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ORIGINAL PAPER

Constructed wetlands for phytoremediation of industrial wastewater in Addis Ababa, Ethiopia

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Abstract

Brewery industries generate large amounts of wastewater rich in organic matter originating from the brewing process, and they are among the major polluting industries. This study aimed to assess the phytoremediation of brewery wastewater using horizontal subsurface flow constructed wetlands (HSFCWs) vegetated with *Typha latifolia* and *Pennisetum purpureum* for organics removal and plant growth analysis. Six parallel pilot-scale HSFCWs were constructed and operated to assess potential of treating wastewater sourced from St. George brewery factory located in Addis Ababa, Ethiopia. Three units were planted with *T. latifolia* and the other three with *P. purpureum* with one control without plants for each species. Primarily settled wastewater was fed evenly to them by gravity. Wastewater quality, plant growth analysis and system efficiency were observed during the experiment following standard methods. Both plants grew and established well, however, *T. latifolia* had more biomass and vigorous growth and showed good phytoremedial capacity to remove organic pollutants. Average removal efficiencies for BOD_5 and COD were significant ($p < 0.05$), up to 87% (inlet BOD_5 of 748–1642 mg l^{-1}) and up to 81% (inlet COD of 835–2602 mg l^{-1}) and *T. latifolia* slightly outperformed *P. purpureum*. Estimated biomass of significant ($p < 0.05$) value (0.61–0.86 kg DW m^{-2}) was produced. HSFCWs are green and environmentally sustainable technology that offers promising alternative wastewater treatment method in developing countries of tropical climate due to its low-tech nature. Integrating treatment and biomass production needs further improvement.

Keywords Biomass · Brewery wastewater · Constructed wetlands · Organics removal · Phytoremediation · Ethiopia

Introduction

Breweries are widespread industries with an important economic value in the agro-food sector, and brewing is intrinsically a water-intensive industry [1]. In Ethiopia, water consumption by breweries reportedly ranges from 9 to 22 m^3 water/m^3 beer, which is far above the accepted international best practice benchmark of 6.5 m^3 water/m^3 beer [2]. This accounts for at least 1.5% of the national consumption of water, impacting on local water services [1]. Due to rapid growth of beer consumption in Ethiopia (24% per year) [3] and the discharge of 70% of the water used by the brewing industry as effluent, the projected expansion of the brewery sector will significantly increase the pressure on the water supply [2, 4]. In general, water and wastewater management in breweries remains a practical problem [5].

Brewery industry generates high amounts of wastewater rich in organic matter originating from the brewing process [4, 6], and it is a major source of environmental and water pollution, particularly in developing countries [7]. This

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means brewery wastewater has to be treated to reduce its environmental impact. Biological treatment methods usually used for brewery wastewater treatment include aerobic sequencing batch reactor, cross-flow ultrafiltration membrane anaerobic reactors and up-flow anaerobic sludge blanket reactors (UASB). These biological treatment processes are particularly effective for wastewater treatment, but they require high energy input and are thus costly.

Phytoremediation is an emerging cleanup technology, aesthetically pleasing and low-cost solution for water pollution. It uses green plants and their associated microorganisms to remove, contain and render harmless environmental contaminants [8]. The remediation technique involves specific planting arrangements, constructed wetlands (CWs), floating plant systems and numerous other configurations. The method is based on a combination of physical, chemical and biological treatment processes to remove organic matter, nitrogen, phosphorus and other substances. The treatment components in the form of vegetation, filter beds and microorganisms contribute both directly and indirectly to the removal of pollutants from wastewater. Dipu et al. [9] reported that constructed wetlands using phytoremediation strategy is the most applicable technology.

Constructed wetlands (CWs) are promising treatment options for domestic and industrial wastewater [10, 11]. They are attractive ecological systems efficiently remove organic pollutants such as biochemical oxygen demand (BOD_5), chemical oxygen demand (COD), suspended solids, pathogens, nutrients and heavy metals [12]. Macrophytes are main biological components in CWs that contribute to wastewater treatment through direct and indirect mechanisms by increasing the environmental diversity in the rhizosphere [13, 14]. Therefore, selection of macrophytes with adequate survival and growth rates in a given ecology with tolerance and efficient pollutant accumulation ability from the media of interest could be a reliable tool for phytoremediation. The rhizosphere (or root zone) of CWs is an active reaction zone. In this reaction zone, various physicochemical and biological processes take place by interaction of plants, microorganisms, media and pollutants. Horizontal subsurface flow constructed wetland (HSFCW) is most widely used system vegetated with *Phragmites australis, T. latifolia*, *Scirpus spp*. and *Phalaris arundinacea* [15].

HSFCW is one of the green treatment technologies which generally produce acceptable effluent qualities without fossil energy input, thus reducing operational costs [16]. Industrial applications of CWS include wastewaters from oil refineries, chemical factories, pulp and paper production, tannery and textile industries, abattoir, distillery and winery industries [17]. Alemu et al. [18] reported use of HSFCW for tannery wastewater with BOD_5 and COD removal efficiencies of 93% and 90%, respectively. Similarly Calheiros et al. [19] indicated BOD_5 and COD removal efficiencies of 41–58% and 41–73%, respectively, by using CWs. Other studies in this field have also shown percentage reduction of BOD_5 (57–78.6%) and COD (58–79%) [20, 21]. Organic removal (96–98% for BOD_5 and 95–98% for COD) from tannery wastewater using CWS in Ethiopia was also reported by Leta et al. [22]. Vymazal and Kröpfelová [23] revealed applications of CWs for treatment of municipal, agriculture and industrial wastewaters. In Kenya, Tanzania, Thailand and many other countries, it was reported that CWs have been successfully used to mitigate environmental pollution by removing a wide variety of pollutants from wastewater, including organic compounds, suspended solids, pathogens, metals and nutrients [12, 24–26].

It is noted that application of CWs has been expanded to the treatment of various industrial effluents. However, the potential in the field is still not established. The search for the potential of CWs in developing countries with tropical climates is particularly urgent [27]. Although they have been successfully used in temperate countries to treat wastewaters, experiences and design criteria employed might not be suitable in tropical countries, including Ethiopia. The potential of CW technology has not been assessed. Climate and other local conditions influence wastewater characteristics, plant growth and evaporation as well as the removal processes in the CW, particularly the microbial processes which may be stimulated by high temperatures [12]. Therefore, there is a need to explore the performance of HSFCW in order to assess the capacity of the systems to treat brewery industrial wastewater and integrate production of plant biomass under tropical climatic conditions of Ethiopia.

The aim of the present study was to assess the potential of using a HSFCW system for organics removal and examine suitability of brewery wastewater to grow valuable biomass in the tropical climate of Addis Ababa, Ethiopia. Cattail (*T. latifolia)* and elephant grass (*P. purpureum*) are locally available macrophytes in the study area but few studies have been carried out on the treatment of brewery wastewater using these plant species [28]. The macrophytes were selected based on plant suitability for use, ecological acceptability, tolerance of local climatic conditions and tolerance of pollutants level, rapid establishment and propagation, and pollutant removal capacity, as recommended by Tanner [29]. The treatment performance of the experimental HSFCW systems was monitored for COD, BOD_5, propagation, growth and biomass production of the two plant species. Thus, the phytoremedial role played by the two plant species for removal of organics in brewery wastewater and production of biomass was assessed.

Materials and methods

Experimental site

The site is located on the premises of Addis Ababa Science and Technology University in Addis Ababa. The city is at an altitude of about 2300 m, and the university is located at the city's southern periphery at 8° 58′ N 38°47′ E. The climate is a subtropical highland climate, with average annual temperature, rainfall and relative humidity of 15.9 °C, 1089 mm and 60.7%, respectively.

A pilot-scale HSFCW treatment system consisting of a primary settling tank (1 m^3), a concrete feed tank (1.5 m^3), two series of triplicated constructed wetland treatment units (CWUs) configured in parallel and a common effluent holding tank was built under the roof of a greenhouse (Fig. 1). There was also one control unit for each series without plants to compare the results and study the role of plants in constructed wetland treatment units. The main experimental materials used were plants, local fine and medium-size gravel and wastewater sourced from St. BGI brewery located in central Addis Ababa. The gravel media, which was predominantly medium size, had porosity of 0.39. Healthy young shoots of *T. latifolia* and *P. purpureum* with a similar state of growth were collected from marshy lands and banks of Akaki and Fanta rivers in the vicinity of the university and transported to the experimental site.

Experimental design and operation of the treatment system

Each treatment unit (2 m long × 0.75 m wide × 0.65 m deep) was filled with gravel of sizes ranging from 8 to 25 mm diameter and aggregate sand and gravel. Average media depths were 0.40 m and 0.50 m for *T. latifolia* and *P. purpureum*, respectively, based on the root potential growth of the macrophytes and level of water surface to keep 0.05 m below the surface of gravel [30]. The height of the treatment units was 0.65 m, with 0.25 m increment to serve as a freeboard for plant safety and monitoring. Inlet and outlet structures were built to each unit for complete wastewater flow within the system. Two perforated 3.8-cm-diameter pipes each 60 cm long were placed inside each CW unit near the inlet to measure wastewater depth and also to serve as inspection box for wastewater level check and for aeration purposes. Required fittings, pipes and valves were used during the installation of the treatment systems.

The roots of young shoots of *T. latifolia* and *P. purpureum* were washed carefully with tap water to remove all adhered soil and sediment prior to use. Then the tops and roots of the selected young and healthy shoots were all pruned to 20 and 10 cm, respectively, and planted in the triplicate treatment units at a density of 16 shoots per m^2 [12]. There were 24 plants in each replicated treatment unit at the beginning of the experiment. Thus, 72 shoots of each species were placed in the support gravel media at the initial planting stage. After planting, the treatment units were flooded with tap water to about 10 cm above the gravel layer and the plants were left to grow 8 weeks to let the system settle to a relatively steady state. Two series (named as TU1, TU2 and TU3 for the triplicate series planted with *T. latifolia* and PU1, PU2 and PU3 for the triplicate series planted with *P. purpureum*) of CWUs were monitored.

A serial exposure of raw brewery wastewater feed was introduced into the treatment units for acclimatization. The wastewater was mixed with 75% tap water, with a gradually increased wastewater/tap water ratio until only wastewater was added after 4 weeks [31]. During the acclimatization period, the roots of both plants were exposed to the diluted wastewater flowed slowly through the entire treatment units. The plants turned green and grew rapidly after a few weeks. The survival conditions were monitored, and dead shoots were replaced after 15 days of transplantation.

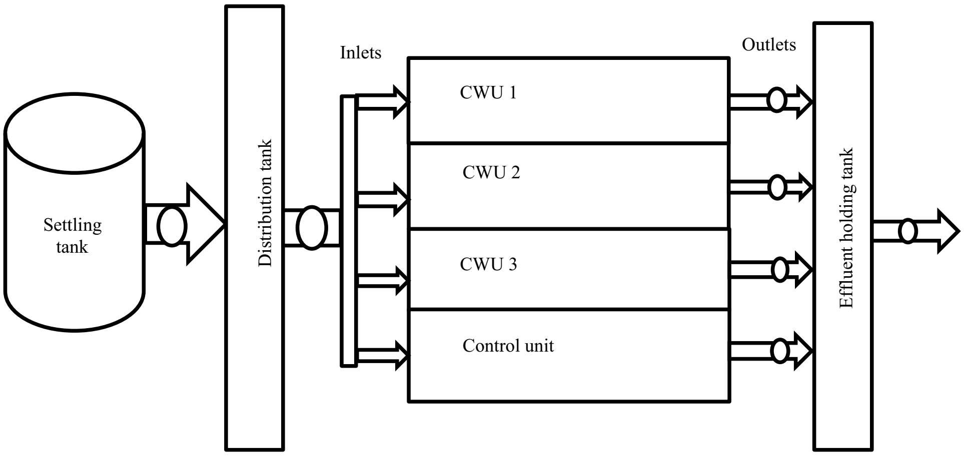

Fig. 1 A pilot-scale HSFCW treatment system consisting of primary settling tank, distribution tank, three parallel constructed wetland treatment units (CWU1, CWU2 and CWU3) with one control unit and a common effluent holding tank

After the acclimatization period of 3 months [31, 32], performance tests were started to investigate the suitability of the CW technology using *T. latifolia* and *P. purpureum* for removal of BOD_5 and COD together with the production of biomass. The CWUs system had a subsurface horizontal and continuous flow mode by receiving brewery wastewater after primary treatment under different loading rates (owing to the natural variation of the wastewater). The wastewater was adjusted to flow by gravity at 0.30 m and 0.45 m deep below the gravel surface for *T. latifolia* and *P. purpureum,* respectively. The system was subjected to BOD_5 and COD loading rates between 26–57 g d^{-1} and 29–91 g d^{-1} per treatment unit, respectively. It was operated at a hydraulic loading rate (HLR) of 0.023 $m^3\ m^{-2} d^{-1}$ and hydraulic retention time (HRT) of 5 days, with corresponding pH and temperature that varied between 4.8–7.80 and 26–40 °C, respectively. The HLR and HRT were based on recommendations for design and intention of the study [33]. Inlet and outlet flow of wastewater (0.035 $m^3\ d^{-1}$) was adjusted to maintain the HRT. The overall activities of the system were accomplished from January 2015 to January 2016.

Wastewater sampling and analysis

Composite samples of untreated wastewater were collected from a manhole placed along a drainpipe that carries wastewater to the existing treatment plant in St. George brewery located at the center of Addis Ababa. Grab wastewater samples were also collected from inlets and outlets of the constructed wetland treatment units on a monthly basis during the study period. Collection, preparation and physicochemical parametric analyses of all samples were carried out as per standard procedures set by American Public Health Association (APHA) [34]. During the entire study period, a total of 52 wastewater samples were analyzed for each plant species for the required water quality parameters. The parameters were biological oxygen demand (BOD_5), chemical oxygen demand (COD), pH and temperature. The samples were prepared and analyzed at the laboratory of Addis Ababa University. Temperature and pH were measured on-site during sample collection using portable digital thermometer and pH meter, respectively.

Plant sampling and analysis

Plant growth parameters such as plant height, number of leaves per plant, number of shoots added and density of plants were observed until the plant was matured on individual marked stems in the center of each experimental unit. The number of leaves per plant and density of plants per square meter were manually counted for each unit. At the end of the experiment, two aboveground biomasses of *T. latifolia* and *P. purpureum* samples from each treatment unit at the inlets, at the middle and at the outlet zones were harvested from the gravel surface and transported to the laboratory for analysis. The monitoring period lasted one vegetative cycle of 7 months for performance tests [35].

Data analysis

Statistical analysis of sample data was performed using SPSS Statistics Package 24 and Microsoft Excel. The data were analyzed using one-way analysis of variance (ANOVA) to compare the performance of HSFCWUs removal of BOD_5 and COD. With 95% confidence interval, multiple comparison tests were performed between inlets versus outlets of HSFCWUs (effect of influent), inlet versus outlet of control (effect of media alone) and HSFCWUs versus control (effect of vegetation) for organic removal. The results of the sample data analyzed were presented by descriptive statistics and percentage removal of BOD_5 and COD measured at the inlets and outlets of the HSFCWUs and the control unit on a monthly basis during the study period. Quantitative linear relationship of loading versus removal was also analyzed.

Results and discussion

Wastewater

The characteristic mean values of BOD_5 and COD of the brewery wastewater varied between 748–1642 mg l^{-1} and 835–2602 mg l^{-1}, respectively. The average wastewater pH and temperature ranged from 5.4–7.0 to 26–38 °C, respectively. The wastewater has high levels of organic matter measured in terms of BOD_5 and COD which might be due to the presence of organic substances such as spent grains, waste yeast, spent hops and grit [36, 37]. It has a COD: BOD_5 ratio of 1.5–1.7, indicating that it is easily degradable [38] and suitable for biological wastewater treatment, including phytoremediation.

Wastewater treatment performance in HSFCWUs

The organic pollution load measured as BOD_5 (up to 1642 mg l^{-1}) and COD (up to 2602 mg l^{-1}) of the brewery wastewater treated in the present study was greater than reported by Simate [7] COD (up to 673 mg l^{-1}) and BOD_5 (up to 786 mg l^{-1}). During treatment of organics from brewery wastewater by phytoremediation planted with *T. latifolia* and *P. purpureum,* wastewater treatment performance in terms of concentration and percentage removal was examined for both plants species. The achieved performances were mainly due to considerable reduction of pollution load by the CWs operated under controlled conditions in a greenhouse using distribution tank to feed the units with homogenized wastewater composition. The CW system removed the organics steadily along the course of study in proportion to the influent composition as has been treated below separately for BOD_5 and COD. High levels of BOD_5 (up to 89%) and COD (up to 86%) removal were recorded for all HSFCWUs series without any significant relationship to plant species ($p > 0.05$).

BOD treatment using *Typha latifolia* and *Pennisetum purpureum*

The organic matter subjected to HSFCWUs for treatment varied between 748 and 1642 mg l^{-1} in terms of BOD_5 during the study period (Table 1). Removal of BOD_5 from HSFCWUs was assessed in triplicate units for both species on the basis of inlet and outlet mean concentrations of the wastewater. As mean concentration of BOD_5 of the influent fed to the triplicate HSFCWUs varied in the range of 748–1642 mg l^{-1}, the mean concentrations at the outlets varied between 92–236 mg l^{-1} and 115–283 mg l^{-1} for *T. latifolia* units (TUs) and *P. purpureum* units (PUs), respectively.

The large value of the standard deviations (Table 1) indicates that inlet mean concentrations in the wastewater varied widely due to the varying nature of brewing operations. Simultaneously, the outlet values of the control units without plants ranged from 412–986 mg l^{-1} and 438–982 mg l^{-1} for TUs and PUs, respectively.

Statistically significant ($p < 0.001$) reductions of the outlet values of TUs and PUs were recorded as compared to the inlet values in the treatment system with 95% confidence interval. The outlet BOD_5 values of the control units also decreased significantly ($p < 0.05$) compared to the same influent. As can be seen from Table 1 and Fig. 2, removal of BOD_5 fluctuated in line with the influent wastewater owing to varied removal efficiencies. The variability of the influent wastewater was due to the various processes that took place during brewing and related cleaning activities. Comparison of BOD_5 variations between the inlet and outlet during the study period showed BOD_5 to be consistently lower at the outlet.

BOD_5 loadings and removal rates were linearly correlated for both plant species (Fig. 3). Varied loadings

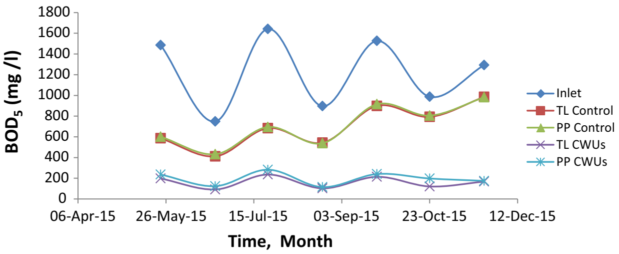

Fig. 2 BOD_5 mean values at the inlet and outlet of the HSFCWs treatment system planted with *T. latifolia* and *P. purpureum* with their control units during the operational period. TL control—*T. latifolia* control unit, PP control—*P. purpureum* control unit, TL CWUs—*T. latifolia* constructed wetland treatment units and PP CWUs – *P. purpureum* constructed wetland treatment units

Table 1 Values of physicochemical parameters at the inlet and outlet of the HSFCWUs planted with *T. latifolia* and *P. purpureum* during the operational periods

Test month	Inlet BOD_5 (mg/l)	Outlet BOD_5 (mg/l)											
		Control unit				HSFCWUs							
	Mean ± SD[a]	Mean ± SD		% R*		Mean ± SD		Min.[b]		Max.[c]		%R	
		TL[d]	PP[e]	TL	PP	TL	PP	TL	PP	TL	PP	TL	PP
May 23, 2015	1486 ± 140	586 ± 58	601 ± 48	61	60	198 ± 16	236 ± 18	184	216	216	251	87	84
June 23, 2015	748 ± 72	412 ± 31	432 ± 25	45	48	92 ± 8	124 ± 9	86	116	101	134	88	83
July 23, 2015	1642 ± 160	684 ± 69	695 ± 34	58	58	236 ± 21	283 ± 21	217	264	259	306	86	83
August 23, 2015	896 ± 85	543 ± 45	538 ± 43	39	40	103 ± 5	115 ± 11	99	107	109	127	89	87
September 23, 2015	1526 ± 147	899 ± 70	915 ± 69	41	40	214 ± 21	241 ± 15	194	224	235	254	86	84
October 23, 2015	987 ± 73	793 ± 78	805 ± 70	20	18	121 ± 8	198 ± 16	112	180	127	209	88	80
November 23, 2015	1293 ± 129	986 ± 88	982 ± 98	24	24	168 ± 14	175 ± 14	155	166	183	191	87	86
		Overall % removal		41	41	Overall % removal						87	84

%R* percentage removal

[a] Standard deviation

[b] Minimum

[c] Maximum

[d] *T. latifolia*

[e] *P. purpureum*

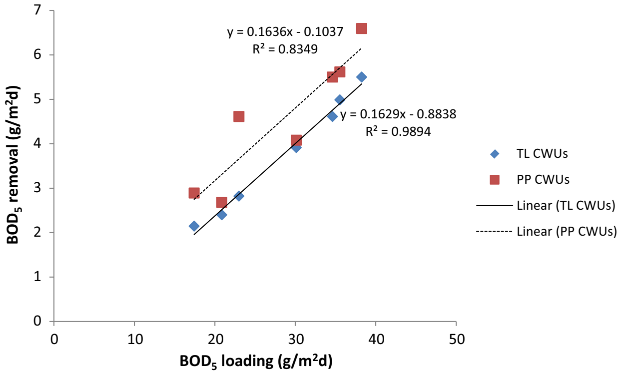

Fig. 3 BOD_5 loading versus removal by the HSFCWs treatment system planted with *T. latifolia* and *P. purpureum*. TL CWUs—*T. latifolia* constructed wetland treatment units, PP CWUs—*P. purpureum* constructed wetland treatment units

(17.42–38.25 g $m^{-2}d^{-1}$) were applied to HSFCWUs under the given conditions of the system and with the natural fluctuations of the wastewater composition. For these loadings, maximum removal of 32.76 and 31.66 g $m^{-2}d^{-1}$ was recorded for TUs and PUs, respectively. The *t* test comparison showed no significant differences ($p > 0.05$) between TUs and PUs treatment series for BOD_5 removal. However, it was noted that the linear relationship of loading versus removal was slightly stronger for TUs ($R^2 = 0.9894$) than the PUs ($R^2 = 0.8349$).

The HSFCWUs presented average BOD_5 removal efficiency of 87 and 84%, reaching at some stages removal levels up to 89 and 87% for TUs and PUs, respectively (Table 1). The removal efficiencies were stable for both plant species during the study period, although there was significant inlet fluctuation as discussed above. This might be attributed to tolerance capacity of the plant species to absorb variations of the input organic matter. The removal efficiencies for the two plant species in regard to BOD_5 were higher for *T. latifolia* (87%) than *P. purpureum* (84%). These differences might be attributed to fast growth condition, high biomass production of *T. latifolia* and its provision of a better conducive environment for associated microorganisms [18, 39]. *T. latifolia* also showed better tolerance to high inlet values of BOD_5 than *P. purpureum*. At high inlet BOD_5 values *P. purpureum* looked stressed and grew more slowly during acclimatization and treatment periods.

The removal efficiencies indicated considerable value for the treatment units. BOD_5 removal of the system might be compared with those achieved by other researchers for different wastewater treatments using *T. latifolia* and other plant species. High level of BOD_5 removal ranging from 70 to 92% was reported for treatment of municipal wastewater planted with *T. latifolia* [39, 40]. Other studies also reported high removal of organics (BOD_5) from tannery wastewater, up to 88% with *T. latifolia* and *P. australis* [17, 18].

The performance of the HSFCWUs (*T. latifolia* (87%) *and P. purpureum* (84%)) was higher than that of the control units (41%). These differences may be due to the contaminant reduction by providing a suitable habitat for microorganisms in the rhizosphere to decompose organics as they play an indirect role in reducing organic matter from wastewater [39, 41]. Other studies also indicated that the reduction of organics was due to the absorption of pollutants by plants roots and mainly by the associated microorganisms that can break down organic compounds in the process of phytoremediation [20, 42].

Among the operating factors, an organic loading rate of 6.7–15.7 g BOD_5 $m^{-2}d^{-1}$ is recommended in the manuals by the United States Environmental Protection Agency [43] to achieve 10–30 mg l^{-1} BOD_5 (emission standard) in the treated effluent for other types of wastewater. For these HSFCWUs systems, much higher loadings (17.45–38.31 g BOD_5 $m^{-2}d^{-1}$) than the recommended were used from the brewery wastewater for testing the system as a stand-alone treatment. Outlet BOD_5 ranged from 92 to 283 mg l^{-1} (Table 1) for both plants, which is beyond the emission standard, although reduction was statistically significant ($p < 0.001$).

COD treatment using *Typha latifolia* and *Pennisetum purpureum*

Patterns of COD changes due to HSFCWs wastewater treatment are presented in Table 2, Figs. 4 and 5. The trends of inlet and outlet values of the system were almost identical. As with the BOD_5 changes, outlet COD concentrations in the HSFCWUs were significantly ($p < 0.01$) lower than those of the inlet values during the monitoring period. Inlet COD fed into triplicate HSFCWs varied between 835 and 2602 mg l^{-1}. This could be due to the nature of beer brewing and associated cleaning practices [44]. The corresponding outlet COD values varied between 221–539 and 258–568 mg l^{-1} on a monthly basis for the TUs and PUs unit series, respectively (Table 2). The outlet values of the control units without *T. latifolia* and *P. purpureum*, by contrast, ranged from 664–1844 and 652–1912 mg l^{-1}, respectively, for the same inlet values.

Assessment of the removal efficiencies during the experiment for all treatment units revealed that COD concentrations were reduced. When outlet wastewater of the treatment units was compared to inlet wastewater, COD percentage reductions by the CW replicate units ranged from 74 to 86%, with average value of 81% for *T. latifolia* and ranged from 64 to 86%, with average value of 79% for *P. purpureum* under the given operating conditions of the system. The highest reduction of COD concentrations by both plants reached 86%, with an initial concentration of 1837 mg l^{-1}, which

Table 2 Descriptive statistics for COD (mg/l) removal by using *T. latifolia* and *P. purpureum* plant in HSFCWs

Test month	Inlet COD (mg/l)	Outlet COD (mg/l)											
		Control unit				HSFCWUs							
	Mean ± SD	Mean ± SD		%R		Mean ± SD		Min.		Max.		%R	
		TL	PP	TL	PP	TL	PP	TL	PP	TL	PP	TL	PP
May 23, 2015	1954 ± 192	1150 ± 106	1198 ± 97	41	39	270 ± 22.61	293 ± 24	251	276	295	321	86	85
June 23, 2015	2381 ± 236	1255 ± 116	1302 ± 81	47	45	418 ± 19.14	396 ± 20	398	377	436	417	82	83
July 23, 2015	835 ± 73	664 ± 54	652 ± 65	20	22	221 ± 17.35	301 ± 19	206	287	240	322	74	64
August 23, 2015	1837 ± 183	755 ± 70	802 ± 67	59	56	252 ± 23.52	258 ± 22	228	234	275	277	86	86
September 23, 2015	2602 ± 248	1844 ± 175	1912 ± 106	29	27	539 ± 49.49	568 ± 37	494	540	592	610	79	78
October 23, 2015	1988 ± 193	810 ± 69	869 ± 87	59	56	383 ± 27.22	411 ± 28	358	379	412	429	81	79
November 23, 2015	2486 ± 248	1618 ± 160	1692 ± 165	35	32	506 ± 44.48	542 ± 50	467	490	555	590	80	78
		Overall % removal		42	40	Overall % removal						81	79

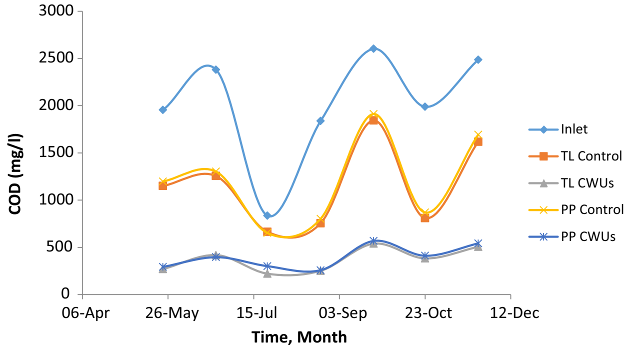

Fig. 4 COD mean values at the inlet and outlet of the HSFCWs treatment system planted with *T. latifolia* and *P. purpureum* with their control units during the operational period. TL control—*T. latifolia* control unit, PP control—*P. purpureum* control unit, TL CWUs—*T. latifolia* constructed wetland treatment units and PP CWUs—*P. purpureum* constructed wetland treatment units

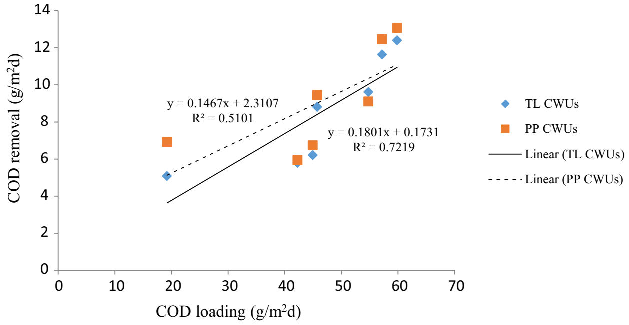

Fig. 5 COD loading versus removal by the HSFCWs treatment system planted with *T. latifolia* and *P. purpureum*. TL CWUs—*T. latifolia* constructed wetland treatment units, PP CWUs—*P. purpureum* constructed wetland treatment units

might be due to suitable wastewater composition among the loadings fed to the system. These macrophyte plants have the ability to remove organic pollutants from the wastewater of the types under study, although average removal efficiencies differed slightly between *T. latifolia* (81%) and *P. purpureum* (79%).

COD removals versus loadings were linearly correlated for both plant species (Fig. 5). Varied loadings (19.20–59.84 g m^{-2} d^{-1}) were applied to HSFCWUs under the given conditions of the system and with the natural fluctuations of the wastewater composition. For these loadings, maximum removal of 47.45 and 46.78 g m^{-2} d^{-1} was recorded for TUs and PUs, respectively. The t test comparison showed no significant differences ($p > 0.05$) between TUs and PUs treatment series for COD removal. However, the linear relationship of removal versus loadings was stronger for TUs ($R^2 = 0.7219$) series than PUs ($R^2 = 0.5101$).

The control units (inlet vs. outlet) also influenced the wastewater treatment as did the HSFCWUs of both species. The ability of gravel alone to reduce COD might also be related to filtration to precipitate the suspended solids, decomposition by microorganisms attached to the surface of the gravel and provision of binding sites on the gravel for adsorption. Nevertheless, the average percentage removals obtained by the controls were lower than those achieved by HSFCWUs as could be expected. COD values of the controls were significantly higher than concentrations of the HSFCWUs during the study period (Table 2) for the same feed. Both TUs and PUs treatment systems showed significantly greater ($p < 0.05$) removal efficiencies for COD (81 and 79% for *T. latifolia* and *P. purpureum,* respectively) than the corresponding control systems (42 and 40%, respectively).

The observed removal efficiencies suggest that organic matter steadily removed from wastewater during the operation period are due to system stability after adaptation of the vegetation to its new habitat and ability of the system to adjust to influent fluctuations. These removal efficiencies are generally within the ranges reported in the literature for similar systems. Snow et al. [45] reported that hydroponically grown macrophytes were able to significantly reduce the pollution load of wastewater COD up to 90%. Hadad et al. [11] reported in a pilot-scale constructed wetland for industrial wastewater that macrophytes removed COD by 79%. Other studies on vegetation-based wastewater treatment technologies revealed organic matter removal rates of 90–98% [46]. The BOD_5 and COD removal efficiencies obtained in this study using *P. purpureum* are corroborated by several other studies which reported removal efficiencies ranging from 70–94% to 57–85.5% for BOD_5 and COD, respectively, in constructed wetlands treating different wastewater types [47, 48]. Calheiros et al. [17] reported that treatment of industrial wastewater with *T. latifolia* and *Phragmites australis* reduced 92% of COD from tannery wastewater.

The results of organics removal showed that outlet concentrations of BOD_5 (92–283 mg l^{-1}) and COD (221–568 mg l^{-1}) obtained by the HSFCWs system exceeded Ethiopia's discharge standards (60 and 250 mg l^{-1} for BOD_5 and COD, respectively) [49]. Although the outlet concentrations did not meet discharge or reuse standards, the hydroponic system of wastewater treatment using *T. latifolia* and *P. purpureum* can be posited as a potentially effective alternative treatment method. This is because the system significantly lowered the levels of BOD_5 and COD from higher initial values, showing satisfying removal efficiencies (Tables 1 and 2) and produced biomass which might be usable for energy and animal forage.

As the main biological component of the HSFCWUs (gravel bed hydroponics), *T. latifolia* and *P. purpureum* are important for purification reactions by enhancing removal processes and the utilization of the pollutants. The reduction of organics is due to the phytoremediation process that relies on the synergistic relationships among the macrophytes, microorganisms, wastewater and supporting gravel media. Phytoremediation takes advantage of the natural processes of macrophytes and their roles in pollutant removal. These processes include water and pollutant uptake, metabolism within the macrophytes and the physical and biochemical impacts of root system [50]. *T. latifolia* and *P. purpureum* were crucial to the functioning of the removal process because they have physical effects due to the plant tissues stabilizing the medium that promotes filtration and absorption, prevent vertical flow systems from clogging and might provide surface area for attachment of microbial growth and supply oxygen to the rhizosphere. In similar studies, Akratos and Tsihrintzis [51] and Shah et al. [50] reported that the main mechanism responsible for organic matter removal could be attributed to the microbial activity of aerobic and anaerobic bacteria possibly establishing a symbiotic relationship with the plants.

Factors associated with removal of organics

The biological removal of organics can be affected by environmental factors (pH, temperature, oxygen) and operating conditions (hydraulic and organic loading, HRT, macrophytes, wastewater type, design, etc.) [17, 48]. The treatment of BOD_5 and COD from brewery wastewater showed that inlet pH throughout the HSFCWUs operation varied between 4.8 and 7.8 and at the outlet ranged from 7.6 to 8.3, with corresponding inlet temperatures ranging 26–40 °C and outlet ranges of 19–21 °C. The pH was slightly higher after treatment, increasing on average from 6.4 in the inlet to 8.1 at the outlets of the treatment units. This increase in pH values might be due to microorganisms consuming some organic acids in the bioremediation process. pH of 6–9 and temperature of 15–38 °C are most favorable for treatment of wastewater by macrophytes [50]. It was noted that the pH and temperatures of wastewater recorded in the present study were in the optimal range. Shah et al. [50] also reported that degradation of organic matter was affected by low temperatures (below 10 °C). The temperature values are within the permissible limit set by the National Environmental Quality Standard for brewery effluent (40 °C) [49].

The DO (dissolved oxygen) at the inlet was low (mean value 0.83 mg l^{-1}) due to high organic content of the wastewater, which requires high levels of oxygen demand. Low levels of DO were also recorded at the outlet (mean value 0.02 mg l^{-1}). The DO concentration drop could also be attributed to biological activity in the root zone of the HSFCWUs, including DO as a source of energy for root respiration and subsequently growing. The other possible source of oxygen required for aerobic removal might be obtained from the atmosphere by diffusion into the *Typha* planted gravel medium and by continuous release of oxygen from the plant internal root zones in the rhizosphere [14].

Factors such as matching plant selection and water depths enhanced the removal by appropriate water depth design depending on the root growth potential of the *T. latifolia* and *P. purpureum.* The gravel bed employed promotes settling of suspended solids and provides surface for biofilm attachment and growth. The loose gravel bed also maximizes hydraulic conductivity and offers little resistance to root growth of the plants. The biological removal of organics with macrophytes is most efficient when residence times are long and water temperatures are high. Recommended range of HRT is 2–5 days and for HLR is less than 0.5 m d^{-1} [33]. For this study, 5-day HRT and 0.023 m d^{-1} were used to achieve optimum treatment performance.

Plant growth and biomass analysis

The changes in growth of the plants as a result of wastewater feed to the CWs treatment units at HLR of 2.3 cm d^{-1} and

HRT of 5 days for each unit are presented in Table 3. After acclimatization, the system had become established and stable to record data for plant growth analysis. The number of shoots (at the beginning of the experiment) was 24 in each replicated treatment unit for both plant species.

The increase in the number of shoots was monitored closely and recorded on a monthly basis. In the initial stage, the plant density was low and gradually the plant growth accelerated. The growth of *T. latifolia* and *P. purpureum* was due to wastewater solution that might provide nutrients and gravel support media designed in the CW system.

Observations during plant establishment showed that new growth initially emerged mainly from rhizomes and rootstock. By the end of the experiment, the number of *T. latifolia* plants had increased to 49 in TU1, 60 in TU2 and 56 in TU3 for the replicates, respectively. In all treatment units, the number of shoots increased and they appeared to be healthy. The growth of *T. latifolia* was better (average 40 shoots per m^2) in TU2 than TU1 and TU3 (average 33 and 37 shoots per m^2, respectively) (Table 3).

The slight difference in the number of plants among treatment units might be due to health conditions of the individual plants that may affect plant multiplication. Sometimes wilting of the shoots was also noticed which might be due to variations of responses of individual plant to high level of organic and/or nutrient loading of the influent that can cause stress to the plants [52].

Similar to *T. latifolia, P. purpureum* showed an increase in shoot density after the initial period of slow growth. The number of plants reached 40 in PU1, 45 in PU2 and 43 in PU3 (Table 3). Among the *P. purpureum* treatment units, PU2 had slightly denser shoots (30 shoots per m^2) than PU1 and PU3. It has tussocky growth with branching upper clumps by extending from the parent plant. Observations during plant establishment showed that new shoots emerged from rhizomes or rootstock and grew well gradually replacing the older shoots in the early stages of growth. *T. latifolia* was slightly better in growth than *P. purpureum* series. During the late stages of monitoring (after 6 months), the shoot density gradually decreased and vertical growth of shoots and leaves became the dominant mode of growth.

Aboveground biomass of *T. latifolia* and *P. purpureum* samples from each treatment unit was harvested from the gravel surface and assessed at the end of the experiment (Table 3). The average aboveground fresh biomass of *T. latifolia* varied from 282 to 324 g per plant in the CWs treatment units. The corresponding average dry weights (DW) ranged from 18.69 to 21.50 g per plant. The estimated fresh and dry biomass ranged from 9.20–12.96 kg m^{-2} and 0.61–0.86 kg DW m^{-2}, respectively.

These results are in agreement with the average aboveground biomass range (0.3–1.8 kg DW m^{-2}) of *T. latifolia* reported by Maddison et al. [53] and Valipour et al. [31]. Better aboveground fresh biomass and DW were recorded (12.96 kg m^{-2} and 0.86 kg DW m^{-2}, respectively) for replicate treatment unit 2. This could be due to higher plant density (40 plant m^{-2}) and individual plant health conditions. Similarly, average aboveground fresh biomass of *P. purpureum* varied from 155 to 180 g per plant. The corresponding average dry weights varied from 10.24 to 11.93 g DW per plant sample. The estimated fresh and dry biomass ranged from 4.35–5.4 kg m^{-2} and 0.288–0.358 kg DW m^{-2}, respectively. PU2 (30 plants m^{-2}) had slightly greater fresh biomass and DW than PU1 and PU3. Comparison between *T. latifolia* and *P. purpureum* indicated that *T. latifolia* had greater average fresh biomass and DW for all treatment units (Table 3).

In all treatment units, the number of shoots per treatment unit and the number of leaves per plant increased for both plant species (Table 3), which appeared to be healthy. The increase in height of plants ranged from 30–190 cm and 28–206 cm for *T. latifolia* and *P. purpureum,* respectively. The ranges varied widely because of emerging young and older shoots. The number of leaves per plant ranged from 5–11 and 4–9 for *T. latifolia* and *P. purpureum,* respectively (Table 3). The density of plants at the inlets of the HSFCWs, where the organic loading could be higher, was lower than the plants in the middle and near the outlets due to wilting

Table 3 Plant growth analysis in the HSFCWUs for *Typha latifolia* and *Pennisetum purpureum*

Parameters	HSFCWUs					
	T. latifolia			*P. purpureum*		
	TU1	TU2	TU3	PU1	PU2	PU3
Initial no. of shoots planted	24	24	24	24	24	24
Final no. of plants	49	60	56	40	45	43
Density of plants per m^2	33	40	37	27	30	29
Height of plants (cm)	50–190	30–175	34–170	36–198	28–206	40–201
Average no. of leaves per stem	5–9	6–10	5–11	4–8	5-9	4-8
Average aboveground fresh biomass per stem (g)	282	324	316	163	180	155
Average aboveground dry mass per stem (g)	18.69	21.5	20.89	10.79	11.93	10.24

and death. In addition, the growth pattern of plants located near the outlet was thick green, robust and taller than plants nearer to the inlet. This could be due to decreased organic and nutrient overloading in water moving toward the outlet of the treatment units, which might decrease stress on plants. However, there were no significant differences observed among the treatments units because of the fact that HSFCWUs were facing similar exposure of the wastewater load and the same environmental conditions in the units. Other research reported that plants used for phytoremediation showed significant growth with regard to height, number of leaves per plant and root length [54]. Generally growth, biomass production, pollutant uptake and tolerance to organic loadings of *T. latifolia* and *P. Purpureum* make them suitable for phytoremediation process in the wastewater treatment.

Conclusion

This study shows that HSFCWs planted with *T. latifolia* and *P. purpureum* significantly removed BOD_5 and COD from brewery wastewater. Average removal efficiency of 87 and 81% was obtained for BOD_5 and COD, respectively, and *T. latifolia* slightly outperformed *P. purpureum.* Both plants grew, propagated and established well in the system. However, *T. latifolia* had more biomass and vigorous growth. The ability of plants to account for the decrease of organic pollutants in wastewater as a function of biomass production plays an important role in wastewater treatment. Analysis of the treatment performance of the system revealed the prospects of CW organics removal using macrophytes with the possibility for further optimization under various conditions to ascertain the suitability of the technology for different wastewater types. Thus, the phytoremedial role played by these two plant species for removal of organics in brewery wastewater is a promising indicator in the search for alternative methods of industrial wastewater treatment. This study further indicates the prospects of effective, sustainable and environmentally friendly phytoremediation of industrial and possibly municipal wastewater using CWs are promising.

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Compliance with ethical standards

Conflict of interest There is no conflict of interest among the Authors.

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