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Performance intensification of constructed wetland technology: a sustainable solution for treatment of high-strength industrial wastewater

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ABSTRACT

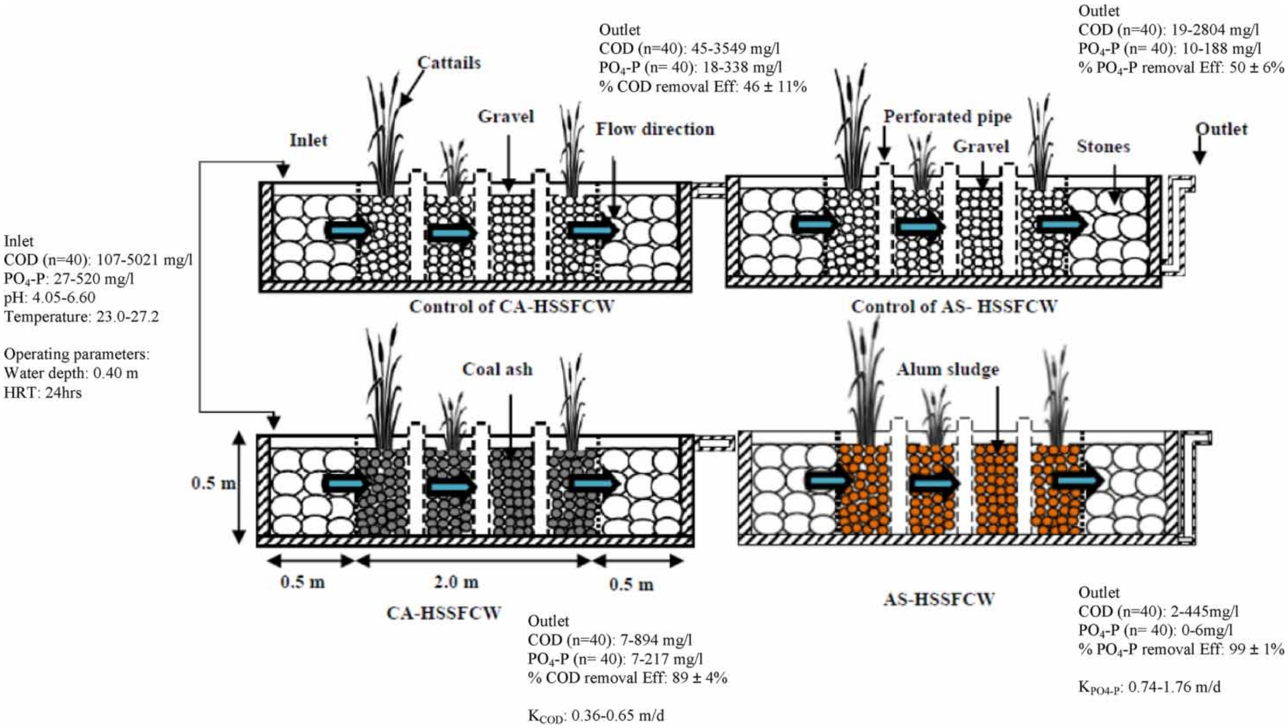
The objectives of this study were to: (1) assess the intensification of chemical oxygen demand (COD) and phosphate (PO_4-P) removal; and (2) generate a set of rate constants of COD degradation (k_{COD}) and phosphate ($k_{PO4\text{-}P}$) removal for the treatment of industrial wastewater (WW) using intensified adsorption beds. Two horizontal subsurface flow constructed wetlands (HSSFCWs) filled with coal ash and alum sludge and two conventional HSSFCWs packed with gravels were operated with different loadings of COD and PO_4-P at a hydraulic retention time (HRT) of 24 hrs at water depth of 0.40 m. The bed performance was analysed for COD and PO_4-P removal efficiency. The intensified HSSFCWs outperformed the control beds by a mean COD and PO_4-P removal efficiency of 43 and 49%, respectively. The progression of COD and PO_4-P removal along the system was fitted into the first-order plug flow model (K-C model). In this study the k_{COD} values ranged from 0.36 to 0.65 m/d with a mean of 0.46 $\pm$ 0.08 m/d ($n = 30$). The $k_{PO4\text{-}P}$ values ranged from 0.74 to 1.76 m/d and averaged to 1.23 $\pm$ 0.37 m/d ($n = 30$), irrespective of the condition applied. Hence, these data can be used for future projects using HSSFCWs to treat industrial wastewater.

Key words: adsorption, constructed wetland, intensified, K-C model

HIGHLIGHTS

- Industrial wastewaters are strong organic sources of pollution.
- Industrial effluents are not commonly treated using conventional HSSFCW.
- Addition of an adsorption mechanism using coal ash and alum sludge in the system intensifies bed performance.
- Such intensified HSSFCWs are a sustainable and low-cost treatment system.
- Rate constants are important design parameters to determine the size of a HSSFCW.

GRAPHICAL ABSTRACT



INTRODUCTION

The discharge of untreated industrial effluents is considered as one of the main sources of water pollution that impact negatively on the environment and human health (Swain *et al.* 2018). Hence, purification of such effluents is necessary, but this may be complicated due to the presence of complex chemical profiles (Yang *et al.* 2018). Constructed wetland (CW), also known as the 'natural biological reactor', is a sustainable alternative for the purification of industrial wastewater (WW) (Khan *et al.* 2009). This nature-based biological system comprises macrophages, substrates and microbial communities and treats wastewater through biological, chemical and physical processes (Saeed & Khan 2019). CWs are effective in contaminant removal, require simple maintenance and do not typically require the input of energy and/or chemicals (Zhuang *et al.* 2019).

Among the various types of constructed wetlands that exist, horizontal subsurface flow CWs (HSSFCWs), the focus of this paper, have obtained much attention globally (Zhou *et al.* 2019). Wu *et al.* (2015) reported that the direct input of effluent having high loads of organic matter (OM) in the system can affect the growth and health of the plants as well as the treatment performance. Rajkumar *et al.* (2010) stated that to implement a conventional biological treatment system, the biological oxygen demand/chemical oxygen demand (BOD/COD) ratio, which signifies the biodegradability of WW, should be greater than 0.6. Effluents having a BOD/COD ratio less than 0.6 cannot be treated effectively by biological purification system alone; additional treatment is typically required.

CWs have developed from passive systems to advance engineered systems, especially those known as intensified wetlands. These have the capacity of degrading contaminants 10–1,000 times faster than conventional CW (Nivala *et al.* 2019). Intensified CWs can be designed differently and built into special configurations to increase treatment performance (Wu *et al.* 2014). The intensified systems provide advantages, such as they require relatively small space to treat WW with an elevated level of pollutants. Among the intensified systems that exist, the integration of adsorption removal mechanisms into CW is believed to be a useful approach because of various benefits such as simple operation, fast elimination of pollutants, low cost and prevention of production of secondary contaminants (Nivala *et al.* 2019).

The substrate is an important component of a CW system especially for subsurface CWs since it allows attachment of microorganisms, serves as a growing platform for the macrophages and has the potential to eliminate pollutants (Wu *et al.* 2015). Yang *et al.* (2018) discuss that the substrate can either be natural (such as gravel) or artificial (such as by-products from industrial processes). Wahyuni *et al.* (2018) found that alum sludge (AS) is a good adsorbent of phosphate (PO_4-P) and coal ash is a potent adsorbent for the removal of COD. However, there has been no study on the integration of coal ash and AS into HSSFCWs purifying high-strength industrial effluents. Also, there is little published data on the COD and PO_4-P removal rate constants (k_{COD} and $k_{PO4\text{-}P}$) for this type of technology treating effluent with elevated levels of COD and PO_4-P. Inaccurate data for k_{COD} and $k_{PO4\text{-}P}$ can lead to over- or underdesign and ultimately can cause poor performance and/or failure of the HSSFCW.

Hence, the main aims of this study are: (1) to investigate the intensification of COD and PO_4-P removal in a coal bottom ash (CBA) and AS integrated HSSFCW; and (2) to develop a range of rate constants of COD/ PO_4-P removal (k_{COD} and $k_{PO4\text{-}P}$) and consequent operational criteria for such system.

METHODOLOGY

Batch experiment

Batch analysis was performed as per the method of Shah *et al.* (2013) to obtain the adsorptive capacity and adsorptive index of coal ash and AS. For the investigation of COD adsorption by activated CBA, a molasses solution having a concentration of 3,435 mg/l of COD was prepared. A phosphate solution of concentration 610 mg/l of PO_4-P was prepared to assess phosphate adsorption by AS.

Batch experiments were performed with sets of beakers comprising 100 ml molasses solution in contact with of 2 g, 4 g, 6 g, 8 g, 10 g and 12 g of activated CBA. The beakers were shaken continuously for 6 hours at 160 rpm until equilibrium was obtained. Samples were taken at an interval of two hours over a period of six hours. A Batch test for the adsorption of phosphate by AS was performed using 0.5 g, 1.0 g, 1.5 g, 2.0 g, 2.5 g and 3.0 g of AS in contact with 100 ml of PO_4-P solution. The samples were then analysed for COD and PO_4-P. The determination of COD was carried out according to APHA (2005) Standard Method of Examination of Water and Wastewater. The USEPA approved Hach DR-2500 light spectrophotometer was utilised to determine PO_4-P by the PhosphoVer3 (ascorbic acid) method.

Continuous column analysis

Continuous column analysis was carried out according to the method described by Wahyuni *et al.* (2018) to obtain the saturation time of the activated CBA and AS. For COD adsorption, 20 g of CBA was placed in a 5 cm diameter glass column and for phosphate adsorption; 15 g of AS was used. Molasses and phosphate solution were fed from the top of the column at a flowrate of 3 ml/min. Samples from the bottom were extracted each 15 minutes over a period of three hours and were then analysed for COD and PO_4-P.

Adsorption isotherms

For better determination of the behaviours, interactions and adsorptive capacity of CBA and AS, Freundlich (Equation (1)), Langmuir (Equation (2)) and Dubinin-Radushkevich (Equation (4)) sorption isotherms were used.

$$ln q_e = ln k_F + \frac{l}{n} ln C_e \tag{1}$$

where q_e is the equilibrium adsorption capacity of adsorbent in mg/g; k_F stands for the Freundlich constant, adsorption capacity in mg/g; n is the Freundlich adsorption intensity, which is dimensionless and it represents favourable adsorption if n-value lies between 0 and 10 (Mirzaei & Javanbakht 2019)

$$\frac{C_e}{q_e} = \frac{C_e}{Q_{max}} + \frac{1}{Q_{max} k_l} \tag{2}$$

C_e denotes the equilibrium concentration in mg/l; Q_{max} is the maximum adsorption capacity of adsorbents in mg/g; k_L is the Langmuir constant in L/mg. The favourability of adsorption can be determined by R_L, which is a dimensionless separation factor as shown in Equation (3). Favourable adsorption occurs when R_L lies between 0 and 1, linear when R_L is equal to 1 and irreversible when R_L is equal to 0 (Gebreegziabher *et al.* 2019).

$$R_L = \frac{1}{1 + bC_o} \tag{3}$$

$$\ln q_e = \ln q_{max} - \beta\varepsilon^2 \tag{4}$$

In Equation (4), β represents the mean adsorption energy in mol^2/kJ^2 and ε symbolises the Polanyi potential, which is calculated using Equation (5).

$$\varepsilon = RT \ln\left(1 + \frac{1}{C_e}\right) \tag{5}$$

R represents the universal gas constant, which is 8.3145 J/mol/K and T is the absolute temperature in Kelvin (^oK). The Dubinin-Radushkevich isotherm was utilised to find out the type of sorption behaviour (Tolić *et al.* 2019). The mean free energy of sorption, denoted by E (kJ mol^{-1}), is associated to the reaction mechanism. A value less than 8 kJ/mol represents physical sorption, while those that lies between 8 and 16 kJ mol^{-1}, indicates chemical sorption. Those greater than 16 kJ mol^{-1} signify a very strong chemical sorption (Yan *et al.* 2014). The mean sorption energy can be calculated by Equation (6).

$$E = \frac{1}{\sqrt{2\beta}} \tag{6}$$

Yoon-Nelson model

Among the models related for the continuous column experiment, the Yoon Nelson model as shown in Equation (7) was found to be a less complex model to determine the saturation time of adsorbents since it does not need any information relating the properties of the adsorbent or adsorbate (Jang & Lee 2019).

$$ln\frac{C_t}{C_o - C_t} = k_{YN}t - \tau k_{YN} \tag{7}$$

In Equation (7), k_{YN} represents the rate constant in min^{-1} and τ stands for the time required for 50% adsorbate breakthrough (in minutes).

Experimental set-up of coal ash and alum sludge HSSFCW

For the experiment, four HSSFCWs were constructed at Reduit, University of Mauritius (20°13′59.9″S 57°29′57.5″E). Two parallel treatment systems were set up whereby each was established with two HSSFCWs in sequence, each having dimensions of 3.0 m × 0.5 m × 0.5 m. One train, denoted as 'intensified beds', comprised of one HSSFCW in which activated coal ash was used as substrate (denoted as CA-HSSFCW), followed by the second train, which was packed with AS (denoted as AS-HSSFCW). Control beds were composed of two conventional HSSFCWs in series. For both experiments, the train was packed with gravel (denoted as CCA-HSSFCW for the coal ash and as CAS-HSSFCW for the AS). The inlet and outlet structures were comprised of stones with a size of 50–200 mm. Cattails (*Typha latifolia*) were planted in all the four beds at a planting density of 8 plants/m^2. Synthetic industrial wastewater simulating COD concentrations ranging from 107 to 5,021 mg/l was prepared by diluting the required amount of molasses in a feed tank having a capacity of 2 m^3. The required amount of potassium dihydrogen orthophosphate and potassium nitrate (nutrients for macrophage growth) to achieve PO_4-P concentration of 27–520 mg/l were diluted in the same feed.

As this study is based on the purification of high-strength effluent, molasses were utilised to imitate a real industrial WW since it consists of hard fractions as well as organic components (Meng *et al.* 2017). Synthetic wastewater was prepared two or three times per week. Furthermore, three perforated pipes were placed within each bed to take samples as well as to allow oxygen to infiltrate the substrate. A ball valve was fitted in the inlet line to control the flowrate of the WW fed to the HSSFCW. The experimental set up is illustrated in Figure 1.

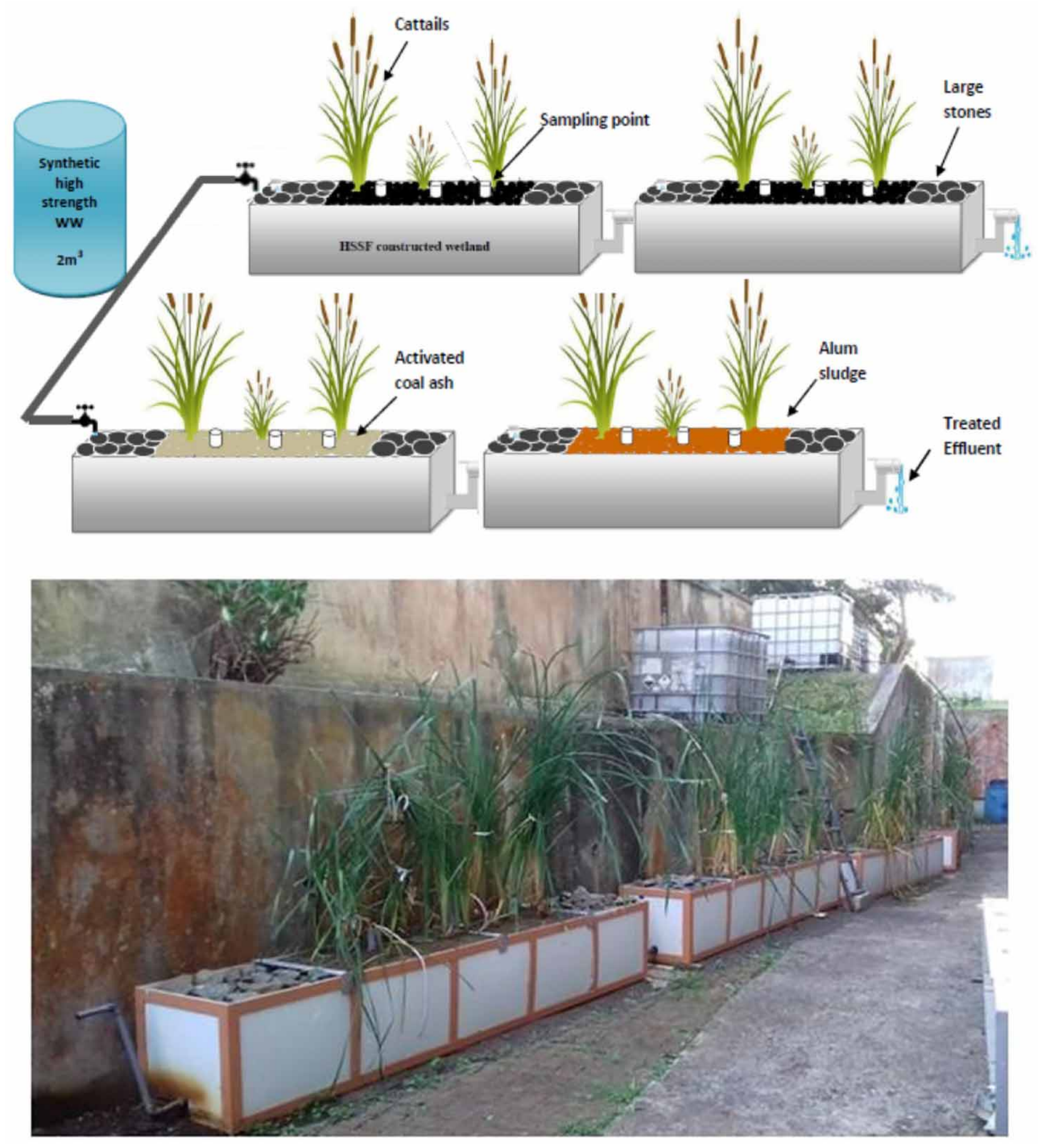


Figure 1 | Intensified and conventional experimental HSSFCWs.

Operating the pilot system

The system was started up for a continuous period of ten weeks with synthetic wastewater of low COD loading (107–130 mg/l) and phosphate loading (23–35 mg/l PO_4-P) at retention time of 24 hrs and a water depth of 0.40 m. After successful start-up, the beds were operated at medium organic loads (912–1,563 mg/l) and phosphate loadings (316–380 mg/l PO_4-P) for a continuous period of 18 weeks. Thereafter, the experimental beds were run at a higher COD loads of range 4,801–5,021 mg/l and phosphate concentrations of range 488–520 mg/l PO_4-P for 12 weeks. Samples were taken from the inlet port, the three intermediate sampling ports and outlet pipes, and were analysed for COD and PO_4-P. The inlet, three intermediates and outlet sampling ports (denoted respectively as S_1, S_2, S_3, S_4 and S_5) are shown in Figure 2. The samples were also monitored for pH and temperature.- The pH was monitored by means of a digital pH meter (model: EUTECH Instrument cyber scan pH 11). A thermometer was used to measure the temperature of the samples.

Kinetics and first-order plug flow modelling

Kinetics for a chemical engineer are considered as an essential element in designing at a professional stage (Sheridan *et al.* 2014).With respect to time, the reaction rate is the speed at which reactants are converted into products. The first-order rate of reaction is proportional to concentration. According to Sheridan *et al.* (2014), the linear reaction rate (first-order rate) is usually used for CW unless they operate beyond saturation conditions. Microbial degradation along the HSSFCW follows a K-C model, commonly known as a first-order plug flow model, from which the Kickuth equation (Equation (8)) was derived.

$$A = \frac{Q(\ln C_o - \ln C_t)}{k_{COD}} \tag{8}$$

where A: bed surface area, m^2; Q: flow rate of effluent, m^3/day; C_o: inlet COD concentration, mg/L; C_t: outlet COD concentration, mg/L and k_{COD}: COD rate constant, m/day.

The first-order plug flow model can be rewritten as:

$$C_t = C_0 e^{-kt} \tag{9}$$

where C_t: effluent concentration of COD/PO_4-P (mg/l); C_o: inlet concentration of COD/PO_4-P (mg/l); k: rate constant, (/d); t: time of degradation (d).

The K-C model fits best for an ideal flow condition and also shows that the exponential decrease of pollutants tends to a zero value. Von Sperling & de Paoli (2013) showed that a HSSFCW does not behave as an ideal flow reactor and an outlet concentration of zero was never found. Therefore, the K-C* model is recognised as a more appropriate model than the K-C model to be used in a HSSFCW since a non -zero outlet concentration is mostly found. Equation (10) shows the K-C* model.

$$C_t - C^* = (C_0 - C^*)e^{-kt} \tag{10}$$

The rate constants k_{COD} and $k_{PO4\text{-}P}$ are important parameters for the sizing of a HSSFCW. This study attempts to propose a range of these rate constants for an intensified bed purifying high-strength industrial WW utilising a first-order plug-flow

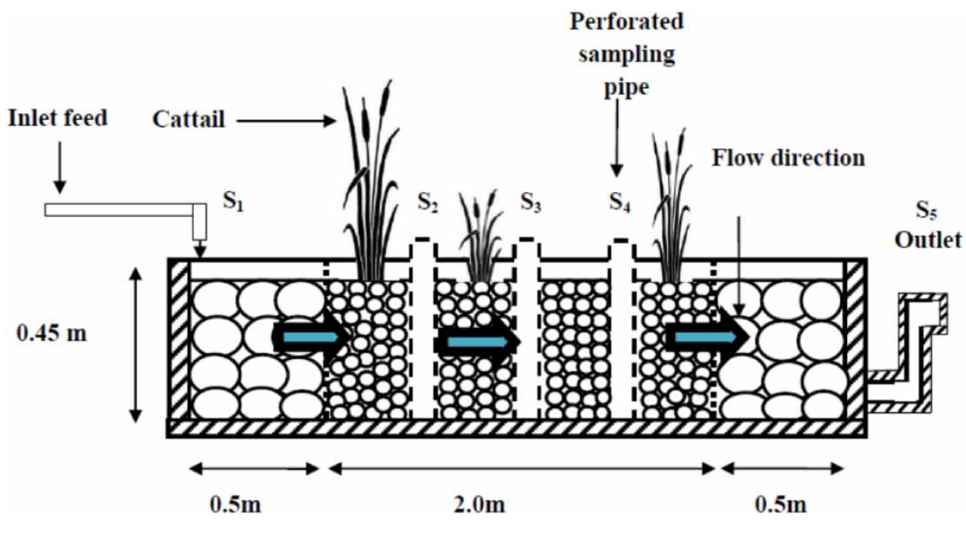


Figure 2 | HSSFCW showing sampling ports.

model. These can be calculated using Equation (11).

$$k_{(CODorPO4-P)} = k_T dn \quad (11)$$

where d is the depth of water (m) and n: porosity of the substrate (dimensionless).

Operating parameters such as hydraulic retention time (HRT), hydraulic loading rate (HLR) and organic surface loading rate (OSLR) affect the efficiency of the system generally. These are represented by Equations (12)–(14), respectively.

$$HRT = \frac{Lwdn}{Q} \quad (12)$$

where HRT = hydraulic retention time; L = length of bed, m; w = width of bed, m; d = average depth of bed, m; n = porosity; %; Q = flow, m^3/d;

$$HLR = \frac{Q}{A_h} \quad (13)$$

where HLR = hydraulic loading rate, m/d; Q = flow rate, m^3/d; A_h = surface area of bed, m^2

$$OSLR = \frac{QxS_o}{A_h} \quad (14)$$

where $OSLR$ = organic surface loading rate, kgCOD/m^2.d; Q = flow rate, m^3/d; S_o = inlet COD, kg/m^3; A_h = surface area of bed, m^2.

Statistical analysis

Standard deviations and mean values of: (1) inlet COD and PO_4-P; (2) outlet COD and PO_4-P; (3) OSLR and phosphate loading rate (PLR); (4) efficiency of COD and PO_4-P removal; and (5) k_{COD} and $k_{PO4\text{-}P}$ were determined using Excel™ software. IBM SPSS Software was used to perform: (1) paired-sample T-test to determine if the mean difference of the characteristic of the inlet and outlet were statistically significant at 95% confidence level. If the characteristic of the inlet and outlet mean are alike, then the null hypothesis tested is not discarded; and (2) Pearson correlation test to assess the statistical significance of the operating parameters such as OSLR and PLR on bed performance. Furthermore, the observed COD and PO_4-P data obtained along each HSSFCW were used in the K-C and K-C* models to confirm the results of this study. The outcomes should fit these two models as the beds function under a plug-flow condition (Von Sperling & de Paoli 2013).

RESULTS AND DISCUSSIONS

Adsorption capacity & time saturation

Table 1 shows the results obtained from the batch analysis using activated CBA and AS.

From Table 1, the adsorption intensity (n value) for AS and coal ash lies between 1 and 10 representing favourable adsorption. Similar results were obtained for Langmuir model where R_L lies between 0 and 1 indicating that coal ash and AS are good adsorbents. The Dubinin-Radushkevich model showed there to be physical sorption since the mean sorption was less than 8 kJ/mol. Table 2 shows the results obtained from the continuous column analysis, which was substituted into the Yoon Nelson Model to determine the saturation time of the coal ash and AS.

From Table 2, a better fit was obtained for Yoon Nelson model (high R^2-values) for both AS and coal ash (CA). AS required 12 minutes per gram to be fully saturated. AS adsorbed phosphate more rapidly than COD showing its higher preference for phosphate. The same explanation can be made for COD removal using coal ash.

Characterization of influent and effluent

The characteristics of the inlet and outlet effluent of the four HSSFCWs are given in Tables 3 and 4, respectively.

Table 1 | Adsorption isotherm parameters for COD and phosphate adsorption onto CBA and AS[a]

		Freundlich isotherm			Langmuir isotherm				Dubinin-Radushkevich isotherm			
Adsorbent	**Adsorbate**	**n**	**k_f**	**R^2**	**Qmax**	**$K_l \times 10^{-3}$**	**R_L**	**R^2**	**B**	**Qmax**	**E**	**R^2**
Alum sludge	PO_4-P	2.26	5.93	0.97	67	24.0	0.10	0.98	0.00003	39	129	0.71
	COD	2.18	3.52	0.75	143	0.1	0.16	0.65	0.009	46	7	0.76
Coal ash	PO_4-P	1.89	0.98	0.82	28	13.0	0.10	0.97	0.00064	22.9	28	0.65
	COD	2.82	3.51	0.97	63	1.3	0.13	0.96	0.04	122	4	0.55

[a]n represents Freundlich adsorption intensity (dimensionless); k_f is the adsorption capacity (mg/g); Qmax is the equilibrium adsorption capacity of adsorbent (mg/g); K_l is the Langmuir constant related to the rate of adsorption (L/mg); R_L is the dimensionless equilibrium parameter; B stands for mean adsorption energy(mol^2/J^2) and E is the mean free energy sorption (J/mol).

Table 2 | Fixed bed adsorption column data and parameters obtained for COD and phosphate removal by using coal ash and AS at different conditions[a]

Adsorbent	**Adsorbate**	**Co**	**Mass**	**v**	**k_{YN}**	**T**	**R^2**
Alum sludge	PO_4-P	610	15	3	−0.030	12	0.848
	COD	1,684	15	3	−0.019	20	0.896
Coal ash	PO_4-P	610	20	3	−0.036	6	0.926
	COD	9,160	20	3	−0.037	9	0.882

[a]Yoon-Nelson parameters where C_o is the initial concentration (mg/L); v is the flowrate (ml/min); k_{YN} is the rate constant (L/min); T is the time saturation for one gram of adsorbate to get saturated (min).

Table 3 | Influent wastewater characteristics

	Influent Parameters							
	COD (mg/l)		PO_4-P (mg/l)		pH		Temp (°C)	
Strength of WW	**Range**	**Mean ± SD**	**Range**	**Mean ± SD**	**Range**	**Mean ± SD**	**Range**	**Mean ± SD**
Low ($n = 10$)	107–130	115 ± 7	27–71	58 ± 13	5.41–6.60	6.14 ± 0.4	23.2–25.2	24.2 ± 0.5
Medium ($n = 18$)	912–1,563	1,150 ± 172	316–380	351 ± 20	4.59–5.77	4.86 ± 0.30	23.2– 27.2	25.4 ± 1.2
Strong ($n = 12$)	4,801–5,021	4,987 ± 60	488–520	506 ± 10	4.05–4.45	4.18 ± 0.12	23–27.0	24.1 ± 1.2

Table 4 | Outlet wastewater characteristics for the intensified and control beds

		Intensified beds				Control Beds			
		CA-HSSCW		AS-HSSCW		CCA-HSSCW		CAS-HSSCW	
Parameters	**N**	**Range**	**Mean ± SD**	**Range**	**Mean ± SD**	**Range**	**Mean ± SD**	**Range**	**Mean ± SD**
COD (mg/l)	40	7–894	279 ± 322	2–445	130 ± 177	45–3,549	1,268 ± 1,429	19–2,804	761 ± 995
PO_4-P mg/l)	40	7–217	126 ± 69	0–6	1 ± 2	18–338	192 ± 97	10–188	95 ± 52
pH	40	5.89–6.99	6.67 ± 0.24	6.40–7.96	7.17 ± 0.4	6–6.9	6.41 ± 0.23	6.39–7.14	6.8 ± 0.19
Temp(°C)	40	22.9–27.1	24.7 ± 1.2	22.6–27.1	24.7 ± 1.2	22.8–27.1	24.7 ± 1.2	23–27.1	24.7 ± 1

The COD, PO_4-P, pH and temperature for the influent ranged from 107 to 5,021 mg/l, 27 to 520 mg/l, 4.05 to 6.60 and 23 to 27.2 °C and averaged to 2,042 ± 2,000 mg/l, 324 ± 170 mg/l, 5.03 ± 0.82, 24.7 ± 1.2 °C respectively. A strong influent having high COD and PO_4-P loadings was used since this study involved the purification of high-strength industrial effluent.

Table 4 shows the range, the mean and the standard deviation of the outlet wastewater for the intensified beds (CA-HSSFCW and AS-HSSFCW) and control beds (CCA-HSSFCW and CAS-HFSSCW) irrespective of the operating conditions applied. Greater values for the standard deviations were obtained for all the influent parameters in contrast with the effluents of the four HSSFCWs since three types of WW were prepared. In addition, the standard deviation for the outlet of the intensified and conventional beds was lower than the inlet showing that in all cases, regardless of remedial activity, the systems moderate the effluent load.

The *p*-value obtained for the means of the inlet and outlet COD and PO_4-P from the paired sample T-test using SPSS Software version 23 was less than 0.05, which means that there was a statistically significant difference between inlet & outlet COD and PO_4-P (at 95% confidence). The null hypothesis was hence rejected. This means that the synthetic industrial WW was treated by the four experimental beds in terms of COD and PO_4-P.

COD removal efficiency

In Figure 3, a comparison of the COD removal efficiency of the CA-HSSFCW and CCA-HSSFCW at a depth of 0.40 m for a retention time of 24 hours is shown.

The COD removal efficiency for the intensified coal ash HSSFCW and conventional HSSFCW ranged, respectively, from 82 to 96% and from 29 to 62% irrespective of the operating conditions that were applied. The mean of the COD removal efficiencies for the CA-HSSFCW and CCA-HSSFCW were 89 ± 4% and 46 ± 11% ($n = 40$), respectively. Therefore, it is concluded that an additional mean (or intensified) removal efficiency of COD of 43% has been accomplished by an intensified CA-HSSFCW bed purifying the high-strength synthetic industrial effluent. Furthermore, the four beds of this study have reached an established state as depicted in Figure 1. Figure 4 depicts the start-up of the systems and Figure 1 shows the bed with well-developed cattails plants.

Table 5 shows the COD removal efficiency for CA-HSSFCW and CCA-HSSFCW. There was an increase in the mean OSLR from 0.024 ± 0.001 ($n = 10$), 0.238 ± 0.035 ($n = 18$) and 1.030 ± 0.013 kgCOD/m^2d ($n = 12$) which represents a range of approximately 9–43 times the loading rate of COD.

Udom *et al.* (2018) discussed that there is a decline in the microbial activities, which leads to a decrease in the degradation of OM when the load increases. Similar results were observed in this study for the CA-HSSFCW and the CCA-HSSFCW (after excluding the start-up period of the system since all the four beds were not yet established at that time and the cattail plants were not well developed). Biodegradation is considered to be the most suitable process for the removal of OM in an effluent having elevated concentration of COD as stated by Stefanakis *et al.* (2016), However, as the OLR increases (as shown in Table 5) a significant decrease in the performance of the conventional system was observed compared to that of the

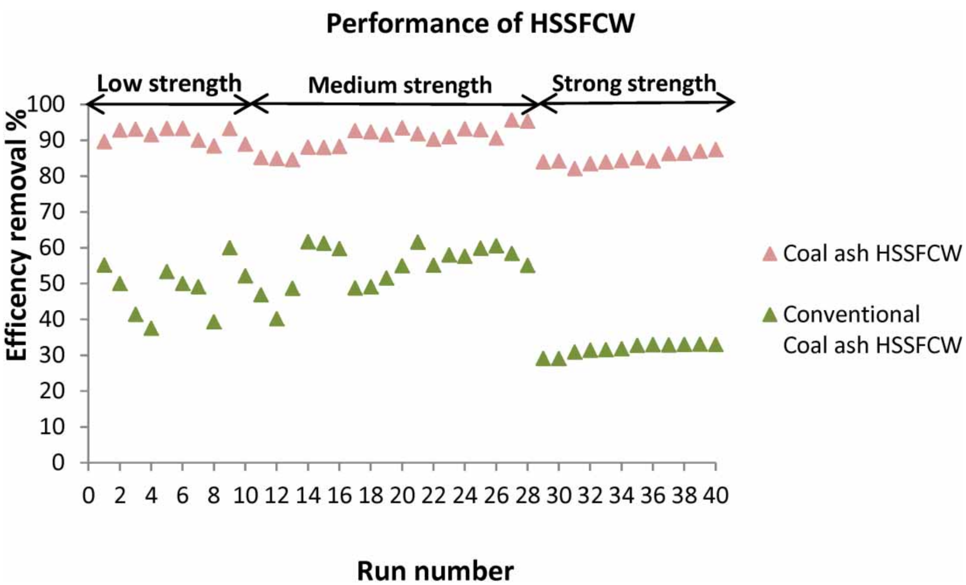


Figure 3 | Comparing COD removal efficiency for CA-HSSFCW and CCA-HSSFCW.

Figure 4 | The four beds (start-up phase in 2019).

Table 5 | Performance of CA-HSSFCW and CCA-HSSFCW at different strength of WW

		CA-HSSFCW			CCA-HSSFCW		
		OSLR (kgCOD/m^2·d)	COD efficiency%		OSLR (kgCOD/m^2·d)	COD efficiency%	
Parameters	N	Mean ± SD	Range	Mean ± SD	Mean ± SD	Range	Mean ± SD
Low	10	0.024 ± 0.001	88–93	91 ± 2	0.023 ± 0.01	38–60	49 ± 7
Medium	18	0.238 ± 0.035	85–96	91 ± 3	0.235 ± 0.035	40–62	55 ± 6
Strong	12	1.030 ± 0.013	82–87	85 ± 2	1.016 ± 0.013	29–33	32 ± 1

intensified beds. This provides evidence that the removal of the elevated amount of COD in the synthetic effluent was substrate dependent. When the OSLR increased from a mean of 0.238 ± 0.035 ($n = 18$) to 1.030 ± 0.013 ($n = 12$) kg COD/m^{2}d, the COD removal efficiency dropped from a mean of 91 ± 3% ($n = 18$) to 85 ± 2% ($n = 12$), that is by an average of 6 percentage points whereas for the conventional HSSFCWs, when the OSLR increased from a mean of 0.235 ± 0.035 to 1.016 ± 0.013 kg COD/m^{2}d, the removal efficiency of the COD decreased significantly from 55 ± 6% to 32 ± 1% that is by 23 percentage points. Mora-Orozco *et al.* (2018) studied a wetland system which was packed with a combination of soil, red volcanic rock and sand planted with *Typha* and *Scirpus* species. They obtained a removal efficiency of 86, 76 and 77% for treating a wastewater having a COD concentration of 400, 800 and 1,200 at an HRT of 10 days.

Since the operating factors HLR, HRT and depth of water remained unvaried during this study, the sole contributor for this intensified performance for the intensified systems was due to the mechanism of adsorption utilising coal ash as a medium. There was a slight decrease in the performance of the intensified beds compared to the conventional one because coal ash was used as media enhancing more removal of COD. It can be concluded that COD from the WW was removed not only by microbial degradation and plant uptake but by adsorption too. Hence, the adsorption mechanism contributes extensively to CW technology purifying high-strength industrial effluent.

Shepherd *et al.* (2001) demonstrated that 97% of COD is expected to be removed if the COD concentration of winery WW is less than 5,000 mg/l. However, when the organic matter concentration increased from 12,800 mg/l to 16,800 mg/l, the purification performance fell significantly, and the wetland plants found at the inlet structure of the system turned yellow and ultimately died. Zingelwa & Wooldridge (2009) studied the tolerance capacity of wetland plants which included *Typhalatifolia, Juncusacutus*, and *Scirpus maritimus*, which were involved in the treatment of winery WW containing elevated levels of organic matter. Their investigation revealed that the macrophytes were strong and healthy at a concentration of less than 5,000 mg/l and when the COD concentration reached 15,000 mg/l, the plants were stressed, yellowed and died. For this study, at a COD concentration ranging from 912 mg/to 1,563 mg/l, healthy plants were detected for both the intensified and conventional systems. Only some yellow shoots and leaves appeared in the control HSSFCW when the system was subjected to a WW having a strong concentration of COD of 4,801–5,021 mg/l. Nonetheless the plants from the CA-HSSFCW were not affected since adsorption mechanism was applied to the system. One more observation that has been made during the investigation was that the cattails grew and developed quickly compared to those from the conventional systems since most of the contaminants were adsorbed and hence, the cattails were under less metabolic stress. It was found that the performance of conventional beds decreased with increasing COD loadings. This indicates that conventional CWs were not able to treat effectively strong organic WWs. Thus, it may not be appropriate to apply CWs with gravel substrate to treat high-strength organic wastewater.

Phosphate removal efficiency

Figure 5 and Table 6 show the performance of the AS intensified systems (AS-HSSFCW) and the conventional AS systems (CAS-HSSFCW) in terms of phosphate removal efficiency at a retention time of 24 hrs and water depth of 0.40 m.

The phosphate removal efficiency of the AS-HSSFCW and CAS-HSSFCW, irrespective of the conditions applied ranged from 97 to 100% and 41 to 60%, respectively. The corresponding means for the phosphate removal efficiency for the AS-HSSFCW and CAS-HSSFCW were 99 ± 1% and 50 ± 6% ($n = 40$), respectively. It can therefore be concluded that a mean additional phosphate removal efficiency of 49% was achieved by an intensified CW purifying synthetic industrial wastewater. Since a low PO_4-P removal efficiency was obtained for the control beds packed with gravels, it is confirmed that gravel did not contribute substantially to the elimination of phosphate. Hence gravel is regarded as a poor adsorbent for phosphate removal (Wu *et al.* 2015).

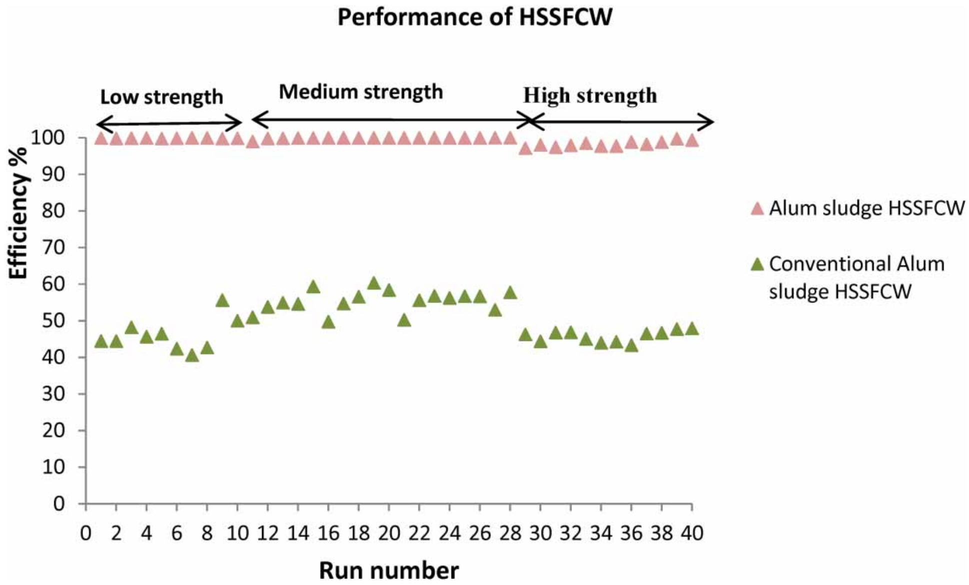


Figure 5 | Comparing PO_4-P removal efficiency for intensified AS-HSSFCW with CAS-HSSFCW.

Table 6 | Performance of AS and conventional HSSFCW at different strength of WW

Parameters	n	AS-HSSFCW PLR (kg PO_4/m^2d) Mean ± SD	AS-HSSFCW PO_4-Pefficiency % Range	AS-HSSFCW PO_4-Pefficiency % Mean ± SD	CAS-HSSFCW PLR (kg PO_4/m^2d) Mean ± SD	CAS-HSSFCW PO_4-P efficiency% Range	CAS-HSSFCW PO_4-P efficiency% Mean ± SD
Low	10	0.0033 ± 0.0011	100–100	100 ± 0	0.0085 ± 0.0022	41–56	46 ± 4
Medium	18	0.030 ± 0.003	99–100	100 ± 0	0.043 ± 0.004	50–60	55 ± 3
Strong	12	0.039 ± 0.003	97–100	98 ± 1	0.060 ± 0.005	43–48	46 ± 2

Results for low strength WW were excluded since it was the start-up period of the systems where the HSSFCWs were not stable, and the cattails were not yet developed. The mean PLR in the CAS-HSSFCW increased from 0.043 ± 0.004 ($n = 18$) to 0.060 ± 0.005 kg PO_4-P/m^2d ($n = 12$). Tilloo (2016) stated that a sudden increase of the PLR causes a decrease in the performance of the system. Similar trends were obtained in this study for the control beds. When the PLR was increased from a mean of 0.030 ± 0.003 ($n = 18$) to 0.039 ± 0.003 kgPO_4-P/m^2d ($n = 12$), the phosphate removal efficiency fell by 2% specifically from an average of 100 ± 0% ($n = 18$) to 98± 1% ($n = 12$). Nevertheless, when the PLR from the conventional systems was increased from 0.043 ± 0.004 ($n = 18$) to 0.060 ± 0.005 kgPO_4/m^2d ($n = 12$) kg PO_4-P/m^2d, the performance of phosphate removal decreased by 9% from 55 ± 3 to 45.8 ± 2%. The performance of the AS-HSSFCW was enhanced or intensified by an average mean of 54, 45 and 52%, respectively, when low, medium and strong synthetic industrial WW was fed to the system. There was a significant decrease in the performance of the conventional AS-HSSFCW compared to the intensified AS-HSSFCW since gravel removes phosphate poorly and AS eliminated phosphate significantly by the process of adsorption. Tilloo (2016) studied two HSSFCWs packed with AS where one system was planted with cattails and the other system was unplanted. The respective performance of the planted and unplanted bed was 99.17 and 99.25%. For this study when the intensified AS-HSSFCW bed was fed with a medium and high PLR, a mean removal efficiency of 100 and 98% was obtained and since the operating factors (HRT and water depth) were kept the same, the main process that caused this intensification was adsorption since AS was used as the substrate. It is evidenced by this study that the adsorption mechanism contributes considerably in HSSFCW in the treatment of a WW highly loaded with phosphate.

Effect of OSLR and PLR on bed performance

The effects of OSLR and PLR on the performance of the bed were assessed statistically by Pearson correlation statistical analysis using the IBM SPPS Software version 23.0. A strong negative correlation was obtained between the OSLR and the removal efficiency of COD for the intensified coal ash HSSFCW ($r = -0.734$, $N = 30$, $p < 0.05$) and the conventional coal ash HSSFCW ($r = -0.912$, $N = 30$, $p < .05$). Hence it can be concluded that as OSLR increases, bed performance decreases. There was a very strong negative correlation between phosphate loading rate and the removal efficiency of phosphate for the AS HSSFCW ($r = -0.716$, $N = 30$, $p < .001$) and conventional alum sludge HSSFCW ($r = -0.814$, $N = 30$, $p < .001$). Therefore, it can be deduced that an increase in the PLR causes a drop in bed performance in terms of phosphate removal efficiency.

A probable reason for a decreased performance in the intensified beds when subjected to increased OSLR/PLR was due to lesser availability of adsorption sites (or more adsorption sites were occupied). However, this is by no means significant as the COD removal efficiency in the intensified bed (CA-HSSFCSW) decreased from 91% to only 85%, when the OSLR was increased from 0.238 to 1.030 kgCOD/m^2·d. Similarly, a paltry reduction of PO_4-P removal efficiency from 100 to 98% was observed in the AS-HSSFCW when the PLR was increased from 0.030 to 0.039 kgPO_4-P/m^2d.

K-C and K-C* model

The removal of contaminants in a HSSFCW normally follows first-order kinetics. Nevertheless, it is stated that the application of the hydraulic model is normally dependent on the aspect ratio of the HSSFCW (Von Sperling & de Paoli 2013). As the four HSSFCWs in this study had a high aspect ratio of 6:1 (length: width) supporting a plug flow regime, the mean observed COD and PO_4-P values should fit the first-order plug flow model. To insert the experimental results in the first-order plug flow model, the method of Von Sperling & De Paoli (2013) was used where a graph of mean observed COD and PO_4-P values were plotted against time and an exponential line was inserted to evaluate the R^2 value by utilising Excel Software. This shows an exponential degradation of COD and PO_4-P along the HSSFCW. Consequently, the progression of COD and phosphate obtained along the systems dropped exponentially from the inlet to the outlet of the bed.

Figure 6 shows the fit of the mean observed COD and PO_4-P values along the intensified and the conventional HSSFCWs into the K-C model at a depth of water of 0.40 m and retention time of 24 hrs for medium and strong WW.

Figure 6 clearly showed a strong correlation as indicated by the high R^2 value for the progression of COD and PO_4-P along both the intensified and control systems for the medium and strong WW. Hence, the experimental outcomes are well suited to modelling with a first-order plug flow model. This confirms the results of this study, as presented in Tables 8 and 9. It clearly demonstrates the COD and PO_4-P elimination along the systems for the intensified and the control beds followed a first-order plug flow model.

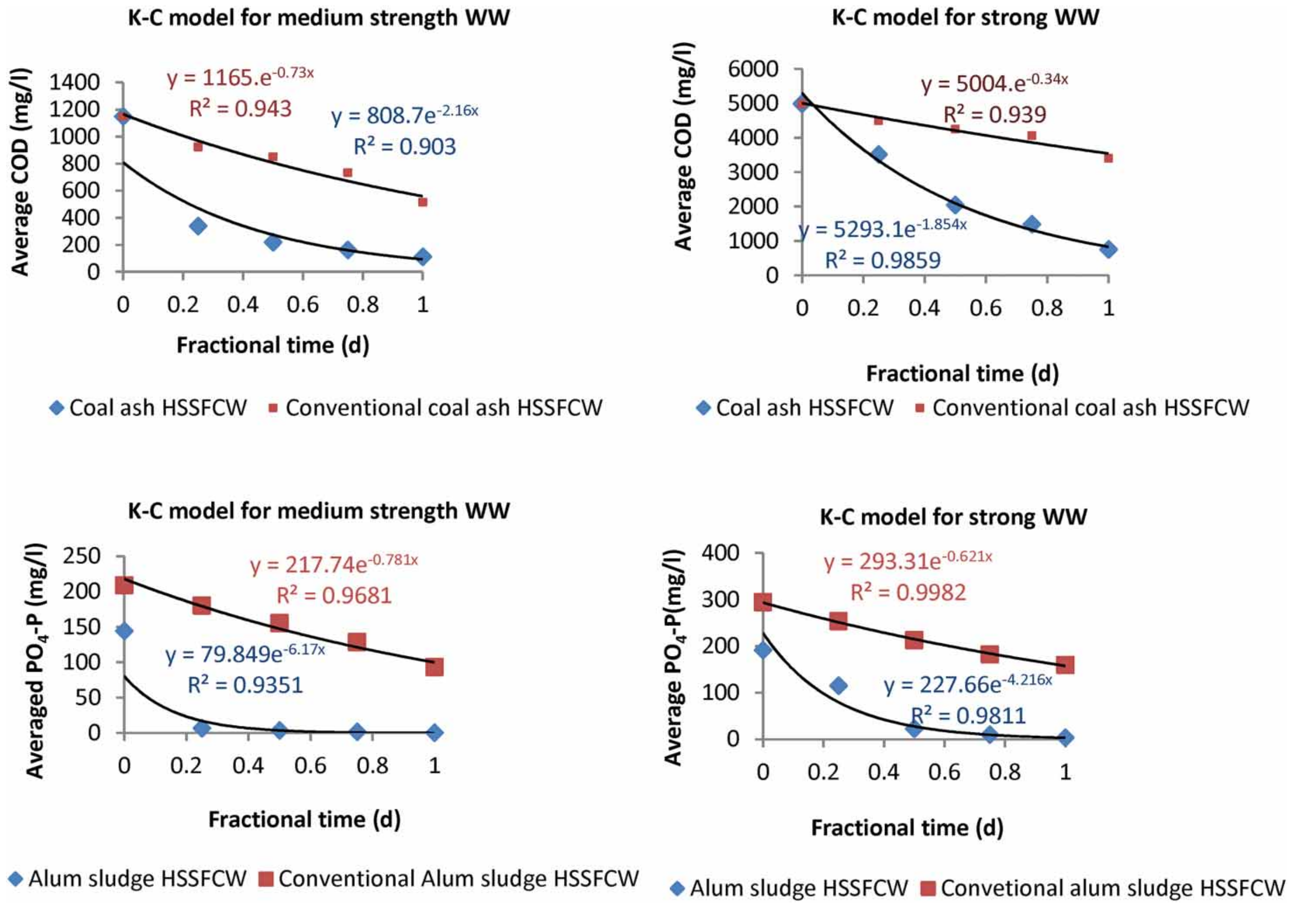


Figure 6 | K-C model for the intensified and conventional HSSFCW for medium and high-strength effluent. Note: The rate constant is higher for an intensified bed than the conventional HSSFCWs that is the intensified bed outperformed the conventional ones.

Kadlec & Wallace (2008), however, discussed that K-C model, which explained the elimination of COD and phosphate along the HSSFCW are regarded as inappropriate model since a zero COD and phosphate can only be obtained for this type of model at infinite HRT. In practice, this does not occur. The K-C model was further developed into the K-C* model, which explained a non-zero COD and PO_4-P and is represented as C*. Von Sperling & de Paoli (2013) stated that the lowest outlet COD and PO_4-P concentration will be the C*. The least COD obtained for the medium and high-strength WW were 43 mg/l and 632 mg/l for the CA-HSSFCW and 410 mg/l and 3,275 mg/l for the CCA-HSSFCW respectively. For the AS-HSSFCW, the lowest PO_4-P was 0.03 mg/l and 0.512 mg/l and for CAS-HSSFCW the C* values 78 mg/l and 138 mg/l for CAS-HSSFCW, when they were subjected to respectively medium and strong WW. The observed mean COD and PO_4-P residual concentrations were fitted into the K-C* model. Figure 7 illustrates the fit of the observed mean COD and phosphate at 24 hrs for a water depth of 0.40 m at different strength.

Trang *et al.* (2010) stated that the Kickuth design equation to evaluate the rate constant (k_{COD} and $k_{PO4\text{-}P}$) could be affected by some parameters such as the wetland configurations and hydraulic loadings. In view of that, other methods were proposed by Kadlec (2003), which were less influenced by these factors. This technique comprised COD/PO_4-P profiles beginning at the start to the end of the HSSFCW. Trang *et al.* (2010) detailed and implemented the K-C* model. The same method was utilised in this study to calculate the k_{COD} and $k_{PO4\text{-}P}$, which is explained here. The rate constants were calculated by using Equation (8).

$$k_{COD}/k_{PO4-P} = k_t.n.d \tag{15}$$

where k_t was obtained from the K-C and K-C* models, n: porosity of media used, d: depth of bed.

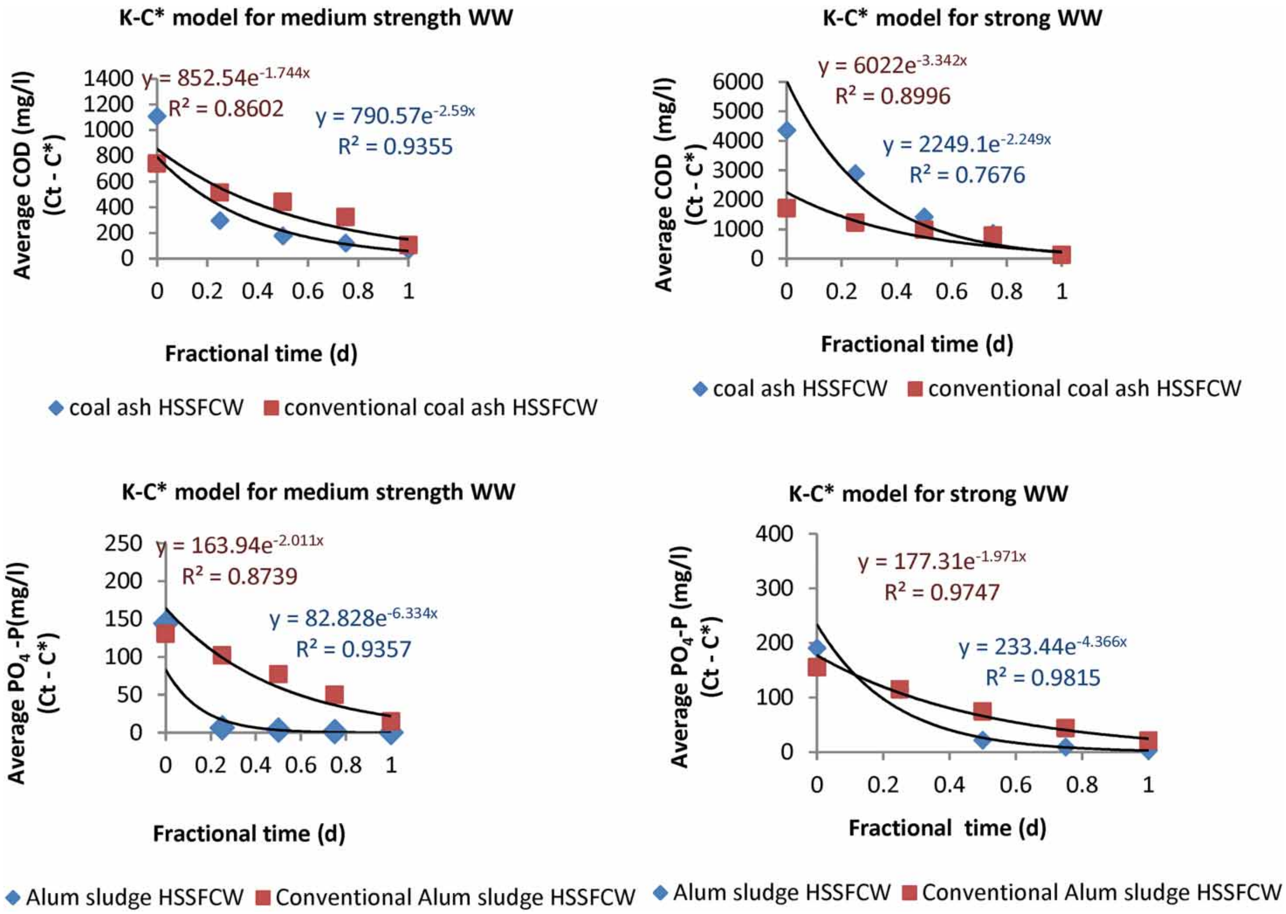


Figure 7 | K-C* model for the intensified and conventional beds at medium and strong strength WW. Note: The rate of constant is higher for an intensified bed than the conventional HSSFCW that is the intensified bed outperformed the conventional one.

In this study the porosity of gravel, coal ash and AS were 0.512, 0.52 and 0.46, respectively. The water depth was 0.40 m. Tables 7 and 8 illustrate the mathematical correlation between concentrations with respect to k_t, k_{COD} and their R^2 value for the K-C and K-C* models for intensified beds (CA-HSSFCW and AS-HSSFCW) and conventional systems (CCA-HSSFCW and CAS-HSSFCW) at medium and high strength.

From Tables 7 and 8, it can be deduced that the removal of COD and PO_4-P for medium and high-strength WW are well described by the K-C and K-C* models as a high R^2 values were obtained for both intensified and control systems. The following finding scan be inferred from Tables 7 and 8.

- Similar fits for both K-C and K-C* model for COD and phosphate elimination at medium and high strength;

Table 7 | Relationship of rate constants of different strength effluent for the K-C and K-C* models for CA-HSSFCW and CCA-HSSFCW[a]

		CA-HSSFCW				CCA-HSSFCW				
Model	**Strength**	**Correlation**	**k_t (/d)**	**k_{COD} (m/d)**	**R^2**	**Correlation**	**k_t (/d)**	**k_{COD} (m/d)**	**R^2**	**Increase**
K-C	Medium	$C_t = 808.7e^{-2.16t}$	2.16	0.45	0.903	$C_t = 1{,}165e^{-0.73x}$	0.73	0.15	0.943	x3
	High	$C_t = 5{,}293\ e^{-1.85t}$	1.85	0.38	0.985	$C_t = 5{,}004e^{-0.34x}$	0.34	0.07	0.939	x5
K-C*	Medium	$C_t\text{-}C^* = 790.5e^{-2.59t}$	2.59	0.54	0.935	$C_t\text{-}C^* = 852.5e^{-1.74x}$	1.74	0.36	0.860	x2
	High	$C_t\text{-}C^* = 6{,}022\ e^{-3.34t}$	3.34	0.69	0.899	$C_t\text{-}C^* = 2{,}249e^{-2.24x}$	2.24	0.46	0.767	x2

[a]The COD rate constant indicates a faster removal of COD for the intensified bed than a conventional HSSFCW for both KC and KC* Model.

Table 8 | Relationship of rate constants of different strength effluent for the K-C and K-C* models for AS-HSSFCW and CAS-HSSFCW[a]

Model	Strength	AS-HSSFCW Correlation	k_t (/d)	$k_{PO4\text{-}P}$ (m/d)	R^2	CAS-HSSFCW Correlation	k_t (/d)	$k_{PO4\text{-}P}$ (m/d)	R^2	Increase
K-C	Medium	$C_t = 79.84e^{-6.17t}$	6.17	1.14	0.935	$C_t = 217.7e^{-0.78t}$	0.78	0.14	0.968	x8
	High	$C_t = 227.6e^{-4.21t}$	4.21	0.77	0.981	$C_t = 293.3e^{-0.62t}$	0.62	0.11	0.998	x7
K-C*	Medium	$C_t\text{-}C^* = 82.82e^{-6.33t}$	6.33	1.16	0.935	$C_t\text{-}C^* = 163.9e^{-2.01t}$	2.01	0.37	0.873	x3
	High	$C_t\text{-}C^* = 233.4e^{-4.36x}$	4.36	0.80	0.981	$C_t\text{-}C^* = 177.3e^{-1.97t}$	1.97	0.36	0.974	x2

[a]The PO_4-P rate constant indicates a faster removal of phosphate for the intensified bed than a conventional HSSFCW for both KC and KC* Model.

- The K-C* model has a high first-order decay rate coefficient for the intensified and control systems at medium and high strength when compared to the K-C model; and
- The rate constants for the control HSSFCW were lower than the intensified beds.

This validated the hypothesis that when implementing the process of adsorption in a HSSFCW, the performance is intensified. This also evidenced that coal ash and alum sludge are good adsorbents when compared to gravel. The intensified HSSFCW removed COD and phosphate at a faster rate than the conventional beds packed with gravels.

Furthermore, the following observations are made in this study:

- The general way in which the COD and phosphate change as the effluent moves through the wetland match with those of the literature review; and
- The removal of the observed COD and phosphate along the systems follow the K-C and K-C* models as stated by Von Sperling & de Paoli (2013), therefore confirm the data obtained experimentally.

Kadlec & Wallace (2008) also noted that the rate constant is normally hydraulic process dependent, and the same observations were made by Trang *et al.* (2010), who mentioned that the rate constant obtained from the K-C* profiles should not be used for designing as a parameter. This is because of climatic conditions, the growth of cattails and other uncertainty in the quality of water and flowrate could produce greater values than expected. Kadlec (2003) mentioned that the inlet and outlet performance outcomes from the horizontal sub-surface flow CW were used to calculate the rate constants. Consequently, the k_{COD} and $k_{PO4\text{-}P}$ were estimated by using the results obtained from the inlet and outlet, flowrate and surface area of the system.

Equation (8) was used to compute the rate constants k_{COD} and $k_{PO4\text{-}P}$ for both intensified and controls HSSFCW, which are shown in Table 9 for medium and high-strength WW.

It can be deduced that the rate constant computed by the models fit in the series obtained by the Kickuth equation. Therefore, the outcomes obtained experimentally are confirmed. When the strength of the WW was increased, a decrease in the rate constant was observed for both CA-HSSFCW and AS-HSSFCW as well as their controls. k_{COD}values ranging from 0.36 to 0.65 m/d and averaging to 0.46 ± 0.08 m/d and $k_{PO4\text{-}P}$ ranging from 0.74 to 1.76 m/d and averaging to 1.23 ± 0.37 m/d were obtained irrespective of the conditions (HRT of 24 hrs and a water depth of 0.40 m) that were applied. The k_{COD} for the conventional HSSFCW was four times less than that of the intensified CA-HSSFCW one and the $k_{PO4\text{-}P}$ was nine times less than that of the AS-HSSFCW.

Table 9 | Rate constant using the Kickuth equation

	k_{COD}(m/d)				$k_{PO4\text{-}P}$ (m/d)				Mean Increase	
	CA-HSSCW		CCA-HSSCW		AS-HSSCW		CAS-HSSCW			
Strength	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	k_{COD}	$k_{PO4\text{-}P}$
Medium ($n = 18$)	0.39–0.65	0.50 ± 0.08	0.10–0.20	0.16 ± 0.03	0.95–1.76	1.47 ± 0.26	0.13–0.17	0.15 ± 0.01	3	10
Strong ($n = 12$)	0.36–0.43	0.39 ± 0.02	0.07–0.08	0.078 ±0.004	0.74–1.22	0.87 ± 0.14	0.10–0.12	0.11 ± 0.005	5	8

Potential application of the intensified constructed wetland technology

The intensified constructed wetland technology addressed in this study is innovative in being developed to be applied mainly in industries in Mauritius (or other similar tropical locations such as Reunion island or Madagascar – within the region). The implementation of such an intensified HSSFCW treating high-strength WW is regarded as a sustainable treatment system where no chemicals or energy are used (Nivala *et al.* 2019). Only natural resources such as plants, gravel and by-products (AS and coal ash) are utilised. Instead of disposing the alum sludge and coal ash to landfill, by using it in CWs for treating effluent (Xu & Shi 2018; Singh *et al.* 2019) it helps to reduce the impact to landfill sites and thereby potentially increases their lifespan – this is particularly important for island countries with limited space) Alum sludge and coal ash, according to research presented here were shown to be good adsorbents for the removal of phosphate and organic matter, respectively (Babatunde *et al.* 2010; Wahyuni *et al.* 2018).

The kind of industries where this new technology can be applied are edible oil refineries, breweries, distilleries, food canning industries, tuna industries, textile industries and sugar industries among others in regions in which similar tropical climatic conditions prevail. Indeed, the objective was to simulate treatment of a wide range of medium to strong industrial WWs, taking cognisance of these industries.

Such a technology is less costly to construct and operate and is definitely more sustainable than other conventional chemical and biological treatment systems, which consume chemicals and fossil fuel-based energy and produce only sludge (Nivala *et al.* 2019). Industrial WWs in Mauritius are currently being treated by conventional physical, chemical and biological treatment systems comprising screening, settling, pH corrections, diffused air floatation, activated sludge process reactors, anaerobic sludge blanket reactors and up flow anaerobic contactors among others. In certain Mauritian industries, which cannot afford on-site treatment (due to high capex and opex) and have limited land space, their WWs are carted away, as per current local norms. Some industries in Mauritius often cannot fully afford treating these effluents through conventional physical, chemical, and biological technologies, which are usually costly. Hence such intensified HSSFCWs can be potentially applied to such industries as green technologies, which will purify the industrial effluent according to the local relevant norms.

CONCLUSION

The main research aspect of this study was to determine a range of rate constants (k_{COD} and $k_{PO4\text{-}P}$) for an adsorption-integrated HSSFCW treating high-strength synthetic industrial WW. These conditions were absent in literature for industries of Mauritius or other countries having similar weather conditions. The following can be concluded from this study:

- The synthetic industrial WW at different concentrations was treated by the four HSSFCWs as evidenced statistically by the paired sample T-test as the mean inlet COD and PO_4-P were different when compared to the outlet COD and PO_4-P.
- The respective COD removal efficiency for the intensified and conventional HSSFCW ranged from 82 to 96% and 29 to 62% having a mean of 89 ± 4 and 46 ± 11% irrespective of the conditions applied. The performance of the intensified bed was enhanced byan efficiency of 43% of removal of COD.
- When the COD concentration of the influent increased from 912 to 1,563 mg/l to 4,801 to 5,021 mg/l, at 24 hours HRT and a depth of water of 0.40 m, the efficiency for CA-HSSFCW decreased from 91 ± 3 to 85 ± 2% and for CCA-HSSFCW from 55 ± 6 to 32 ± 1% respectively.
- Few yellow plants were obtained in the conventional HSSFCW when the strength of the WW was increased. Nevertheless, there was no effect on the plants of the intensified beds when it was subjected to an increase in the concentration of the WW.
- The respective removal efficiency of phosphate ranged from 97 to 100% and 41 to 60% having a mean of 99 ± 1 and 50 ± 6% for the AS-HSSFCW and CAS-HSSFCW irrespective of the condition applied. An additional mean elimination efficiency of phosphate of 49% was achieved for the AS-HSSFCW.
- As the phosphate concentration increased from 316 to 380 mg/l to 488 to 520 mg/l, there was a slight decrease in the phosphate removal efficiency for the AS-HSSFCW from 100 ± 0 to 98 ± 1%. While for the conventional AS-HSSFCW the removal efficiency fell from 55 ± 3 to 46 ± 2%.
- Pearson correlation analysis demonstrated that the operational parameters such as OSLR and PLR had an effect on the performance of both intensified and control beds in terms of COD and phosphate. When the strength of the influent

was increased, a strong negative correlation was achieved. This showed that when the concentration was increased, the removal efficiency decreased.
- COD and phosphate removal along the four HSSFCWs fit the K-C and K-C* models, which are accepted worldwide. Hence, the COD and phosphate removal was deduced to follow a first-order reaction.
- The experimental outcomes of this study were statistically significant since high R-squared values were obtained for the removal of COD and phosphate.
- The respective COD removal rate constant for the intensified coal ash bed ranged from 0.39 to 0.65 m/d and 0.36 to 0.43 m/d for a medium and high WW and for the control beds the COD rate constant ranged from 0.10 to 0.20 m/d and 0.07 to 0.08 m/d. The PO_4-P rate constant for AS HSSFCW ranged from 0.95 to 1.76 m/d and 0.74 to 1.22 m/d for a medium and high-strength WW and for the control AS bed, the rate constant for phosphate ranged from 0.13 to 0.17 m/d and 0.10 to 0.12 m/d, respectively.

Hence, the main element of research of this study has successfully been achieved and a new range of k_{COD} and $k_{PO4\text{-}P}$ were developed for an adsorption integrated HSSFCW using coal ash and AS as substrate.

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DECLARATION OF INTEREST

The authors of ‘Performance intensification of constructed wetland technology: A sustainable solution for treatment of high strength industrial wastewater’’, Nazeemah Nurmahomed, Arvinda Kumar Ragen and Craig Michael Sheridan certify that they have NO affiliations with or involvement in any organisation or entity with any financial interest (such as honoraria; educational grants; participation in speakers’ bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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