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Effect of Attached Growth on Treatment Performance in Waste Stabilization Ponds

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Abstract: Waste stabilization ponds (WSPs) rely upon natural biochemical reactions for treatment and are used widely across the world. However, WSPs often fail to meet treatment performance expectations due to insufficient hydraulic performance. Installation of baffles can improve hydraulic performance of WSPs by increasing the mean residence time, reducing dead zones, and short circuiting, thus improving pond treatment performance. Theoretically, baffles with the ability to sustain attached growth will increase the possible attachment area of microorganisms and further contribute to nutrient removal. However, to date there have been no full-scale studies exploring attached growth baffles in WSPs. The main objective of this study was to investigate and quantify the effect of attached growth baffles on WSP treatment performance, specifically in terms of improvements in treatment performance provided by attached biofilm compared with hydraulic improvement. A first-order kinetic model was used to predict biological oxygen demand (BOD) removal efficiency, including suspended and biofilm biomass reactions, to determine whether attached growth or hydraulics had the most influence on performance improvement. At the operational WSP scale, we found that although the presence of attached growth on baffles results in a modest (~0.6%) improvement in treatment performance, the most influential factor for improving treatment was improved hydraulics (~5.3%). In model generalization, the change in biofilm thickness and biofilm area had less effect on treatment in WSPs in higher organic loading scenarios; however, a considerable improvement (~12%) in treatment efficiency could be achieved by doubling the total biofilm area. Overall, this study shows that baffles can not only improve WSP hydraulics but can also be used as a medium for increasing biofilm area to improve WSP biological treatment efficiency.

Keywords: baffles; waste stabilization ponds; modelling; treatment performance; first-order kinetics; attached growth

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1. Introduction

Waste stabilization ponds (WSPs) are widely used for wastewater treatment due to their robust natural treatment processes and low energy consumption [1–3]. Hydraulic performance of WSPs has long been the focus of research due the vital role it plays in the treatment efficiency, as reviewed in Passos et al. [4]. However, due to a number of factors, including sludge accumulation and distribution, WSPs are notoriously hydraulically inefficient, leading to poor treatment performance [4,5]. Previous reviews of 41 WSP modelling studies have shown that the majority of the models developed concentrated purely on modelling either pond hydraulics or water quality [4,6]. Very few existing studies include the analysis of both hydraulic performance and water quality, and overall, there are a lack of studies to quantify the interaction between hydraulics and treatment efficiency or impact of hydraulic performance on the WSP treatment performance.

The installation of baffles—solid partitions to confine or direct flow—is a solution to improve pond hydraulics, with laboratory experiments, computer modelling, and operational-scale studies all having shown the advantage of baffles in WSPs for improving hydraulic and treatment performance [7–9]. However, the majority of the research to date has either simply explored the impact of baffles on pond hydraulic performance or assumed that only suspended biomass is responsible for nutrient removal. Biofilm growth on streambeds has been identified as a contributor to degrading organic matter [10]. Furthermore, a previous study on self-purification in storage tanks has shown that biofilm biomass growing on tank walls was effective in enhancing rainwater purification [11]. As WSPs normally have low velocities and higher organic loadings compared with freshwater systems, biofilm can be expected to grow on the pond walls and floor, which could contribute to the wastewater treatment process.

Kinetic analysis can used for describing and predicting the performance of biological treatment systems, as it can bridge the gap between the parameters of different and intertwined processes [12]; it could be used to provide further information for WSP design or validate the experimental data. Kinetic models can be used to predict biological oxygen demand (BOD) removal efficiency, including the first-order reactions of both suspended microorganisms and biofilm biomass under the plug flow and completely mixed conditions [12]. Several studies have attempted to incorporate first-order kinetics into computational fluid dynamics modelling (CFD) to estimate BOD and *Escherichia coli* (*E. coli*) removal [13–20]; however, no further development has occurred in this space in recent years. Figure 1 shows a conceptual illustration of a biofilm model in a facultative pond [21] where the biofilm growing on the pond walls and floor is assumed to have constant thickness and density [21,22]; the liquid sublayer acts as a link between the bulk liquid flow and the biofilm [21]. In addition, it also assumed that the diffusion and consumption of the substrate occurs simultaneously inside the biofilm [21]. During these processes, as illustrated in Figure 1, the coefficients of diffusion and kinetics are also assumed to be constant throughout the pond length [23]. A first-order kinetic model, including both suspended microorganisms and biofilm biomass attached to pond floor and walls, has been used to predict the treatment efficiency of facultative ponds [21,23]. The results of these two studies showed that the estimated treatment efficiency was close to the observed value when the kinetic model included both suspended and biofilm biomass reactions; when only suspended biomass was considered, there was a discrepancy of 20% between the estimated and observed values [21].

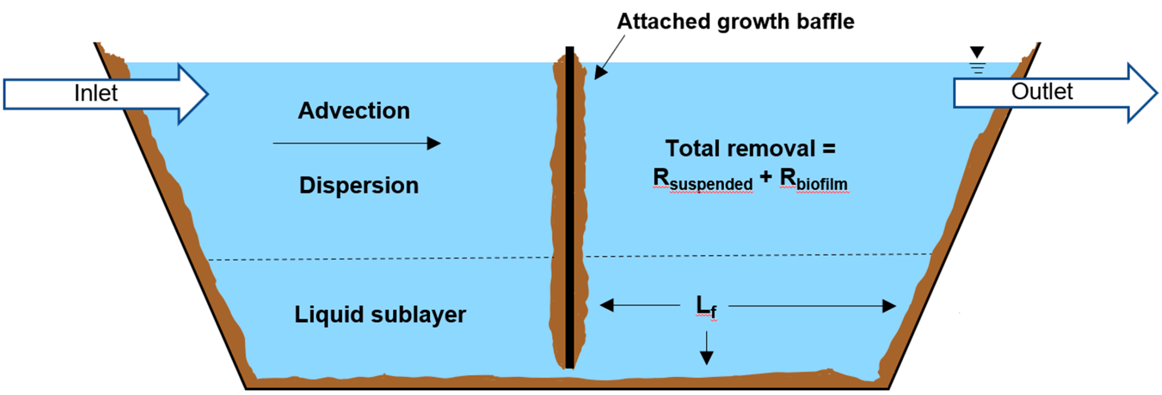

Figure 1. A conceptual illustration of a biofilm model in a facultative pond. This model involves substrate mass balances in bulk liquid flow and biofilm. Waste transportation is governed by diffusion and dispersion [21,22]. $R_{suspended}$ and $R_{biofilm}$ represent the biological oxygen demand (BOD) removal efficiency when considering suspended or biofilm biomass only, respectively. L_f refers to the biofilm attached on baffles and pond floor and walls. In this study, the total removal is considered as the sum of $R_{suspended}$ and $R_{biofilm}$.

Previous studies largely overlook that baffles may provide additional surface area for biofilm growth, which might further contribute to pond treatment performance. In theory, baffles with the ability to sustain biofilm, hereafter referred to as attached growth baffles, will increase the attachment area for biofilm growth beyond the pond walls and floor, result in improved pond treatment performance and efficiency, and increase the concentration of microorganisms in pond [22]. A study by Shin and Polprasert [24] showed that an increase in biofilm attachment area could have a positive effect on pond performance; however, their study did not consider hydraulic performance factors. A laboratory scale study of attached growth baffles in WSPs showed that although total biofilm biomass increased with an increasing number of baffles, the average biofilm thickness decreased as more baffles were added [22]. Additionally, a pilot scale study by the same authors found the increased biofilm surface area may improve nitrogen removal efficiency [25]. Although these findings show some potential for the improvement of WSPs' performance, further investigation of attached growth baffles is required to ascertain the role of biofilm in WSP treatment performance, especially at the operational WSP scale. To date, there has been no research that has studied attached growth and hydraulics at the same time, despite the possibility that it could improve the treatment performance of WSPs.

A study on optimization of baffle configuration and hydraulics was presented in Coggins et al. [26], which proved that installing baffles improved short-circuiting and mean residence time in a full-scale operational WSP. Therefore, the overall objective of this study is to quantify the effect of biofilm attachment on the treatment performance of an operational WSP with attached growth baffles. More specifically, the aims are to: (1) determine which factor, hydraulic improvement or increased attached growth, has more influence on WSP performance, and (2) determine whether attached growth baffles are a viable solution in the long term to improve the organic matter removal treatment performance of WSPs.

2. Materials and Methods

2.1. Study Site

The study site is a wastewater treatment plant located in a town 86 km of south of Perth, Western Australia. The site experiences a Mediterranean climate, with an average rainfall of 690 mm and yearly average temperatures of between 10–30 °C [27]. This site was selected as it has two parallel primary facultative ponds: Pond 1 and Pond 2 (the trial system). Each pond has dimensions of 120 × 60 × 1.3 m, and equally share a total inflow of up to 770 m^3 day^{-1}. An aerial map of the two ponds is shown in Figure 2.

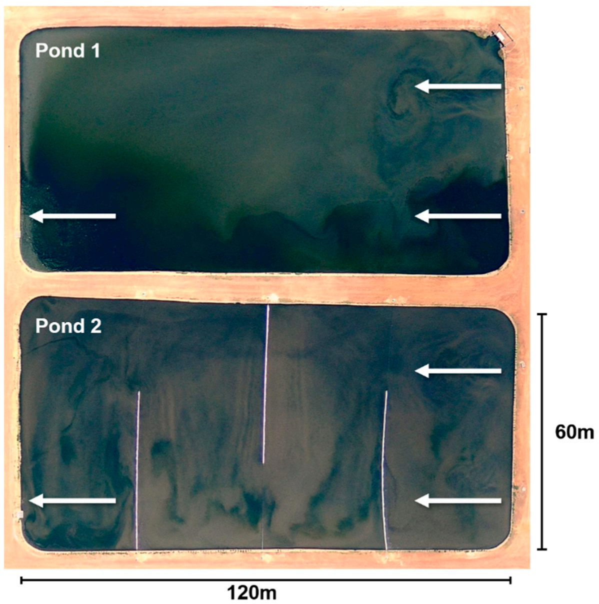

Figure 2. Aerial view of the study site with two facultative ponds (Image: NearMap). Three perpendicular baffles were installed in Pond 2 in 2014. Inlets and outlets are indicated by white arrows, and the ponds equally share a total inflow of up to 770 m^3 day^{-1}.

2.2. Attached Growth Baffle Selection and Installation

Instead of traditional non-porous baffle media, a geotextile material was selected to promote biofilm growth or attached growth. Sample strips of several geotextiles were tested in-pond on site for two months to determine the material that promoted the highest amount of growth (Figure 3). Scanning electron microscopy (SEM) was used to determine whether biofilm attached to the baffle material. Total attached mass was calculated after suspension in water as total suspended solids (TSS), volatile suspended solids (VSS), attached carbon content (C), and chlorophyll-*a* (chl-*a*) to determine the level of biofilm growth on baffles [28].

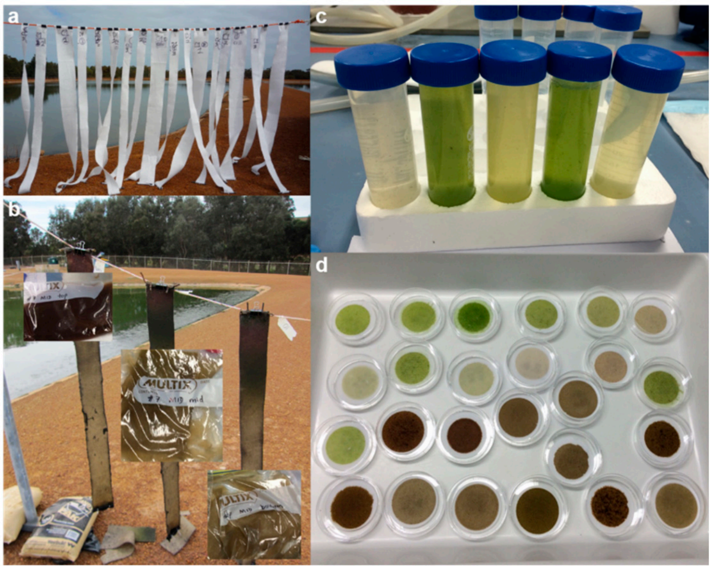

Figure 3. Process for selecting the material for the attached growth baffles used in this study. (**a**) Strips of different geotextiles were deployed in pond for two months, and then samples (**b**) were taken of each to determine which had the best adhesion ability. In determining best adhesion ability, samples were subject to (**c**) nutrient and chlorophyll-*a* analysis and (**d**) biomass analysis after filtration.

The material chosen for the baffles and experimental strips was a non-woven polypropylene geotextile (TerraTex D1 PP, Polyfabrics Australia, Kingsgrove, NSW, Australia). Using the results of pond hydrodynamic modelling, in October 2014 three attached growth baffles were installed perpendicular to water flow in Pond 2 [26]. Each baffle was 40 m long, equal to two-thirds the width of the pond. The study of the hydraulic performance of these two ponds, including the design configuration of the baffles is presented in Coggins et al. [26]. Water quality data were collected monthly during 2014–2016 at the inlet and outlet, i.e., before and after the baffle installation. The experimental strips were installed in the same location as the baffles and taken out after seven months for further microscopic, nutrient, and biomass analysis.

Tracer testing was carried out to determine the mean residence time of the two facultative ponds. The testing was conducted with Rhodamine WT (Product code: 703-010-27, Keystone Aniline Corporation, Chicago, IL, USA) that contains 20% true dye, and the fluorescence signal was monitored at the outlet at 15 min intervals over a period of six weeks [26]. As reported in Coggins et al. [26], the installation of baffles in Pond 2 increased the mean residence time from 14 to 17 days, despite an increase in sludge infill during the experimental period, and the effect of wind on the mean residence time was not significant [26]. For the present study, effective volume ratio (e) is selected as another indicator of hydraulic performance, calculated as in Persson and Wittgren [5]:

$$e = \frac{V_{effective}}{V_{total}} = \frac{t_m}{t_n} \quad (1)$$

where t_m (the mean residence time) has been reported in Coggins et al. [26] and t_n (the nominal residence time) was calculated by the ratio of pond volume and ideal flow.

2.3. Application of First-Order Kinetics to Determine the Contribution of Baffles on Pond Performance

The BOD concentration in effluent (C_e, mg L^{-1}) was estimated by three forms of first-order kinetic models. Model validation was carried out by comparing the modelled results with observed data, then the equation with both accuracy and a simpler calculation formula could be used for the further analysis. Model formulas are as shown in Polprasert and Agarwalla [21], Muttamara and Puetpaiboon [22], and Polprasert and Bhattarai [29]:

$$C_e = \frac{4a_1 \times e^{\frac{1-a_1}{2d}}}{(1+a_1)^2} \times C_i \tag{2}$$

$$C_e = \frac{4a_1 \times e^{\frac{1}{2d}}}{(1+a_1)^2 \times e^{\frac{a_1}{2d}} - (1-a_1)^2 \times e^{\frac{-a_1}{2d}}} \times C_i \tag{3}$$

$$C_e = \frac{2a_1 e^{\frac{1}{2d}}}{(1+a_1) \times e^{\frac{a_1}{2d}} - (1-a_1) \times e^{\frac{-a_1}{2d}}} \times C_i \tag{4}$$

where C_i is influent BOD concentration (mg L^{-1}), the value of a_1 is calculated as $a_1 = \sqrt{1+4ktd}$, where a_1 is one of the terms in kinetic model expression, k (day^{-1}) is the overall reaction rate, t (day) is residence time, and d is dispersion number. Equation (2) is a more simplified version and suitable for estimating BOD reduction if dispersion number (d) is less than 2 [29]. In this study, the value of d was determined by the range obtained from two different formulas in Polprasert and Agarwalla [21] and Polprasert and Bhattarai [29]:

$$d = 0.10201\left(\frac{U_*}{u}\right)^{-0.81963} \times \left(\frac{h}{L}\right)\left(\frac{h}{W}\right)^{-\left(0.98074+\frac{1.38485h}{W}\right)} \tag{5}$$

$$d = \frac{0.184 \times [tv \times (W+2h)]^{0.489} \times W^{1.511}}{(L \times h)^{1.489}} \tag{6}$$

where U_* is shear velocity, which is equal to $u\sqrt{f/8}$; flow velocity (u, m day^{-1}) is obtained from measured data; f, friction factor is equal to $24/Re$; Re, Reynolds number, could be calculated by $(4LWh)/[(W+2h)tv]$, where L, W, h are pond dimensions; kinematic viscosity, v (m^2 day^{-1}) was obtained from Von Sperling [30] and determined by $0.325 \times T^{-0.450}$ for T_{median} = 20 °C.

We calculated the reaction rate, k (day^{-1}) in this study as:

$$k = k_{fs} + a_s \times \frac{\alpha\beta}{\alpha+\beta} \tag{7}$$

where k_{fs} (day^{-1}) is the first-order rate constant of suspended biomass, and can be estimated when there is lack of harmful industrial pollutants in ponds as:

$$k_{fs} = k_{fss} \times \left\{1 - \frac{0.083\left[log\left(\frac{67.2}{L_0}\right)\right]}{k_{fss}}\right\} \tag{8}$$

where k_{fss} (day^{-1})is the standard first-order rate constant, which is equal to 0.056 per day at 20 °C; and L_0 (kg ha^{-1} day^{-1}) is organic loading rate.

Specific surface area, a_s (m^2 m^{-3}) was calculated for biofilm attached area without attached growth after Polprasert and Agarwalla [21] and Polprasert and Bhattarai [29] as:

$$a_s = \frac{2}{W} + \frac{1}{h} + \frac{2}{L} \tag{9}$$

and with attached growth as:

$$a_s = \frac{2}{W} + \frac{1}{h} + \frac{2}{L} + n \times \left(\frac{2h_1 l_1}{WhL}\right) \tag{10}$$

α is calculated as $\alpha = \frac{D_w}{L_s}$, where the ratio of D_f/D_w was chosen as 0.5, that is, between the reported ratio of aerobic and anaerobic biomass, which is similar to pond conditions [21]. The ratio of D_f/D_w is used for determining the values of D_f and D_w, which were taken as 24.45×10^{-6} m^2 day^{-1} and 48.9×10^{-6} m^2 day^{-1}, respectively. D_f is used for the calculation of the characteristic biofilm parameter $\varnothing$.

β is calculated as $\beta = \frac{\tan h(\varnothing)}{\varnothing} \times k_{fa} \times L_f$, where the liquid sublayer thickness (L_s, m), biofilm thickness (L_f, m), and first-order constant rate of biofilm biomass (k_{fa}) are derived from Polprasert and Agarwalla [21]. k_{fa} is adjusted based on the biofilm density range of 0.027–0.115 g cm^{-3} [31]. L_f is adjusted based on the phenomenon found in Muttamara and Puetpaiboon [22], that is, an increase in baffle number would lead to an increase in total biofilm biomass but a decrease in biofilm thickness. θ is the temperature coefficient, with Mara [32] and Thirumurthi [33] calculating the value of θ as 1.05 and 1.036, respectively, and this is used for adjusting k_{fa} and k_{fs}. All equations are summarized in the Supplementary Material in Table S1.

2.4. Baffle Impacts Analysis and Generalization of Kinetic Model

Once the best model formula was selected, the substrate removal efficiencies of the scenarios shown in Table 1 were used to analyse the influence of the installation of attached growth baffles. Some of the scenarios are derived from Coggins et al. [26]. Comparing the results of scenarios allows for the determination of whether the improvement in treatment efficiency of the WSP comes from the hydraulic improvement or the increase in attached biofilm area.

Table 1. BOD removal scenarios for the application of kinetic model of Pond 2. Some of the scenarios are derived from Coggins et al. [26].

Scenario	Characteristics
1	No baffles (control pond)
2	Three perpendicular baffles, no attached growth
3	Three perpendicular baffles with attached growth
4	One island + three perpendicular baffles, no attached growth
5	One island + three perpendicular baffles with attached growth

The organic loading during the experiment was 104 kg ha^{-1} day^{-1}. To extend the application of the kinetic model for a more generalized expression of pond performance, three loading rates, shown in Table 2, were used to further analyse the impacts of hydraulics and biofilm on BOD reduction. Equation (5) was utilised for calculating the dispersion number (d) as previously validated with full-scale pond data [21]. The hydraulic retention time was modelled from 0 to 30 days.

Table 2. Scenarios for generalising the kinetic model in BOD removal efficiency prediction. There are three organic loading rates and each were tested with three distinct sets of characteristics, making nine scenarios.

Organic Loading Rate	Characteristics
As in the experiment (1×)	With hydraulic improvement but no attached growth.
Three times that of the experiment (3×)	With hydraulic improvement and change in biofilm thickness.
Half that of the experiment (0.5×)	With hydraulic improvement and increased in biofilm area.

The value of biofilm thickness used in this study is that used by Polprasert and Agarwalla [21]. To determine whether the change in biofilm thickness impacted pond performance, the analysis was divided into two parts: (1) to increase the biofilm thickness from the baseline of 1.54×10^{-3} m, and (2) to reduce the thickness of the biofilm. The increment uses for both increased and decreased conditions selected in this study was 5×10^{-5} m.

2.5. Statistical Analysis

Principle component analysis (PCA) can be used for identifying the dominant factors in large data sets that have a number of variables and observations [34]. In water quality analysis, this method is mainly used to identify the correlation between water quality indicators and the main factors that cause water quality change [34–36]. PCA was also performed to identify the dominant component of the effluent in this research [34]. Six water quality parameters were considered: temperature (T), total nitrogen (TN), total phosphorus (TP), total suspended solids (TSS), chemical oxygen demand (COD), and biological oxygen demand (BOD).

3. Results

3.1. Hydraulics and Attached Growth

Attached growth baffles increased the effective volume of the experimental WSP and act as a medium for microbial attachment. The data used for effective volume ratio calculation was derived from Coggins et al. [26], and the effective volume ratio (*e*) of Pond 2 before and after baffle installation was 0.73 and 0.94, respectively, suggesting that baffle installation results in a ~29% increase in *e*.

The results of the SEM, as shown in Figure 4, show the temporal comparison of biofilm matrix of strips which were collected at two and seven months, and it is evident that there is a significant difference in biofilm structure between the two images. After two months, the biofilm displays greater microbial diversity and different morphologies can be identified, such as coccus, bacillus, and spirochete, whereas the biofilm at seven months is rich in bacillus and extracellular polymer substances (EPS). Similarly, Figure 5 shows vertical spatial SEM images of the biofilm attached at the top, middle, and bottom of the water column collected after seven months. A large number of microorganisms can be clearly seen in the images at the three depths but differ in diversity. There is clearly more EPS and a thicker biofilm developed towards the top of the water column (Figure 5). The linear relationship and spatial distribution of selected parameters are represented in Figure 6; there is a strong positive correlation between volatile suspended solids (VSS) and carbon content (C) with the concentration of TSS (from TSS samples and TSS measured from samples collected for particulate carbon and nitrogen analysis) and between chlorophyll-*a* (chl-*a*) and VSS. The VSS, C, and chl-*a* are more concentrated at the top of the water column.

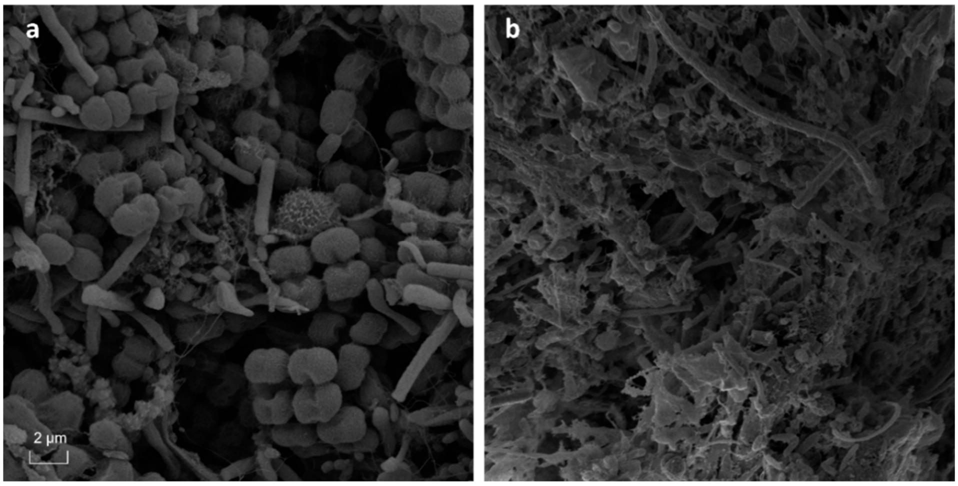

Figure 4. Temporal comparison of biofilm matrices formed on the test baffle strips using scanning electron microscopy (SEM) collected after (**a**) two and (**b**) seven months. After two months the biofilm shows greater microbial diversity, whereas the biofilm at seven months is rich in extracellular polymer substances (EPS).

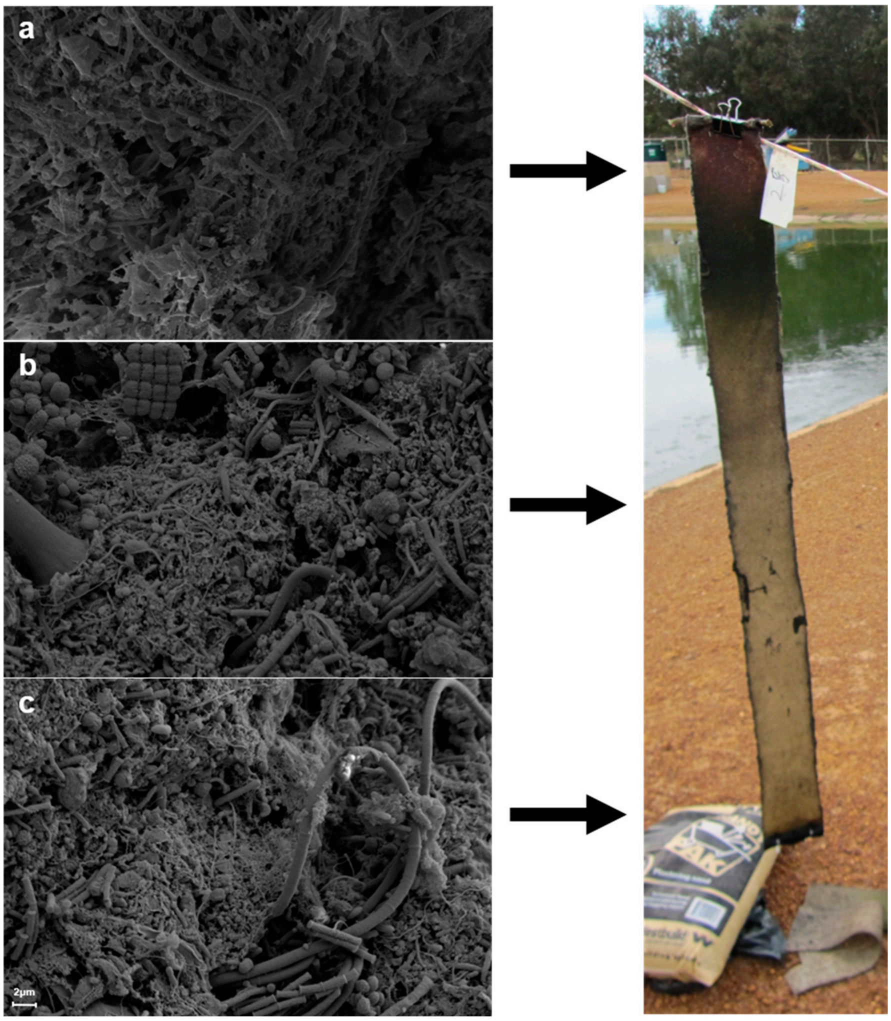

Figure 5. Spatial change in biofilm matrix in the water column (along the baffle test strip) after seven months, shown using scanning electron microscopy (SEM). At the top of the water column (**a**) a thicker biofilm developed that decreased towards the middle (**b**) and bottom (**c**) of the water column.

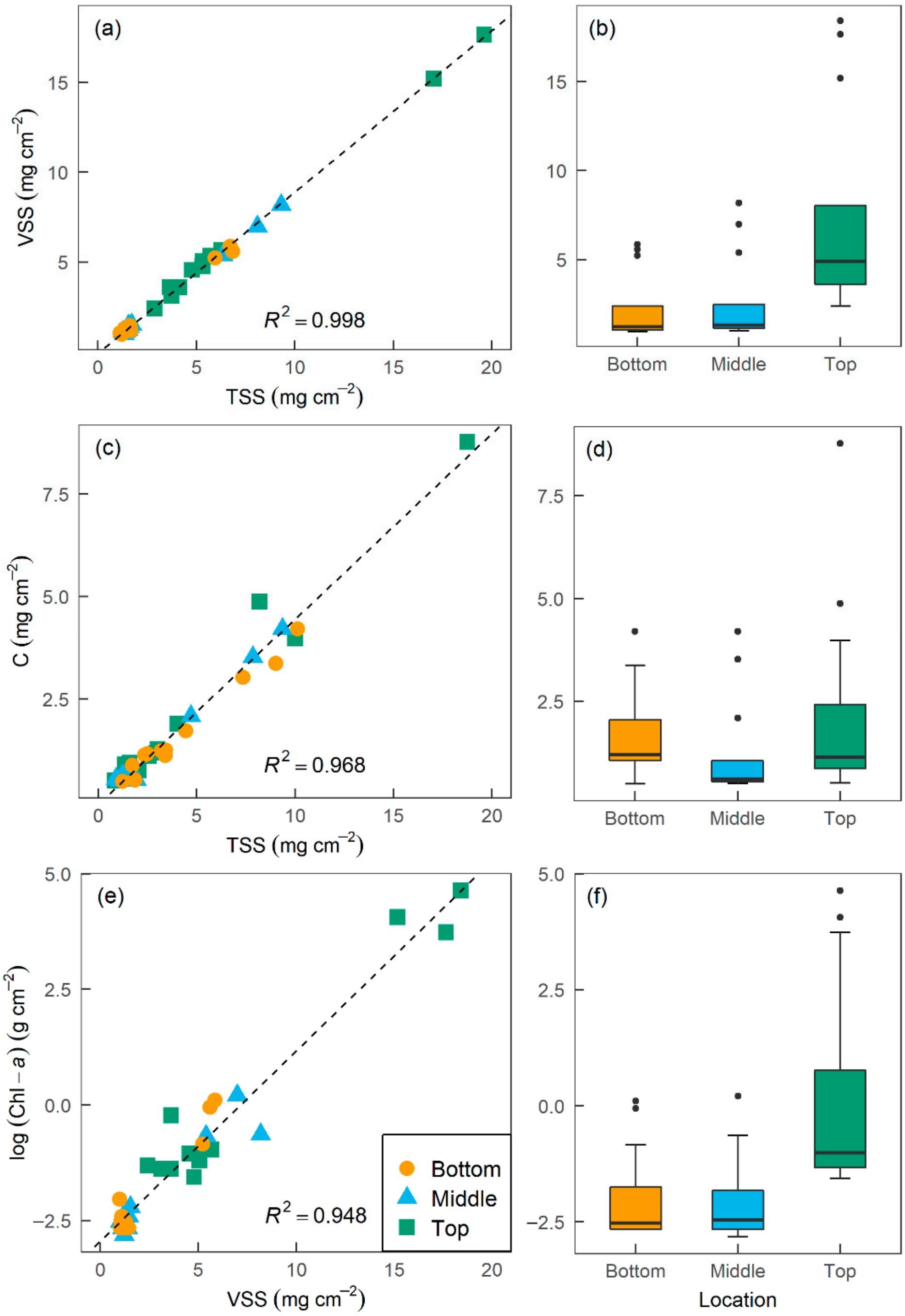

Figure 6. Relationship between selected water quality variables and the baffle samples taken at three points through the water column. Five variables were selected for analysis: volatile suspended solids (VSS); total suspended solids (TSS), including TSS samples and TSS values recorded from particulate carbon and nitrogen samples; attached carbon content (C); and chlorophyll-*a* (chl-*a*). Here, it can be seen that (**a**) VSS, (**c**) C, and (**e**) chl-*a* are more abundant and have a larger range in the upper water (top) column (b, d, f, respectively). Samples collected in the middle and lower parts of the water column (middle and bottom) have similar interquartile ranges for VSS (**b**) and chl-*a* (**f**) content. In the linear analyses (**a**,**c**,**e**), dashed lines represent the line of best fit and R^2 reflects the strength of fit. Log transformations for chl-*a* values are natural log. In boxplots (**b**,**d**,**f**), boxes show quartiles 1 (25%) and 3 (75%) with the median, whiskers show the minimum and maximum values, and dots indicate outliers.

3.2. *Application of Kinetic Model*

3.2.1. Model Validation

The best kinetic model formula was determined by comparing the calculated BOD concentration of effluent with observed data. Using the data collected, BOD removal efficiency was calculated using Equations (2)–(4); the model output that was closest to what was observed was then chosen as the best model. Compared with the outputs from Equations (2) and (3), the predicted result of Formula (4) was the furthest away from the field data. The BOD concentration obtained via Equations (2) and (3) was similar to the observed data and the value of d for all modelled scenarios was less than 2. As Equation (2) is relatively simple in structure it was chosen for the subsequent analysis.

The validation results are shown in Table 3 and the parameters used for validation are shown in Table 4. It can be seen the calculated BOD concentration of effluent with biofilm activity was lower than when considering suspended biomass only and agreed well with the field data. In addition, under the condition of considering suspended biomass activity only, Pond 2 showed better performance in BOD reduction than Pond 1, 42% versus 34%, respectively. The comparison of three models is shown in the Supplementary Material (see Table S2).

Table 3. Best computed (using Equation (2)) and observed BOD concentration of effluent (mg L^{-1}) and removal efficiency (%).

Parameter	Pond 1	Pond 2 (Baffle)
Raw water	200	200
Observed	89	64
Estimated	89.68	65.48
Suspended biomass only	134	116
Actual treatment efficiency	55.5%	68%
Estimated treatment efficiency	55.16%	67.3%
BOD reduction by suspended biomass only	34%	42%

Table 4. The calculation of model parameters at 20 °C.

Parameters	Unit	Pond 1	Pond 2 (Baffled)	Comment
d	-	0.35	0.39	Equations (5) and (6) give the range of 0.336–0.445 for Pond 1 and 0.383–0.400 for Pond 2.
a_s	$m^2\ m^{-3}$	0.822	0.858	Equations (9) and (10)
k_{fs}	day^{-1}	0.0366	0.0366	Equation (8)
k_{fa}	day^{-1}	199	199	Adjusted based on the assumed biofilm density 0.03 g cm^{-3}.
D_f	$m^2\ day^{-1}$	24.45×10^{-6}	24.45×10^{-6}	Assumed based on reasonable principle.
D_w	$m^2\ day^{-1}$	48.9×10^{-6}	48.9×10^{-6}	Assumed based on reasonable principle.
L_f	m	1.54×10^{-3}	1.386×10^{-3}	Assumed based on reasonable principle.
L_s	m	200×10^{-6}	200×10^{-6}	Assumed based on reasonable principle.

3.2.2. Calculating BOD Reduction of Different Scenarios

More scenarios were modelled to identify the most influential factor between hydraulics and attached growth; the results of BOD removal efficiency of each scenario are shown in Table 5. Pond 2 without baffles is considered the control pond in this study and BOD removal efficiency was estimated as 60.4%. Scenarios with three perpendicular baffles with or without attached growth have the same residence time and only differ in biofilm attached area, with BOD removal efficiency improvement compared with the control pond being 6.9% and 5.3%, respectively. Similarly, compared with the control pond, ponds with an island and three perpendicular baffles with or without attached growth also had the same residence time and resulted in BOD reduction of 15.3% and 14.1%, respectively. By comparing scenarios with the same residence time with or without attached growth, the treatment efficiency improvement brought by the attached growth was 1.6% and 1.2%, respectively.

Table 5. The application of kinetic model to estimate BOD reduction of different scenarios.

Scenario	Residence Time (days)	Specific Surface Area (m^2/m^3)	BOD Removal Efficiency	Compared to Control Pond	Comparison of with and without Attached Growth
No baffles	14	0.822	60.4%	0	—
Three perpendicular baffles, no attached growth	17	0.822	65.6%	+5.3%	0
Three perpendicular baffles with attached growth	17	0.858	67.3%	+6.9%	+1.6%
One island + three perpendicular baffles, no attached growth	22.4	0.822	74.4%	+14.1%	0
One island + three perpendicular baffles with attached growth	22.4	0.882	75.7%	+15.3%	+1.2%

3.2.3. Generalisation of Kinetic Model

To generalise the kinetic model, scenarios of different organic loading (0.5×, 1× and 3×) were used to simulate which factors, i.e., hydraulics and biofilm thickness or biofilm area, most influenced the BOD removal efficiency (Figures 7 and 8).

In terms of hydraulics and decreasing biofilm thickness (Figure 7), overall treatment capacity decreases with the increase of residence time (Figure 7a,b), and the impact of biofilm reduction becomes more obvious. The pond becomes more efficient in treating influent with higher (3×) organic loading, whereas the difference of the treatment efficiency between 1× and 0.5× scenarios is negligible. Overall, the efficiency curve of the 3× organic loading scenario is higher. Furthermore, the decrease in biofilm thickness does not have a significant impact on pond performance initially (Figure 7b,c). For the 1× scenario, the decrease in biofilm thickness begins to have an obvious impact on pond performance with the residence time over 20 days and is reduced by ~18% by the 30th day. Similarly, under the 0.5× loading scenario, the decrease in biofilm thickness starts to have an obvious effect on pond performance and reaches a maximum value of ~12% on the 30th day. In addition, as shown in Figure 7b, the trend of the removal efficiency in the 3× organic loading is similar to the others but with a higher removal percentage; the biofilm reduction only has a minor effect on this scale, which reaches the maximum decreased value of 4% at day 30. The scenario of increasing biofilm thickness was also modelled but did not have a significant effect on pond performance. Overall, the impact on pond performance brought by a change in biofilm thickness gradually increases when biofilm thickness decreases past a certain threshold.

For the comparison of hydraulics and increasing specific biofilm area (Figure 8) the interval for increasing the specific biofilm area used was 0.01 m^2 m^{-3}, which is approximately equal to the area of one installed baffle. The degradation efficiency of BOD increases with the increasing residence time but the rate gradually slows down (Figure 8b). Overall, the pond is more efficient in treating influent with higher organic loading, and the increasing biofilm area has a more obvious effect in ponds with lower organic loadings. The largest efficiency improvement is in the 0.5× loading scenario, which is about 6%. The maximum values for improvement under 1× and 3× organic loading are ~4% and ~1.4%, respectively.

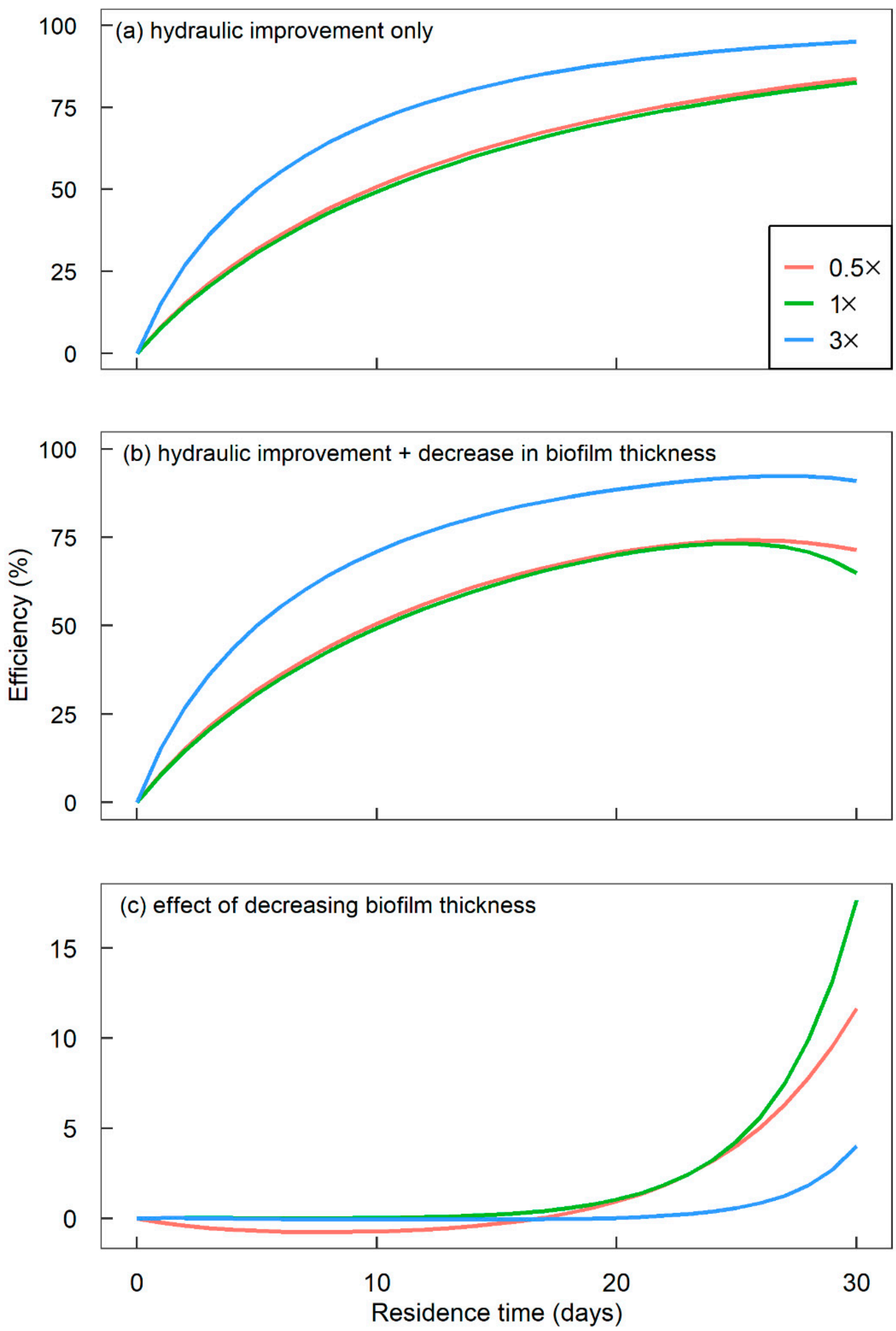

Figure 7. Comparison of biological oxygen demand (BOD) removal efficiency under three scenarios, 0.5× (red), 1× (green), and 3× (blue) loading, shown as: (**a**) hydraulic improvement only, (**b**) hydraulic improvement with a decrease in biofilm thickness, and (**c**) effect of decreasing biofilm thickness only, calculated as the difference between scenarios shown in (**a**,**b**). The reduction of biofilm thickness within a certain range does not have a significant impact on final effluent quality.

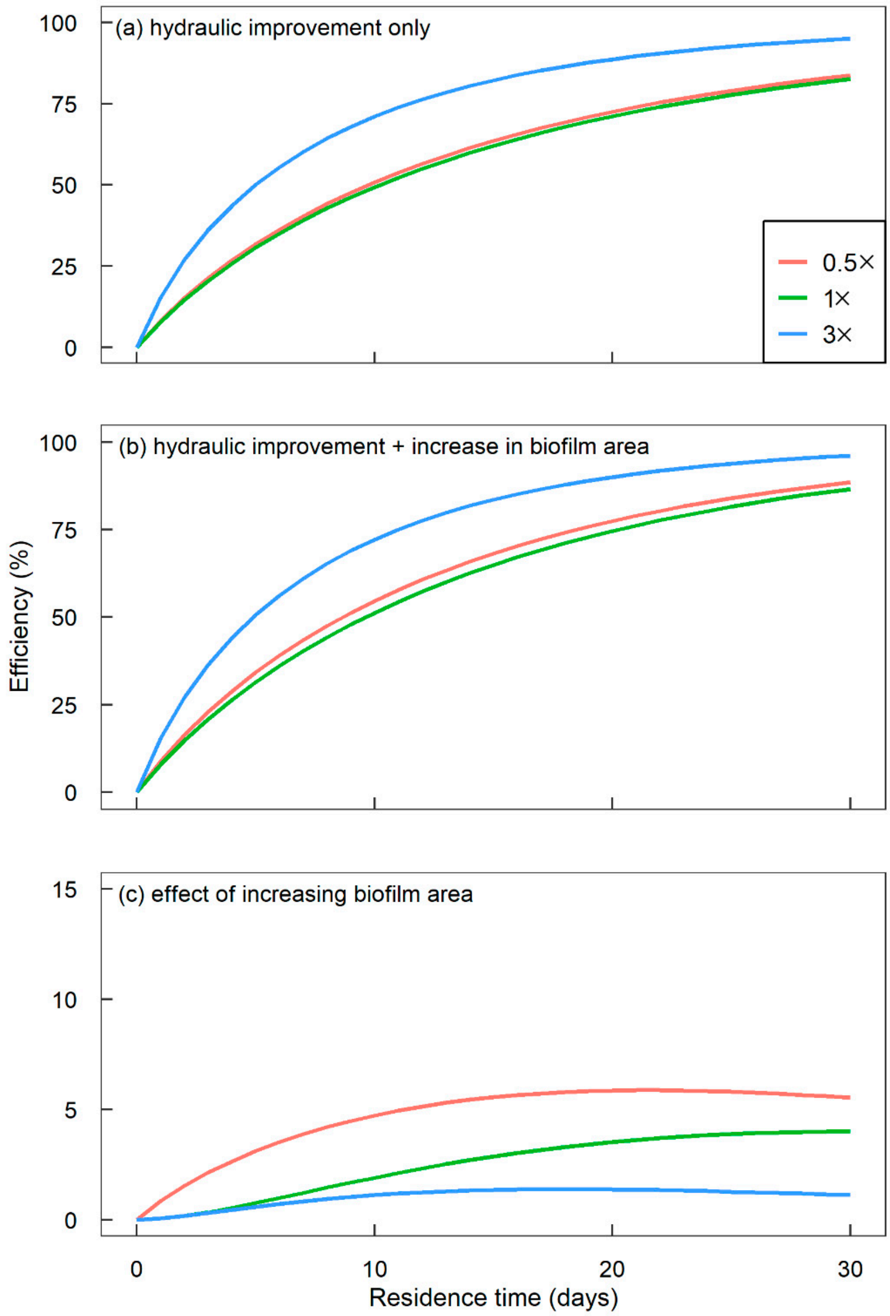

Figure 8. Comparison of biological oxygen demand (BOD) removal efficiency under three scenarios, 0.5× (red), 1× (green), and 3× (blue) loading, shown as: (**a**) hydraulic improvement only, (**b**) hydraulic improvement with an increase in biofilm area, and (**c**) effect of increasing biofilm area only, calculated as the difference between scenarios shown in (**a**,**b**). The increase in biofilm area has a more obvious impact on ponds receiving lower organic loading.

3.3. Water Quality

Six parameters of Pond 1 and Pond 2 (baffled) were included as independent variables for PCA analysis. In Figure 9, the horizontal and vertical coordinates represent the largest and second largest variability among all combinations. All the selected water quality parameters are negatively correlated with PC1 value in Pond 1, and the interaction among various factors are not obvious except all parameters are positively correlated with each other. Furthermore, based on the results of varimax rotations, high loadings of PC1 and PC2 can mostly be attributed to TSS and temperature, respectively. For Pond 2, TN, TP, and TSS have larger weightings for PC1 and have an inverse relationship with PC1 value. Similarly, PC2 has higher loadings for temperature, BOD, and COD, and shows a positive relationship. The high loadings of PC1 and PC2 result from TP and COD, respectively. Moreover, the distribution of water samples collected in Pond 1 and Pond 2 is shown in Figure 10; compared with Pond 1, it is clear to see that the samples collected in the baffled pond have a smaller weighting in PC1 and similar significance in PC2.

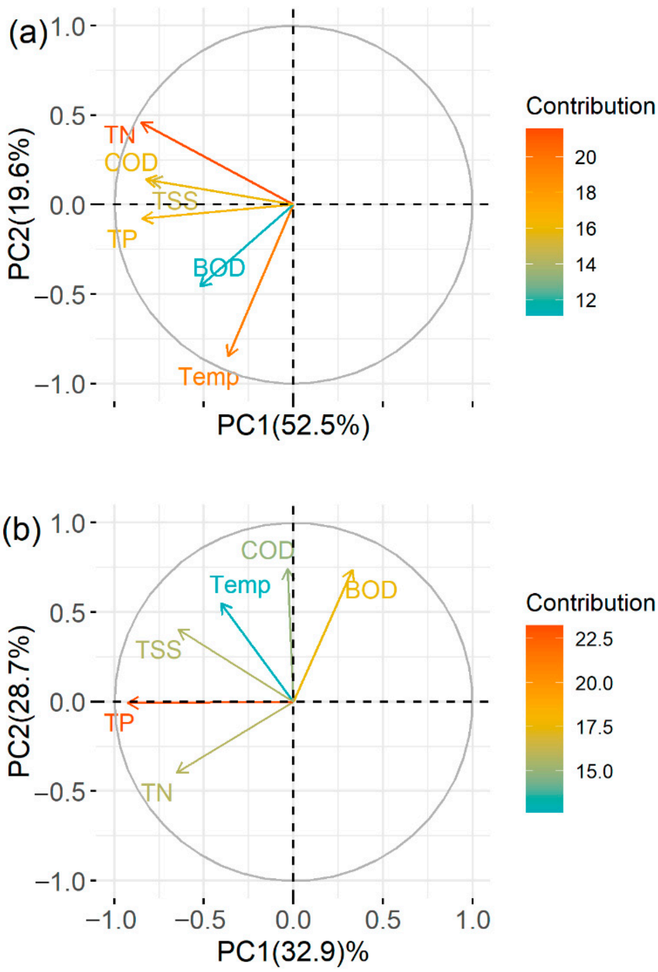

Figure 9. Biplots of results of principle component analysis (PCA) for (**a**) Pond 1 and (**b**) Pond 2 (baffled pond). In Pond 1 (**a**), all the selected water quality parameters are negatively correlated with PC1 but positively correlated with each other. In Pond 2 (**b**), TN, TP, and TSS show an inverse trend along PC1, whereas PC2 has higher loadings in temperature, BOD, and COD and shows a positive relationship.

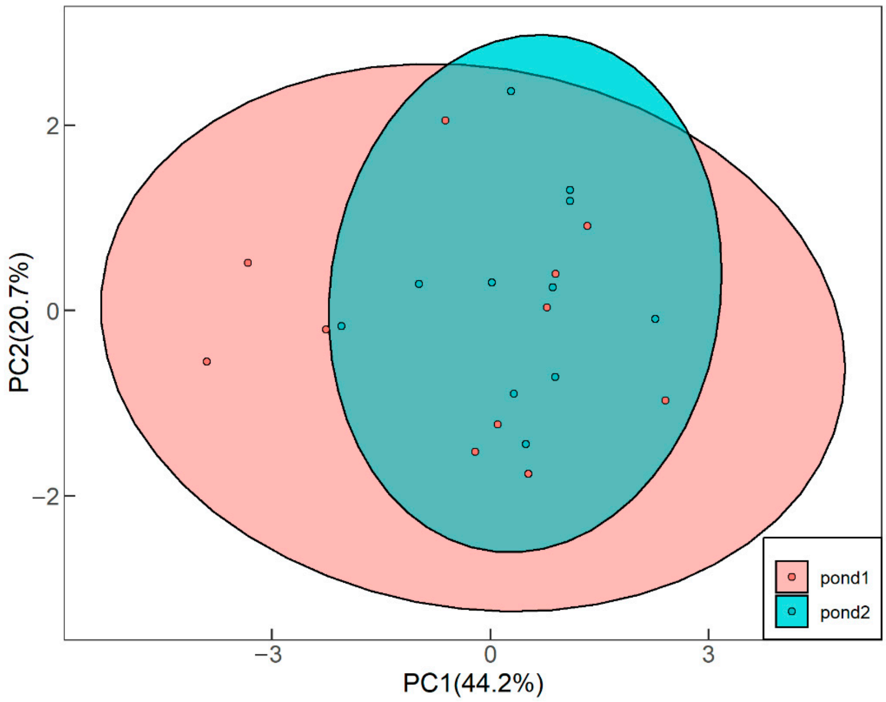

Figure 10. Biplot from PCA analysis of the distribution of water samples collected in Pond 1 and Pond 2. Compared with Pond 1, samples collected in the baffled pond have a smaller weighting in PC1 and similar significance in PC2.

4. Discussion

4.1. Hydraulic Improvement and Attached Growth

The optimal baffle configuration resulted in an increase in residence time in Pond 2 from 14 to 17 days, and the effective volume ratio of both conditions was calculated as 0.73 and 0.94. Based on Equation (1), the results indicated that the installation of baffles resulted in the mean residence time being closer to the nominal residence time. A study by Persson and Wittgren [5] on how hydraulics influence pond performance suggests that the smaller the difference between the mean residence time and nominal residence time, the fewer dead zones and short-circuit conditions in pond. Moreover, the results demonstrated in Persson and Wittgren [5] also suggested that effective volume ratio (e) had a clear positive influence on pond performance.

The biofilm at two and seven months showed high diversity and EPS content, respectively, which is consistent with previous research; there is evidence that less EPS would be produced due to the rapid growth of microorganisms at the early stage [37] and more EPS will appear while the inner biomass is growing slowly [37–39]. EPS plays an important role in the stabilization of the biofilm structure and the communication among cells [37]. Furthermore, the structure of the biofilm will be influenced by the organic loading, hydraulics (especially the shear stress), and the composition of the physiological group [40]. Some researchers have also indicated that the steady-state conditions could lead to the balance between biofilm growth and the detachment [40] and shedding of biomass would be reflected in the sudden increase in total COD [41]. The increased attached growth is regarded as relatively stable after seven months considering the steady-state organic loading, the abundance of EPS, and no significant changes in the average COD in effluent. Over time, we could expect the change in the biofilm structure is negligible.

SEM images and selected indicators show evidence of biofilm growth on the baffle surface and the diversity and concentrations are different at different growing depths. One explanation could be that the upper area of baffles is more likely to receive sufficient light [42]. The abundance of chl-*a* content in the upper area is consistent with algal biofilms having the ability to colonise where light and nutrients are available [42]. Moreover, Figure 4 suggests the richness in EPS on the upper surface. This is in accordance with earlier studies that indicated that EPS production is highly correlated with photosynthesis [43,44].

4.2. Analysis of the Improvement of Pond Performance

The BOD reduction by the suspended biomass only for Pond 1 and baffled Pond 2 was found to be 34% and 42%, respectively, which were both more than 20% lower than the observed overall BOD removal efficiency. These results reflect that the biofilm attached on the pond walls and floor plays an important role in substrate reduction. Furthermore, the calculated BOD removal efficiency of Pond 2 without the baffles installed was ~60%, which is in between the removal efficiency for Pond 1 and Pond 2 with baffles (~56% for Pond 1 and ~68% for Pond 2 with baffles). This outcome is to be expected owing to the better hydraulic performance of Pond 2 compared with Pond 1. Compared with the control pond, the removal efficiency only increased marginally with the increase in biofilm-attached area, which is equivalent to the surface area of three or five baffles. Conversely, the treatment efficiency increased by 5.3% when the residence time increased from 14 to 17 days, and a more pronounced increase was found (up to ~15%) when the residence time increased to ~22 days. In this study, it is clear that hydraulics is the dominant factor for improving treatment efficiency and increased biofilm has a minor effect on pond performance at the considered time and experimental scale.

The treatment performance of the pond receiving a higher organic loading is significantly higher than the other two scenarios. However, the general trend of all scenarios is similar: the rate of increase in BOD removal efficiency gradually slows down and plateaus. Previous research states that BOD removal is similar to first-order kinetics, that is, the removal efficiency of BOD at any time is proportional to the remaining concentration of BOD [1]. With the increase in hydraulic retention time, the remaining BOD concentration in pond decreases, resulting in the BOD removal efficiency slowing down. Another explanation for this phenomenon is that the increase in influence concentration improves the stability of the pond system against hydraulic loads, resulting in better performance [24].

4.3. The Influence of Biofilm Thickness Changes on Pond Performance

The reduction of biofilm thickness within a certain range will not have a significant impact on final effluent quality (Figure 7). Previous studies reported that only 6% of the total biomass was active in mobile bed biofilm and also the existence of a minimum active biofilm thickness [45,46]. Therefore, the variation within a certain range of biofilm thickness will not have a large effect on the final effluent and the influence only starts to become obvious after the thickness decreases to a certain amount. In addition, a pond receiving lower organic loading being more affected by the change in biofilm thickness may be explained by the insufficient transport of substrate into the deeper parts of the biofilm and the correlation between active thickness and substrate concentration [47–50]. However, in the current study, the scenario with the 1× organic loading was more affected by the decrease in biofilm thickness than the 0.5× loading scenario. One possible explanation could be that the difference between these two organic loads is not obvious enough to reflect the general trend. Furthermore, the above results are based on the premise that the biofilm thickness is uniform, however, it is obvious that the thicker biofilm is developed at the top of water column (Figure 5). Overall, the structure of first-order kinetic models for wastewater treatment need to be improved by considering biofilm structure and development [47], which can be achieved by further studying the role of biofilm in wastewater treatment and improved models for predicting the treatment efficiency of WSPs.

4.4. The Influence of Increasing Biofilm Area on Pond Performance

The removal performance of ponds receiving large organic loading is less affected by an increase in biofilm area, and the significance of the effect will decrease as the biofilm area continues to increase. A previous study showed that WSPs with attached growth media could have a better treatment efficiency for organic matter, nutrients, and suspended solids, where the improvement in COD removal efficiency could be increased by up to 10% but the treatment efficiency would not continue to increase significantly with the increase of attached growth media percentage [24]. The possible explanation could be either that the laboratory-scale pond cannot simulate the real situation or the benefit by the continuous growth of biofilm gradually decreases. Polprasert and Khatiwada [51] found that the BOD removal efficiency of water hyacinth ponds could increase by ~16% if the specific surface area was doubled. In order to confirm whether this phenomenon is theoretically feasible for a typical WSP, the specific surface area of control pond was increased from 0.82 $m^2\ m^{-3}$ to 1.64 $m^2\ m^{-3}$ and it was found that BOD treatment efficiency increased by ~12%. This result shows that it is possible to have an obviously positive effect on pond performance if the specific surface area is increased a significant amount. However, for a water hyacinth pond unit as presented in Polprasert and Khatiwada [51], the increased specific surface area is related to the stocking density of the water hyacinth plant [51], which is rare in operational WSPs. Additionally, in the study pond, an increase in biofilm area from 0.82 $m^2\ m^{-3}$ to 1.64 $m^2\ m^{-3}$ is equivalent to the surface area of more than 82 baffles—this is very unrealistic. In general, although the increase in biofilm area does not significantly improve the treatment efficiency at the experimental scale of this study, a considerable improvement could be achieved when the biofilm area is significantly increased. Thus, further investigation is required to determine how this could be realistically applied in WSPs to maximize improvement in pond performance while improving hydraulics.

4.5. The Analysis of First-Order Kinetic Model Formula

The dispersion number (d) in all simulated scenarios was in the range 0.30–1.30, and within the same scenario, a smaller the dispersion number the higher the treatment efficiency. Polprasert and Bhattarai [29] demonstrated that the value of the dispersion number reflected the flow conditions of the pond: in ideal plug flow conditions $d = 0$ and BOD degradation efficiency is highest, whereas large values of d correspond to more dead zones and short circuits in ponds (i.e., decreased residence time), resulting in less time for biochemical reactions to take place and a lower degradation efficiency. In this study, whereas the dispersion number calculated for Pond 1 is lower than both Pond 2 scenarios (with or without three perpendicular baffles), this did not translate to better treatment efficiency. However, this could be due to the greater effective volume ratio of Pond 2 [5], and the prediction of nitrogen removal through first-order kinetics conducted by Polprasert and Bhattarai [29] showed that the dispersion is of minor importance for pond performance compared with the effective volume. As some of the parameters applied in the kinetic model were obtained from the literature, a sensitivity analysis was conducted to determine the effect of their variation on BOD treatment efficiency. The parameters k_{fa}, D_w, D_f, L_f, and L_s were varied by $\pm$10%, and d was adjusted based on the range of two different formulas. The results showed that d and k_{fa} are the most sensitive parameters to the model results but the differences with the initial results are not significant and both are within the range of $\pm$1.5%. The parameters D_w, D_f, L_f, and L_s produced less variation (within $\pm$0.5%) in BOD removal efficiency. Furthermore, the effluent concentration will vary within $\pm$1 mg L^{-1} depending on the precision of the values used in the calculation. The results of the sensitivity analysis provide a direction for the further improvement of the kinetic model, such as adjusting the d value more carefully and measuring the value of k_{fa} in experiments.

4.6. The Relationship among Water Quality Indicators

For both ponds, all indicators are significant for water quality and show variational correlations. To date, temperature has been proven to have a significant impact on treatment efficiency [1,52,53] and there is a correlation between BOD and COD [54]; however, the relationship between other water quality indicators is unclear [55]. Moreover, the projection of water samples collected in the baffled pond along PC 1 is significantly smaller than that of Pond 1, resulting from the fact that PC 1 of Pond 1 mainly reflects other water quality indicators except for T and further suggests that installed baffles could improve the water quality of effluent. In general, PCA is useful for pointing out dominant variables and the interaction within them, resulting in simplifying large data sets [56]. Although this study only included a few indicators, it provides potential direction for future research with large water quality data sets.

4.7. Recommendations

From this study, several directions that could be explored in further research include, but are not limited to, the following:

- Exploring and considering the interplay between hydraulics and suspended biomass in the kinetic model.
- Including the correlation between biofilm structure and development in the model formula.
- Performing longer field experiments and increasing the frequency of water quality data collection. In this study, the attached growth baffles were installed in Pond 2 at the study site for 13 months, however, the water quality data was limited after removing the outliers.
- Measuring all the result-sensitive values in the first-order kinetic model formula (e.g., k_{fa}).
- The choice of baffle material would be more flexible: choosing baffles without the ability of attached growth, which will lower their cost.
- Exploring methods and materials to greatly increase attached growth area to maximize the effect of both hydraulics improvement and attached growth.

5. Conclusions

In this study, a first-order kinetic model was applied to predict the BOD removal efficiency of WSPs with and without attached growth baffles. The results demonstrate that the biofilm growing on the pond walls and floor contributes to the degradation of BOD. In the full-scale experimental WSP, most of the improvement to pond performance brought by attached growth baffles was related to hydraulic improvement (i.e., increasing residence time). However, a considerable improvement in BOD treatment efficiency can be achieved (~12%) by doubling the total biofilm area. Overall, the treatment efficiency of conventional WSPs can be effectively improved by the installation of simple baffles. Increasing biofilm area on baffles has the potential to significantly improve treatment performance, however, further studies are required to explore how this could be achieved at the operational scale.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/w14203245/s1, Table S1: List of equations; Table S2: Modelled effluent results (mg L^{-1}) of Equations (2)–(4).

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