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# Effectiveness of two-stage horizontal subsurface flow constructed wetland planted with *Cyperus alternifolius* and *Typha latifolia* in treating anaerobic reactor brewery effluent at different hydraulic residence times

Ermias Alayu[1*] and Seyoum Leta[2]

## Abstract

**Background:** Agro-industrial wastewaters in Ethiopia cause severe environmental pollution problems. Research evidence showed that anaerobic reactors are good options for the treatment of these wastewaters. But, their final effluent does not meet the discharge standards. Conversely, a series stage horizontal subsurface flow constructed wetland (HSSFCW) system is encouraging for the polishing of anaerobic reactor effluents. However, its treatment efficiency is dependent on hydraulic residence time (HRT). *Cyperus alternifolius* and *Typha latifolia*-based wastewater treatment showed good removal efficiencies individually. However, data on their combined treatment effectiveness is negligible. Therefore, this study assesses HRT influences on the treatment effectiveness of a two-stage HSSFCW system planted with these two macrophytes for the polishing of anaerobic reactor brewery effluent. A series connected two-stage HSSFCW unit planted with *Cyperus alternifolius* and *Typha latifolia* was built to treat Kombolcha brewery anaerobic reactor effluent. Then, the macrophytes were endorsed to grow with continuous application of diluted brewery effluent from a reservoir tank using gravity force. After dense stand formation, the experiment was initiated to determine the influence of HRTs on the removal efficiency of a complete wetland system. The system was operated sequentially by supplying fixed influent inflow rates of 2791, 1395, 930, 698, and 558 L $day^{-1}$, respectively, for 1, 2, 3, 4, and 5 days HRT. Both the influent and effluent of the two-stage HSSFCW system were analyzed following common procedures for main brewery pollutants.

**Result:** Results showed that as HRT increased from 1 to 5 days, the effluent pH and temperature were decreased along with enhanced pollutant removals ranging from 47.8–87.2%, 29.2–90.1%, 32.9–77.7%, 16.8–75.4%, and 18.4–76.8% with decreased influent mass loading rates ranging from 26.4–2.1, 64.5–7.3, 11.5–0.8, 5–0.6, and 3.8–0.4 $gm^{-2}$ $day^{-1}$, respectively, for total suspended solids, chemical oxygen demand, total nitrogen, total phosphorous and orthophosphate. However, better and steadier pollutant removals were achieved at higher HRTs.

**Conclusion:** For better nutrient removals, the 4 and 5 days HRT can serve as a good benchmark.

**Keywords:** Efficiency of two-stage HSSFCW, Effect of hydraulic residence times, *Cyperus alternifolius*, *Typha latifolia*, Brewery wastewater

*Correspondence: ermias.alayu@gmail.com
[1] Department of Chemistry, College of Natural and Computational Sciences, Assosa University, Assosa, Ethiopia
Full list of author information is available at the end of the article

## Background

Agro-industrialization is a key development priority goal for developing countries, since in developed countries, 15% of their national income is generated from these sectors (Kingsley et al. 2013). Analogously, after the 2000s, the Ethiopian governments were also devoted to applying an active industrial policy (Oqubay 2018), and the rapid expansion of agro-industries increased the economic growth substantially (Firdissa et al. 2016). However, in eastern African countries, agro-industrial wastes have become a major encountered pollution problem (Njau et al. 2011). Among the subdivisions, beverage industries in Ethiopia cause high pollution problems by discharging their untreated or partially treated effluents into nearby water bodies and irrigated soils (Oljira et al. 2018). Since brewery wastewater contains variable pollutants such as carbohydrates, alcohols, suspended solids, yeast (Mohan et al. 2018); proteins, ethanol, and volatile fatty acids (Raposo et al. 2010); nutrients, refractory organics, and pathogens (Hultberg and Bodin 2017).

Nevertheless, an up-flow anaerobic sludge blanket (UASB) reactor-based the treatment is being implemented currently by a few brewery industries. UASB reactor-based treatment is an economically feasible and sustainable treatment approach for the generation of energy from wastewaters (Bhatti et al. 2014; Aiyuk et al. 2004). However, the final effluent of this reactor does not meet the national discharge guidelines due to the occurrence of high residual organic matter, nutrients, and pathogens (Yasar and Tabinda 2010; Foresti et al. 2006). Similarly, Ethiopia´s brewery sectors also face a major challenge in achieving the national discharge limit (Firew et al. 2018). Hence, UASB reactor effluent requires further treatment to reduce pollutants for safe disposal or sustainable reuse (Yasar and Tabinda 2010; Chernicharo, 2006). There are several polishing choices, but the tertiary-level treatment should be environmentally friendly, efficient, and low cost. Self-adaptive and naturally working wetland-based treatment was often recommended as a polishing alternative to meet the requirements because of its cost-effective ability to remove pollutants efficiently (Vymazal 2009; Mesquita et al. 2018; Sultana et al. 2017). Constructed wetland (CW) system is divided into two groups that rely on the direction of water flow; horizontal and vertical, but the HSSFCW treatment system has been successfully applied in developed countries for different types of wastewater treatment (Albalawneh et al. 2016), and widely serves as an excellent option for the efficient removal of pollutants from the UASB reactor effluent to reach a high effluent quality (Sedaqua 2013; Jamashidi et al. 2014). Many authors have also recommend that the UASB–CW integrated treatment system has a high potential to improve water quality (Badejo et al. 2014; Raboni et al. 2014), since the UASB reactor removes most of the organic matter (Morino-Solis et al. 2015), reduces the large wetland area requirement (Alvarez et al. 2008), avoids wetland clogging problems, and increases the life cycle of CW (Kyambadde et al. 2005). While the CW further mitigates the remaining organics, solids, nutrients, and pathogens (Balleseros et al. 2016; Jinadasa et al. 2013), and heavy metals (Sedaqua 2013). Moreover, the application of this integrated treatment technology is promising for resource-limited countries due to its superior advantages mentioned elsewhere (Jamashidi et al. 2014; Kyambadde et al. 2005). Treatment of various types of wastewaters using a single-stage HSSFCW at secondary or tertiary level is increasing globally (dela Varga et al. 2013; Zeb et al. 2013). However, high-strength agro-industrial wastewaters are difficult to treat using this stage, so various hybrid HSSFCW systems have been hosted in a series (Morino-Solis et al. 2015; Cheng et al. 2010) for effective removal of agro-industrial wastewaters via controlling pollutant load variations (Karathanasis et al. 2003). Considering the economic limitations and strict national discharge guidelines, CWs are good options to treat UASB reactor brewery effluents in Ethiopia without any operational complexity and high-cost investment. Because previous research evidence showed that anaerobic reactor integrated with HSSFCW treatment has a high potential to improve brewery wastewater quality (Badejo et al. (2014). The HSSFCW post-treatment system offers enhanced pollutant removal efficiency through interaction between wetland components and is mostly influenced by macrophyte species and media types (Saraiva et al. 2018).

Different studies reported that *Cyperus alternifolius* and *Typha latifolia*-planted HSSFCW treatment system showed good removal efficiencies of organic matter and nutrients; *Cyperus alternifolius*-planted HSSFCW showed promising removal performance of 95% COD at 12 days HRT, and 93% TSS at 6 days HRT (Sa'at et al. 2017), while *Typha latifolia* achieved 92% TSS and 79% COD at HRT of 4.7 days (Ciria et al. 2005). In the case of nutrient removals, Bilgin et al. (2014) reported that the *Cyperus alternifolius*-based treatment system removed 59.84% of TN, while *Typha latifolia* achieved 28–46% removal of TN from raw brewery wastewater at 5 days (Worku et al. 2018). Similarly, *Cyperus alternifolius* achieved 73% TP removal efficiency from aerobic palm oil mill effluent at 21 days HRT (Rezaie and Salehzadeh 2014), and *Typha latifolia* removed 39% of TP at HRT of 4.7 days (Ciria et al. 2005). However, single macrophyte-planted CW treatment system is not efficient at removing nutrients (Zurita et al. 2009). Hence, a combination of macrophytes may contribute to better treatment efficiency than alone (Rezaie and Salehzadeh 2014). Macrophyte species richness provides increased biomass production, which may greatly enhance nutrient reductions due to the increased nutrient retentions, microbial activity, and macrophytes uptake (Geng et al.

2017). Although HRT plays a significant role in the effectiveness of nutrient removal in a CW, the longer HRT can provide the best formation of microbial communities and adequate contact time to remove nutrients (Mora-orozco et al. 2018). Research evidence showed that shorter HRTs of 2–4 days, achieved over 90% of TSS and COD, 75% of TN and TP, respectively (Cheng et al. 2010). Similarly, Abou–Elela et al. (2017) evaluated the application of CWs for domestic wastewater treatment, and concluded that enhanced nutrient removal was achieved as the HRT increases, despite too long HRT would reduce the treatment efficiency (Guo et al. 2017). Therefore, choosing the most suitable operating HRT for the efficient removal of pollutants is the main issue. Hence, this study assesses the treatment effectiveness of a two-stage HSSFCWs by subjecting with different HRTs to identify the most suitable benchmark that incorporates efficient pollutant reductions for future long-term evaluation of the treatment system.

## Materials and methods

### Experimental design and operating systems

A series-connected two-stage HSSFCW unit (size: 15.12 m × 1.52 m × 0.45 m (length × width x depth) was constructed near the premises of Kombolcha brewery factory wastewater treatment plant located at 11°04′ 42.43″ N 39°43′ 34.45″ E and at an altitude of 1833 m above sea level. The average temperatures and rainfall of the experimental site varied from 8 to 25.7 °C and 0 to 51.2 mm during the entire monitoring period. Preliminary evaluation of the UASB reactor performance showed that pollutant reductions varied between 62.7–70.1% of TSS, 72–84% of COD, 12.7–41.5% of TN, 13–32.4% of TP, and 15.5–36.1% of orthophosphate ($PO_4$-P) with corresponding average residual concentrations of 192.2, 415.5, 42.5, 18.4 and 12.8 mg/L, respectively for TSS, COD, TN, TP, and $PO_4$-P, which exceeded the national discharge guideline of EEPA (2003). But, scientific reported evidence showed that the HSSFCW system has a high potential to improve anaerobic reactor brewery wastewater quality (Badejo et al. 2014). Thus, the authors decide to polish UASB reactor brewery effluent using series-connected two-stage HSSFCWs, whose design is indicated in Fig. 1a. The HSSFCW body was made from concrete and the interior region was well insulated. Then, a 3000-L reservoir tank was connected to the HSSFCW with a 4-inch-diameter high-density polyvinyl pipe (HDP). But, the 4-inch HDP was reduced to 2-inch HDP and the UASB reactor effluent was delivered into the HSSFCWs using gravity force through regulating its hydraulic flow rate by a 2-inch gate valve. Similarly, the 2-inch HDP was fitted at the outlet end for effluent collection. Then, both cells were filled with 15–25 mm size of clay rock composed of 76.36% $SiO_2$, 13.69% $Al_2O_3$, 4.24% $Fe_2O_3$, 1.52% CaO, <0.1% MgO obtained from Mitikolo to the depth of 0.45 m with an effective pore space of 26.99% (Fig. 1b). Locally available young macrophytes were collected from Borkena River and identified at Addis Ababa University National Herbarium. After identification, the macrophytes were planted orderly in the first and second cells in August 2018. The plantation order was preceded by *Cyperus alternifolius* due to its high pH resistance (Miyazaki et al. 2004), high productivity, relatively strong root system, easy adaptation to organic load changes, and salinity tolerance along with high nutrient absorption capacity (Bilgin et al. 2014), followed by *Typha latifolia* due to its short root length (Bonanno and Cirelli, 2017), active carbon-producing potential around the rhizosphere for denitrifiers (Fahlgren, 2017), less salinity tolerance, and ability to mitigate nutrient-rich wastewater (Mollard et al. 2013). Besides, their biomass usage for making floors, animal feed, roofs, and mattresses (Assefa et al. 2013) and low evapotranspiration rates (Leto et al. 2013) being considered as a selection criterion. Then, these macrophytes were endorsed to grow with continuous application of diluted (75:25, brewery effluent to pipe water ratio) brewery effluent from a reservoir tank. After acclimatization (i.e., dense stand formation), the experiment was initiated on November 1st, 2018 to select the most advantageous HRT for the better treatment efficiency of the wetland. The UASB reactor effluent was flown from the tanker into the series-connected HSSFCW system and operated sequentially by changing HRT starting from 1 to 5 days at 3 days free hydraulic inflow gaps between the HRTs for 2-month. The UASB reactor effluent inflow rates into the HSSFCW were fixed at 2791, 1395, 930, 698, and 558 L $day^{-1}$**,** respectively, for 1, 2, 3, 4, and 5 days. These fixed daily hydraulic flow rates for each HRTs were calculated by considering the theoretical HRT (Reed et al. 1995) Eq. (1), and the incoming mass loading rate (MLR) effect for each HRTs was computed for each specific flow rate considering the hydraulic loading rate (HLR) and influent concentration using Eq. (2) (Juang and Chen 2007):

$$\mathrm{HRT(day)} = \frac{V}{Qi} = \frac{\mathrm{Adp}}{Qi} \tag{1}$$

$$\mathrm{MLR}\left(\mathrm{gm^{-2}d^{-1}}\right) = \frac{C_\mathrm{i}Q_\mathrm{i}}{\mathrm{A}} = C_\mathrm{i} \times \mathrm{HLR} \tag{2}$$

where Q (L $day^{-1}$) is the determining influent flow rate measured by bucket using a stopwatch, V ($m^3$) is the volume of the two-stage HSSFCW unit, A ($m^2$) is the surface area of the two-stage HSSFCW unit, d (m) is the influent flow depth, and p is the porosity (%) of the media used.

**Table 1 General characteristics of UASB reactor effluent throughout each monitoring period**

| Operation | Pollutants concentration (mg/L) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Temp.(°C) | pH | TSS | COD | TN | TP | $PO_4$-P |
| 1 day HRT | 31.2 ± 2.81 | 7.7 ± 0.24 | 217.1 ± 7.21 | 531 ± 47.81 | 94.8 ± 12.17 | 41.1 ± 8.46 | 31 ± 7.95 |
| 2 days HRT | 29.8 ± 2.83 | 7.6 ± 0.17 | 167.5 ± 39.03 | 409.4 ± 54.61 | 70.3 ± 13.46 | 35.3 ± 7.94 | 21.2 ± 6.03 |
| 3 days HRT | 28.2 ± 3.52 | 7.4 ± 0.15 | 128.6 ± 29.62 | 381.9 ± 53.05 | 51.1 ± 10.61 | 31.2 ± 6.56 | 19.4 ± 8.79 |
| 4 days HRT | 28.6 ± 2.65 | 7.5 ± 0.26 | 95.1 ± 15.34 | 381.2 ± 33.59 | 44.9 ± 7.29 | 29.3 ± 6.48 | 16.9 ± 5.29 |
| 5 days HRT | 26.1 ± 3.89 | 7.3 ± 0.25 | 86.2 ± 17.04 | 298.6 ± 38.55 | 34.1 ± 6.48 | 24.8 ± 5.11 | 16.4 ± 3.41 |
| p-value | >0.05 | >0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 |
| Standard[a] | 40 | 6–9 | <50 | <250 | <40 | - | <5 |

[a] EEPA (2003) brewery effluent discharge standards

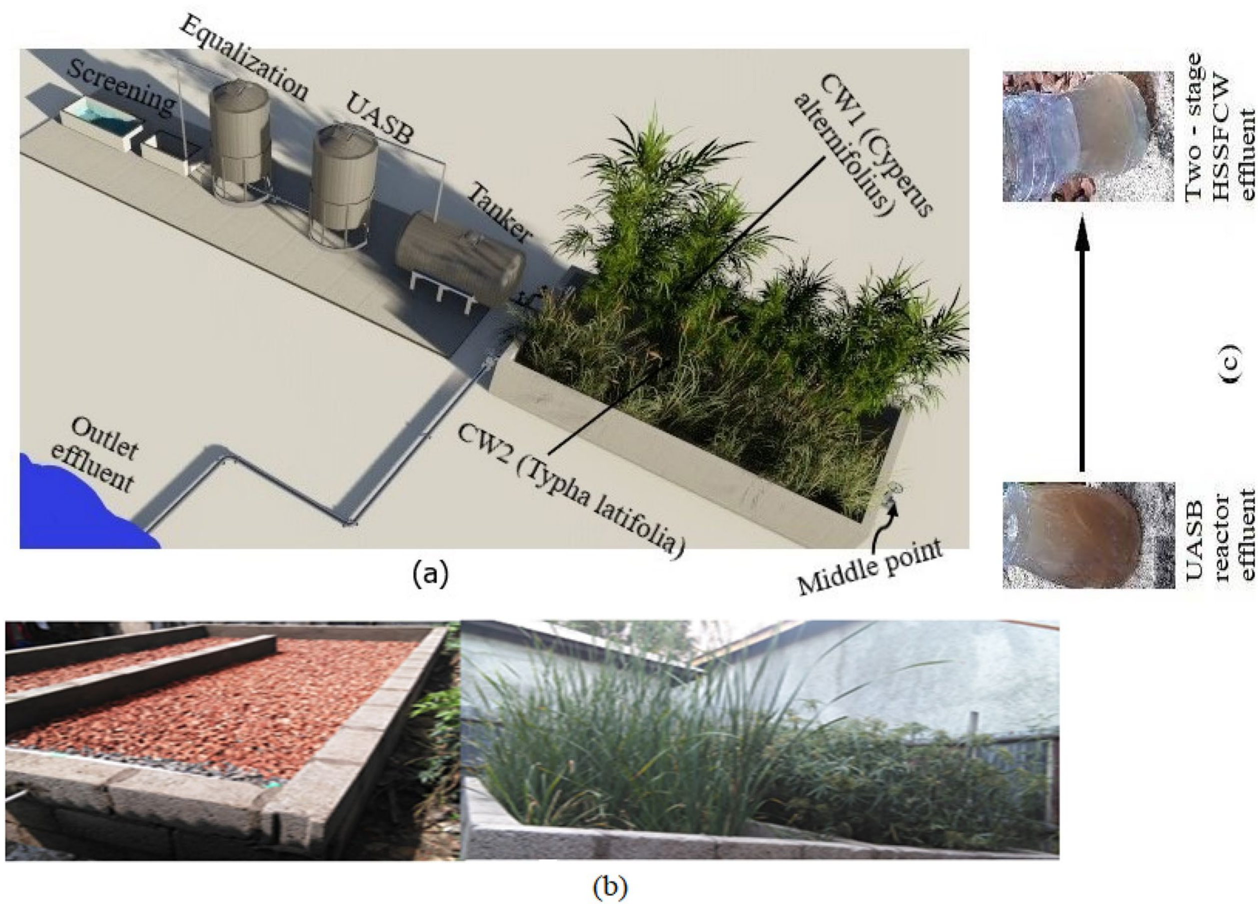


**Fig. 1** **a** Design of the two-stage HSSFCWs in treating UASB reactor effluent, **b** field view of the two-stage HSSFCW unit before planting and after plantation and **c** two-stage HSSFCW treated effluent quality

**Wastewater quality and data analysis**

Five rounds of wastewater samples were collected from the inlet and outlet of the two-stage HSSFCW unit throughout the 2-month study periods using well-cleaned polyethylene bottles and transported to the laboratory for immediate analysis. Temperature and pH were measured in triplicate on-site using the IntelliCAL™ pH/temperature digital probe (HACH® HD30d Flexi, Loveland, USA), whereas TSS (oven-dry method), COD (reactor digestion method), TN (persulfate digestion method), TP and $PO_4$-P (Phosphover®3 with acid persulfate digestion method) were measured in triplicate using HACH spectrophotometer following HACH instructions in the laboratory (APHA 1998). The average specific pollutant removal efficiency (RE) was calculated using the following common equation (3):

$$\mathrm{RE}(\%) = \left[1 - \frac{C_e}{C_i}\right] \tag{3}$$

where $C_e$ and $C_i$ are the effluent and influent concentrations.

## Data analysis

Both descriptive and inferential statistics were analyzed using Origin Pro2017 and Microsoft Excel 2010. Correlation and normality distribution were also performed using Origin Pro2017 software.

## Results

### Characteristics of the UASB reactor effluent

Characterization of the UASB reactor brewery effluent (photo indicated in Fig. 1c) showed that the effluent has a high level of TSS, COD, TN, TP, and $PO_4$-P between HRT operations (Table 1), signifying its substantial fluctuations. However, the on-site-measured average pH and temperature of the effluent showed an insignificant variation ($p>0.05$) throughout the study periods. Overall, the high organic residuals and nutrients remained in the UASB reactor brewery effluent has to be further treated to minimize its environmental impact.

### Treatment efficiency of a two-stage HSSFCW system

After treatment with the two-stage HSSFCW system, the average pH was decreased insignificantly ($p > 0.05$) from 7.7 to 7.6 for 1 day HRT; from 7.6 to 7.3 for 2 days HRT; from 7.4 to 7.2 for 3 days HRT; from 7.5 to 7.3 for 4 days HRT; from 7.3 to 7.1 for 5 days HRT. The inlet, as well as outlet pH values of the two-stage HSSFCW effluent, meet the suggested optimum pH discharge rate, and a strong-to-moderate linear correlations were observed between the influent and effluent pH values during the study periods as HRT extends from 1 day to 5 days (Fig. 2a), indicating that the HSSFCW outlet pH has a dependency on the initial pH of the effluent. On the other hand, the insignificant inlet water temperature ranged from 26.1 to 31.2 °C, and its further treatment showed a significant reduction in the range of 18.2 to 23.2 °C (Fig. 2b).

### TSS and COD removals

Table 2 shows the average result of TSS and COD outlet concentrations, mass loading rates, and removal efficiencies after treated with a series-connected two-stage HSSFCW system under five different HRTs. The two-stage HSSFCW treatment system at these operation conditions resulted in variable removal efficiency of these pollutants from the influent. However, higher HRTs or lower MLRs yielded a decreased TSS and COD effluent concentrations (Table 2). From the data, the 1 day HRT TSS removal efficiency varied from 45 to 49.3% with MLR fluctuation of 25 to 27.4 $gm^{-2}\ day^{-1}$ (Fig. 3a) with respective average removal of 47.8% TSS at average

**Table 2 Average (±SD) effluent concentration, mass loading rate and percentage removal of pollutants in a series-connected two-stage HSSFCW system at different HRTs**

| Operation | Effluent concentration (mg/L) | | | | | Mass loading rate ($gm^{-2}d^{-1}$) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | TSS | COD | TN | TP | $PO_4-P$ | TSS | COD | TN | TP | $PO_4$-P |
| 1 day HRT | 113.3 ± 5.82 | 376.2 ± 31.86 | 63.6 ± 11.27 | 34.2 ± 7.93 | 25.3 ± 5.3 | 26.4 ± 0.78 | 64.5 ± 5.2 | 11.5 ± 1.31 | 5 ± 0.91 | 3.8 ± 0.87 |
| 2 days HRT | 70.7 ± 16.81 | 155.9 ± 20.77 | 37.3 ± 7.67 | 23 ± 5.89 | 12.4 ± 4.01 | 10.2 ± 2.11 | 24.9 ± 2.97 | 4.3 ± 0.75 | 2.1 ± 0.45 | 1.3 ± 0.34 |
| 3 days HRT | 31.1 ± 9.57 | 63.8 ± 11.3 | 19.9 ± 3.6 | 15.1 ± 3.99 | 7.8 ± 3.39 | 5.2 ± 1.07 | 15.5 ± 1.91 | 2.1 ± 0.37 | 1.3 ± 0.47 | 0.8 ± 0.32 |
| 4 days HRT | 15.7 ± 4.85 | 37.8 ± 9.97 | 10.1 ± 1.82 | 9.1 ± 3.66 | 3.81 ± 1.58 | 2.9 ± 0.42 | 11.6 ± 0.94 | 1.4 ± 0.19 | 0.9 ± 0.17 | 0.5 ± 0.15 |
| 5 days HRT | 11 ± 3.79 | 29.7 ± 7 | 7.6 ± 1.75 | 6.1 ± 2.46 | 3.83 ± 1.25 | 2.1 ± 0.37 | 7.3 ± 0.83 | 0.8 ± 0.16 | 0.6 ± 0.12 | 0.4 ± 0.09 |
| p value | *<0.05* | *<0.05* | *<0.05* | *<0.05* | *<0.05* | *<0.05* | *<0.05* | *<0.05* | *<0.05* | *<0.05* |

| Operation | Percentage removal efficiency (%) | | | | |
|---|---|---|---|---|---|
| | TSS | COD | TN | TP | $PO_4$–P |
| 1 day HRT | 47.8 ± 1.52 | 29.2 ± 2.11 | 32.9 ± 3.08 | 16.8 ± 1.91 | 18.4 ± 5.44 |
| 2 days HRT | 57.8 ± 1.65 | 61.9 ± 4.26 | 46.9 ± 4.94 | 34.8 ± 3.39 | 41.5 ± 5.44 |
| 3 days HRT | 75.8 ± 4.75 | 83.3 ± 1.55 | 61.1 ± 3.88 | 51.6 ± 3.13 | 59.8 ± 3.49 |
| 4 days HRT | 83.5 ± 2.89[a] | 90.1 ± 2.64[a] | 77.5 ± 2.56[a] | 68.9 ± 5.9[a] | 77.5 ± 2.84[a] |
| 5 days HRT | 87.2 ± 2.54[a] | 90.1 ± 2.25[a] | 77.7 ± 1.71[a] | 75.4 ± 6.14[a] | 76.8 ± 4.97[a] |
| p value | *<0.05* | *<0.05* | *<0.05* | *<0.05* | *<0.05* |

[a] Average mass removal efficiencies of the same parameter in columns are insignificantly different

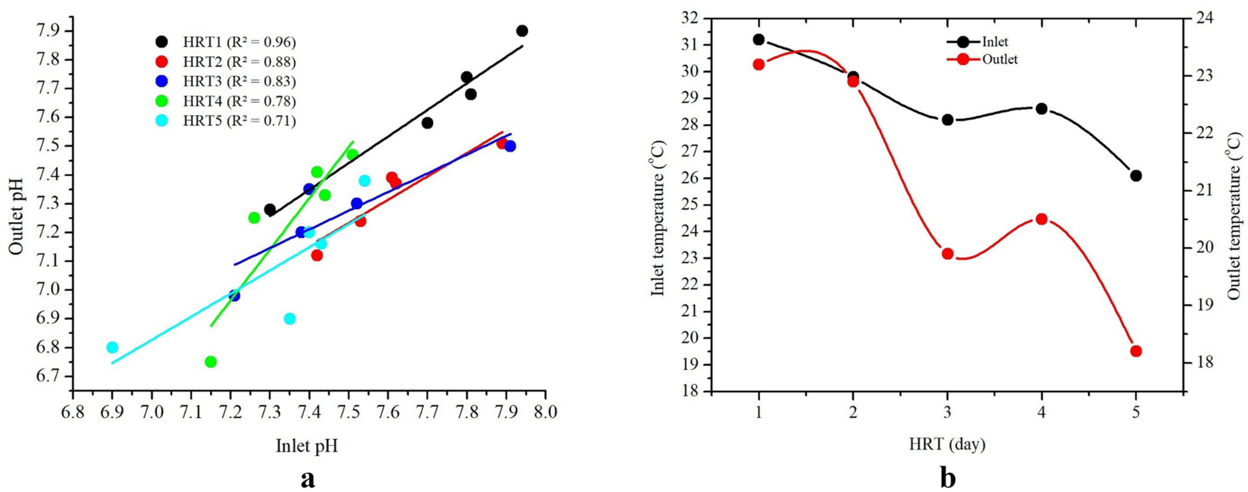


**Fig. 2** **a** Correlation of inlet and outlet pH and **b** inlet and outlet temperature changes for each HRTs in the series-connected two-stage HSSFCW during the monitoring period

MLR of 26.4 $gm^{-2}$ $day^{-1}$ (Fig. 3b). Further increase of HRT to 5 days doubled the removal efficiency of TSS in the range of 84.4% to 91.2%, at lower MLRs ranged from 1.7 to 2.6 $gm^{-2}$ $day^{-1}$ with an average removal efficiency of 87.6% TSS at 2.1 $gm^{-2}$ $day^{-1}$. Analogously, the 1-day COD removal efficiency varied from 25.8% to 32.3% with MLR variation of 57.9 to 71.7 $gm^{-2}$ $day^{-1}$ (Fig. 3c) and resulted in average removal efficiency of 29.2% COD at 7.3 $gm^{-2}$ $day^{-1}$ (Fig. 3d), which was less than by twofold and threefold as compared to the 2 and 5 days average efficiencies, respectively. These higher TSS and COD removal variability implies that lower HRT causes a shorter contact time of pollutants with the wetland component and favors easy escape of these pollutants. On the other hand, the 4 and 5 days HRT exhibited higher and steadier TSS and COD removal efficiencies (Fig. 3a, c) throughout the monitoring periods, which verifies that an increased HRT, providing solid particles to have more tendency to settle inside the treatment system as well as more degradation of organic matter. Overall, the use of the two-stage HSSFCW planted with *Cyperus alternifolius* and *Typha latifolia* in treating UASB reactor brewery effluent was able to achieve higher TSS and COD removals that meet the national standards at higher HRTs.

### TN removal

Figure 4a shows TN removal efficiency variation in the two-stage HSSFCW treatment system operated at different HRTs. As perceived in this figure, the TN removal efficiency varied from 28.2% to 36.9% with MLR of 9.5 to 13.2 $gm^{-2}$ $day^{-1}$ on 1 day HRT, and further increased HRT to 2 days increased its removal by double with almost triple reduction of the MLR (Fig. 4b). Overall, increased HRT operations increased the TN removal efficiency significantly from 32.9 to 77.7% (Table 2). However, maximum and steadier removal efficiencies were achieved at 4 and 5 days HRT with a decrease in MLRs (Fig. 4a), and able to achieve efficient TN concentration that meets the EEPA standard, < 40 mg/L.

### TP and $PO_4$-P removals

The TP and $PO_4$-P contents in both the influent and effluent of a two-stage HSSFCW treatment system showed a variable reduction between HRTs (Table 2). Minimum TP and $PO_4$-P removal efficiencies ranged from 13.8% to 19.2% for TP, and from 9.1 to 24.3% for $PO_4$-P were obtained at 1 day HRT with respective MLR of 4.1 to 6.6 $gm^{-2}$ $day^{-1}$ and 2.8 to 4.5 $gm^{-2}$ $day^{-1}$ (Fig. 5a, b). The corresponding average TP and $PO_4$-P removals were 16.8% TP and 18.4% $PO_4$-P with average MLR of 5 $gm^{-2}$ $day^{-1}$ and 3.8 $gm^{-2}$ $day^{-1}$, respectively. However, further increase of HRT from 1 to 2 days doubles the 1-day TP and $PO_4$-P removal efficiencies, and at the end, the fourfold increment was observed for both pollutants as HRT extends from 1 to 5 days (Table 2; Fig. 5c, d), and able to achieve efficient $PO_4$-P concentration reductions that meet the EEPA standard, < 5 mg/L.

## Discussion

### UASB reactor brewery effluent characteristics

Several studies reported that the treatment efficiency of UASB reactor in the reduction of COD was promising with high methane production potential, and less in removing BOD and TSS. However, the reactor is limited

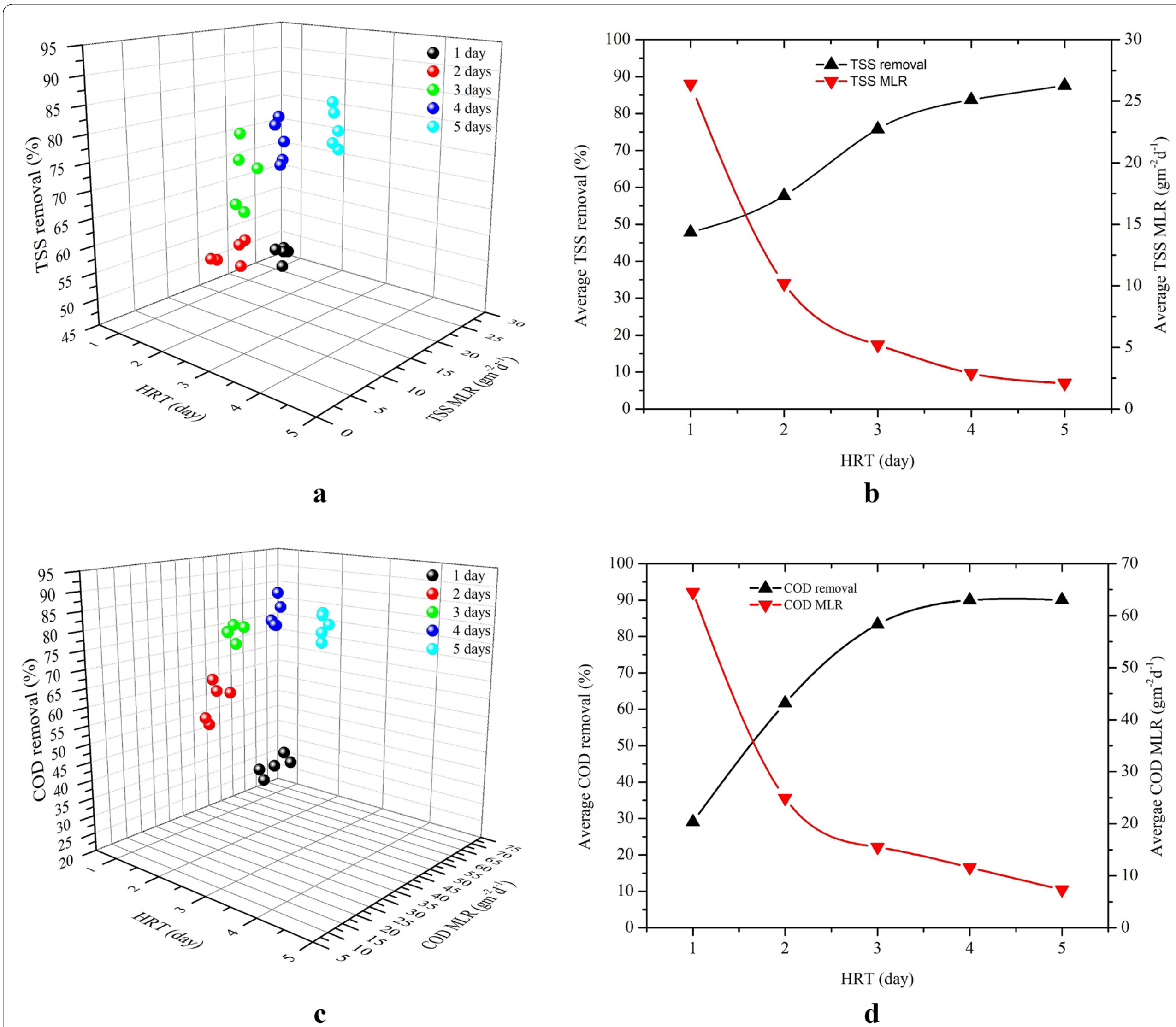


**Fig.3** **a** Effect of HRT and MLR on TSS removal, **b** average TSS removals at different HRTs and MLRs, **c** effect of HRT and MLR on COD removal, and **d** average COD removals at different HRTs and MLRs in a series-connected two-stage HSSFCW. Dots indicate five rounds of sampling removals for each HRT

in nutrient removal due to mineralization of compounds in the effluent like ammonia, and orthophosphate (El–Khateeb and El–Bahrawy 2003; Bruno and Oliveira 2013). Additionally, the overall performance efficiency of the reactor sometimes deteriorates due to poor maintenance and operations, various factors such as temperature, pH, the composition of wastewater, HRT, organic loading rate (Mirsepasi et al. 2006), and incidence of other toxic substances (Rajagopal et al. 2019). Overall, many studies reported that anaerobically treated effluents are characterized by high levels of residual organics and nutrients (Yasar and Tabinda 2010; Foresti et al. 2006). Similarly, in the present study, the UASB reactor brewery effluent suspended solids, organic matter and nutrient contents indicated substantial fluctuations, which exceeded the national discharge standard limits, while the brewery effluent pH showed an insignificant variation throughout the study periods, probably due to certain pH adjustments made in the equalization tank for the proper functioning of the UASB reactor microorganisms. Most of the times, these highly variable compositions of brewery TSS, COD, TN, TP, and $PO_4$-P concentrations may be due to the various dynamic processes carried out in the beer production process (Bakare et al. 2017; Raposo et al. 2010). The brewery effluent nutrient contents are mainly influenced by the nature and quantity of external

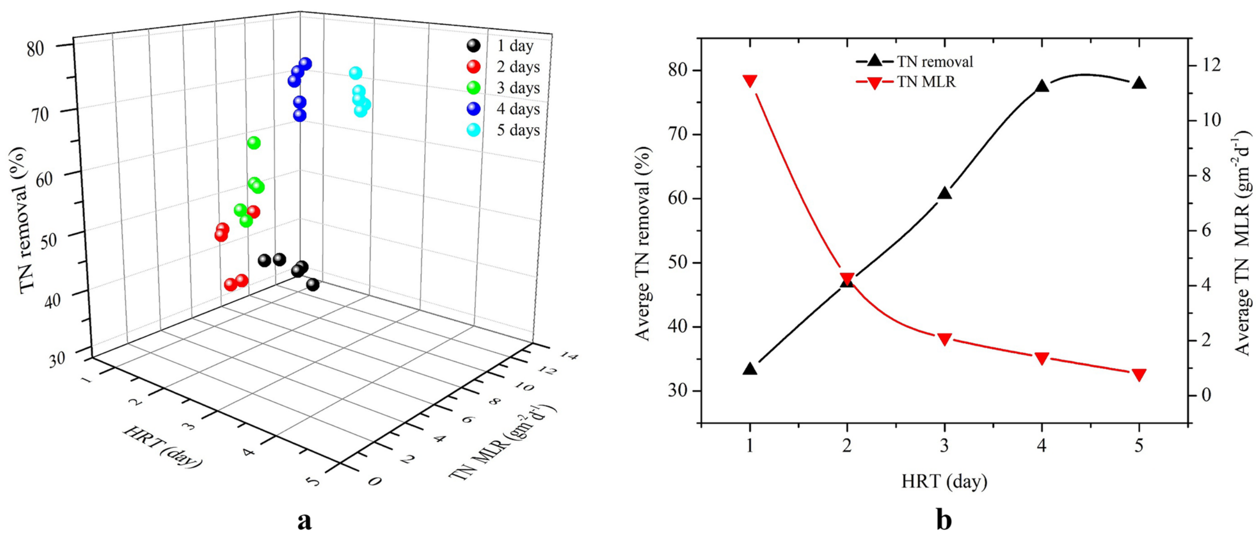


**Fig.4** **a** Effect of HRT and MLR on TN removal, and **b** average TN removal at different HRTs and MLRs in a two-stage HSSFCW system

chemicals used for cleaning and sanitizing agents such as caustic soda, phosphoric acid, and nitric acid (Belay and Sahile 2013; Simate et al. 2011; Driessen and Vereijken 2003), including raw materials; whereas, the high suspended solids and organic matter contents mainly arise from spent grains, kieselguhr, yeast cells (Driessen and Vereijken 2003), and the presence of soluble starch, sugars, ethanol, volatile fatty acid, proteins and amino acids (Raposo et al. 2010). Scientific evidence showed that disposal of brewery effluents without proper treatment causes severe environmental pollution problems (Oljira et al. 2018*;* Abrha and Chen 2017*)*, particularly to the downstream receiving surface water, and soil, due to the great variety of contaminants mentioned above, including oil and greases, salts, refractory organics, and sometimes pathogens (Hultberg and Bodin 2017; Singh and Agrawal 2012). For instance, in Ethiopia, results of Belay and Sahile (2013) study revealed that the release of brewery effluent affects water bodies to pong, discoloration, and oily nature. In the same argument, our study area downstream community faces a great problem with brewery effluents.

Presently, the treatment of UASB reactor brewery effluent using the two-stage HSSFCW system plays an important role in reducing the in situ pH value to the neutral condition at all HRT operations. Similar to this study, Winanti et al. (2018) observed a pH decline from 8.15 to 6.6 at 1 day HRT and increased to 7.0 at 3 days HRT, but again decreased into 6.8 at 5 days HRT operation with a working temperature range of 27–28°C in a HSSFCW system planted with *Canna* sp. during the treatment of grey wastewater. Analogously, Merino-Solís et al. (2015) reported that HSSFCW effluent showed pH variability between HRTs, and overall dependence on the influent pH value. Likewise, the series-connected two-stage HSSFCW effluent pH value showed a strong-to-moderate linear correlations, implying that the series-connected two-stage HSSFCW effluent pH value is dependent on the UASB reactor brewery effluent pH. All HRT operation pH values are insignificant and normally play an important role for better degradation of organic matter removals, since it meets the recommended pH ranging from 6.5 to 8.5, which is the optimum range for improved degradation of organic matter by microorganisms in the HSSFCW unit (Merino-Solís et al. 2015). Likewise, pH > 8.0 disturbed the nitrogen removal processes by decreasing the activity of microorganisms in the nitrification and denitrification processes (Vymazal 2007). Along with pH, many studies reported that temperature has a significant effect on the removal mechanism of organic matter and nitrogen in CWs (Saeed and Sun 2012; Zhu et al. 2017). The recorded average UASB reactor effluent temperature entering the HSSFCW ranged from 26.1 to 31.2 °C, and meets the normal temperature range (16.5–32 °C) of nitrifying microorganisms (Saeed and Sun 2012). But much decline of the metabolic rate and activities of nitrifying and denitrifying bacteria was below 10 °C (Yan and Xu (2013), and completely ceased < 6 °C (Zhu et al. 2017). Overall, the present study showed a significant reduction of inlet temperature as compared to the outlet temperature when passing through the HSSFCW treatment system, and the HSSFCW acts as a refrigerating box.

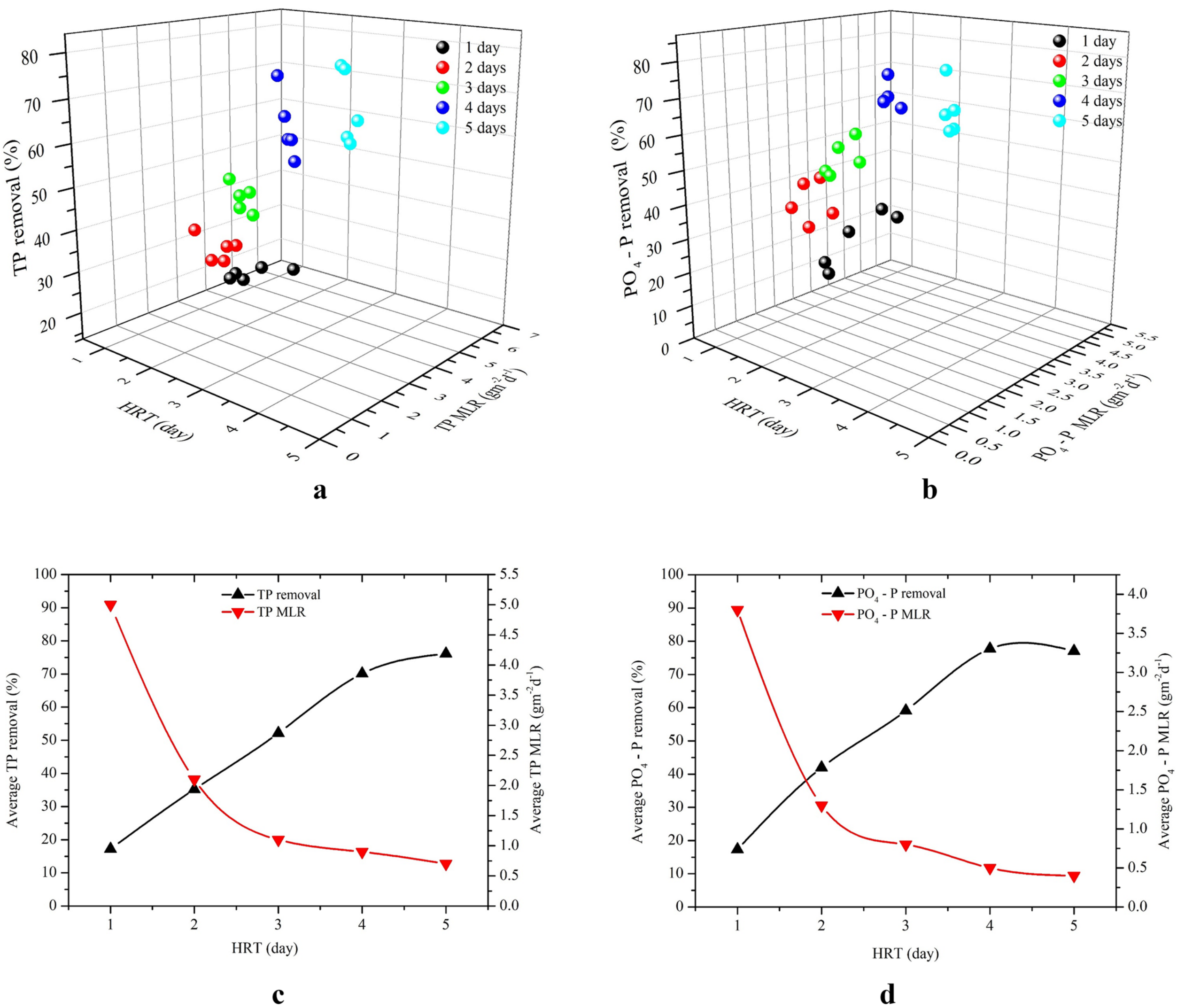


**Fig. 5** Effect of HRT and MLR on **a** TP and **b** $PO_4$-P at different HRTs and MLRs, **c** TP average removal efficiency, and **d** $PO_4$-P average removal efficiency at different HRTs and MLRs in a two-stage HSSFCW

**Pollutant removal efficiency**

The efficiency of the series-connected two-stage HSSFCW treatment system showed a variable removal efficiency of TSS and COD, despite the significant influent fluctuations. Other studies also reported that the degradation and sedimentation of pollutants in the HSSFCW treatment system are mostly affected by hydraulic factors (i.e., HRT or HLR) and mass loading rate (Ghosh and Gopal 2010; Jinadasa et al. 2013; Shuib and Baskaran 2015). Furthermore, the treatment efficiency of a HSSFCW system is dependent on the nature of the original wastewater supply, selection of media types, feeding mode, and aeration conditions (Stefanakis and Tsihrintzis, 2012). For instance, the higher HRT or lower pollutant loading rate brings pollutants to have a higher rate of contact time within the treatment system (Reed et al. 1995); favors greater chances of TSS sedimentation, and COD degradation, including high nutrient absorption and retention (Guo et al. 2017), and improves the effluent quality. According to Mora-orozco et al. (2018), a comparison of different treatment wetland efficiencies with the removal of solids and organic matter at different HRTs revealed that the 10 days HRT showed the highest removal efficiency than 5 days HRT. Sultana et al. (2016) also showed that 8 days HRT removed 100% COD. Likewise, in the present study, higher HRTs bring enhanced TSS and COD removals. From Table 2, it is apparent that the 1 day HRT TSS removal efficiency was minimum, but a further increase of HRT to 5 days, double the TSS removal efficiency to the steadier removal range (84.4 to 91.2%) at lower MLRs ranged from 1.7 to 2.6 $gm^{-2}$ $day^{-1}$. While the 1-day COD removal efficiency was less than twofold and threefold as compared to the 2 and 5 days average efficiencies, respectively. This higher removal variability implies that a lower HRT brings a shorter contact time of pollutants with the wetland components and causes easy escape of these pollutants. On the reverse, increased HRT enhances solid particles to have more tendency to settle and organic matter to undergo more degradation inside the treatment system, and achieves better abatement of TSS and COD. In agreement with this study, Ghosh and Gopal (2010) investigated that the removal of TSS and COD at 1 day HRT was 78.55 and 15.96%, respectively, with corresponding MLR of 0.73 and 34.48 $gm^{-2}$ $day^{-1}$. Later on, as HRT doubles, the removal of TSS and COD were increased to 84.07 and 46.01% with a further reduction of MLR from 0.73 to 0.28 $gm^{-2}$ $day^{-1}$, and 34.48 to 13.25 $gm^{-2}$ $day^{-1}$, respectively for TSS and COD. But, the maximum of 92.91% TSS and 89.28% COD removal efficiencies were achieved at 4 days HRT with corresponding MLR of 0.18 and 8.62 $gm^{-2}$ $day^{-1}$. Angassa et al. (2019) have also investigated the effect of HRT and MLR on the performance efficiency of a gravel-based pilot-scale HSSFCW in treating municipal wastewater using *Vetiveria zizanioides*, and the results indicated that an increased treatment efficiency from 90.8 to 95% COD was observed as MLR is decreased from 22.5–25.8 $gm^{-2}$ $day^{-1}$ to 11–12.7 $gm^{-2}$ $day^{-1}$, respectively, at 3 and 6 days HRT.

Many studies have also recognized that sufficient contact time has a significant role in the TSS and COD removal since it offers both particles to settle, and improve the removal efficiencies through adhering rather than absorbed by vegetation (Mora-orozco et al. 2018; Winanti et al. 2018). Also, the biological process has a main role in the removal of organic matter through microbiological degradation with bacteria attached to macrophyte roots (Winanti et al. 2018). A study conducted by Sa'at et al. (2017) using *Cyperus alternifolius*-based treatment of aerobic palm oil mill effluent showed 96% COD and 91% TSS removals at 21 days HRT; whereas, the *Typha latifolia*-based treatment of wastewater achieved 92% TSS and 79% COD at HRT of 4.7 days with a loading rate of 150 $kgha^{-1}day^{-1}$ (Ciria et al. 2005). Different study results evidenced that the massive root systems of *Cyperus alternifolius* and *Typha latifolia* provide a larger surface area for accumulation of various microbial communities that helps for high organic matter and solids removals via degradation and sedimentation processes (Ciria et al. 2005; Aziz et al. 2015). Overall, the use of two-stage HSSFCW planted with these two combined macrophytes in UASB reactor effluent treatment at 4 and 5 days HRT were able to achieve higher COD removal in the present study as compared with 86% of COD removal reported by Merino-Solís et al. (2015) during treatment of anaerobic reactor effluent of domestic wastewater using a two-stage HSSFCW planted *Canna hybrids* and *Strelitzia reginae* at 3 days HRT and HLR of 11 $m^3$/day. The study TSS and COD results are also close to Alemu et al. (2016) who reported removal efficiencies of 95.6% TSS and 90.8% COD in treating anaerobic-sequencing batch reactor effluent of tannery wastewater using a *Phragmites karka* cultured series HSSFCWs at 5 days HRT and hydraulic flow rate of 23.625 $m^3day^{-1}$. In another report, HSSFCW TSS removal efficiency and mostly oscillated between 56.62 to 91.04% (Albalawneh et al. 2016). Likewise, Zamora et al. (2019) observed significant TSS removal efficiency differences between *Canna* and *Cyperus* species planted CWs due to the difference in the root volume production of these macrophytes, and the author studied that a hybrid CW treatment system showed better TSS removal. Generally, in this study, promising results that meet the national standards were achieved at higher HRTs and lower MLRs. This may be probably due to the combined action of macrophytes and the longer HRT operations, which creates good cooperation of the physical and biochemical processes in which the former favors more tendencies for solid particles to settled, adsorbed, and attached to the substrate media, while the latter for better chemical and biological degradation and consumption of COD by root zone accumulated immense microorganisms (Leto et al. 2013; USEPA 2000).

The TN removal efficiency was also significantly varied between HRTs except between the 4 and 5 days. For instance, the 1 day HRT removed below 40%, but further increased HRT to 2 days increased its removal by double. Overall, increased HRT operations increased TN removal efficiency. However, maximum and steadier TN removal efficiency was achieved at 4 and 5 days HRT with a decreased in MLRs. Similarly, Ghosh and Gopal (2010) have explored nearly threefold TN removal efficiency (21.86% to 63.66%) with HRT doubling from 1 to 2 days, along with a threefold reduction of MLR from 4.99 to 1.92 $gm^{-2}$ $day^{-1}$. But, the highest of 83.99% TN removal was achieved at $\geq$ 4 days HRT. Another study by Angassa et al. (2019) have also reported higher removal efficiency of TN at 6 days HRT with corresponding MLR ranged from 1.9 to 2.5 $gm^{-2}$ $day^{-1}$ as compared to the 3 days HRT in treating municipal wastewater using HSSFCW planted with *Vetiveria zizanioides*. This indicates that HRT has a key role in the effective nutrient removal process in a CW. Since optimal HRT can provide the best formation of microbial communities and adequate contact time to remove nutrients in the CW (Mora-orozco et al. 2018). Overall, increased HRT produced substantial increases in the TN removal efficiency (Sa'at et al. 2017). Overall, this research evidence showed that the better decline of the TN effluent concentration in the constructed wetland was obtained by increasing the HRT. This directs that hydraulic operational factors played a more significant role in the removal of nitrogen in the HSSFCW treatment system (Guo et al. 2017; Shuib and Baskaran, 2015; Cheng et al. 2010). However, Vymazal (2005) reported that nutrient removal is usually insignificant in CW, does not greater than 50%. On the contrary, Son et al. (2010) reported a nitrogen removal efficiency of 53%, and overall observed a two-stage vertical subsurface flow constructed wetland (VSSFCW) performed better nitrogen removal as compared to a single-stage VSSFCW. Accordingly, in the present study, the two-stage HSSFCW treatment achieved over 77.5% of TN removal at higher HRTs. This may be due to the combined effect of macrophytes probably due to their effective nutrient uptake potential as well as their extensive rhizosphere growth that serves as an important attachment site for various microorganisms and thus allowing more nitrification and denitrification processes. Many other contributing factors such as the aeration ability of the wetland macrophyte could also exert a resilient environment for the nitrogen transformation through providing large surface area for bacterial growth and the release of organic carbon as an energy source for more denitrification process (Nivala et al. 2013; Akratos and Tsihritzis 2007), and plant attached microbial uptakes (Theophile et al. 2011; Winanti et al. 2018). Another research evidence also indicated that macrophytes serve as the key component of nitrogen removal in CW acting as a reaction medium for purification by enhancing a variety of removal processes (Wu et al. 2015). Nitrogen removal in CW relied on the nitrification–denitrification, oxygen-carrying capacity macrophytes, which related to root growth, and macrophyte adsorption processes. CW macrophytes root and media are also provide a larger surface area to microorganisms attachment for better nitrogen removal via nitrification - denitrification processes; whereas, a decrease in nitrogen removal efficiency was likely due to oxygen deficiency in HSSFCW filter media as a result of saturated conditions (Winanti et al. 2018). Overall, both species richness and community composition constitute a greater effect on the nitrogen removal efficiency of a CW through increased biomass production, retention time, and microbial activity (Geng et al. 2017).

As studies reported that the *Cyprus alternifolius* has a higher TN removal without pH inhibition (Miyazaki et al. 2004), and *Typha latifolia* has also greater nitrogen absorption capacity in its below- and above-ground biomasses than *Cyperus alternifolius*. Additionally, Mollard et al. (2013) have also reported that *Typha latifolia* is effective in mitigating nutrient-rich wastewater. Another TN removal effectiveness study of these macrophytes was investigated separately, and the *Cyperus alternifolius*-based treatment of sewage wastewater was able to remove 59.84% of TN at MLR of 940 $mgm^{-2}\ day^{-1}$ in a vertical constructed wetland treatment system (Bilgin et al. 2014), while *Typha latifolia*-based treatment of raw brewery wastewater able to achieve 28–46% of TN at 5 days HRT and HLR of 10 $cmday^{-1}$ (Worku et al. 2018). Generally, the present result had higher than Jamshidi et al (2014) reported TN removal efficiency of 71.9% at ½ day HRT operation using a bio-rack wetland planted with *Phragmites sp.*, and *Typha sp.* in treating anaerobic baffled reactor effluent of domestic wastewater, but lower than Alemu et al. (2016) reported value (i.e., 82% TN) using three series-connected HSSFCW systems planted with *Phragmites karka* in treating anaerobic–aerobic sequence reactor effluent of tannery wastewater at 5 days HRT. Another research study evidence indicated that approximately 60–70% of TN is removed by the denitrification process, whereas 20–30% can be removed by plant uptake (Wang et al. 2016). Overall, many researchers investigated that macrophytes play a significant role in the removal of nutrients. Hence, two or more macrophytes combination may contribute to better removal efficiency than alone (Sa'at et al. 2017). On the contrary, the HSSFCW treatment system planted with *Canna sp.* showed maximum removal efficiency of nitrogen that varied between 91 and 95%. This may be due to the conversion of organic nitrogen into ammonia by ammonification, followed by adequate nitrification into nitrates by Nitrosomonas bacteria, and then nitrates converted to nitrogen molecular nitrogen by chemoautotrophic bacteria and/or subsequently absorbed by *Canna sp.* (Winanti et al. 2018).

Removal of TP and $PO_4$-P in the series-connected two-stage HSSFCW system showed significant fluctuations among HRTs except for the 4 and 5 days in line with the influent variations. This may be due to the anaerobic reactor condition, which typically produces a high amount of orthophosphate through mineralization (Jamshidi et al. 2014), and its removal in the constructed wetland is dependent on the HLR, type of macrophytes root and substrate medium used (Sigh et al. 2014). In this study, the UASB reactor effluent TP and $PO_4$-P concentrations that drained into the two-stage HSSFCW showed a significant variation, and treatment using a two-stage HSSFCW showed a variable removal efficiency. The minimum TP and $PO_4$-P removal efficiencies were obtained at 1-day HRT operation due to the relatively high loading of these pollutants as compared to other

HRTs. In the time being, an increase of HRT from 1 to 2 days, doubles these pollutant removal efficiencies, and further increases HRT from 1 to 5 days HRT showed a fourfold increment for both pollutants. A similar trend of $PO_4$-P removal was reported by Ghosh and Gopal (2010), which initially touched 11.61% at 1 day HRT with corresponding MLR of 1.08 gm$^{-2}$ day$^{-1}$. When the HRT doubles, the removal of $PO_4$-P showed a nearly double increment from 11.61 to 21.11% at MLR 0.42 gm$^{-2}$ day$^{-1}$, and so on. However, relatively higher percentage removal of 46.36% $PO_4$-P was obtained at 4 days HRT with corresponding MLR of 0.27 gm$^{-2}$ day$^{-1}$; and Angassa et al. (2019) observed 95.2% of phosphorus removal at 6 days HRT with corresponding MLR that varied from 0.9–1.25 gm$^{-2}$ day$^{-1}$ as compared 88.5% of phosphorus obtained at 3 days and MLR of 2–2.5 gm$^{-2}$ day$^{-1}$ in *Vetiveria zizanioides* planted HSSFCW unit during treatment of municipal wastewater. In another study by Ewemoje et al. (2015), a *Coix*-based treatment resulted in 69% of phosphorus removal in 3 days HRT and further increasing the HRT to 5 days, increased its removal to 76.8%, and maximum removal of 89.1% of TP and 84.4% of $PO_4$-P were attained in 7 days HRT with HLR of 19.91 m$^3$m$^{-2}$ day$^{-1}$. Moreover, Sehar et al. (2013) reported much decline of $PO_4$-P concentration at increased HRT from 4 to 20 days and attained a substantial reduction of 89.35% $PO_4$-P at 20 days. Another work optimized CWs using 3 and 7 days of HRT for treating wastewater from a pig farm, and the 7 days HRT resulted in removal efficiency of about 100% of TP (Muñoz et al. 2016). Likewise, in this study, higher HRT operations along with lower MLRs were able to achieve the national discharge standard limit of < 5 mg/L. The accomplishment of this promising result may be due to the use of a mineral-rich clay rock that adsorbs phosphorus, as well as the combined effect of both macrophytes, which enhances phosphorus removal by filtration, sedimentation, macrophytes direct uptake and microbial assimilation. Likewise, Akratos and Tsihrintzis (2007) reported that vegetation uptake and media adsorption are the dominant phosphorous removal mechanisms. Macrophytes play a significant role in the phosphorous recycling in CW (Vymazal 2007). In addition to the physical and chemical processes, direct macrophyte absorption and macrophyte-mediated microbial processes account for a significant phosphorous removal in CWs. Hence, macrophytes owe substantial removal of phosphorous in their tissues (Geng et al. 2017). *Cyperus alternifolius*-based treatment of aerobic palm oil mill effluent showed 73% of TP removal efficiency at 21 days HRT (Rezaie and Salehzadeh 2014), while *Typha latifolia*-based treatment of wastewater was able to achieve 39% of TP removal at HRT of 4.7 days with MLR of 150 kgha$^{-1}$day$^{-1}$ in the 1-year operation period (Ciria et al. 2005). As a whole, the present study result is comparable with Cheng et al. (2010) reported result of 77.9% phosphorus at 4.5 days in treating pilot-scale UASB reactor effluent of mixtures of sewage and partially treated swine wastewater using a series-connected two-stage constructed wetland planted with reeds and water lettuce, and close to Badejo et al. (2014) reported result of 82.73% $PO_4$-P removal using a *Phragmites karka* planted HSSFCWs in treating anaerobic effluent of brewery wastewater at 10 days HRT. In another review report, TP removal may be as high as 31–99% in various CWs (Geng et al. 2017). This author also observed that an increased abundance of macrophyte species decreased the concentration of influent TP. For instance, four species cultured CWs able to reduce TP concentration approximately 78% relative to monocultures (Geng et al. 2017). Overall, phosphorous removal was improved by higher contact time between the media and wastewater for its better media adsorption and precipitation, degradation of bacteria and macrophyte absorption (Albalawneh et al. 2016).

## Conclusion

This study assessed the effect of HRT variations on the treatment efficiency of a two-stage HSSFCW in treating UASB reactor brewery effluent, and the result indicated that HRT has a major impact on the removal of main brewery pollutants and optimization of this factor is a key for effective treatment efficiency of the series connected two-stage HSSFCW. Overall, the complete treatment system showed a substantial reduction of pollutants that ranged from 47.8–87.2% for TSS, 29.2–90.1% for COD, 32.9–77.7% for TN, 16.8–75.4% for TP and 18.4–76.8% for $PO_4$-P with decreased MLR ranged from 26.4–2.1, 64.5–7.3, 11.5–0.8, 5–0.6, and 3.8–0.4 gm$^{-2}$ day$^{-1}$, respectively, for TSS, COD, TN, TP, and $PO_4$-P as HRT extends from 1 to 5 days. However, insignificantly differed better and steadier pollutant removals were achieved at 4 and 5 days HRT and can be established as a good benchmark for the polishing of anaerobically treated brewery effluent.

### Abbreviations

HRT: Hydraulic residence time; HLR: Hydraulic loading rate; MLR: Mass loading rate; TSS: Total suspended solids; COD: Chemical oxygen demand; TN: Total nitrogen; TP: Total phosphorous; $PO_4$-P: Orthophosphate; APHA: American Public Health Association; HDP: High-density polyvinyl pipe; UASB: Up-flow anaerobic sludge blanket; CW: Constructed wetland; HSSFCW: Horizontal subsurface flow constructed wetland; VSSFCW: Vertical subsurface flow constructed wetland;; EEPA: Ethiopian Environmental Protection Authority; RE: Removal efficiency; V: Volume; A: Area; $Q_i$: Influent flow rate; d: Depth; p: Porosity; $C_i$: Influent concentration; $C_e$: Effluent concentration.

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**Authors' contributions**

All authors have made equal contributions to this study. EA designed the study, conducted the experiments, contributed to data collection and analysis, and wrote the manuscript. SL was involved in the study design, advised the experiment and offered comments and suggestions during the entire work. All authors read and approved the final manuscript.

**Authors' information**

EA is currently a student at Addis Ababa University, Center for Environmental Science, College of Natural Science, and lecturer at Assosa University, Chemistry department. He has given different inorganic chemistry and industrial chemistry courses and also contributes to community services and research activities and has published articles in the international peer-reviewed journals. SL is an Associate Professor of environmental biotechnology and works at the Center for Environmental Sciences, Addis Ababa University. He has given various multidisciplinary courses in the stream. He is also supervising several MSc. and Ph.D. students in the areas of environmental biotechnology, wastewater treatment, and water quality studies, waste to energy (resource recovery and climate change mitigation and adaptation studies), bioremediation, phytoremediation, microbial ecological studies, environmental sanitation, etc. He has published several peer-reviewed papers in different international reputable journals. He has also served at different positions like as Department chairman, executive Director for Horn of Africa Regional Environmental Centre and Network (HOARECN), a regional program manager for the Bio-resources innovation network for Eastern Africa development, a principal investigator for several research projects such as "Development of innovative technologies for the sustainable treatment of high-strength wastewater in East Africa" and a regional research program involving Ethiopia, Kenya, Tanzania and Uganda funded by Sida.

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**Availability of data and materials**

The data and materials used in this manuscript are available at hand and can be shared whenever necessary. Data were gathered by the principal author from the experimental site through sample collection and laboratory analysis.

**Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare no conflict of interest

**Author details**

[1] Department of Chemistry, College of Natural and Computational Sciences, Assosa University, Assosa, Ethiopia. [2] Center for Environmental Science, College of Natural Science, Addis Ababa University, Addis Ababa, Ethiopia.

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