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Treatment of Synthetic Dairy Wastewater Using Constructed Floating Wetlands Planted with Canna indica and Cyperus papyrus: A Comparative Phytoremediation Study

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Abstract

Dairy processing generates 2.5–10 L of high-strength wastewater for every litre of milk handled, and small and medium dairy units in developing countries frequently lack the capital to install conventional treatment infrastructure. This study evaluated constructed floating wetlands (CFWs) as a low-cost, nature-based treatment for synthetic dairy wastewater, comparing two macrophytes Canna indica and Cyperus papyrus against an unplanted control. Synthetic wastewater was formulated from milk powder, glucose and mineral salts to give an initial BOD of 1300 mg/L, COD of 2200 mg/L, total Kjeldahl nitrogen (TKN) of 112 mg/L and phosphate of 140 mg/L. Three floating units, each holding four planted or empty pots in a cocopeat-vermicompost substrate, were floated on 20 L buckets of wastewater and monitored on Days 1, 7, 15 and 21. As a complementary bioaugmentation strategy, lipolytic bacteria isolated from dairy-contaminated soil were immobilised in sodium alginate beads and tested for their ability to degrade fats in the wastewater over 15 days. By Day 21, the Canna indica unit achieved the best overall performance (BOD 34%, COD 33%, TKN 34%, phosphate 33% removal), while Cyperus papyrus performed comparably for COD, TKN and phosphate (30–31%) but showed lower BOD removal (24%) and superior sulphate removal (20% vs 12%). Both planted units removed two- to four-fold more pollutants than the unplanted control (14% BOD/COD removal) and maintained dissolved oxygen above 4.8 ppm, compared with a decline to 2.8 ppm in the control, confirming active radial oxygen release from the roots. In the bioaugmentation trial, alginate beads loaded with lipolytic bacteria reduced turbidity by 57.3% (25 beads) and 46.1% (50 beads) over 15 days, versus 11.5% in the control. Removal efficiencies were lower than those reported from tropical or summer trials (60–90%), consistent with the winter conditions under which this study was conducted, but the results confirm that CFWs remain functional in cold weather and can serve as a practical, low-cost pre-treatment or stand-alone option for small dairy operations.

Keywords: *Constructed floating wetlands; synthetic dairy wastewater; Canna indica; Cyperus papyrus; phytoremediation; lipolytic bacteria; sodium alginate immobilisation.*

1. Introduction

Global milk production reached approximately 935 million metric tonnes in 2024, driven by population growth, urbanisation and changing dietary habits (Slavov, 2017). India has been the world's largest milk producer since 1998, and its output crossed 239 million tonnes in 2024 around

24% of global production of which only about 53 million tonnes passes through organised dairy plants (Department of Animal Husbandry and Dairying, 2024). Milk processing is, however, a water-intensive activity: roughly 2.5–10 L of wastewater is generated for every litre of milk processed, depending on the product line and the water-management practices in place (Kushwaha et al., 2011).

Dairy wastewater is a complex mixture of proteins (chiefly casein), lactose, fats and cleaning chemicals, and its composition varies widely with the product being manufactured. Reported BOD values range from 40 to 48,000 mg/L and COD from 80 to 95,000 mg/L, with nitrogen up to 830 mg/L, phosphorus up to 280 mg/L and pH swinging between 4 and 12 due to acidic and alkaline cleaning agents (Kushwaha et al., 2011; Demirel et al., 2005; Slavov, 2017). When discharged untreated, this effluent rapidly depletes dissolved oxygen in receiving waters, triggers eutrophication and algal blooms, and can compromise both aquatic life and downstream drinking-water quality (Kushwaha et al., 2011; Slavov, 2017).

Conventional treatment options activated sludge, sequencing batch reactors, membrane bioreactors, coagulation–flocculation and advanced oxidation can achieve high removal efficiencies but are capital- and energy-intensive, require skilled operation, and generate chemical sludge that must be separately disposed of (Kushwaha et al., 2011; Vymazal, 2010). These constraints place such systems out of reach for the large number of small and medium dairy units, particularly in rural and semi-urban India, that continue to discharge partially treated or untreated effluent despite discharge standards set by the Central Pollution Control Board under the Environment (Protection) Rules, 1986 (CPCB, 2021).

Constructed wetlands (CWs) offer a nature-based alternative in which macrophytes, substrate and microbial communities act together to remove pollutants (Vymazal, 2010). Constructed floating wetlands (CFWs), or floating treatment wetlands, extend this concept by growing macrophytes on a buoyant mat with roots suspended directly in the water column rather than in a soil or gravel bed (Headley and Tanner, 2012). This design needs little additional land, tolerates fluctuating water levels, and exposes roots directly to the wastewater, increasing the surface area available for microbial biofilm attachment (Pavlineri et al., 2017; Afzal et al., 2019). Removal in CFWs proceeds through sedimentation and adsorption at the root mat, direct nutrient uptake by the plant, and microbial transformation supported by radial oxygen loss (ROL) from the roots, which creates paired aerobic/anaerobic micro-zones that drive nitrification–denitrification and organic-matter breakdown (Faulwetter et al., 2009; Rehman et al., 2019).

Species selection strongly influences CFW performance. Canna indica (Cannaceae), valued for its fast growth, dense root system and ornamental flowers, has achieved BOD removal of 59–98% and COD removal of 47–99% across various CW studies (Kumar and Dutta, 2019; Zurita et al., 2009; Konnerup et al., 2009). Cyperus papyrus (Cyperaceae), a tropical African sedge with an extensive fibrous root network, has performed particularly well for ammonium-nitrogen and phosphorus removal, including in substrate-free and floating configurations (Kyambadde et al., 2004, 2005; Benrahmane et al., 2022). Despite the individual promise of both species, CFW research has concentrated on municipal wastewater and stormwater; very few studies have examined high-strength industrial effluents such as dairy wastewater, and no published study has directly compared Canna indica and Cyperus papyrus in a floating configuration treating dairy effluent under identical conditions (Pavlineri et al., 2017; Zurita et al., 2009).

This study addresses that gap. Its objectives were to (i) evaluate CFW performance for synthetic dairy wastewater across BOD, COD, TKN, phosphate, TDS, pH, dissolved oxygen and sulphate; (ii) directly compare the phytoremediation ability of Canna indica and Cyperus papyrus under identical experimental conditions; and (iii) assess, through a complementary lipolytic-bacteria bioaugmentation trial, whether immobilised lipid-degrading bacteria could serve as a useful pre-treatment step for fat-rich dairy effluent.

2. Materials and Methods

2.1 Preparation of Synthetic Dairy Wastewater

Synthetic dairy wastewater was prepared in the laboratory to reproduce the physicochemical profile of real dairy effluent under reproducible conditions. A 200 mL batch was made up in distilled water using milk powder as the primary source of organic carbon, protein and fat; glucose as a supplementary carbon source; sodium bicarbonate for alkalinity and pH buffering; calcium chloride dihydrate and magnesium sulfate heptahydrate to supply divalent cations; ammonium chloride as the nitrogen source; and potassium dihydrogen phosphate as the phosphorus source (Table 1).

Table 1: Composition of synthetic dairy wastewater used in the present study (per 200 mL)

Component	Quantity (mg)	Quantity (g)
Milk powder (Nestlé Everyday)	300	0.30
Glucose	20	0.02
Sodium bicarbonate ($NaHCO_3$)	70	0.07
Calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$)	1	0.001
Magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$)	1.6	0.0016
Ammonium chloride (NH_4Cl)	56	0.056
Potassium dihydrogen phosphate (KH_2PO_4)	40	0.04

2.2 Physicochemical Characterisation

The prepared wastewater was characterised in triplicate for pH, dissolved oxygen (DO), total dissolved solids (TDS), electrical conductivity (EC) and salinity using a multiparameter probe,

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and the mean of three readings was taken as representative. Biochemical oxygen demand was determined by the Winkler azide-modification titration method (5-day BOD), and chemical oxygen demand by the closed-reflux dichromate titration method, with COD calculated as COD (mg/L) = [(A − B) × N × 8000] / (sample volume × dilution factor), where A and B are the titre values of the blank and sample and N is the normality of ferrous ammonium sulfate (0.25 N).
Sulfate was estimated turbidimetrically by the barium chloride method, with absorbance read at 420 nm and concentration calculated as X = [(Y − 0.0388)/0.0089] × dilution factor. Phosphate was estimated colorimetrically by the stannous chloride–ammonium molybdate method, with absorbance read at 690 nm. Total hardness was determined by EDTA titrimetry using Eriochrome Black T indicator. Total Kjeldahl nitrogen (TKN) was determined by the standard three-step digestion–distillation–titration procedure, using a potassium persulfate/potassium sulfate–copper sulfate catalyst for digestion, sodium hydroxide for alkaline distillation into boric acid, and titration against 0.04 N H_2SO_4, with TKN (mg/L) = [(A − B) × 0.04 N × 280] / mL of sample. Total solids, total suspended solids and total dissolved solids were determined gravimetrically following standard drying (105 °C, overnight) and weighing procedures (APHA, 2017).

2.3 Macrophyte Selection and Floating Wetland Construction

Canna indica and Cyperus papyrus were selected as test macrophytes, with an unplanted treatment serving as the control. Plants were grown in pots containing a 1:1 cocopeat–vermicompost substrate, with cocopeat pre-soaked and conditioned for 48 hours before planting. Four pots of each species, and four unplanted control pots, were used, giving three treatment groups. The floating wetland system comprised three separate 20 L buckets filled with tap water (Table 2). Pot bases were perforated so that roots could extend into the water column, and the pots were floated on the water surface with shoots above and roots submerged, replicating a floating treatment wetland configuration. The assembled units were dosed with the synthetic dairy wastewater and monitored over a 21-day batch period, with samples drawn on Days 1, 7, 15 and 21 for the parameters listed in Section 2.2.

Table 2: Constructed floating wetland (CFW) system assembly

Bucket	Treatment	No. of pots/plants
Bucket 1	Cyperus papyrus	4 plants
Bucket 2	Canna indica	4 plants
Bucket 3	No plants (control)	4 pots

2.4 Isolation and Immobilisation of Lipolytic Bacteria

Because dairy wastewater is rich in fats and lipids, lipolytic bacteria were isolated from soil collected near a dairy wastewater runoff site using serial dilution and the spread-plate technique on tributyrin nutrient agar (a selective/differential medium in which lipase-producing colonies form clear hydrolysis haloes). Well-isolated colonies exhibiting clear zones were purified by quadrant streaking and maintained for further work.

A pure colony was grown in nutrient broth for 24 hours at 37 °C with shaking to maintain aerobic conditions, harvested by centrifugation (5000 rpm, 10 min), washed in 0.85% saline, and resuspended to give a concentrated bacterial pellet. This suspension was mixed with sterile sodium alginate solution and added drop-wise into a stirred calcium chloride solution, where each droplet gelled instantaneously into a bead entrapping the bacterial cells within a calcium–alginate matrix. Beads were cured, washed, and used in a bioaugmentation trial in which 25 beads or 50 beads were added to separate 50 mL flasks of synthetic dairy wastewater, alongside an untreated control. Turbidity (OD_{600}) was monitored on Days 1, 2, 4 and 15 as a proxy for fat/lipid breakdown, with distilled water used as the blank.

3. Results and Discussion

3.1 Initial Wastewater Characteristics

The synthetic wastewater had an initial BOD of 1300 mg/L, COD of 2200 mg/L, TKN of 112 mg/L, phosphate of 140 mg/L and TDS of 795 mg/L, with pH 7.3 and DO 6.35 ppm (Table 3). These values fall within the range reported for moderately high-strength dairy effluent (Kushwaha et al., 2011; Slavov, 2017), confirming that the synthetic formulation adequately reproduced the pollutant profile of real dairy wastewater. The Day-1 BOD:COD ratio of approximately 0.59 exceeded the 0.5 threshold generally taken to indicate wastewater that is predominantly biodegradable and therefore amenable to biological treatment (Pavlineri et al., 2017).

Table 3: Physicochemical parameters of the synthetic dairy wastewater sample (Day 1)

Parameter	Unit	Value
BOD	mg/L	1300
COD	mg/L	2200
TKN	mg/L	112
Phosphate	mg/L	140
TDS	mg/L	795
DO	ppm	6.35
pH	–	7.3
EC	µS/cm	1593
Salinity	%NaCl	3.1

Parameter	Unit	Value
Hardness	mg/L	<5
Sulphate	mg/L	4

3.2 BOD and COD Removal

In the Canna indica unit, BOD fell from 1300 mg/L to 854 mg/L by Day 21 (34% removal), and COD fell from 2200 to 1476 mg/L (33% removal). The Cyperus papyrus unit reduced BOD to 993 mg/L (24% removal) and COD to 1542 mg/L (30% removal). The unplanted control achieved only 14% removal for both BOD (1118 mg/L) and COD (1892 mg/L) (Table 4, Figure 1). A slight early rise in BOD was observed in the Canna indica and control units during the first week, most likely reflecting initial leaching of organic matter from the cocopeat–vermicompost substrate before biological treatment fully established a pattern also reported in constructed-wetland studies using organic substrates (Vymazal, 2010).

Canna indica consistently outperformed Cyperus papyrus for organic-matter removal, consistent with reports that its rhizosphere supports a denser, more active heterotrophic bacterial community (Kumar and Dutta, 2019; Konnerup et al., 2009). Removal in both planted units was 2–2.5 times higher than in the control, confirming that the macrophytes and their associated root microbiota were the principal drivers of treatment. The 24–34% removal obtained here is markedly lower than the 60–95% commonly reported from tropical or summer trials, an outcome consistent with the reduced microbial metabolic activity expected at the lower ambient temperatures under which this study was conducted (Faulwetter et al., 2009).

Table 4: Comparative water-quality results after 21 days of treatment

Parameter	Unit	Day 1	Canna indica	Cyperus papyrus	Control
BOD	mg/L	1300	854 (34%↓)	993 (24%↓)	1118 (14%↓)
COD	mg/L	2200	1476 (33%↓)	1542 (30%↓)	1892 (14%↓)
TKN	mg/L	112	74 (34%↓)	77 (31%↓)	100 (11%↓)
Phosphate	mg/L	140	94 (33%↓)	98 (30%↓)	129 (8%↓)
DO	ppm	6.35	4.8	5.7	4.8
pH	–	7.3	7.0	6.9	7.0
TDS	mg/L	795	685 (14%↓)	686 (14%↓)	771 (3%↓)

3.3 TKN and Phosphate Removal

TKN fell from 112 mg/L to 74 mg/L (34% removal) in the Canna indica unit and to 77 mg/L (31% removal) in the Cyperus papyrus unit, against 11% removal in the control. Phosphate followed a similar pattern: 33% removal (140 → 94 mg/L) for Canna indica, 30% removal (140 → 98 mg/L) for Cyperus papyrus, and only 8% removal in the control, which briefly showed a rise in phosphate on Day 7, likely reflecting release of phosphorus from decomposing organic matter before net removal began.

Canna indica performed marginally better than Cyperus papyrus for both nutrients in this study, which contrasts with reports from tropical settings where Cyperus papyrus outperformed comparator species for nitrogen and phosphorus removal (Kyambadde et al., 2004, 2005). This divergence may reflect the fact that Cyperus papyrus, a tropical species, is more sensitive to the winter temperatures under which the present trial was conducted, constraining its metabolic and uptake capacity. TKN removal in the planted units is attributable to a combination of direct plant uptake and microbial nitrification–denitrification supported by root-zone oxygenation, while phosphate removal in the absence of any adsorptive substrate depended primarily on direct plant uptake, a recognised limitation of floating (versus substrate-based) wetland systems (Headley and Tanner, 2012).

3.4 Dissolved Oxygen and pH

Dissolved oxygen in the control fell sharply from 6.35 ppm to 2.8 ppm by Day 21, indicating unchecked aerobic consumption with no compensating oxygen supply. In the Canna indica unit, DO declined more gradually to 4.8 ppm, with a comparatively fast initial drop (to 5.2 ppm by Day 7) consistent with higher early microbial activity. The Cyperus papyrus unit maintained the highest DO throughout, ending at 5.7 ppm. Both planted units therefore sustained DO well above the 4.5 ppm level maintained in the control, supporting the role of root-mediated radial oxygen loss and shoot photosynthesis in maintaining aerobic conditions conducive to biological treatment (Faulwetter et al., 2009).

pH remained within the 6.5–8.5 range considered suitable for both biological treatment and plant growth throughout the study, moving from an initial 7.3 to 7.0 (Canna indica), 6.9 (Cyperus papyrus) and 7.0 (control) by Day 21. A small rise in pH on Day 7 in the planted units may reflect photosynthetic CO_2 drawdown; the mild subsequent acidification is consistent with CO_2 from microbial respiration and organic acids released as root exudates.

3.5 TDS, EC, Salinity, Hardness and Sulphate

TDS fell by 14% in both planted units (795 → 685 mg/L for Canna indica; 795 → 686 mg/L for Cyperus papyrus) against only 3% in the control. EC decreased by 10.2% in the Cyperus papyrus unit and 9% in the Canna indica unit, compared with 3% in the control; salinity fell from 3.1% to 2.5% NaCl in both planted units with negligible change in the control. These reductions reflect biological breakdown and assimilation of dissolved organic material into plant and microbial biomass, a process that occurred only marginally in the unplanted control.

Hardness remained below 5 mg/L in all three units throughout, consistent with the low initial hardness of the synthetic formulation. Sulphate removal was modest but consistently higher in the Cyperus papyrus unit (20%, 4 → 3.2 mg/L) than in the Canna indica unit (12%, 4 → 3.5 mg/L) or the control (5%, 4 → 3.8 mg/L), suggesting somewhat greater sulphate-reducing microbial activity within the anaerobic micro-zones of the Cyperus papyrus root mat.

3.6 Lipolytic Bacteria Bioaugmentation

Turbidity (OD_{600}) in both bead-treated flasks was initially higher than in the control on Day 1 (1.240 and 1.115 vs 0.904), attributable to transient release of gel fragments and loose cells from the beads during the first 24 hours of contact. From Day 2 onward, both flasks showed a steady decline, consistent with active lipase production breaking fat globules down into soluble fatty acids and glycerol, which no longer scatter light (Table 5). By Day 15, the 25-bead flask had reached an OD_{600} reduction of 57.3%, and the 50-bead flask a reduction of 46.1%, compared with only 11.5% in the bead-free control confirming that immobilised lipolytic bacteria actively degraded fats that otherwise persisted largely unchanged.

The better performance of the lower bead loading (25 beads) is attributed to improved diffusion of substrate to the immobilised cells and greater dissolved-oxygen availability per bacterium in the less crowded flask, both of which would be constrained at the higher bead density.

Table 5: Optical density (OD_{600}) of synthetic dairy wastewater treated with alginate-immobilised lipolytic bacteria over 15 days

Sample	Day 1	Day 2	Day 4	Day 15	% Reduction (Day 1→15)
Control (no beads)	0.904	0.935	0.880	0.800	11.5%
Flask 1 (25 beads)	1.240	0.785	0.612	0.529	57.3%
Flask 2 (50 beads)	1.115	0.955	0.649	0.601	46.1%

3.7 Overall Discussion

Both macrophyte-planted CFW units clearly outperformed the unplanted control across every major pollutant parameter, confirming that plants and their root-associated microbiota rather than passive physical or chemical processes were the principal drivers of treatment in this system. Canna indica delivered the strongest overall organic-matter and nutrient removal (BOD 34%, COD 33%, TKN 34%, phosphate 33%), consistent with literature describing its dense root system and capacity to sustain diverse heterotrophic microbial communities (Kumar and Dutta, 2019;

Konnerup et al., 2009). Cyperus papyrus, while somewhat weaker for BOD removal (24%), maintained higher dissolved oxygen throughout (5.7 vs 4.8 ppm at Day 21) and achieved superior sulphate removal, pointing to a distinct root-zone oxygenation and microbial profile consistent with earlier reports (Kyambadde et al., 2004).

The 24–34% removal efficiencies obtained are lower than the 60–90% typically reported from warm-climate studies, an outcome attributable to the winter conditions of this trial, which suppress both microbial metabolism and root activity (Vymazal, 2010; Faulwetter et al., 2009). Nonetheless, even under these sub-optimal conditions the planted units removed two to four times more pollutant load than the control, supporting the case for CFWs as an effective nature-based treatment option even in cooler seasons, with performance likely to approach the 60–80% range reported from tropical or summer trials once temperatures rise (Benrahmane et al., 2022; Konnerup et al., 2009). The complementary success of the lipolytic-bacteria bead trial further suggests that a bioaugmented pre-treatment step targeting fats and oils could usefully be integrated with a CFW system to improve overall dairy-wastewater treatment performance.

4. Conclusions

Constructed floating wetlands planted with Canna indica or Cyperus papyrus substantially outperformed an unplanted control in treating synthetic dairy wastewater over a 21-day winter trial. Canna indica achieved the best overall performance (34% BOD, 33% COD, 34% TKN and 33% phosphate removal), while Cyperus papyrus performed comparably for nutrients (30–31%) and showed superior sulphate removal (20%) and dissolved-oxygen maintenance. The unplanted control achieved only 14% BOD/COD removal and its dissolved oxygen collapsed from 6.35 to 2.8 ppm, underscoring the central role of macrophytes and their root-associated microbiota including root-mediated oxygen transfer in driving pollutant removal. Although removal efficiencies (24–34%) were lower than those reported from tropical or summer studies (60–90%) because of reduced microbial and plant activity in winter, the planted units still removed two to four times more pollutant load than the control, demonstrating that CFWs remain functional under cold conditions. A complementary trial confirmed that lipolytic bacteria immobilised in sodium alginate beads can substantially reduce wastewater turbidity (up to 57.3% in 15 days), indicating potential as a bioaugmented pre-treatment step for fat-rich dairy effluent. Taken together, these findings support constructed floating wetlands particularly when planted with Canna indica or Cyperus papyrus and combined with immobilised lipolytic bacteria as a practical, low-cost, nature-based treatment option for small and medium dairy units lacking access to conventional treatment infrastructure.

Future work should test the system under summer conditions, evaluate a polyculture of both macrophytes, extend the retention time or move to continuous-flow operation, use real (rather than synthetic) dairy effluent, integrate the lipolytic bacteria beads directly within the CFW units as an in-situ pre-treatment step, and assess performance at pilot scale together with quantification of pollutant accumulation in plant tissue.

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