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Original article

Performance evaluation and microbial profiling of integrated vertical flow constructed wetland (IVFCW) for simultaneous treatment of domestic and pulp and paper industry waste water

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ABSTRACT

The present study demonstrates the potential of an integrated vertical flow-constructed wetland (IVFCW) for simultaneously treating black liquor and domestic wastewater. IVFCW was operated and monitored for 12 samples with the frequency of one sample per week with the following specifications viz,4 L of wastewater, a blend of 1:1 of pulp and paper industry effluent (black liquor BL), and domestic wastewater, was fed daily in a continuous mode with organic loading rate (OLR) of 1230 mg COD/L-Day, at a temperature range of 40-45℃ (natural temperature of the workstation). Valves controlled each chamber's hydraulic retention time (HRT) of 3 days and flow rate of 10 mL/minute. The IVFCW showed remarkable efficiency in removing various pollutants, including total suspended solids (TSS) and total dissolved solids (TDS), by 100 % and 83 %, respectively, and organic contaminants such as chemical oxygen demand (COD) and biological oxygen demand (BOD) by 80 % and 81 %, respectively. Moreover, the IVFCW efficiently reduced nutrients such as sulfates (SO_4^{-2}), phosphates (PO_4^{-3}), and total nitrogen by about 81 %, 63 %, and 61 %, respectively. The treatment also led to the reduction of lignin content by 83 %. Microbiological analysis revealed a significant reduction in fecal coliforms, and microbial profiling of *Typha latifolia* roots confirmed the presence of bacteria involved in lignin degradation. Seed germination and seedling survival were found to be negativelyaffected by untreated wastewater in a phytotoxicity study, suggesting that the wastewater's toxic chemicals could be harmful to plant life.This study highlights the effectiveness of IVFCW as a sustainable, economically viable, and resilient wastewater treatment system for mitigating environmental concerns related to the release of untreated wastewater.

1. Introduction

Rapid industrialization has resulted in a significant interruption in the natural ecosystem in the last few decades. The paper industry is of great importance both economically and ecologically (Gupta & Gupta, 2019). Wastewater pollution and freshwater utilization are the major environmental concerns of this industry (Negi & Suthar, 2018). About 250–300 m^3 of water is required in different steps of the pulp and papermaking process produce 1ton of paper (Chaudhry & Paliwal, 2018). Thus, a considerable amount of effluent is produced in the form of sludge and wastewater containing about 250 different chemicals such as lignin, chlorinated phenols, organic acids, various natural polymers, organic halogens, and metallic ions (Abedinzadeh et al., 2018; Kumar et al., 2018). This dark-colored effluent, commonly known as black liquor (BL), augments the number of hazardous materials in the freshwater (Oller et al., 2011). BL has an exceptionally high concentration of contaminants that cause the coloration, slime, and scum. Furthermore, contaminants in the BL affect aquatic life and decrease the dissolved oxygen content and loss of aesthetics in the surroundings (Haq & Raj, 2020).

Answering these environmental concerns and protecting natural freshwater reservoirs from contamination has received much attention

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(Sehar et al., 2015). There is a dire need for an eco-friendly, green, cost-effective, and energy-independent wastewater treatment system. Among available wastewater treatment techniques, constructed wetlands are showing promising results for different industrial effluents like tannery (Saeed et al., 2012), food and related products-based industries (Comino et al., 2011), fertilizers and other chemical synthesizing units (Domingos et al., 2007), refinery (Wallace & Kadlec, 2005), elimination of organic waste of emerging nature (Ávila et al., 2013) pulp and paper industry (Setiawan et al.; H., 2020) and household wastewater (Song et al., 2009). At the same time, wetlands provide aesthetic value, for instance, support wildlife and produce usable plant biomass (Poe et al.,2003; Solano et al., 2004; Shalaby et al., 2008; Carty et al., 2008).

Constructed wetlands (CWs) are artificial replicas of natural wetlands in a laboratory or an open environment of different beds, such as clay/soil, sand or pebbles/gravels/stones, and plant vegetation. The rhizosphere and packing media contain an enormous population of microbial inhabitants necessary for mineralizing water contaminants (EPA, 2000). Based upon the direction of water flow, subsurface flow constructed wetlands (SSF-CW) are classified as vertical flow (VF) and horizontal flow (HF). All these types effectively remove contaminants; however, excessive evaporative loss limits water reuse (Saher et al., 2015). To overcome this challenge, hybrid constructed wetlands (HCWs) are getting much attention due to low evaporative loss (Masi & Martinez, 2007) and high efficiency of contaminant removal, particularly total nitrogen (TN) concentration (Sayadi et al., 2012). For industrial effluents like PPI (having a low degradability factor) through CWs, another critical problem is substrate clogging. This clogging causes the quick failure of all types of CWs, which may be due to the reduced infiltration of oxygen into the support media (Nivalva et al., 2012) or due to the excessive solids in the wastewater (Mosse et al., 2011; Knowles et al., 2011). This concern can also be solved using pretreatment with settling, screening, air flotation, and anaerobic digestion (Poggi-Varaldo et al., 1999). Such types of CWs integrated with pre-and post-treatment of the effluent are considered more appropriate options due to low maintenance and operating cost, ability to operate with low and high organic loading rates, and green and sustainable nature (de la Varga et al., 2013).

The current study is focused on constructing, operating, and evaluating a lab-scale integrated vertical flow-constructed wetland (IVFCW) for the efficient treatment of wastewater. The term integrated means the VFCW is fitted with a primary sedimentation tank (PST), anaerobic chamber as pretreatment and aerobic chambers, as well as sand beds for post-treatment. The BL contains chemicals lignin and other aromatic recalcitrant; an equal proportion of domestic wastewater was mixed to enhance the biodegradability and microbial population. Co-digestion of various streams comprising a variety of substrates has the potential to balance and reduce toxicity, potentially resulting in a stronger microbial community. Additionally, the microbial population can acclimate and adjust to unfavorable circumstances. A well-adapted community might manage instances of stress like sudden influxes of toxins or short-term organic overload. In contrast, an unadapted community could experience disruptions in the process. Hence, when dealing with wastewater containing high levels of inhibitors or toxic substances like lignin or its derivatives, it is advisable to commence the treatment with a period of acclimation/adaptation, which could extend from a few days to weeks.

2. Materials and methods

2.1. Designing and construction of IVFCW for the treatment of wastewater

A mesocosm IVFCW integrated with anaerobic and aerobic chambers fitted with a sand bed for treating wastewater was constructed in the Applied Environmental Microbiology Lab at Quaid-i-Azam University Islamabad, Pakistan. This mesocosm IVFCW consisted of five plexiglass chambers (W = 120 & H = 220 mm) arranged on a tabletop at decreasing heights (1 foot) to maintain water flow under natural gravitational flow. These chambers were designated as one primary sedimentation tank (PST) with a loading capacity of 4 L, one anaerobic chamber (ANC), one VFCW, one aerobic chamber (AC), and one sand bed chamber. Anaerobic conditions were maintained using paraffin wax, while aquarium pumps were used for oxygenation. In mesocosm, IVFCW, four layers were placed from top to bottom (8 cm soil, 4 cm sand,4 cm gravel, and 4 cm stones or pebbles), and the *Typha latifolia* was planted in the uppermost layer (soil). All these chambers were connected through polyvinyl chloride (PVC) pipes (having ½ inch diameter), and the flow rate was maintained using peristaltic valves. Before being exposed to working conditions, the middle two units underwent a soaking process with BL obtained from the pulp and paper industry in Lahore, Punjab-Pakistan. This soaking process lasted for 3 to 4 weeks, intending to facilitate the growth of plants and the development of biofilm in both aerobic and anaerobic chambers on filter/packing media. The IVFCW system was implemented and supervised for 12 samples (W1-W12), with a consistent HRT of 3 days for each sample. The temperature of the workstation was consistently monitored and was observed in the range of 40-45℃. Fig. 1 illustrates a schematic depiction of the IVFCW system's various components.

2.2. Operational setup of IVFCW for wastewater treatment

Samples of BL were collected from the pulp and paper industry, Lahore, Punjab-Pakistan, in bulk quantity and stored at 4 °C in the laboratory. Fresh domestic wastewater was collected from the residential colony of Quaid-i-Azam University Islamabad, Pakistan. This lab-scale vertical flow constructed wetland (working volume of 4 L) was fed with 4 L of (a blend of 1:1 of BL and domestic wastewater) in a continuous mode with an organic loading rate (OLR) of 1230 mg COD/L-Day. Valves controlled each chamber's hydraulic retention time (HRT) and flow rate of 10 mL/minute. The BL was fed to the first chamber, the PST, at a 10 mL/minute flow rate. Then, wastewater was retained in the PST for 12 h to ensure the settling of suspended particles and particulate materials and then fed to the remaining chambers so that wastewater remained in contact with anaerobic, aerobic, and wetland for the rest of the time (60 h) for the efficient treatment. Each sample of wastewater completed 60 h in the following sequence: anaerobic treatment for 24 h and then passed sequentially to VFCW for 24 h, aerobic treatment of 12 h, and finally, the sand bed for filtration. A retention time of 72 h was completed from PST to the sand bed for each sample. This IVFCW was monitored for 12 weeks, from June 2022 to August 2022. The inlet (influent) and effluent samples were taken in sterilized disposable polyvinyl bottles before and after treatment and subjected to different physicochemical and microbiological parameter analyses. All these analyses were conducted in triplicates, and mean values were recorded (±SD).

2.3. Estimation of physicochemical parameters of wastewater

Untreated and treated wastewater samples were collected and analyzed for physicochemical and microbiological parameters. The pH of both inlet and outlet was determined using a pH meter PHS-3C (Shanghai Puchun Measure Instrument Co., Ltd. Shanghai, China). COD, TDS, and TSS were measured by Spectroquant® cell 114,541 (Merck, Germany) and Spectro Quant Pharo 300 (Merck, Germany), the standard method 5210B (APHA, 1926), and by standard methods of 2540C and 2540D with a spectrophotometer (T60VU-UVIS spectrophotometer, Beijing, China) at 465 nm respectively. The lignin content

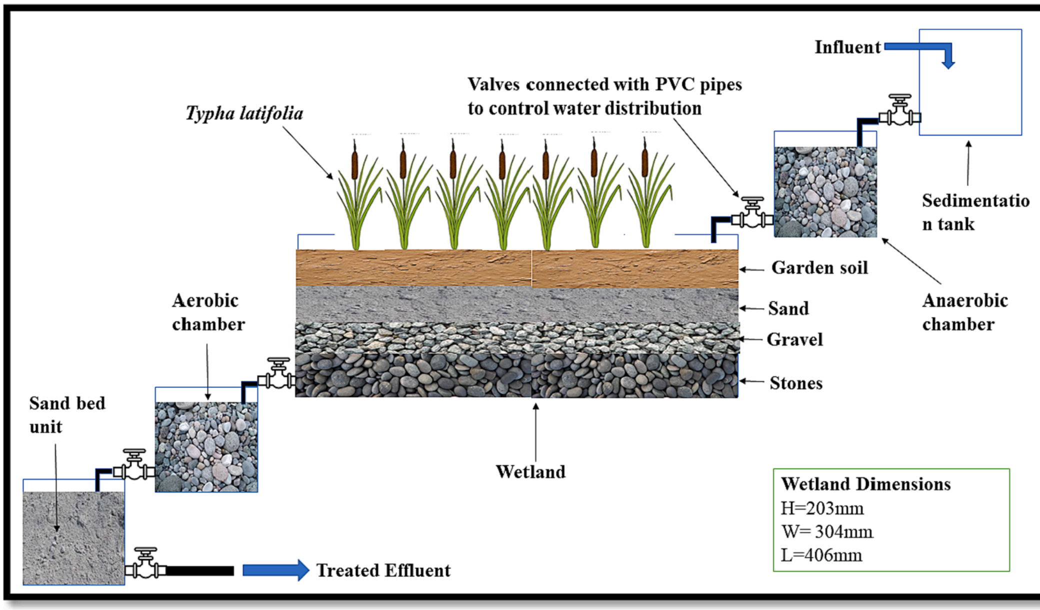

Fig. 1. Schematic illustration of vertical flow constructed wetland (VFCW) planted with *Typha latifolia.*

was measured using the standard Biorefreniary Test Method L2:2016 (Costa et al., 2017). The procedure relies on sulphuric acid hydrolysis of the samples, enabling the determination of total lignin content by summing up the acid-insoluble matter (AIM) and acid-soluble matter (ASM) concentrations after the hydrolysis. Similarly, D.O. and total nitrogen (T.N.) were also determined by using a D.O. meter (MM60R, TOA-DKK, Tokyo, Japan) and SpectroquantPharoo 300(Merck, Germany), respectively. In contrast, sulfates, color, and phosphates were determined using standard EPA methods (APHA, 1926). Table 1 represents physicochemical characteristics of wastewater before treatment.

2.4. *Microbiological analysis of blended wastewater*

Microbiological analyses of both treated and treated wastewater were done for CFU/mL and MNP/100 mL indices using standard microbiological protocols described by Rasool et al., (2018).

2.5. *Microbial profiling of rhizosphere of Typha latifolia*

The soil sample was taken from the rhizosphere of *Typha latifolia.* Different dilutions (10^{-1}-10^{-6}) of the sample were made with autoclaved distilled water, spread on the nutrient agar plates under sterile conditions in a laminar flow hood, and incubated at 37 °C for bacterial growth. Colonies were picked after 24–48 h based on differences in

Table 1
Physicochemical characteristics of wastewater before treatment.

Duration	Physicochemical characteristics of wastewater before treatment										
(weeks)	COD (mg/L)	BOD (mg/L)	DO (mg/L)	pH	EC (µs/cm)	TDS (mg/L)	TSS (mg/L)	PO_4^{3-}(mg/L)	SO_4^{2-}(mg/L)	TN (mg/L)	Lignin (mg/ L)	Color (Pt-Co)
1	1086.67 ± 47.14	823.3 ± 33.99	1.87 ± 0.07	9.46 ± 0.18	1925.3 ± 38.65	1566 ± 16.97	2571.6 ± 58.92	3.68 ± 0.17	892 ± 33.94	133 ± 4.2	158.6 ± 6.3	270.3 ± 24.9
2	1194 ± 52.32	811.3 ± 23.09	1.94 ± 0.03	8.33 ± 0.18	1778.6 ± 47.14	1575 ± 18.38	2559.6 ± 74.01	3.29 ± 0.14	834.33 ± 23.57	139 ± 5.6	157.6 ± 2.3	272 ± 19.7
3	1190.66 ± 42.89	821.6 ± 27.78	2.05 ± 0.05	8.51 ± 0.14	1789.3 ± 61.28	1671.33 ± 22.15	2448.3 ± 65.99	3.12 ± 0.05	895 ± 28.28	124.5 ± 4.9	145 ± 4.8	259.3 ± 21.8
4	1196.66 ± 25.92	787.3 ± 26.59	2.79 ± 0.16	8.56 ± 0.09	1872.3 ± 76.83	1489.66 ± 22.15	2462.6 ± 73.06	3.16 ± 0.10	866 ± 32.52	132.1 ± 4.4	162.3 ± 8.0	233.3 ± 23.2
5	1364 ± 59.39	680 ± 29.43	2.13 ± 0.08	8.73 ± 0.37	1913 ± 53.74	1524.33 ± 16.49	2483 ± 91.92	3.25 ± 0.16	908 ± 11.313	151.4 ± 3.5	138 ± 6.1	245.3 ± 16.5
6	1288.66 ± 27.34	775 ± 26.77	1.85 ± 0.02	8.10 ± 0.28	1889.3 ± 50.44	1515 ± 18.38	2555.3 ± 78.72	3.89 ± 0.14	937 ± 25.45	133.1 ± 5.7	150.3 ± 7.7	202 ± 17.2
7	1397.33 ± 44.31	744.6 ± 31.11	1.66 ± 0.04	8.40 ± 0.14	1939 ± 43.84	1450.67 ± 13.19	2489.3 ± 47.14	3.65 ± 0.03	840.33 ± 29.22	123.3 ± 4.7	138.3 ± 5.7	233 ± 14.7
8	1264.33 ± 44.78	835 ± 24.05	1.74 ± 0.06	8.50 ± 0.14	1925.3 ± 45.72	1560.33 ± 14.61	2508.3 ± 65.52	3.66 ± 0.06	921.33 ± 19.32	138.3 ± 5.1	151.3 ± 7.7	259 ± 20.9
9	1211.33 ± 43.36	799.6 ± 33.72	2.06 ± 0.05	8.50 ± 0.14	1793.3 ± 51.85	1567 ± 11.31	2438.3 ± 66.93	3.43 ± 0.13	859.73 ± 21.02	134 ± 5.6	144 ± 6.1	231 ± 18.4
10	1175.33 ± 53.26	792.3 ± 23.32	1.89 ± 0.03	8.70 ± 0.14	1785.6 ± 47.14	1376.33 ± 16.02	2499 ± 48.08	3.73 ± 0.10	878.33 ± 16.49	139.6 ± 7.5	149.3 ± 6.9	229.3 ± 21.8
11	1296 ± 32.52	802.3 ± 17.24	1.55 ± 0.10	8.63 ± 0.18	1879.3 ± 49.96	1376.66 ± 14.61	2559.6 ± 74.01	3.4 ± 0.22	927.33 ± 24.51	135 ± 4.2	164.6 ± 4.7	281 ± 16.3
12	1212 ± 43.36	730.6 ± 12.76	1.69 ± 0.21	8.93 ± 0.04	1829.3 ± 42.89	1435 ± 14.14	2480.6 ± 64.11	3.543 ± 0.15	877.66 ± 16.02	137.8 ± 4.4	148.6 ± 7.7	230.6 ± 22.0

Values are given as mean with S.D (n = 3)

morphology, size, coloration, marginal shapes, and pigmentation. Gram staining, microscopic, and biochemical tests were performed to profile the isolates in the rhizosphere *Typha latifolia* that were involved in the contaminate removal from the wastewater (Holt et al., 1994).

2.6. Phytotoxicity analysis

A pot experiment was conducted to assess the toxicity of untreated and treated wastewater on the germination of wheat seeds (Akbaret al., 2019). Healthy seeds (three seeds per pot) were planted in tray pots. These seeds were irrigated with untreated, treated wastewater and tap water. The germination rate in terms of percentage was recorded on the 10th day of sowing and the 20th day of the date of sowing, while the second rate of germination was recorded at post-emergence death.

3. Results

3.1. Color, odor, and pH

Wastewater has a pungent odor and a relatively black color. The wastewater was given a retention time of 3 days (72 h) in IVFCW to facilitate biodegradation of different organic contaminants that impart color to wastewater, like lignin, thus causing a reduction in the intensity of odor and color (approximately 56 %) as shown in Fig. 2.

3.2. Removal of total solids

TSS and TDS in the wastewater before treatment were around 2,500 and 1500 mg/L, respectively, which are higher than the values recommended by WHO, 2004. After the treatment through IVFCW, a 100 % reduction in TSS and an 84 % reduction (0 mg/L and 150 mg/L) in TDS were observed, as shown in Fig. 3 (a) and 3 (b). TDS also influences EC; in untreated wastewater samples, the average EC was about 1860μS/cm, while after treatment with IVFCW, EC was reduced to 408μS/cm. The current study showed a maximum reduction of 85 % EC values of blended wastewater after treatment with IVFCW (Fig. 3c) at an HRT of 3 days.

3.3. Removal of organic contaminants

In the present study, a remarkable reduction in COD (1237 mg/L to 240 mg/L) and BOD (780 mg/L to 143 mg/L) (80 % and 81 %) was observed, with a significant increase in DO content (about 317 times), as shown in Fig. 4 (a), (b) and (c) respectively.

3.4. Removal of nutrients (sulfates, phosphates, nitrates)

In the blended wastewater (used as influent for the IVFCW), the average concentrations of SO_4^{2-}, PO_4^{3-}, and T.N. (in terms of NO^{-3}) were 886 mg/L, 3.4 mg/L, and 135 mg/L, respectively. IVFCW showed a

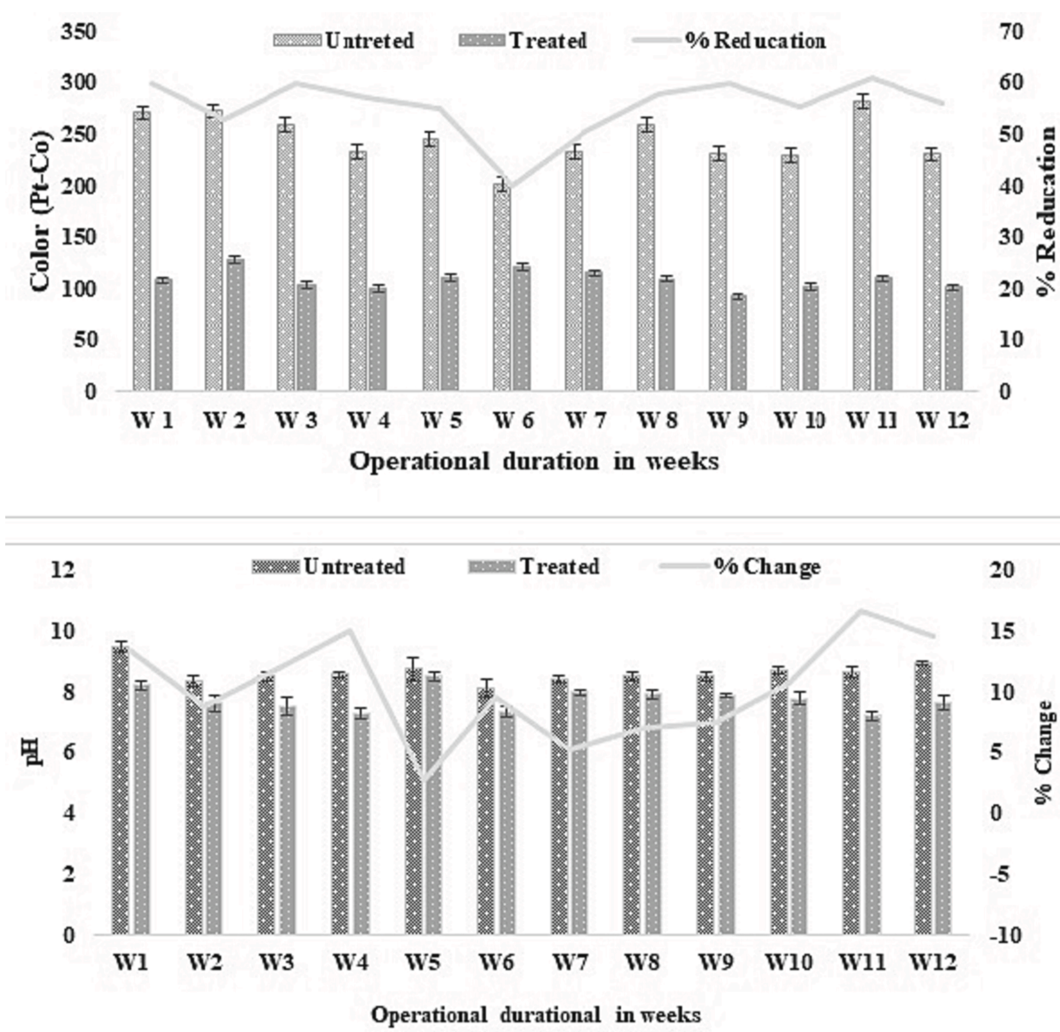

Fig. 2. Color reduction (a) and pH variationin (b) the influent and effluent during 12 weeks of operation through vertical flow constructed wetland (IVFCW)planted with *Typha*.

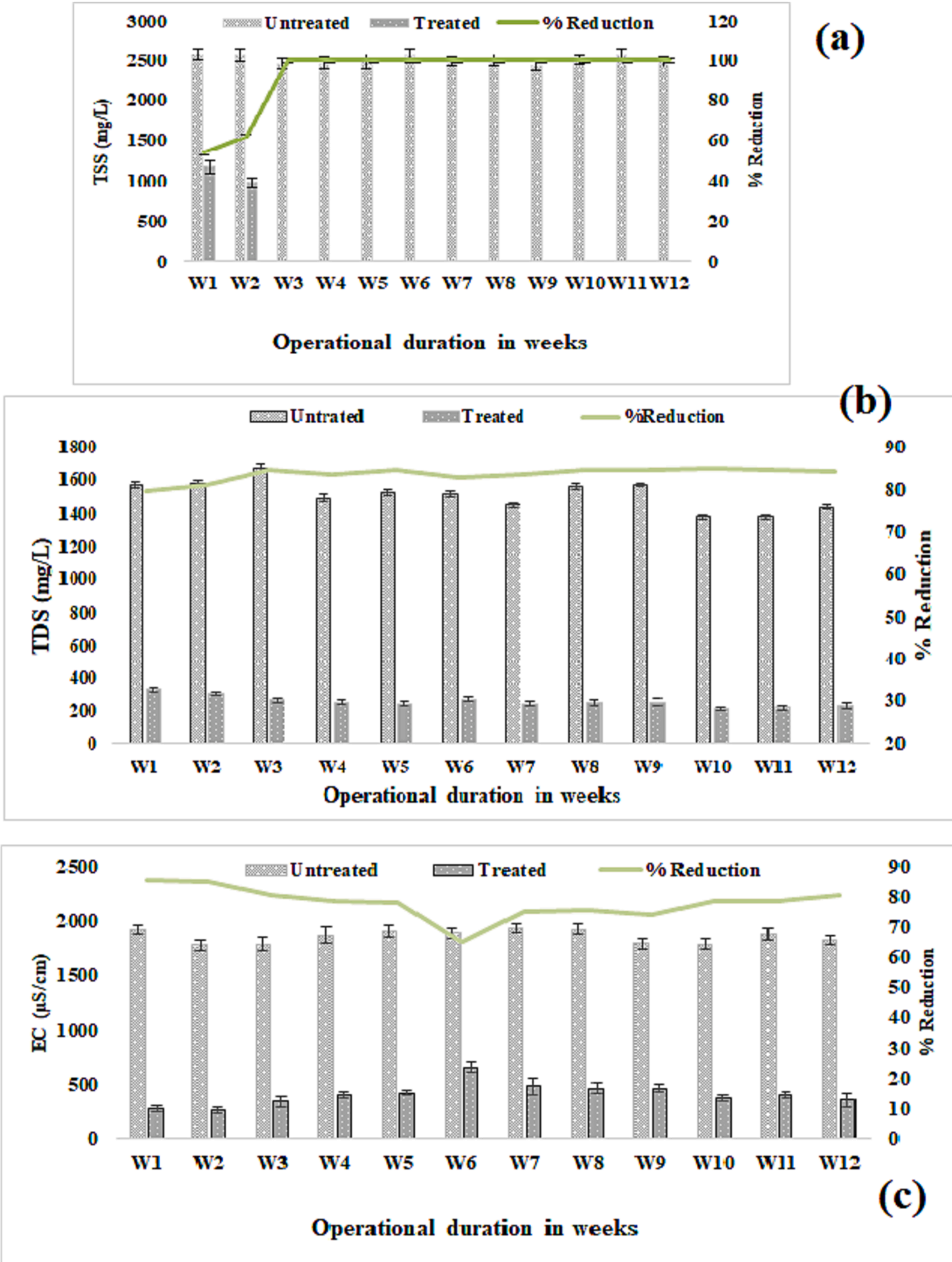

Fig. 3. Comparative variations in TSS (a), TDS (b) and EC (c) influent and effluent during 12 weeks of operation through vertical flow constructed wetland (VFCW) planted with *Typha latifolia.*

significant reduction in removing these nutrients, indicating that the Typha latifolia, along with aerobic and anaerobic treatment through microbial biofilm, played a significant role. Fig. 5 (a) shows the efficiency of IVFCW for the treatment of blended wastewater. On average, about 81 % reduction in sulfates (Fig. 5a), 63 % reduction in phosphates (Fig. 5b), and about 61 % reduction (Fig. 5c) in total nitrogen were observed.

3.5. *Removal of lignin*

Lignin is well recognized as the primary contaminant in effluent generated by the pulp and paper industries and from domestic sources. The wastewater sample has a maximum lignin content of 164 mg/L in the influent and about 40 mg/L in the effluent, as shown in Fig. 6. The IVFCW was run for 12 weeks, with an HRT of 3 days; the current investigation saw a maximum reduction of around 88 % in the lignin concentration, while the average efficiency of the IVFCW for 12 samples

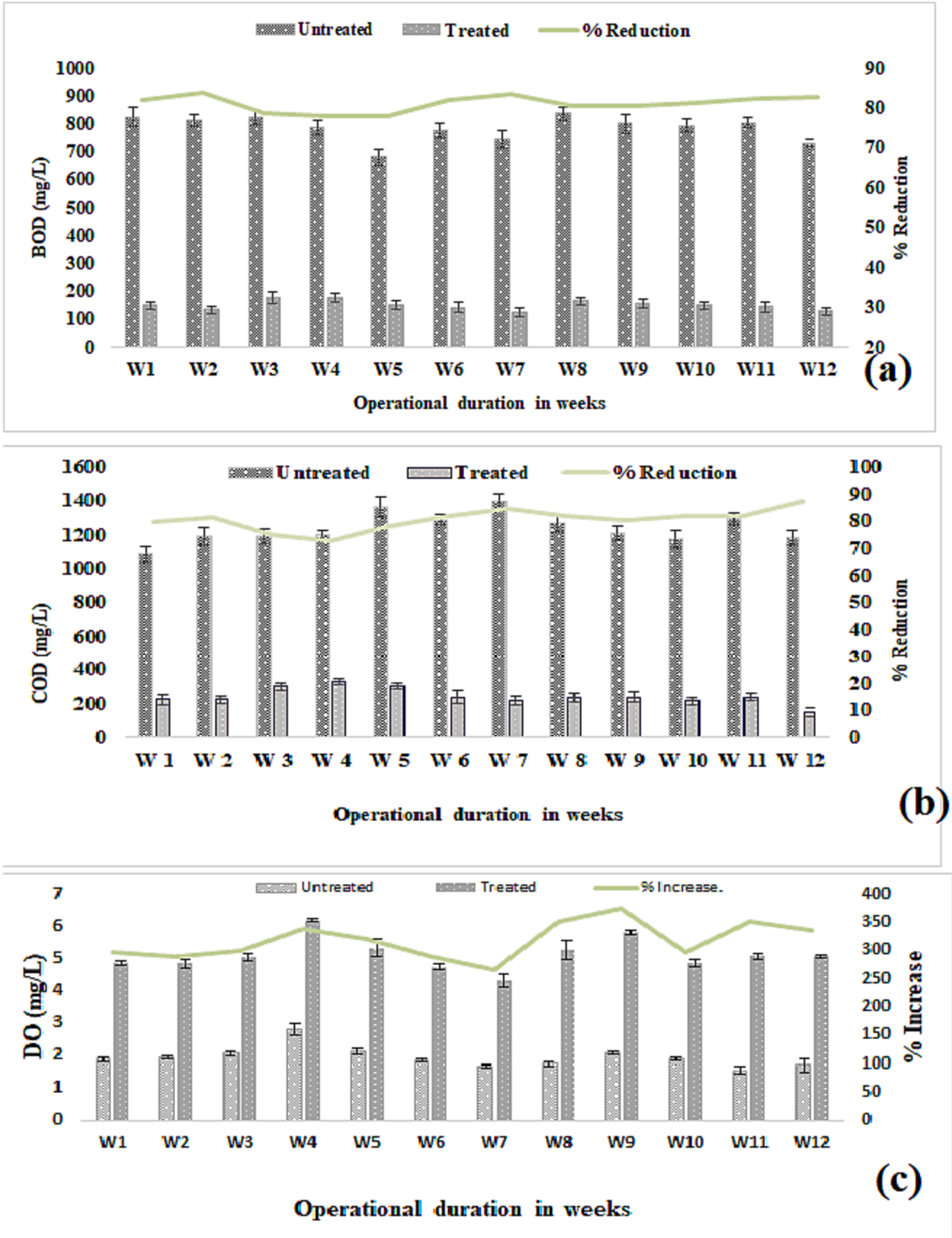

Fig. 4. Comparative variations in BOD_5 (a), COD (b), and DO (c) during 12 weeks of operation through IVFCWplanted with *Typha latifolia.*

was 83 %. These results affirm the potential IVFCW to reduce the lignin concentration.

3.6. Microbiological analysis

Two important microbiological parameters, i.e., CFU/mL and MPN/100 mL indices of all samples (W1-W12) were conducted. The results of these parameters are shown in Fig. 7 (a) and 7 (b). Initially treated samples do not show a significant reduction in MPN/100 mL; however, after plant growth and maturation of biofilm on stone media in aerobic and anaerobic chambers, a significant reduction, about 90 %, was observed. Likewise, in CFU/mL, the average reduction was about 55 % and showed a maximum reduction of 67 % in the 8th sample (W8). MPN index is used to determine the number of *Fecal coliforms* in wastewater. As the black liquor sample was mixed with domestic wastewater/sewage water, the influent contained a high *Fecal coliform* population. According to WHO guidelines (2004), *fecal coliforms* should not exceed 1000 per 100 mL in irrigation water. Similarly, the CFU/mL of the influent showed a higher microbial concentration in the blended wastewater.

3.7. Microbial profiling of rhizosphere

In the present study, *Typha latifolia* rhizosphere microbial profiling was done (unidentified strains were given code S1-S17), and the results of biofilm characterization, i.e., microscopy and biochemical identification, are shown in Table 2.

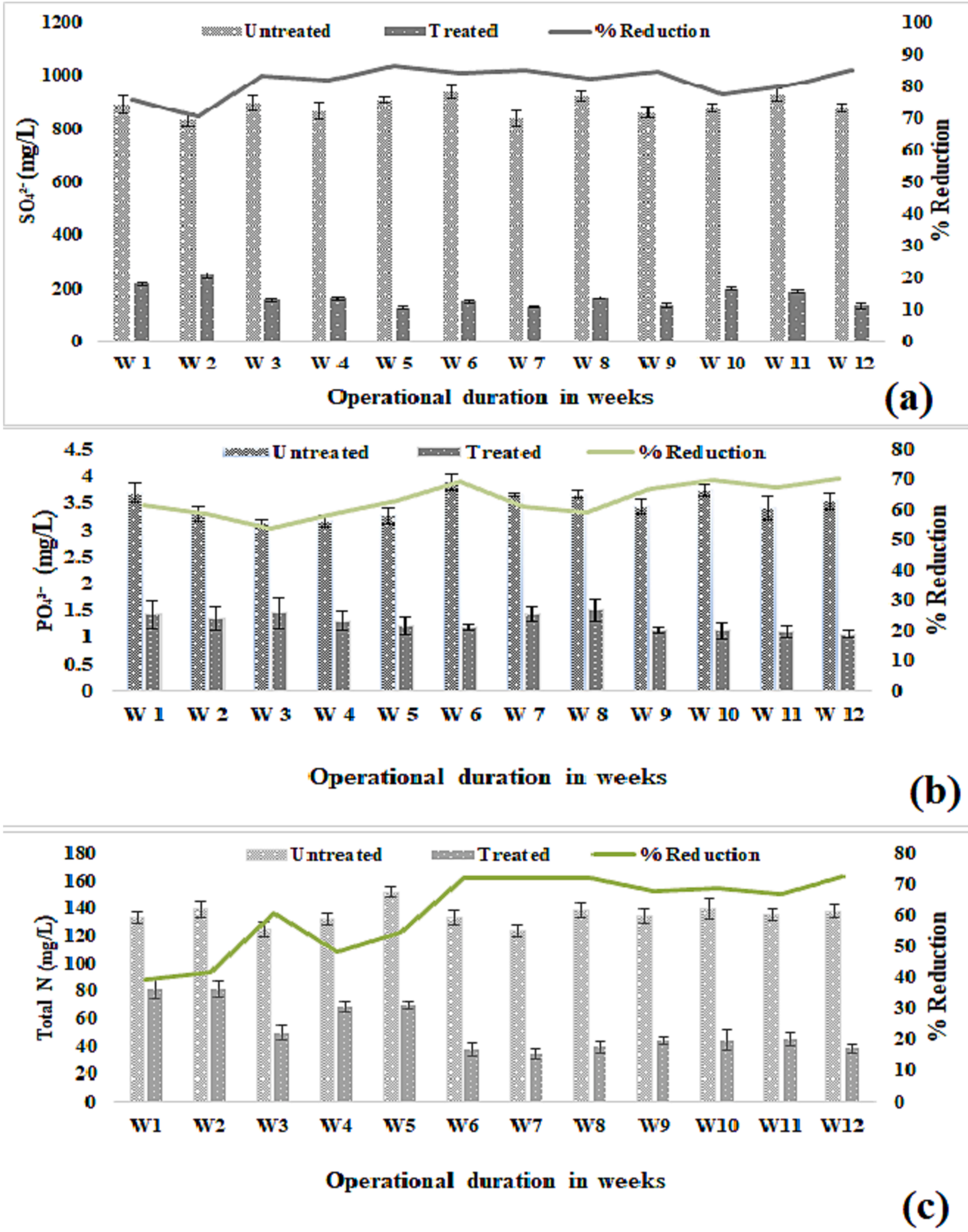

Fig. 5. Comparative variations in sulfates (SO_4^{2}-) (a), phosphates (PO_4^{3-}) (b), and nitrates (NO^{-3}) (C) during 12 weeks of operation through IVFCW planted with *Typha latifolia*.

3.8. Phytotoxicity analysis

In the present study, phytotoxicity analysis was carried out to investigate the potential effects of untreated black liquor on seed germination and seedling growth. The findings revealed that pots irrigated with untreated black liquor had a significantly lower percentage of germination and a delayed seed germination process. Furthermore, 11.11 % of the seedlings experienced post-emergence death, indicating the potential detrimental effects of the black liquor on the seedlings' viability and survival and these results (shown in Table 3).

4. Discussion

4.1. Color, odor, and pH

Any color or odor is evidence of contaminated water. Several factors may be responsible for the odor in water, like excessive nutrients, sulfur-containing aromatics, and the chemical oxidation of ketonic or aldehyde compounds. Similarly, there are compounds attributed to the coloration of wastewater. In PPI, during different steps of the product-making process, a considerable quantity of these compounds is released and cause coloration and a foul smell of the effluent. Biofilm present in anaerobic and aerobic chambers and microbial communities in wetland rhizospheres performed a crucial role in hazardous chemical degradation, color reduction, and odor reduction. Similar types of results in

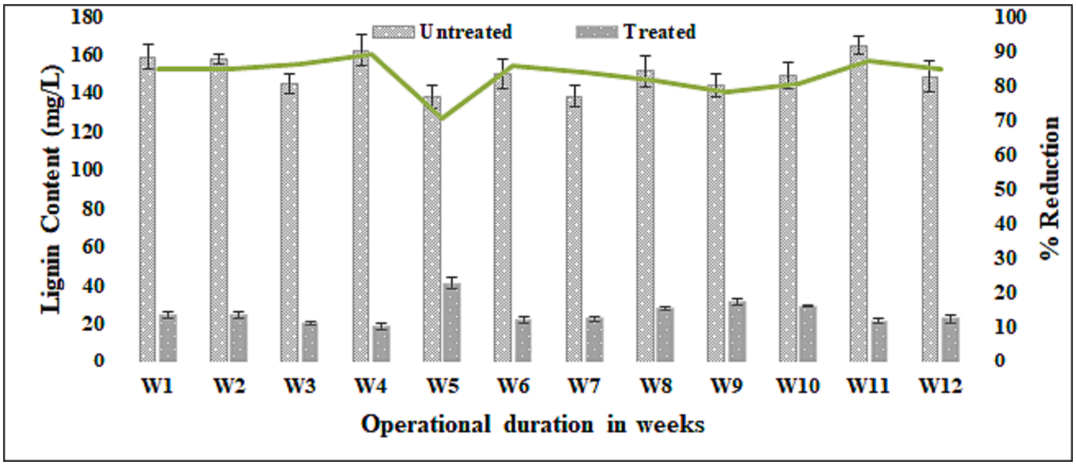

Fig. 6. Reduction in lignin contents in the effluent during 12 weeks of operation through IVFCW.

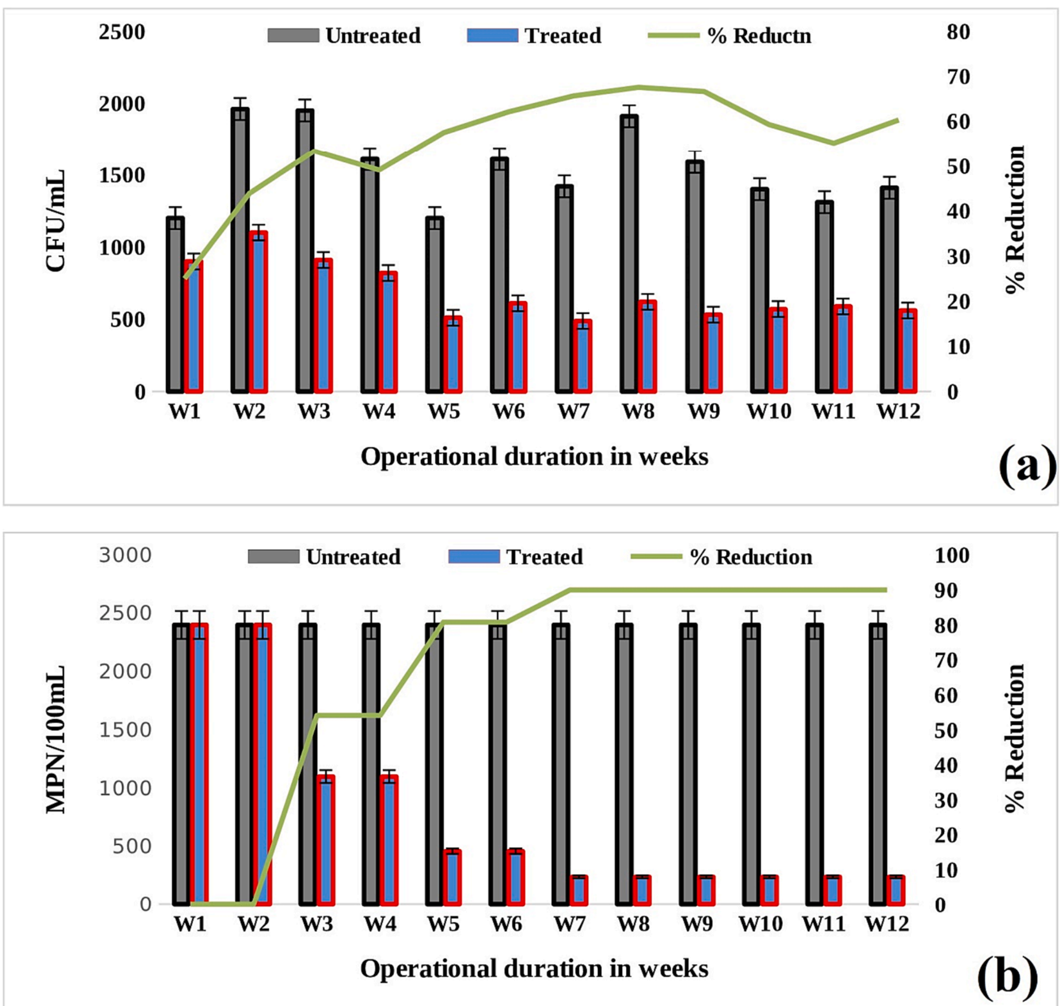

Fig. 7. Reduction in CFU/mL(a) and MPN index/100 mL (b) in the effluent during 12 weeks of operation through IVFCW.

terms of odor and color reduction were observed by (Rasool et al., 2018). The pH value of influent and effluent was recorded and varied from 7.2 to 9.6. However, after treatment through IVFCW, it significantly reduced, as shown in Fig. 2. Although pH has no direct or indirect influence on terrestrial or aquatic life, it is one of the critical and essential parameters determining wastewater quality. The decrease in pH in the IVFCW system may be because of nitrate conversion to molecular nitrogen and phosphate and sulfate metabolism (Sakuma et al.,2008).

Table 2
Microscopic and biochemical characterization of isolated bacterial strains from the rhizosphere of *Typha latifolia*.

Strain Code	Gram's Staining	Morphology	Oxidase	Catalase	Urease	MR	VP	Indole	Nitrate	Citrate	TSI	H_2S	Identified Genera
S1	G^+	Rods	–	+	+	–	+	–	+	–	A	+	*Klebsiella*
S2	G^+	Rods	+	+	–	–	+	–	+	+	A	+	*Bacillus subtilis*
S3	G^+	Chain	–	+	–	+	+	–	–	–	A	–	*Micrococcus variens*
S4	G-	Chain	+	+	+	+	+	+	+	+	A	+	*Salmonella*
S5	G^-	Chain	+	+	–	–	+	–	+	+	A	–	*Bacillus cereus*
S6	G^+	Rods	–	+	–	+	–	–	+	–	A/K	–	*Aeromonas hydrophilla*
S7	G^+	Chain	+	+	–	+	+	–	+	+	A	–	*Proteus vulgarius*
S8	G^-	Cocci	–	+	+	+	+	–	–	–	–	+	*Acenetobacter* sp.
S9	G^+	Rods	–	+	–	–	+	–	+	–	K	–	*Listeria* sp.
S10	G^+	Rods	–	+	–	+	–	–	+	–	A/K	–	*Corynebacterium*
S11	G^+	Cocci	–	–	–	+	–	–	+	–	A	+	*Sterptococcus*
S12	G^-	Rods	–	+	–	–	–	–	+	–	A	+	*Neisseria* sp.
S13	G^-	Rods	+	+	–	+	–	–	+	+	–	–	*P. aeruginosa*
S14	G^-	Rods	–	+	–	–	–	+	+	–	K/A	–	*S. dysentery*
S15	G^-	Rods	–	+	–	–	–	–	+	+	K/A	–	*Enterobacter aerogenes*
S16	G^-	Cocci	+	+	–	–	–	–	–	+	–	–	*Alcaligensfaecals*
S17	G^-	Rods	–	+	–	+	–	+	+	–	A	–	*E. coli*

(Key: + = positive test; - = negative test; ± = Variables; AG = Acid and gas production; K = alkaline; A = Acidic; NC = No color change; K/A, H_2S = Red/yellow with black precipitation; K/A = Red/yellow; A/NC = Acid/no color change).

Table 3
Phytotoxicity analysis of treated and untreated waste water.

Treatments	**Total No. of seeds planted**	**Germination (%) at 10th day of sowing**	**Germination (%) at 20th day of sowing**	**Post-emergence death (No. of seedlings died)**
Untreated Wastewater	45	40 %	63 %	5
Treated Wastewater	30	83 %	83 %	1
Tap water	30	90 %	93 %	0

4.2. Removal of total solids

Total solids are the sum of TSS and TDS. Previous studies have documented similar findings for TDS, and TSS concentrations reduction. The removal efficiencies achieved with *B. reptans* were 58 % (201.88 mg/L) and 63.42 % (212.30 mg/L), respectively, while with *T. portulacastrum*, they were 70.03 % (144.06 mg/L) and 74 % (150.93 mg/L) in the constructed wetlands. These outcomes were seen when the hydraulic retention period was 20 days. The increased rates of removal observed in *T. portulacastrum* can be attributed to possessing a well-developed network of roots and root hairs. Sehar et al. (2015) have witnessed that increased HRT increases the interaction time between pollutants and bacteria in the rhizosphere. The proper selection of macrophytes is critical in removing TSS and TDS. Significant expansion of root systems and root hairs, which effectively increase the accessible surface area for microbial adhesion and colonization, and a more significant number of microbial populations leads to effective removal of total solids by biomineralization and bioaccumulation. The IVFCW system, employed for wastewater treatment, can be ascribed to the concurrent processes of physical filtration, adsorption, and microbial degradation to reduce TDS and TSS. The macrophytes' extensive root network and root hairs increase the surface area for microbial attachment. Settlement in PST and IVFCW allows enhanced particulate matter (TSS) and solutes (TDS) removal through biological interactions and absorption onto root surfaces. This interplay of physical and biological mechanisms effectively reduces TDS and TSS in the effluent.

Multiple reasons for reducing EC include reduction in TSS, TDS, and conversion of nitrate or nitrite into molecular nitrogen. Another study showed a decrease of EC by about 29.4 % due to an increase in retention time of 48 h in a trickling sand filter (Khan et al., 2014).

4.3. Removal of organic contaminants

Organic pollutants, such as BOD and COD, are linked to the concentration of dissolved organic contaminants in wastewater. The high BOD and COD values in pulp and paper industry effluent can be attributed to organic compounds derived from various processes involved in paper production. These compounds include lignin, cellulose, hemicellulose, and other organic substances in the raw materials. During manufacturing, these organic compounds are released into the wastewater, resulting in elevated BOD and COD levels. The effluent from PPI often consists of complicated organic molecules resistant to natural degradation, making it challenging to treat and reduce BOD and COD effectively. Similarly, in 2007, Akratos&Tsihrintzisreported that 91.9 % of COD and BOD were reduced with HRT of 8 days with a gradual increase in dissolved oxygen. This gradual increase can be related to root development over time, which increases the pore formation in the soil and allows more oxygen to reach down (Hench et al., 2003). An increase in DO content (1.9 mg/L to 5.1 mg/L) confirms the reduction of organic containments and better water quality. Thus, the present design of IVFCW proved very efficient in reducing organic contaminants. As reported by Taylor in 2011 and Ciria in 2005, plants can transfer oxygen from leaves to roots and father into the rhizome, where bacteria take it up as an electron donor to degrade the pollutants.

Wetlands promote the growth of aerobic microorganisms, such as bacteria and algae, responsible for the breakdown and decomposition of organic pollutants (Moazzem et al., 2023). As these microorganisms metabolize the organic pollutants present in the wastewater, they consume oxygen. This microbial activity and macrophytes in the wetlands promote oxygenation through photosynthesis (Babuponnusami et al., 2023). Macrophytes release oxygen as a byproduct of photosynthesis, further contributing to increased D.O. levels. Additionally, the physical processes in wetlands, such as aeration and exposure to air–-water interfaces, facilitate the transfer of atmospheric oxygen into the water, enhancing D.O. levels. As a result, wastewater treatment through wetlands can lead to increased dissolved oxygen concentrations (Mittal et al., 2023).

4.4. Removal of nutrients (sulfates, phosphates, nitrates)

Essential nutrients such as sulfates (SO_4^{-2}), phosphates (PO_4^{-3}), and nitrates (NO^{-3}) (total nitrogenT.N.) are commonly found in domestic wastewater and pulp and paper industry effluent. However, their concentrations are much higher in the latter due to detergents, agriculture runoff, and the use of sodium sulfite during the pulping process (Khan et al., 2022). Several variables contribute to the decrease in sulfates. First, dissolved oxygen concentration rises; second, sulfate-reducing bacteria reduce it (Stein et al., 2007). Third, some of the sulfates are trapped by the different layers of the wetland; fourth, the chemical reduction of sulfates into elemental Sulphur. Fifth, some Sulphur is taken up by the plants into their body through roots (Bottrell et al., 2010). Roots also increase the efficiency of removing ions by entrapping sulfate ions on their surface, providing additional time for bacterial reduction (Moazzem et al., 2023).

Phosphates are also found to be significant in wastewater. Our study showed a significant reduction in the phosphates level, consistent with prior results (Chung et al., 2008), as depicted in Fig. 5 (b). Phosphorous is removed in two ways, i.e., plant uptake as a nutrient and phosphorous utilizing bacteria in biofilms (Sehar et al., 2013) and (Mateus et al., 2014). Furthermore, for phosphates removal, filtration and precipitation are also employed (Arias et al., 2001). An integrated system exhibits superior performance due to its utilization of alternating anaerobic and aerobic conditions, facilitating microorganisms' degradation of organic phosphates (Bonomo, 1997).

Nitrogen, when mingled with phosphate in an aquatic environment, may generate algal blooms. The sources of these nitrogenous wastes in PPI wastewater are hydrolytic products of plants' cell walls and detergents used during pulp washing (Bhandari et al., 2023). Before the treatment, the average concentration and the results of before and after treatment are shown in Fig. 5 (C). The reduction in T.N. content was due to the assimilation of NO^{-3} by the plant roots and the presence of denitrifying bacteria, which convert these ions into atmospheric nitrogen. The results of the present study are similar to Chang et al., 2013.

4.5. Removal of lignin

The paper's quality is contingent upon the quantity of lignin in the ultimate completed product. To optimize the overall quality of the ultimate paper product, a substantial portion of the lignin content is typically eliminated during the washing and bleaching procedures. During the stages mentioned earlier, the process of lignin hydrolysis gives rise to the formation of phenolic chemicals, which subsequently contribute to alterations in pH levels and the development of a dark brown hue in the effluent. Lignin and phenolics significantly impact aesthetic value and pose a substantial hazard to the environment. Singh and Chandra (2019) have shown evidence of the effects of lignin and its derivatives, specifically chloropicrin, on fish's reproductive system. These effects manifest as delayed maturity, decreased levels of sex hormones, and a reduction in gonad size. Within terrestrial ecosystems, introducing these contaminants results in their incorporation into the food chain, presenting potential carcinogenic and genotoxic hazards to both human beings and other animal species (Savant et al., 2006; Khan et al., 2022).

The leading cause of lignin breakdown in IVFCWs is commonly linked to bacterial biofilms. Bacterial organisms possess the inherent ability to metabolize lignin and its derivative aromatic compounds, resulting in the development of intricate lignocellulosic biomass that sustains their proliferation. Furthermore, this metabolic pathway promotes the creation of ligninolytic enzymes, which are critical in lignin degradation (Rinaldi et al., 2016). Numerous bacterial species have been studied for their ability to detoxify lignin and produce ligninolytic enzymes. Bacterial strains were obtained from PPI sludge; notably *Bacillus sp*. and *Paenibacillus sp*. their ability to degrade lignin was validated by evaluating degradation products (Chandra et al., 2007). Laccase-producing bacteria, such as *Azotobacter*, *B. megatarium*, and *Serratia*, were also obtained from soil samples (Lai et al., 2023). These bacteria can break down lignin, and their ability to degrade lignin is associated with their laccase production (Xu et al., 2018). A recent study conducted by Khan et al. (2022) observed that *B. altitudinis SL7* exhibited a notable capacity for efficient breakdown of lignin, particularly when exposed to increased concentrations of this compound.

4.6. Microbiological analysis

Constructed wetlands are also reported (Rehman et al., 2020) to reduce microbiological parameters, i.e., MPN index and CFU, making the wastewater suitable for irrigation. Rasool et al., 2018 reported similar results of about 92 % CFU and 90 % MPN/100 mL in reducing microbial and pathogenic populations. The reduction in MPN/100 mL and CFU/mL after treatment through IVFCW is attributed to several factors, such as optimal habitat conditions for diverse microbial communities in aerobic and anaerobic chambers. These microbial communities are essential in organic pollutant reduction, organic matter decomposition, and a considerable reduction in MPN and CFU (Shakira et al., 2023).Wetlands substrate(stone/pebbles/gravels) provides a physical filter to trap solids and microbes. In the present study, the sand bed as the final filtration unit enhanced the physical filtration, leading to a 90 % reduction of the MPN index. *Typha latifolia*, present as a wetland plant, not only oxygenates the rhizosphere but also affects nutrient uptake, thus affecting microbial populations indirectly. Other important factors such as competition, predation (by protozoa), and nutrient removal also significantly reduce MPN and CFU.

4.7. Microbial profiling of rhizosphere

A significant number of bacterial species have been reported attached to soil particles Torsvik &Ovreas (2002), stones media (Rehman et al.2022,) and roots of the *Typha latifolia* and are involved in biofilm formation as well as the removal of nutrients/containments from wastewater (Saher et al., 2015). In the present study, microbial profiling of Typha latifolia was done and presented in Table 2. Several bacterial species were identified, and their involvement in lignin degradation was also reported several times, such as *Bacillus* sp. and *Paenibacillus* sp. (Rinaldi et al., 2016), (Chandra et al., 2007), *Azotobacter*, *Bacillus* spp. and *Serratia* were reported by (Xu et al., 2018).

4.8. Phytotoxicity analysis

The phytotoxicity analysis conducted in this study revealed that untreated black liquor negatively affects seed germination and seedling survival. The death of seedlings is likely attributed to toxic compounds like lignin, phenols, and other toxic compounds in the black liquor, which disrupt essential physiological processes and restrict the growth and development of the seedlings. The current study's findings align with previous research, supporting the notion that these harmful components might be responsible for the observed adverse effects on seedlings. Our findings are consistent with previous investigations (B. Ravindran et al., 2016; Oleszczuk, P., 2008) and reinforce the need for an appropriate treatment method of black liquor and domestic wastewater to mitigate the adverse implications on the environment, seed germination, and early seedling growth.

5. Conclusion

In presnt srudy an innovative bioreactor was designed in order to treat two different waste waters simultaneously. The IVFCW effectively removed most of the contamianants from the waste water after treatment such as BOD (81%), COD (80%), and TSS (100%). The IVFCW exhibited its capacity as an environmentally friendly, economically efficient, and self-sustaining wastewater treatment system for addressing the environmental issues associated with wastewater release. The results of our study indicated that the IVFCW system, when combined with suitable vegetation and retention time, has excellent potential for implimentation at large scale.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Abedinzadeh, N., Shariat, M., Monavari, S.M., Pendashteh, A., 2018. Evaluation of color and COD removal by Fenton from biologically (SBR) pre-treated pulp and paper wastewater. Process Saf. Environ. Prot. 116, 82–91.
American Public Health Association, 1926. Standard methods for the examination of water and wastewater, 6. American Public Health Association.
Arias, C.A., Del Bubba, M., Brix, H., 2001. Phosphorus removal by sands for use as media in subsurface flow constructed reed beds. Water Res. 35 (5), 1159–1168.
Ávila, C., Reyes, C., Bayona, J.M., García, J., 2013. Emerging organic contaminant removal depending on primary treatment and operational strategy in horizontal subsurface flow constructed wetlands: influence of redox. Water Res. 47 (1), 315–325.
Babuponnusami, A., Sinha, S., Ashokan, H., Paul, M.V., Hariharan, S.P., Arun, J., Pugazhendhi, A., 2023. Advanced oxidation process (AOP) combined biological process for wastewater treatment: A review on advancements, feasibility and practicability of combined techniques. Environ. Res. 116944.
Bhandari, M., Kumar, P., Bhatt, P., Simsek, H., Kumar, R., Chaudhary, A., Prajapati, S.K., 2023. An integration of algae-mediated wastewater treatment and resource recovery through anaerobic digestion. J. Environ. Manage. 342, 118159.
Bonomo, L., Pastorelli, G., Zambon, N., 1997. Advantages and limitations of duckweed-based wastewater treatment systems. Water Sci. Technol. 35 (5), 239–246.
Bottrell, S.H., Hatfield, D., Bartlett, R., Spence, M.J., Bartle, K.D., Mortimer, R.J., 2010. Concentrations, sulfur isotopic compositions and origin of organosulfur compounds in pore waters of a highly polluted raised peatland. Org. Geochem. 41 (1), 55–62.
Carty, A., Scholz, M., Heal, K., Gouriveau, F., Mustafa, A., 2008. The universal design, operation and maintenance guidelines for farm constructed wetlands (FCW) in temperate climates. Bioresour. Technol. 99 (15), 6780–6792.
Chandra, R., Raj, A., Purohit, H.J., Kapley, A., 2007. Characterisation and optimisation of three potential aerobic bacterial strains for kraft lignin degradation from pulp paper waste. Chemosphere 67 (4), 839–846.
Chang, J.J., Wu, S.Q., Dai, Y.R., Liang, W., Wu, Z.B., 2013. Nitrogen removal from nitrate-laden wastewater by integrated vertical-flow constructed wetland systems. Ecol. Eng. 58, 192–201.
Chung, A.K.C., Wu, Y., Tam, N.Y., Wong, M.H., 2008. Nitrogen and phosphate mass balance in a sub-surface flow constructed wetland for treating municipal wastewater. Ecol. Eng. 32 (1), 81–89.
Ciria, M.P., Solano, M.L., Soriano, P., 2005. Role of macrophyte *Typha latifolia* in a constructed wetland for wastewater treatment and assessment of its potential as a biomass fuel. Biosyst. Eng. 92 (4), 535–544.
Comino, E., Riggio, V., Rosso, M., 2011. Mountain cheese factory wastewater treatment with the use of a hybrid constructed wetland. Ecol. Eng. 37 (11), 1673–1680.
Costa, S., Dedola, D.G., Pellizzari, S., Blo, R., Rugiero, I., Pedrini, P., Tamburini, E., 2017. Lignin biodegradation in pulp-and-paper mill wastewater by selected white rot fungi. Water 9 (12), 935.
De la Varga, D., Ruiz, I., Soto, M., 2013. Winery wastewater treatment in subsurface constructed wetlands with different bed depths. Water Air Soil Pollut. 224, 1–13.
Domingos, S., Germain, M., Dallas, S., Ho, G., 2007. Nitrogen removal from industrial wastewater by hybrid constructed wetland systems. In *2nd IWA-ASPIRE Conference and Exhibition*.
Gupta, A., Gupta, R., 2019. Treatment and recycling of wastewater from pulp and paper mill. Adv. Biol. Treatment Indus. Waste Water Their Recycl. Sustain. Future 13–49.
Haq, I., Raj, A., 2020. Pulp and paper mill wastewater: ecotoxicological effects and bioremediation approaches for environmental safety. Bioremed. Indus. Waste Environ. Saf.: Vol. II: Biol. Agents Methods Indus. Waste Manage. 333–356.
Hench, K.R., Bissonnette, G.K., Sexstone, A.J., Coleman, J.G., Garbutt, K., Skousen, J.G., 2003. Fate of physical, chemical, and microbial contaminants in domestic wastewater following treatment by small constructed wetlands. Water Res. 37 (4), 921–927.
Holt, J.G., Krieg, N.R., Sneath, P.H.A., Ataley, J.T., Williams, S.T., (1994). Bergey's Manual of Determinative Bacteriology, ninth ed. Lippincott, Williams and Wilkins Co., Baltimore, pp.1860–1937.
Khan, S.I., Zarin, A., Ahmed, S., Hasan, F., Belduz, A.O., Çanakçi, S., Shah, A.A., 2022. Degradation of lignin by *Bacillus altitudinis SL7* isolated from pulp and paper mill effluent. Water Sci. Technol. 85 (1), 420–432.
Knowles, P., Dotro, G., Nivala, J., García, J., 2011. Clogging in subsurface-flow treatment wetlands: occurrence and contributing factors. Ecol. Eng. 37 (2), 99–112.
Kumar, D., Gaurav, V.K., Sharma, C., 2018. Ecofriendly remediation of pulp and paper industry wastewater by electrocoagulation and its application in agriculture. Am. J. Plant Sci. 9 (12), 2462.
Lai, C. M. T. (2023). Production and Characterisation of Lignin Peroxidase from Newly Isolated Thermophilic Bacillus licheniformis from Empty Fruit Bunch (Doctoral dissertation, Curtin University).
Masi, F., Martinuzzi, N., 2007. Constructed wetlands for the Mediterranean countries: hybrid systems for water reuse and sustainable sanitation. Desalination 215 (1–3), 44–55.
Mateus, D.M., Vaz, M.M., Capela, I., Pinho, H.J., 2014. Sugarcane as constructed wetland vegetation: Preliminary studies. Ecol. Eng. 62, 175–178.
Mittal, M., Bhuwal, A., Sharma, P., Aggarwal, N.K., 2023. Utilization of pulp and paper industrial wastewater for production of polyhydroxybutyrate by *Bacillus sonorensis* NAM5. Syst. Microbiol. Biomanuf. 1–14.
Moazzem, S., Bhuiyan, M., Muthukumaran, S., Fagan, J., Jegatheesan, V., 2023. Microbiome wetlands in nutrient and contaminant removal. Curr. Pollut. Rep. 1–16.
Mosse, K.P.M., Patti, A.F., Christen, E.W., Cavagnaro, T.R., 2011. Winery wastewater quality and treatment options in Australia. Aust. J. Grape Wine Res. 17 (2), 111–122.
Oleszczuk, P., 2008. Phytotoxicity of municipal sewage sludge composts related to physico-chemical properties, PAHs and heavy metals. Ecotoxicol. Environ. Saf. 69 (3), 496–505.
Oller, I., Malato, S., Sánchez-Pérez, J., 2011. Combination of advanced oxidation processes and biological treatments for wastewater decontamination—a review. Sci. Total Environ. 409 (20), 4141–4166.
Poe, A.C., Piehler, M.F., Thompson, S.P., Paerl, H.W., 2003. Denitrification in a constructed wetland receiving agricultural runoff. Wetlands 23 (4), 817–826.
Poggi-Varaldo, H. M., Gutierrez-Saravia, A., Fernández-Villagómez, G., Martínez-Pereda, P., & Rinderknecht-Seijas, N. (1999, November). A full-scale system with wetlands for slaughterhouse wastewater treatment. In *Wetlands & Remediation II: Second International Conference on Wetlands & Remediation* (pp. 213-223).
Rasool, T., Rehman, A., Naz, I., Ullah, R., Ahmed, S., 2018. Efficiency of a locally designed pilot-scale trickling biofilter (TBF) system in natural environment for the treatment of domestic wastewater. Environ. Technol. 39 (10), 1295–1306.
Ravindran, B., Kumari, S.S., Stenstrom, T.A., Bux, F., 2016. Evaluation of phytotoxicity effect on selected crops using treated and untreated wastewater from different configurative domestic wastewater plants. Environ. Technol. 37 (14), 1782–1789.
Rehman, A., Ayub, N., Naz, I., Perveen, I., Ahmed, S., 2020. Effects of Hydraulic Retention Time (HRT) on the performance of a pilot-scale trickling filter system treating low-strength domestic wastewater. Pol. J. Environ. Stud. 29 (1).
Rinaldi, R., Jastrzebski, R., Clough, M.T., Ralph, J., Kennema, M., Bruijnincx, P.C., Weckhuysen, B.M., 2016. Paving the way for lignin valorisation: recent advances in bioengineering, biorefining and catalysis. Angew. Chem. Int. Ed. 55 (29), 8164–8215.
Saeed, T., Afrin, R., Al Muyeed, A., Sun, G., 2012. Treatment of tannery wastewater in a pilot-scale hybrid constructed wetland system in Bangladesh. Chemosphere 88 (9), 1065–1073.
Sakuma, T., Jinsiriwanit, S., Hattori, T., Deshusses, M.A., 2008. Removal of ammonia from contaminated air in a biotrickling filter–denitrifying bioreactor combination system. Water Res. 42 (17), 4507–4513.
Savant, D.V., Abdul-Rahman, R., Ranade, D.R., 2006. Anaerobic degradation of adsorbable organic halides (AOX) from pulp and paper industry wastewater. Bioresour. Technol. 97 (9), 1092–1104.
Sayadi, M.H., Kargar, R., Doosti, M.R., Salehi, H., 2012. Hybrid constructed wetlands for wastewater treatment: A worldwide review. Proc. Int. Acad. Ecol. Environ. Sci. 2 (4), 204.
Sehar, S., Aamir, R., Naz, I., Ali, N., & Ahmed, S. (2013). Reduction of contaminants (physical, chemical, and microbial) in domestic wastewater through hybrid constructed wetland. *International Scholarly Research Notices*, *2013*.
Sehar, S., Naeem, S., Perveen, I., Ali, N., Ahmed, S., 2015. A comparative study of macrophytes influence on wastewater treatment through subsurface flow hybrid constructed wetland. Ecol. Eng. 81, 62–69.
Setiawan, Y., &Hardiani, H. (2020, December). The potential of anaerobic-constructed wetland system for wastewater treatment of rice straw pulping. In *IOP Conference Series: Materials Science and Engineering* (Vol. 980, No. 1, p. 012070). IOP Publishing.
Shakira, A., Mullah, A., Lubis, S.S., 2023. Efektivitas Metode Multi Soil Layering (MSL) dalamPenurunan Total KoliformLimbahCairDomestik. Dampak 20 (2), 83–92.
Shalaby, I.M., Altalhy, A.D., Mosallam, H.A., 2008. Preliminary field study of a model plant for sewage water treatment using gravel bed hydroponics method. World Appl. Sci. J. 4 (2), 238–243.
Singh, A.K., Chandra, R., 2019. Pollutants released from the pulp paper industry: Aquatic toxicity and their health hazards. Aquat. Toxicol. 211, 202–216.
Solano, M.L., Soriano, P., Ciria, M.P., 2004. Constructed wetlands as a sustainable solution for wastewater treatment in small villages. Biosyst. Eng. 87 (1), 109–118.
Song, H.L., Nakano, K., Taniguchi, T., Nomura, M., Nishimura, O., 2009. Estrogen removal from treated municipal effluent in small-scale constructed wetland with different depth. Bioresour. Technol. 100 (12), 2945–2951.
Stein, O.R., Borden-Stewart, D.J., Hook, P.B., Jones, W.L., 2007. Seasonal influence on sulfate reduction and zinc sequestration in subsurface treatment wetlands. Water Res. 41 (15), 3440–3448.
Taylor, C.R., Hook, P.B., Stein, O.R., Zabinski, C.A., 2011. Seasonal effects of 19 plant species on COD removal in subsurface treatment wetland microcosms. Ecol. Eng. 37 (5), 703–710.
Torsvik, V., Øvreås, L., 2002. Microbial diversity and function in soil: from genes to ecosystems. Curr. Opin. Microbiol. 5 (3), 240–245.

Wallace, S., Kadlec, R., 2005. BTEX degradation in a cold-climate wetland system. Water Sci. Technol. 51 (9), 165–171.

World Health Organization. (2004). *Guidelines for drinking-water quality* (Vol. 1). World Health Organization.

Xu, Z., Qin, L., Cai, M., Hua, W., Jin, M., 2018. Biodegradation of kraft lignin by newly isolated Klebsiella pneumoniae, Pseudomonas putida, and Ochrobactrumtritici strains. Environ. Sci. Pollut. Res. 25, 14171–14181.