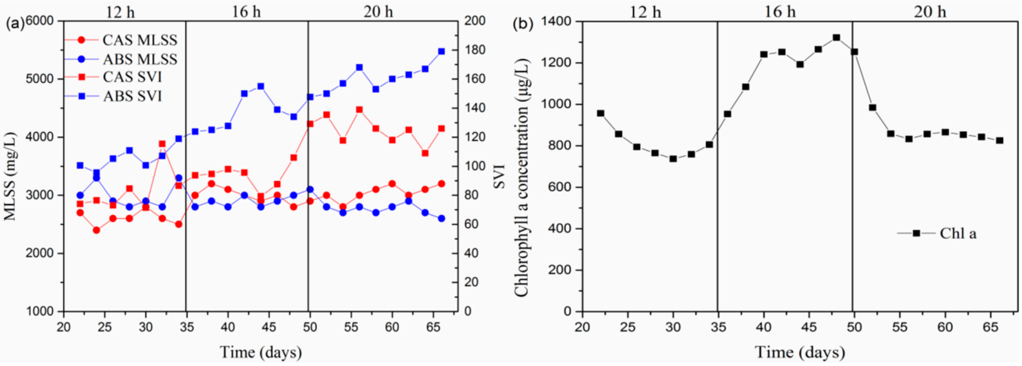

Figure 7. Biochemical indicators of the CAS system and ABS system (**a**), and chlorophyll a concentration of ABS system under different HRTs (**b**).

3.3. Performance of the ABS System under Different Aeration Conditions

After determining the optimal hydraulic retention time, the treatment performance of the two systems for high-load printing and dyeing wastewater under different aeration rates was studied, and MLSS, SVI and chlorophyll a were measured as shown in Figure 8.

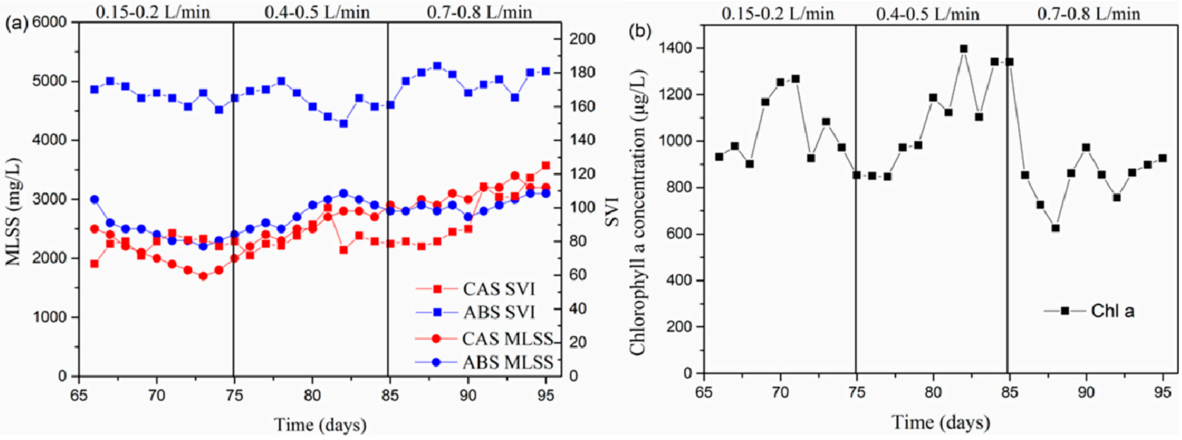

Figure 8. Biochemical indicators of the CAS system and ABS system under different aeration conditions: 0.15–0.2 L/min, 0.4–0.5 L/min, 0.7–0.8 L/min (**a**) and chlorophyll a concentration of ABS system under different aeration conditions: 0.15–0.2 L/min, 0.4–0.5 L/min, 0.7–0.8 L/min (**b**)

As shown in Figure 9a,b, when the aeration rate was 0.15–0.2 L/min, the average removal rate of COD in the ABS system was 74%, which represented a larger advantage than the CAS system, when the aeration rate was 0.4–0.5 L/min, and the average removal rate of COD in both reaction systems was improved. As the rate of aeration continued to rise, the average COD removal rate of the ABS system decreased, but it was still higher than that of the CAS system. The results showed that when the aeration rate was 0.4–0.5 L/min, the average removal rate of COD in the ABS system was higher than in the two systems under other aeration rates, which reached 85%. The reason for the analysis of MLSS, SVI and chlorophyll a, as shown in Figure 8a,b, was that when the aeration rate was 0.15–0.2 L/min, the dissolved oxygen content of the CAS system was only 0.45 ± 0.2 mg/L, as shown in Table 3. The activity of activated sludge is inhibited under low dissolved oxygen conditions [29]. However, low aeration can promote algal growth and produce oxygen through photosynthesis, which can be used by bacteria to promote bacterial growth and nutrient removal [10]. On the other hand, the growth of algae also promotes the removal of organic matter [34]. When the aeration rate was 0.4–0.5 L/min (DO at 2.85 ± 0.2), the algal content increased to 1400 μg/L, and MLSS was stable, between 2600–3100 mg/L, as shown in Figure 8a,b. The synergistic role of the sludge and algae was improved under symbiotic conditions and the COD removal rate was also improved. However, at higher aeration rates, the growth of algae was reduced and photosynthesis was inhibited [29]; when the aeration rate was 0.7–0.8 L/min (DO at 3.95 ± 0.2), the algal content decreased to 800 µg/L. The increase in aeration rate led to a decrease in algae content and a corresponding reduction in the removal of contaminants. The synergistic effect of activated sludge and algae was degraded.

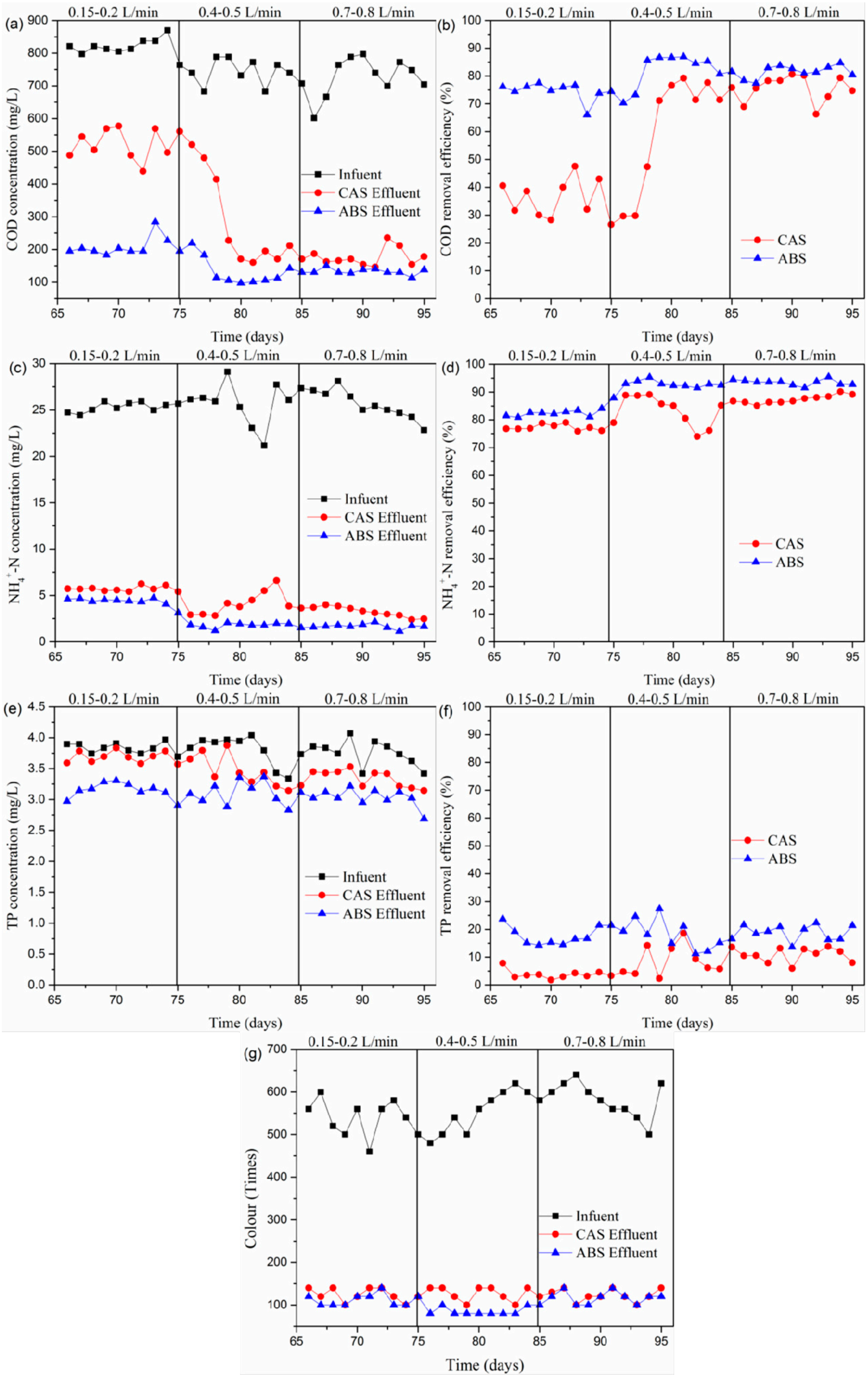

Figure 9. Removal performance of COD (**a**,**b**), NH_4^+-N(**c**,**d**), TP (**e**,**f**) and colour (**g**) in the CAS system and ABS system under different aeration: 0.15–0.2 L/min, 0.4–0.5 L/min and 0.7–0.8 L/min, and the same HRT: 16 h.

As shown in Figure 9c,d, the NH_4^+-N removal rate of the ABS system under the same aeration operation conditions was higher than that of the CAS system. The average NH_4^+-N removal rate of the ABS system under different aeration conditions were 82.9%, 93.1% and 93.4%, respectively. It has previously been found that microalgae can preferentially use ammonia nitrogen and organic nitrogen [10]. Furthermore, the addition of algae has a significant improvement in the removal of other forms of nitrogen compared to conventional activated sludge [10]. Therefore, for printing and dyeing wastewater, algae are more capable of removing nitrogen species present in dye.

As shown in Figure 9e,f, the average TP removal rate of the ABS system under different aeration conditions was maintained at approximately 18.0%. Although the removal rate of TP was not improved, it was still superior to that of the CAS system.

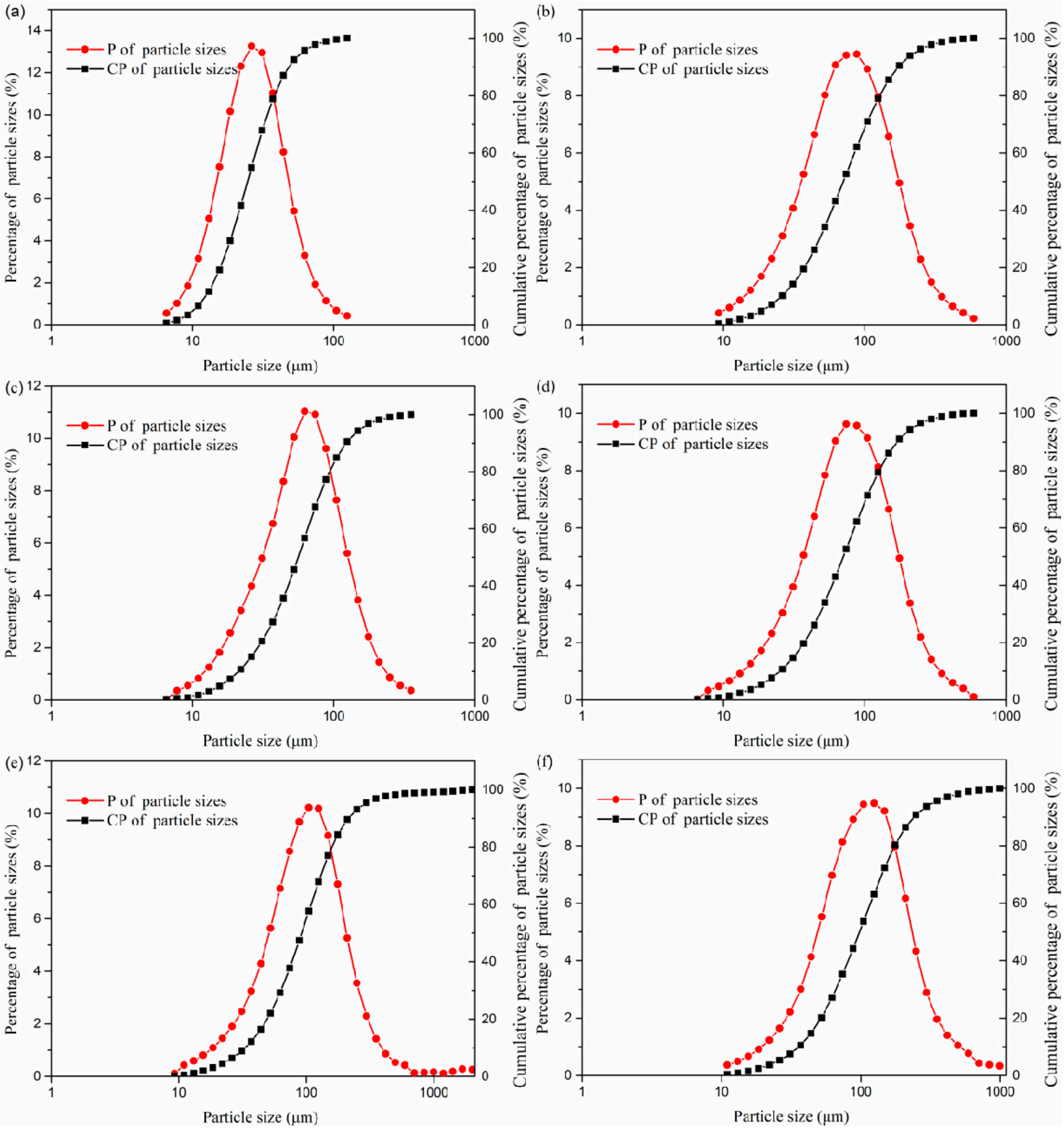

Figure 10. Percentage of different particle size distributions and cumulative percentage of different particle size distributions for the CAS system (**a**,**c**,**e**) and ABS system (**b**,**d**,**f**) under different aeration conditions: 0.15–0.2 L/min, 0.4–0.5 L/min and 0.7–0.8 L/min.

As shown in Figure 9g, when the aeration rate was 0.4–0.5 L/min, the effluent colour of the ABS system was between 80–120 times, which presented an obvious advantage compared with the CAS system. However, although the effluent colour was reduced, the desired effect was not achieved.

While investigating the effects of different aeration conditions on wastewater treatment performance, the stability of the ABS system was also studied. The sludge particle size distribution in CAS system and the sludge and algae particle size distribution in ABS system under different aeration rates were analysed as shown in Figure 10.

The ABS system exhibited a stable particle size distribution under different aeration rates, with values between 52.32–124.40 µm. The average particle size under each aeration rate was 89.60 µm, 88.36 µm and 127.60 µm, respectively, as shown in Figure 10b,d,f. For the CAS system, the particle size increased with increasing aeration. When the aeration rate was 0.15–0.2 L/min, the particle size was only 22.00–37.00 µm, as shown in Figure 10a. Sludge growth is inhibited under low dissolved oxygen conditions. As a result, the ABS system maintained high stability and better synergism under different aeration rates. Tang et al. [10] reported that the algae can increase the activity of microorganisms and that unicellular algae are adsorbed on the sludge floc by the extracellular polymer substances (EPS), which can maintain the algae accumulation and prevent its loss [2].

In summary, the addition of algae can provide more oxygen and leads to a higher removal effect on COD and NH_4^+-N. This system is superior to printing and dyeing wastewater treatment plants in terms of various water effluent indicators.

3.4. Analysis of Effluent Organic Matter and Colour Quality

Since some COD and colour components remained in the effluent and were not effectively removed, the organic matter and colour of the influent water were analysed via UV-vis spectroscopy. In the near-ultraviolet region, there was a strong absorption peak between 200–220 nm, which is the absorption peak when a hydrogen atom is replaced by certain ions; there was a strong absorption peak between 220 and 250 nm, indicating that this compound had a conjugated double bond; there was an absorption peak between 250 and 290 nm, indicating the presence of a benzene ring; and there was an absorption peak between 300 and 400 nm, indicating that the conjugated system was larger. It was observed that there were many absorption peaks in the range of 200–350 nm. On the one hand, there were some small molecular compounds in the wastewater. After treatment, the decrease of the absorption peaks in the range of 250–350 nm indicated that these substances were degraded. On the other hand, the increase in the range of 200–250 nm was mainly due to the degradation products of dye degradation. When the conjugated system reached a certain amount, an absorption peak appeared in the visible light wavelength range, and a coloured substance therefore appeared [35]. In printing and dyeing wastewater, this coloured substance is a dye molecule. It was found that the printing and dyeing wastewater treated by the ABS system still contained certain dyes and dissolved organic matter that were not degraded, as shown in Figure 11a. To investigate whether algae could further remove the colour and organic matter content of printing and dyeing wastewater the effluent was further processed by collecting a certain volume of effluent and adding algae to it. As shown in Figure 11b, the dye's concentration was slowly and continuously decreasing. Five days were required to achieve the maximum removal of dye. There were two reasons for this finding: First, autotrophic microbial algae take longer to degrade macromolecular dyes. Second, in the process of wastewater treatment, algae will give priority to the use of small molecular organic substances that are easily degraded, thereby reducing the ability to degrade macromolecular dyes.

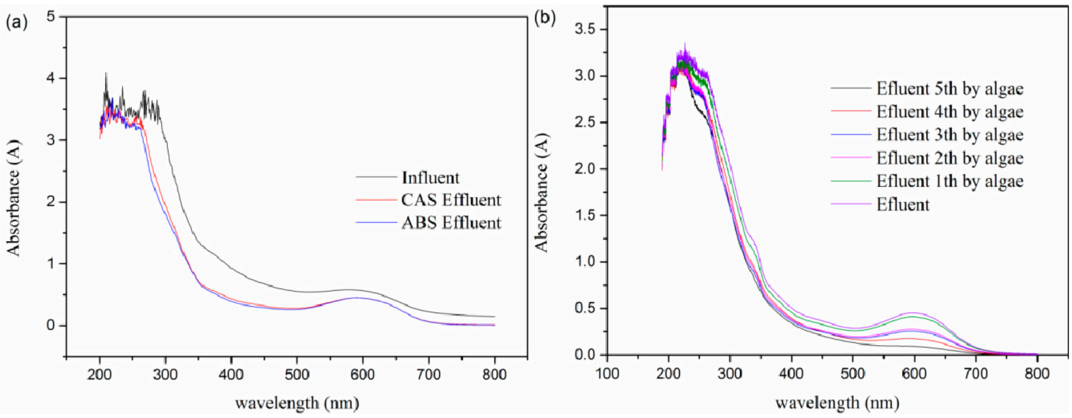

Figure 11. Influent and effluent UV full-wavelength scanning of processed using the ABS and CAS systems (**a**), and effluent UV full-wavelength scanning of processed using mixed algae (**b**).

3.5. Advantages of Algae Addition

Printing and dyeing wastewater is a complex and refractory industrial wastewater. With the continuous improvement of emission standards in the textile printing and dyeing industry, the difficulty in handling such wastewater is also increasing. Usually, the combined treatment of physical, biological and chemical processes are used in the printing and dyeing wastewater treatment plant. However, the complex processes lead to more shortcomings. The traditional activated sludge process requires not only a large footprint but also sufficient aeration. Chemical methods, such as ozone oxidation, usually require significant processing costs. For the ABS system, the addition of algae not only provides additional aeration for the activated sludge but also not changing the structure of the original biochemical pool while further reducing the pollutants. At the same time, the decrease of organic matter concentration in effluent could reduce the cost of subsequent chemical treatment. The difficulties of transforming the existing printing and dyeing wastewater treatment plant has been greatly reduced.

4. Conclusions

When the hydraulic retention time was 16 h and the aeration rate was 0.4–0.5 L/min, the ABS system not only showed the best removal performances for COD, NH_4^+-N and TP, which were increased by 12.5%, 23.1% and 10.5%, respectively, but also reduced colour 60–80 times compared with the printing and dyeing wastewater treatment plant. The addition of algae could provide more dissolved oxygen and could cause the ABS system to exhibit a higher, stable removal rate for nutrients. On the other hand, algae could further degrade COD and the colour quality under conditions of no external carbon source and longer hydraulic retention time.

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