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ORIGINAL ARTICLE

Comparing The Ability to Treat Artificial Cow Wastewater by Constructed Wetland Model Using *Sorghastrum nutans* and *Brachiaria humidicola*

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Abstract

In addition to meat production, the cattle industry generates significant waste, including bedding materials, wastewater, animal manure, and losses related to feed. If not managed correctly, these byproducts can have adverse environmental impacts. Constructed wetlands (CWs) offer a cost-effective and eco-friendly solution for sustainable wastewater treatment. By virtue of their extensive root systems and filtration matrices, CWs effectively reduce pollution by eliminating suspended particles, organic matter, heavy metals, and pathogens from wastewater. This research aims to assess pollutants present in cattle wastewater and evaluate the efficacy of *Sorghastrum nutans* and *Brachiaria humidicola* in purifying contaminants within constructed wetlands (CWs). CWs planted with *B. humidicola* exhibited higher removal rates for nutrient pollutants compared to CWs utilizing *S. nutans*. After a week of treatment, *B. humidicola*-based CWs demonstrated removal percentages of 94.07% for total nitrogen and 91.58% for phosphate (PO_4^{3-}). Constructed wetlands also prove effective in eliminating biological contaminants like *Escherichia coli* and *Shigella sp.* This study highlights that the CW model incorporating *B. humidicola* outperforms the *S. nutans* model, achieving 100% removal of *E. coli* and 97.37% removal of *Shigella sp.* In conclusion, cow wastewater contains nutrient and biological pollutants, both effectively mitigated by CWs using selected plant species. Notably, *B. humidicola* surpasses *S. nutans* in its capacity for pollutant removal.

Keywords: Constructed wetlands, Cow wastewater, Wastewater treatment, *Sorghastrum nutans, Brachiaria humidicola*

Introduction

Human societies derive numerous advantages from streams and rivers. These water bodies play diverse roles, such as transporting waste materials, facilitating hydropower generation, supplying drinking water, and supporting crop irrigation. Furthermore, rivers serve as crucial components of the water cycle by functioning as conduits for the drainage of surface water. Both rivers and streams offer valuable sustenance and habitats for a wide array of Earth's organisms. Consequently, any agricultural or industrial wastewater necessitates proper treatment before its discharge into these watercourses. Constructed wetlands represent systems for wastewater treatment that effectively eliminate pollutants and process sewage, greywater, stormwater runoff, and industrial wastewater via natural mechanisms. These mechanisms encompass the interplay of plants, substrates, and microbes, which collectively play a pivotal role in ensuring the system's optimal functionality. Wetlands serve multifarious purposes, including water filtration, storage, and the digestion and recycling of carbon as well as various micro and macronutrients.

According to a study by Vymazal (2010), the initial exploration of wetland macrophytes for wastewater treatment dates back to the early 1950s in Germany. Since then, constructed wetlands have evolved into a practical approach for treating diverse wastewater categories. Across the globe, constructed wetlands have been employed to treat various types of wastewaters over the years. The expanding global population, augmented water demand, and the trends associated with the industrial revolution have collectively contributed to environmental degradation, notably due to the discharge of inadequately treated or untreated effluents into aquatic systems.

Materials and Methods

Cow Wastewater Preparation

Cattle manure was gathered from Ladang Pasir Akar in Terengganu, situated at coordinates 5°38'38.1"N 102°28'17.5"E (Figure 1). Subsequently, the cow manure was mixed with tap water at a 10-fold dilution ratio to generate synthetic wastewater resembling that of a cow farm. This artificial wastewater was employed to operate the constructed wetlands (CWs). The wastewater within the CWs was retained for one week without recycling.

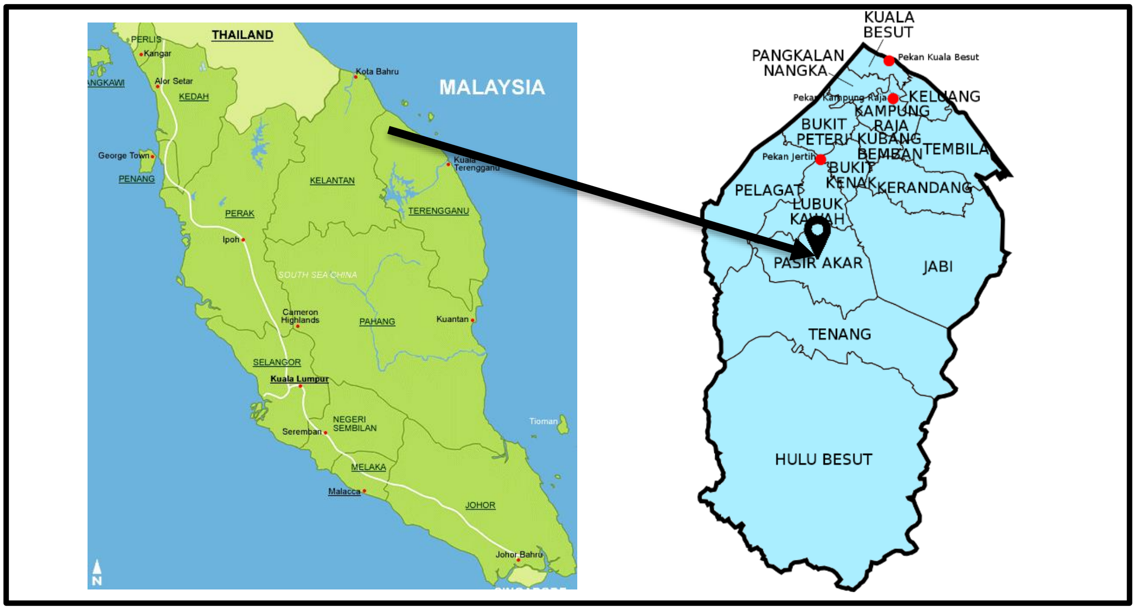


Figure 1. Location of cattle manure collection.

Plant Collection

S. nutans and *B. humidicola* specimens were gathered from Universiti Sultan Zainal Abidin, Besut Campus, located at coordinates 5°45'34.3"N 102°38'00.8"E. Subsequently, both plant species were introduced into individual experimental containers containing tap water, and this setup, maintained for 7 days, allowed the plants to establish new root systems and facilitated the natural adaptation of microorganisms to the wetland environment (Hisham et al., 2022). Following this acclimatization period, the formal experiment commenced by replacing the tap water with synthetic cattle wastewater.

Experimental Set-Up

The design of the constructed wetlands (CWs) models (Figure 2) was influenced by the research conducted by Maharjan et al. (2020), which utilized vertical flow-built wetlands due to their demonstrated capacity for nitrification and their effectiveness in treating wastewater containing elevated ammonium concentrations. Additionally, the choice of substrate material was informed by a study by Tsihrintzis (2017), incorporating layers of natural rocks to facilitate appropriate wastewater drainage.

Three distinct CWs models were established and designated as A, B, and C. Following the experimental design, container A was populated with *S. nutans* (Indiangrass), while container B was populated with *B. humidicola* (Koronivia grass). Container C, on the other hand, served as a control with no plant present.

During the experiments, approximately 60 mL water samples were collected every two days over the course of a week from the drainage outlets of each model. These samples were subsequently subjected to parameter analysis, the details of which will be elaborated upon later.

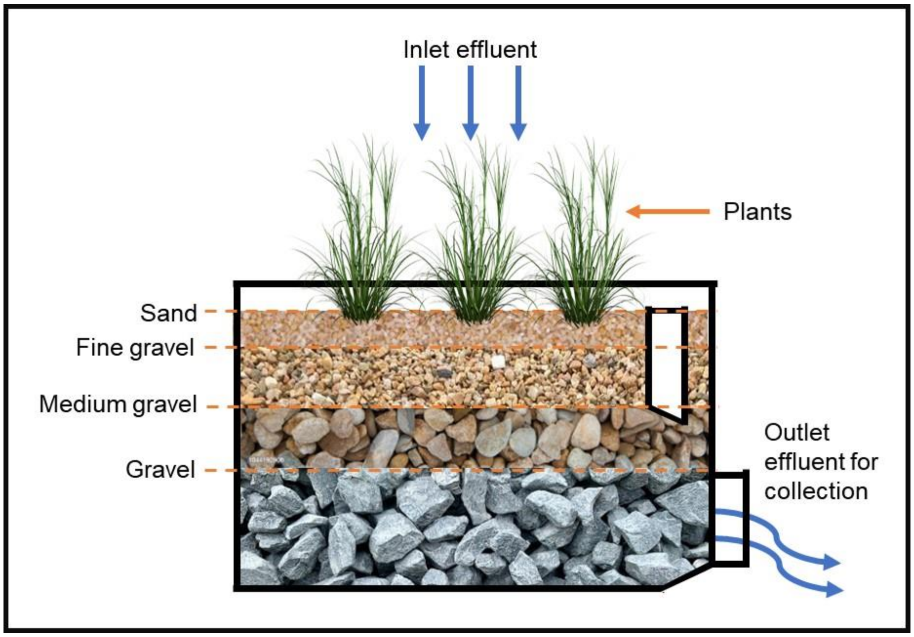


Figure 2. Experimental model of constructed wetlands

Pollutant Analysis

Following the passage of artificial wastewater through the CWs models, the treated wastewater was gathered and subjected to analysis, targeting parameters indicative of high pollution levels. The examination of these collected samples from the CWs took place within a laboratory setting.

For the quantification of total nitrogen and total phosphate, the Hach DR 900 Colorimeter was employed. The collected nitrogen samples underwent a heating process in an oven, reaching a temperature of 105°C within 30 minutes, while the phosphorus samples were heated to 150°C within the same time frame. Conversely, measurements of pH, temperature, conductivity, total dissolved solids, and salinity were carried out using the YSI multiparameter instrument.

Bacteria Isolation

Samples were subjected to initial culturing and subsequent sub-culturing to ensure purity. The cultured samples were placed onto Xylose Lysine Deoxycholate (XLD) agar to ascertain bacterial results, and onto Eosin Methylene Blue (EMB) agar for bacterial identification. The identification process involved visual assessment of colony morphology on the agar plates.

For isolating bacteria, only the initial and final wastewater samples from the three CWs were streaked onto isolation agar plates. Following streaking, the plates underwent incubation at 37 °C for a duration of 24 hours. Manual counting was employed to determine the number of colonies formed on the agar, quantified, and expressed in terms of colony forming units (CFUs).

Statistical Analysis

To assess potential statistically significant variations in pollutant concentrations across all three CWs models, descriptive analysis was employed using the Microsoft Excel add-in. The entirety of data collected during this research endeavour was harnessed to compute the efficacy of both plant species in purging pollutants, with the outcomes subsequently illustrated in the graphical representations.

$$E = \frac{\text{Input concentration} - \text{Output concentration}}{\text{Input concentration}}$$

(Sperling et al., 2020)

Results and Discussion

Overall Results Comparison

Following the acquisition of environmental parameter data, the values for pH, dissolved oxygen (DO), total dissolved solids (TDS), salinity, conductivity, temperature, total phosphate, total nitrogen, as well as the total colony-forming units for *E. coli* and *Shigella sp.* were established. The outcome of these determinations is presented in Table 1, showcasing the measurement results for parameters across the three CWs models.

Table 1(a). Results for model A (*S. nutans*)

Parameters	Day 0	Day 2	Day 4	Day 6	Day 8
pH	7.47	8.35	8.16	8.03	7.68
DO (mg/L)	8.77	10.88	10.2	10.07	8.43
TDS (mg/L)	663	298.8	282.1	287.3	377
Salinity (%)	0.5	0.22	0.21	0.21	0.28
Conductivity (µs/cm)	1098	460.7	442.2	451.4	620
Temperature (°C)	29.1	26	26	26.1	28.5
Total phosphate PO_4^{3-} (mg/L)	>100 (ND)	12.5	14.1	9.8	12.3
Total nitrogen N (mg/L)	25.3	2.2	2.2	2.9	2.7
Total *E. coli* (CFU)	12	-	-	-	0
Total *Shigella* sp. (CFU)	266	-	-	-	51

Table 1(b). Results for model B (*B. humidicola*)

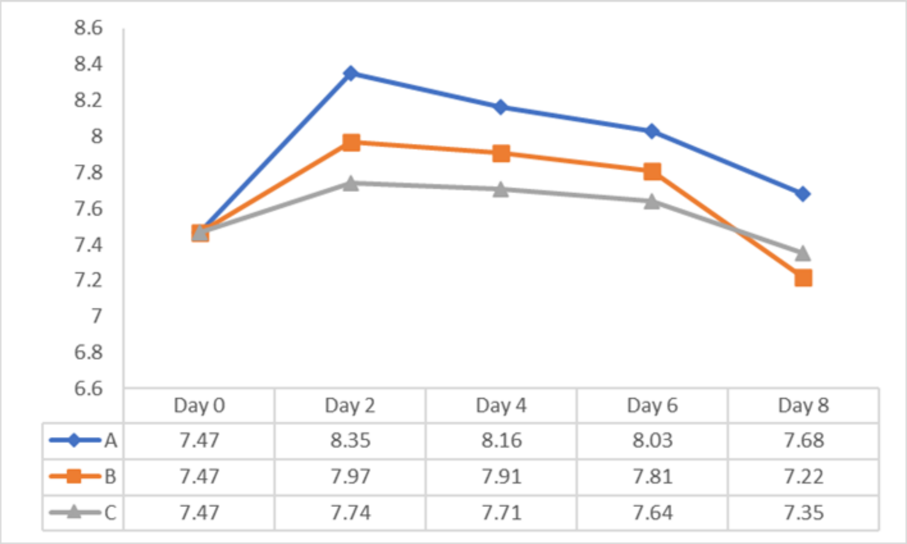
Parameters	Day 0	Day 2	Day 4	Day 6	Day 8
pH	7.47	7.97	7.91	7.81	7.22
DO (mg/L)	8.77	12.36	12.81	12.37	9.3
TDS (mg/L)	663	344.5	265.85	302.25	278.2
Salinity (%)	0.5	0.25	0.2	0.22	0.2
Conductivity (µs/cm)	1098	506	388.6	444.2	457.8
Temperature (°C)	29.1	23	22.4	22.7	28.6
Total phosphate PO_4^{3-} (mg/L)	>100 (ND)	20.9	15	11.8	8.5
Total nitrogen N (mg/L)	25.3	2.5	1.5	2.7	1.5
Total *E. coli* (CFU)	12	-	-	-	0
Total *Shigella* sp. (CFU)	266	-	-	-	7

Table 1(c). Results for model C (Blank)

Parameters	Day 0	Day 2	Day 4	Day 6	Day 8
pH	7.47	7.74	7.71	7.64	7.35
DO (mg/L)	8.77	9.94	9.56	10.28	8.94
TDS (mg/L)	663	364	266.5	325	357.5
Salinity (%)	0.5	0.27	0.2	0.24	0.26
Conductivity (µs/cm)	1098	569	418.4	510	586
Temperature (°C)	29.1	26	26.1	26	28.9
Total phosphate PO_4^{3-} (mg/L)	>100 (ND)	2.6	4	0.8	1.6
Total nitrogen N (mg/L)	25.3	1.4	2.1	5.6	1.8
Total *E. coli* (CFU)	12	-	-	-	0
Total *Shigella* sp. (CFU)	266	-	-	-	4

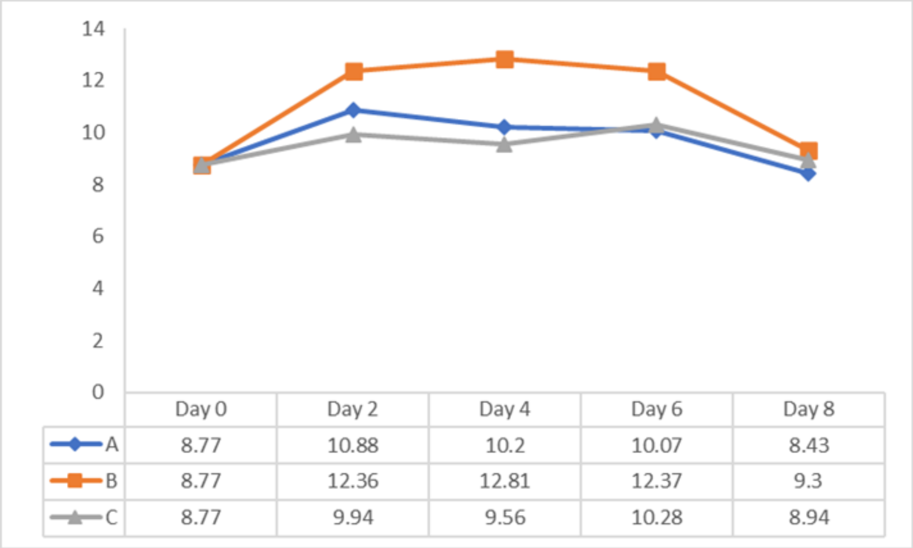
Changes In Parameters and Pollutants

To assess numerical data, quantitative data analysis was employed to examine variations in parameters over time. The relationships between pH, temperature, and dissolved oxygen (DO) are illustrated in Figures 3(a) to 3(c).



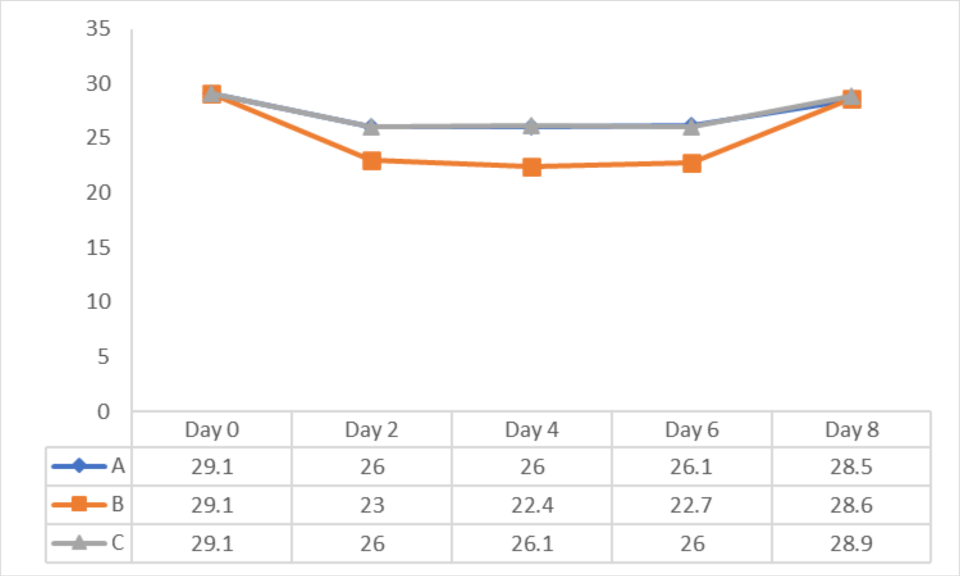
	Day 0	Day 2	Day 4	Day 6	Day 8
A	7.47	8.35	8.16	8.03	7.68
B	7.47	7.97	7.91	7.81	7.22
C	7.47	7.74	7.71	7.64	7.35

Figure 3(a). Concentration results of pH between *S. nutans, B. humidicola* and blank



	Day 0	Day 2	Day 4	Day 6	Day 8
A	8.77	10.88	10.2	10.07	8.43
B	8.77	12.36	12.81	12.37	9.3
C	8.77	9.94	9.56	10.28	8.94

Figure 3(b). Concentration results of DO (mg/L) between *S. nutans, B. humidicola* and blank

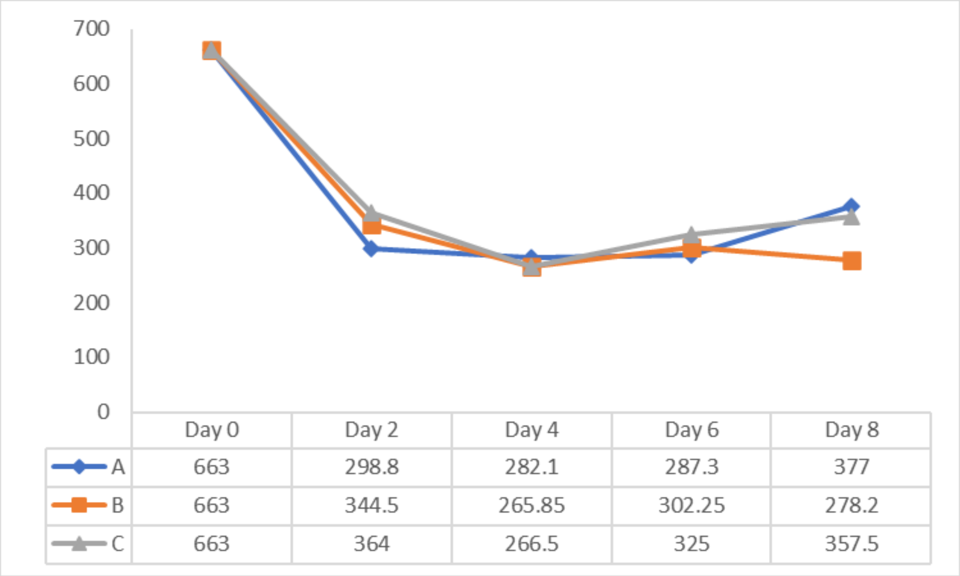


	Day 0	Day 2	Day 4	Day 6	Day 8
◆ A	29.1	26	26	26.1	28.5
■ B	29.1	23	22.4	22.7	28.6
▲ C	29.1	26	26.1	26	28.9

Figure 3(c). The results of Temperature (°C) between *S. nutans, B. humidicola* and blank

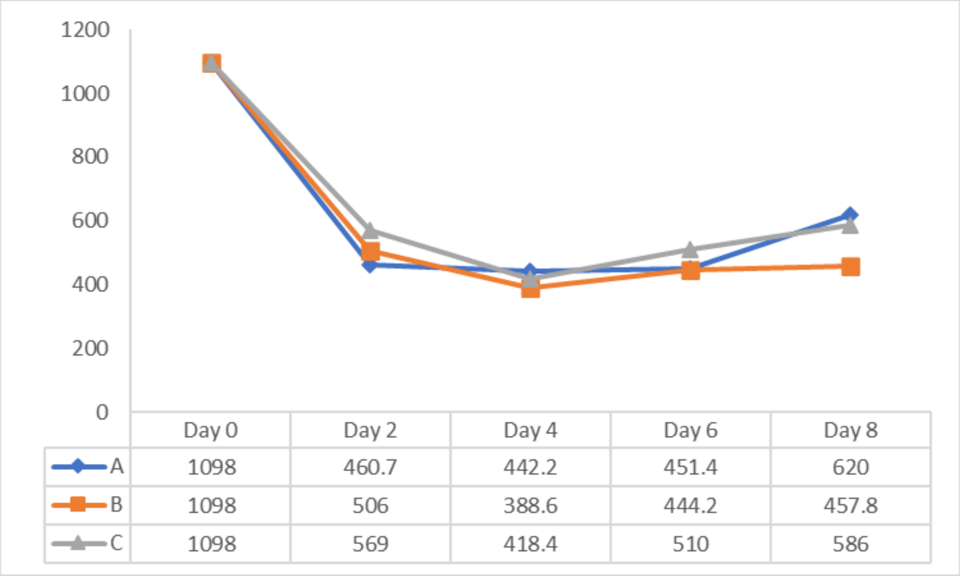
Referring to Figures 3(a) to 3(c), it was observed that the dissolved oxygen (DO) and pH exhibited an inverse relationship with the temperature of the samples. For instance, on day 0, with a temperature of 29.1 °C, the DO and pH measurements were lower than those on day 2, with a temperature range of 23-26 °C. Cold water has the capacity to retain greater amounts of dissolved oxygen and exhibits higher pH levels compared to warm water.

For water quality deemed satisfactory, the dissolved oxygen should not fall below 7 mg/L, and the pH level should remain above 7. Nonetheless, it's important to acknowledge that colder water temperatures could potentially impact the functioning of the constructed wetland (CW) process. As highlighted in a review by Varma et al. (2021), microorganisms and plants tend to operate less efficiently in colder environments, especially when the average temperature drops to around 10°C or lower. This limitation can hinder the effectiveness of treatment procedures. Thus, maintaining an optimal temperature level is crucial.



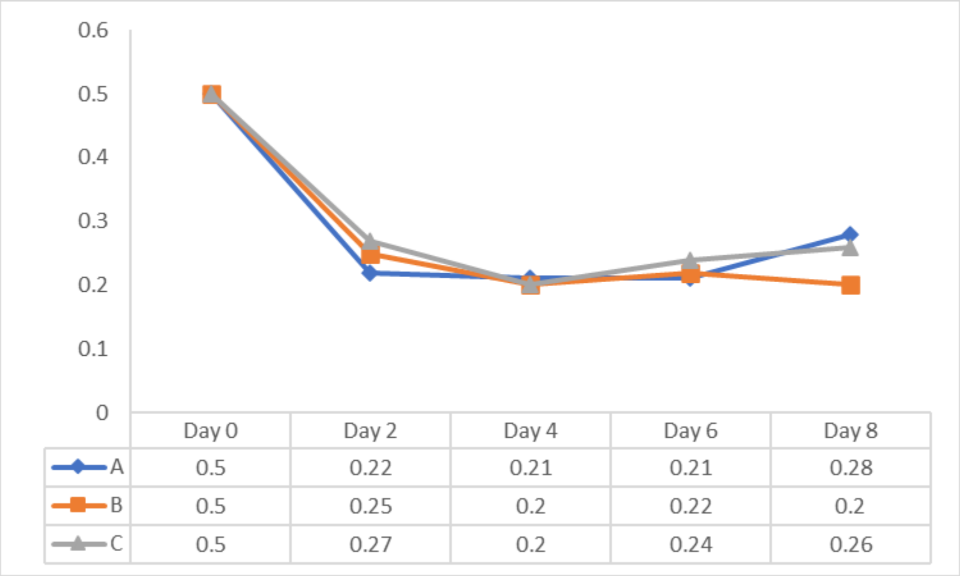
	Day 0	Day 2	Day 4	Day 6	Day 8
◆ A	663	298.8	282.1	287.3	377
■ B	663	344.5	265.85	302.25	278.2
▲ C	663	364	266.5	325	357.5

Figure 3(d). Concentration results of TDS (mg/L) between *S. nutans, B. humidicola* and blank



	Day 0	Day 2	Day 4	Day 6	Day 8
◆ A	1098	460.7	442.2	451.4	620
■ B	1098	506	388.6	444.2	457.8
▲ C	1098	569	418.4	510	586

Figure 3(e). Concentration results of conductivity (µs/cm) between *S. nutans, B. humidicola* and blank



	Day 0	Day 2	Day 4	Day 6	Day 8
◆ A	0.5	0.22	0.21	0.21	0.28
■ B	0.5	0.25	0.2	0.22	0.2
▲ C	0.5	0.27	0.2	0.24	0.26

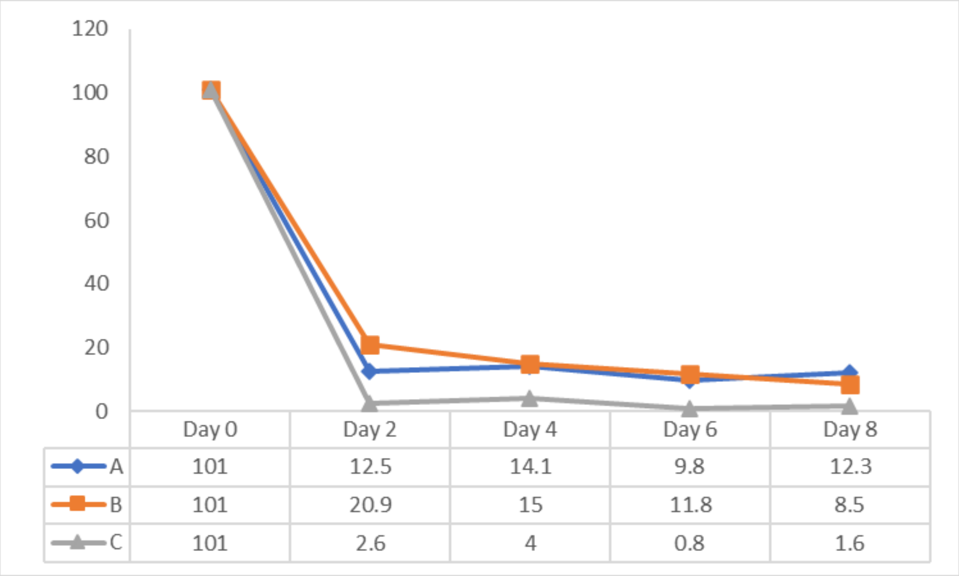
Figure 3(f). Concentration results of salinity (%) between *S. nutans, B. humidicola* and blank

The concentration of total dissolved solids (TDS) exhibited a notable reduction across all three CWs models following a week of treatment, despite slight increases in TDS concentration observed on day 8. According to findings presented in the study by Mikalsen (2005), heightened TDS concentrations can stem from increased alkalinity and calcium levels attributed to processes like weathering, efficient transportation, and rapid calcium carbonate degradation in urbanized streams. Elevated sulphate concentrations likely result from enhanced transport of deposited or spilled sulphur compounds originating from impermeable surfaces, wastewater leaks, or unauthorized discharges. Additionally, elevated chloride concentrations can arise from various sources, including wastewater leakage, runoff from lawn fertilizers, as well as discharges or releases of chloride-containing chemicals like road salts.

The Figure 3(d) illustrates that TDS levels within the CWs model containing *B. humidicola* demonstrated the most effective reduction of this parameter. A lower TDS level signifies high-quality water that poses no detrimental impact on human health, although it may exhibit a somewhat neutral taste due to the limited presence of minerals.

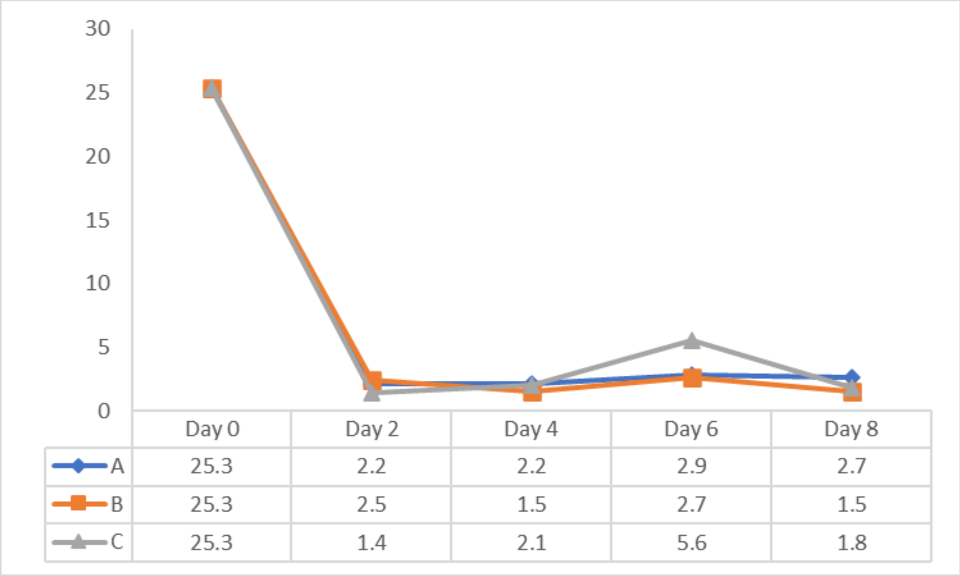
Figures 3(d) to 3(f) show visual similarities. Notably, significant reductions were observed from day 0 to day 4, followed by slight increases from day 4 to day 8. This pattern arises due to the inherent correlation between conductivity (C) and total dissolved solids (TDS), which are commonly employed measures to assess salinity levels. This relationship is substantiated by the research conducted by Choo (2019), which underscores the strong correlation between conductivity and total dissolved solids. Similarly, Rusydi (2018) also noted that water quality indicators such as C and TDS serve as reliable indicators of salinity levels.

Furthermore, Figures 3(d) to 3(f) underscore the constructed wetlands' capacity to effectively diminish and maintain TDS and conductivity levels within treated wastewater. Additionally, the data suggests that *B. humidicola* outperforms *S. nutans* as a treatment plant in CWs, as demonstrated by its superior performance.



	Day 0	Day 2	Day 4	Day 6	Day 8
◆ A	101	12.5	14.1	9.8	12.3
■ B	101	20.9	15	11.8	8.5
▲ C	101	2.6	4	0.8	1.6

Figure 3(g). Concentration results of total phosphate PO_4^{3-} (mg/L) between *S. nutans, B. humidicola* and blank



	Day 0	Day 2	Day 4	Day 6	Day 8
A	25.3	2.2	2.2	2.9	2.7
B	25.3	2.5	1.5	2.7	1.5
C	25.3	1.4	2.1	5.6	1.8

Figure 3(h). Concentration results of total nitrogen N (mg/L) between *S. nutans, B. humidicola* and blank

The Figures 3(g) and 3(h) illustrate the reduction of total phosphate and total nitrogen within wastewater following a week of treatment in the three CWs. Notably, the reduction of total phosphate is evident across all three models, even within the first 2 days of treatment. However, on day 2, the CWs with *S. nutans* exhibit lower total phosphate levels compared to the CWs with *B. humidicola*. Phosphate is typically found in water as a part of phosphorus, a key component of soil nutrients, as well as the biological makeup of animals and plants. The available amount of this nutrient governs the rate of growth of algae and aquatic vegetation. Among the three models, CWs planted with *B. humidicola* consistently maintain a reduction in total phosphate, unlike the models with *S. nutans* and the blank model, which exhibit slight increases in total phosphate from day 2 to day 4 and again from day 6 to day 8. Even small increments in phosphorus concentration can significantly impact water quality and ecological conditions. In certain aquatic environments, low phosphorus concentrations are critical in limiting algae blooms and aquatic plant growth. Despite this, water bodies with elevated total phosphorus levels are categorized as highly eutrophic, characterized by abundant nutrients and heightened algae presence (MPCA, 2007).

Figure 3(g) and Figure 3(h) demonstrate a substantial reduction in total nitrogen, with CWs for model B showcasing the lowest total nitrogen content on day 8. Modest increases in total nitrogen across all CWs models from day 4 to day 6 likely stem from fluctuations in water temperature and oxygen levels. Over time, elevated wastewater temperatures (a common trait of desalter effluent) and inadequate oxygen levels within various stages of the wastewater process can lead to an uneven hydrolysis and ammonification of organic nitrogen molecules, resulting in the conversion to ammonia.

Culture Result

In the cultivation process on XLD and EMB plates, the morphology of the bacteria was subjected to observation. On XLD plates, the growth of red to pink colonies indicated the presence of *Shigella sp.* within the cow manure samples. Meanwhile, the growth of yellow colonies pointed to the presence of *E. coli*, also originating from the cow manure. The inability of Shigella species to ferment xylose, lactose, or sucrose resulted in red colonies. While the colonies themselves appeared colourless and translucent, the medium's hue caused them to assume a red appearance. Conversely, other unrestricted microorganisms capable of fermenting lactose or sucrose generated yellow colonies, attributed to consistent acid production and subsequent pH alterations (Fluids & Guideline, 2015).

On EMB plates, the observation revealed the presence of metallic green colonies with dark centres, confirming the morphology of *E. coli*. In acidic environments, *E. coli* forms blue-black colonies with a distinct green metallic sheen, owing to the amide bonding of dyes. On the other hand, non-lactose fermenters like *Shigella sp.* produce transparent, colourless, or amber colonies (Remel, 2010).

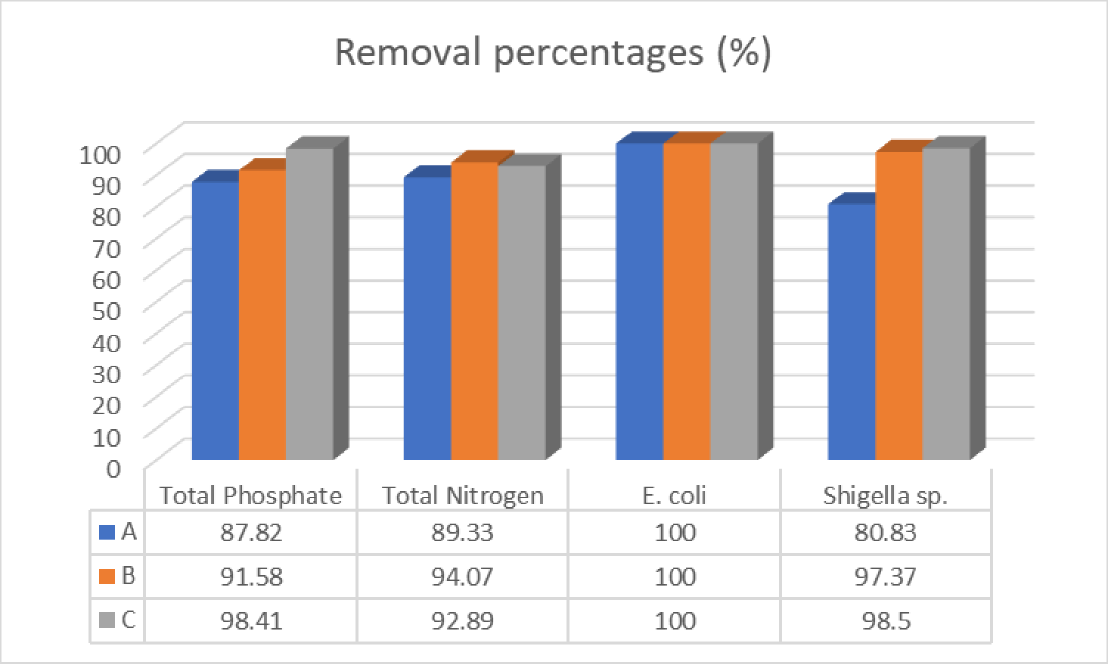
Table 2. Total CFU of *E. coli* and *Shigella* sp.

	Before treatment	After treatment		
		A (*S. nutans*)	B (*B. humidicola*)	C (Blank)
Total E. coli	12	0	0	0
Total Shigella sp.	266	51	7	4

Considering Table 2, the total colony-forming units (CFUs) of *E. coli* and *Shigella sp.* have significantly diminished, although the total CFUs of *Shigella sp.* in the constructed wetlands model using *S. nutans* exhibited a higher count compared to that of *B. humidicola*. Constructed wetlands have demonstrated their capacity to effectively eliminate an array of contaminants, including bacterial pollutants. The removal of pathogenic microorganisms from constructed wetlands is facilitated through a combination of intricate chemical processes (such as oxidation, exposure to plant biocides, adsorption to organic matter, and interaction with biofilm), physical mechanisms (including filtration and sedimentation), and biological factors (like predation, biolytic processes, antibiosis, and natural die-off). These factors often interact in tandem to ensure efficient removal (Ansari et al., 2016).

Removal Percentages

Figure 4 illustrates the removal percentages of pollutants, computed using the removal efficiency formula.



	Total Phosphate	Total Nitrogen	E. coli	Shigella sp.
■ A	87.82	89.33	100	80.83
■ B	91.58	94.07	100	97.37
■ C	98.41	92.89	100	98.5

Figure 4. Removal percentages of pollutants in all three CWs models

By comparing the two species, it becomes evident that model B, characterized by *B. humidicola*, demonstrates a superior removal efficiency compared to *S. nutans.* However, when all three models are contrasted, it is apparent that model C, without any plant, predominantly exhibits the highest pollutants removal efficiency. This scenario likely arises due to the treatment duration. The potential of both plant species to remove pollutants could surpass that of the blank model with an extended treatment duration, allowing ample time for the interaction between roots and microorganisms to effectively reduce pollutants.

Conclusion

In this study, the presence of bacterial contaminants, such as *E. coli* and *Shigella sp.* that found from cattle wastewater, was determined through streaking polluted and treated samples on XLD and EMB agar plates. The other pollutants and water quality parameters were assessed in this study, and the DOE's Water Quality Index (WQI) was employed to facilitate a comparison of removal efficiency across the three CWs models.

The interrelation between pH, DO, and temperature values were identified, with acknowledgment of their potential impact on wetland processes. Conductivity was found to correlate with TDS, both of which serve as indicators of water salinity. Regarding to the nutrient removal, all three CWs models demonstrated the capability to effectively lower total phosphate and total nitrogen content to safer levels. Notably, the CWs model incorporating *B. humidicola* exhibited superior pollutant removal compared to the model with *S. nutans*.

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