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Remediation of acid whey from the cheese industry in Libres, Puebla, Mexico, using a subsurface horizontal flow wetland

Remediación de lactosuero ácido de la industria quesera en Libres, Puebla, México, utilizando un humedal de flujo horizontal subsuperficial

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Abstract

This study evaluated the feasibility of a subsurface horizontal flow wetland as treatment of acid whey produced by artisanal cheese manufactury in Libres, Puebla, Mexico. A laboratory-scale wetland prototype (area = 2.88 m^2) was designed and built using coarse gravel, sand, and red tezontle as filtering substrates, while *Phragmites australis* was used as macrophyte species. The projected full-scale wetland area was estimated using a simplified Kickuth model considering a whey discharge of 25 m^3 day^{-1}. The untreated acid whey exhibited an acidic pH (4.86 ± 0.06) and high organic pollution indicators, as the chemical oxygen demand (COD) and biochemical oxygen demand (BOD_5) values of 76,002.12 ± 4,675.49 mg L^{-1} and 39,917.74 ± 888.85 mg L^{-1}, respectively. After 125 days of wetland adaptation with discontinue discharges and 6 days with whey discharges of 51.5 L day^{-1}, the wetland achieved COD and BOD_5 removal values of 97.36% and 97.14%, respectively. Significant reductions in fats and oils, total solids, and turbidity were also observed, while the effluent pH reached neutral values (7.10 ± 0.14). These results demonstrate the potential of constructed wetlands as a sustainable alternative for preliminary acid whey treatment in rural artisanal cheese production systems.

Keywords: remediation, whey, wetland, *Phragmites australis*.

Resumen

Este estudio evaluó la viabilidad de un humedal de flujo horizontal subsuperficial para el tratamiento del suero ácido producido por la fabricación artesanal de queso en Libres, Puebla, México. Se diseñó y construyó un prototipo de humedal a escala de laboratorio (área= 2.88 m^2) utilizando grava gruesa, arena y tezontle rojo como sustratos filtrantes, mientras que *Phragmites australis* se utilizó como especie macrófita. El área proyectada del humedal a escala real se estimó utilizando un modelo Kickuth simplificado considerando una descarga de suero de 25 m^3 $día^{-1}$. El suero ácido sin tratar exhibió un pH ácido (4.86 ± 0.06) y altos indicadores de contaminación orgánica, como los valores de demanda química de oxígeno (DQO) y demanda bioquímica de oxígeno (DBO_5) de 76,002.12 ± 4,675.49 mg L^{-1} y 39,917.74 ± 888.85 mg L^{-1}, respectivamente. Tras 125 días de adaptación del humedal con descargas discontinuas y 6 días con descargas de suero de 51.5 L $día^{-1}$, el humedal alcanzó valores de eliminación de DQO y DBO_5 de 97.36% y 97.14%, respectivamente. También se observaron reducciones significativas en grasas y aceites, sólidos totales y turbidez, mientras que el pH del efluente alcanzó valores neutros (7,10 ± 0,14). Estos resultados demuestran el potencial de los humedales artificiales como una alternativa sostenible para el tratamiento preliminar del suero ácido en sistemas rurales de producción de queso artesanal.

Palabras clave: remediación, lactosuero, humedal, *Phragmites australis*.

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1 Introduction

The dairy industry generates large volumes of wastewater characterized by high concentrations of organic matter, suspended solids, fats, and nutrients, which represent a significant environmental concern when discharged without adequate treatment (Mateo-Díaz *et al.*, 2023; Obayomi *et al.*, 2026; Valencia-Denicia and Ramírez-Castillo, 2009). In Mexico, approximately 40 varieties of cheese are currently recognized, many of which are produced through artisanal processes under small-scale rural conditions (González-Córdova *et al.*, 2016). During cheese production, whey is generated as the principal byproduct, representing nearly 85-95% of the original milk volume and retaining approximately 55% of milk nutrients, including lactose, proteins, minerals, and soluble organic compounds (Azkarahman *et al.*, 2025; Mahmoudi *et al.*, 2024; Obayomi *et al.*, 2026). Consequently, the generation and disposal of whey have become critical environmental challenges for the dairy sector.

Mexico produces around 13 billion liters of milk per year and seeks to increase this value to 15 billion before 2030, in order to reduce dependence on imports (SADR, 2025). A substantial fraction of this production is destined for cheese manufacturing; however, the contribution of artisanal cheese factories is difficult to quantify because many production units operate informally or under traditional manufacturing schemes (Alvarado-Castillo *et al.*, 2024; González-Córdova *et al.*, 2016; Mazorra-Manzano and Moreno-Hernández, 2019). Under these conditions, acid whey is frequently discharged directly into soils, drainage canals, or water bodies without previous treatment, especially in rural regions where wastewater treatment infrastructure is limited or unavailable (Obayomi *et al.*, 2026).

This situation is particularly relevant in Libres, Puebla, Mexico, where an important artisanal cheesemaking tradition based on milk processing prevalence. According to information provided by local producers, approximately 30-40 artisanal cheese factories operate in the region, collectively generating several hundred thousand liters of acid whey daily. Figure 1 shows one of the drainage channels receiving untreated acid whey near the study area (own photograph). Continuous disposal of this effluent has promoted foul odors, microbial proliferation, soil acidification, and deterioration of local water quality, representing a potential environmental and public health concern for nearby communities.

Acid whey generated during artisanal cheesemaking is characterized by extremely high concentrations of chemical oxygen demand (COD), biochemical oxygen demand (BOD_5), fats, suspended solids, and nutrients (Mateo-Díaz *et al.*, 2023). These characteristics confer high biodegradability to the effluent, but simultaneously generate severe oxygen depletion and eutrophication risks when discharged into natural ecosystems. Additionally, the acidic nature of the wastewater may alter soil physicochemical properties and negatively affect aquatic microbial communities and surrounding vegetation (Azkarahman *et al.*, 2025; Obayomi *et al.*, 2026).

Generally, the treatment technologies for dairy wastewater, including membrane filtration, advanced oxidation, reverse osmosis, anaerobic digestion, and aerobic activated sludge systems, require elevated operational costs, specialized infrastructure, and high energy consumption. Such limitations restrict their implementation in small rural agroindustrial systems, where economical and low-maintenance treatment alternatives are required (Azkarahman *et al.*, 2025; Chen *et al.*, 2014; Kravtsov *et al.*, 2026; Obayomi *et al.*, 2026; Sehar and Nasser, 2019; Wu *et al.*, 2013). In this context, artificial wetlands have emerged as a promising environmentally sustainable technology for wastewater treatment due to their low energy requirements, reduced operational costs, simple maintenance, and capacity to integrate physical, chemical, and biological pollutant removal mechanisms (Gogoi *et al.*, 2026; Jóźwiakowski *et al.*, 2026; Kaur *et al.*, 2026; Sehar and Nasser, 2019).

Among the different wetland configurations, subsurface horizontal flow wetlands have shown technical feasibility for the treatment of agroindustrial wastes with elevated organic loads (Jóźwiakowski *et al.*, 2026; Kaur *et al.*, 2026). Organic matter removal in these systems occurs through sedimentation, filtration, adsorption, microbial degradation, and plant-associated rhizospheric processes (Kaur *et al.*, 2026). In addition, the use of locally available substrates and adapted macrophytes may improve treatment efficiency while reducing construction costs (Mahmoudi *et al.*, 2024; Mateo-Díaz *et al.*, 2023).

The treatment efficiency of constructed wetlands depends on several factors, including hydraulic retention time, organic loading rate, substrate characteristics, climatic conditions, and plant species selection (Kadlec and Wallace, 2009; Maine *et al.*, 2007; Nocetti *et al.*, 2020). Macrophytes such as *Phragmites australis* have been extensively used in constructed wetland systems due to their high adaptability to wastewater exposure, tolerance to fluctuating environmental conditions, and ability to stimulate microbial activity within the rhizosphere (López Cruz *et al.*, 2022; Mahmoudi *et al.*, 2024; Zhou *et al.*, 2024).

Previous studies have demonstrated the feasibility of constructed wetlands for dairy wastewater treatment. Sultana *et al.* (2014) demonstrated that pilot-scale horizontal subsurface flow wetlands with *Phragmites australis* could treat dilute whey wastewater with COD removal efficiencies above 90% under optimized hydraulic retention times. Likewise, Nocetti et al. (2020) observed efficient reductions of organic matter and nutrients in horizontal subsurface flow wetlands treating cheese factory dilute wastewater under microcosm conditions, and quantified the influence of plant species and substrates. Besides, Mahmoudi *et al.* (2024) reported high removal efficiencies of COD, BOD_5, suspended solids, and nutrients using horizontal subsurface flow wetlands planted with *Phragmites australis* for dairy dilute wastewater treatment. The authors reported removal efficiencies of 99.6% for total suspended solids (TSS), 80% for chemical oxygen demand (COD), 97% for biochemical oxygen demand (BOD_5), 90.4% for total nitrogen (TN), and 99.6% for total phosphorus (TP).

However, most previous studies have evaluated diluted dairy wastewater or effluents with considerably lower organic loads than those typically generated during artisanal cheesemaking processes. Information regarding the treatment of undiluted acid whey under conditions representative of rural artisanal cheese production remains limited, particularly in Latin American agroindustrial systems.

Therefore, the objective of this study was to design, construct, and evaluate a laboratory-scale subsurface horizontal flow constructed wetland as a preliminary treatment alternative for undiluted acid whey generated by an artisanal cheese factory in Libres, Puebla, Mexico. Additionally, the study aimed to estimate the technical feasibility of implementing a projected full-scale wetland under real operating conditions through topographic assessment, hydraulic scaling criteria, and physicochemical characterization of influent and effluent wastewater.

2 Materials and methods

2.1 *Residual whey sampling*

Acid whey samples were obtained from an artisanal cheese factory located in Libres, Puebla, Mexico. This production unit manufactures traditional artisanal cheeses and generates acid whey as the main residual byproduct of the process. Samples were collected directly from the whey storage tank using containers prepared according to NMX-AA-003-1980 guidelines. After collection, the samples were transported to the Environmental Laboratory of the Technological Institute of Tehuacán in insulated containers at 4-8 °C and stored at 4 ± 2 °C until physicochemical analysis.

2.2 *Topographic survey and estimation of the projected wetland area*

Prior to the design of the treatment system, a topographic survey was conducted within the cheese production unit to determine the available area for the potential installation of a subsurface horizontal flow constructed wetland under real operating conditions. In addition, the average daily acid whey discharge generated by the factory was estimated at approximately 25,000 L day^{-1}.

Subsequently, the required wetland area was estimated using a simplified form of the Kickuth model (Equation 1), derived from a first-order kinetic model for organic matter removal (Kadlec and Wallace, 2009; Saeed and Sun, 2011; Weerakoon *et al.*, 2020):

$$A_w = \frac{Q_d(\ln C_i - lnC_e)}{K_{BOD}} \tag{1}$$

where A_w is the wetland area (m^2), Q_d is the design flow rate (m^3 day^{-1}), C_i is the BOD_5 value in the influent (mg L^{-1}), C_e is the target BOD_5 concentration in the effluent (mg L^{-1}), and K_{BOD} is the overall removal constant for horizontal subsurface flow wetlands (0.15 m day^{-1}).

A design flow rate of 25 m^3 day^{-1} and a target effluent BOD_5 concentration of 60 mg L^{-1} were considered. The purpose of this calculation was to estimate the area required for a full-scale treatment system and evaluate the technical feasibility of installing the wetland within the available land area.

2.3 *Scaling and construction of the wetland prototype*

A laboratory-scale prototype was developed as a preliminary approach to evaluate the hydraulic behavior and pollutant removal capacity of the projected full-scale wetland under controlled operating conditions. The scaling criterion was based on maintaining the relationship between flow rate and wetland surface area, according to Equation 2 (Kadlec and Wallace, 2009):

$$\frac{Q_d}{A_w} = \frac{Q_{lab}}{A_{lab}} \tag{2}$$

where Q_{lab} and A_{lab} correspond to the flow rate (m^3 day^{-1}) and surface area (m^2) of the laboratory-scale prototype, respectively, while Q_d and A_w represent the design flow rate and estimated area of the projected full-scale wetland.

The linear dimensions were estimated using Equation 3.

$$L_{lab} = \frac{L_w}{19} \tag{3}$$

where L_{lab} is the laboratory-scale linear dimension and L_w is the corresponding dimension of the projected full-scale wetland.

The prototype was constructed using 3 mm thick transparent acrylic sheets. The wetland was divided longitudinally into three sections (A, B, and C) with proportions of 25:50:25. Coarse gravel (particle size: 30-50 mm) was placed in the inlet and outlet zones (sections A and C). The central section (B) was filled with a sand (particle size: 1-2 mm) and red tezontle (particle size: 1-5 mm); the sand was placed as the first layer and red tezontle as the surface layer, in proportions of 1:1. The selection of filtering materials was based on preliminary operational and hydraulic stability tests (data not shown).

Before incorporation into the system, all filtering materials were washed and disinfected using a 0.1% chlorine solution (1000 ppm). In addition, the prototype was covered laterally with shade mesh to minimize the entry of insects and external particulate matter.

The macrophyte used was *Phragmites australis*, which was established in the central section of the wetland. Eleven cuttings were planted with a spacing of approximately 4-7 cm between plants. The cuttings were collected from areas previously exposed to wastewater to facilitate their adaptation to the experimental conditions.

2.4 Start-up and operation of the wetland prototype

Prior to the experimental stage, the system underwent a 30-day acclimation period using tap water to promote stabilization and adaptation of the macrophytes. After this period, the wetland was operated for 131 days using undiluted acid whey. During the first 125 days of operation, the wetland prototype was fed with 51.5 L of acid whey every five days in order to promote the acclimation of the macrophytes and the stabilization of the biological processes within the system. Subsequently, from day 126 to day 131, the feeding regime was adjusted to 51.5 L day^{-1} to satisfy the hydraulic loading criterion established by the Q_d/A_w scaling relationship (Equation 2) (Kadlec and Wallace, 2009). This operational modification allowed the system to operate under conditions closer to those projected for the full-scale wetland.

To reduce the concentration of fats and settleable solids before entering the wetland, a 60 L gravity grease trap was incorporated into the treatment system. The unit consisted of three consecutive compartments designed to promote the separation of floating and sedimentable materials.

Acid whey first entered the initial compartment, where floating fats were retained. The flow then passed through a second compartment to facilitate sedimentation of suspended solids before reaching the final compartment, from which the pretreated whey was conveyed into the wetland prototype.

Influent acid whey samples were characterized at three different operational times (days 1, 60, and 120) in order to evaluate variations in wastewater composition during the experimental period. Effluent samples were collected at the end of the operational period (day 131) and analyzed in triplicate. Reported values correspond to mean values ± standard deviation obtained from analytical replicates.

2.5 Wetland performance assessment

The treatment performance of the wetland was evaluated through the removal efficiency of physicochemical parameters according to Equation 4 (Kadlec and Wallace, 2009).

$$Removal\ (\%) = \left[\frac{V_1 - V_2}{V_1}\right] \times 100 \tag{4}$$

where V_1 represents the initial value of the physicochemical parameter and V_2 corresponds to the value measured in the treated effluent.

2.6 Physicochemical analysis

2.6.1 pH

pH was measured using a HANNA HI98130 potentiometer according to the Mexican standard NMX-AA-008-SCFI-2016 for pH determination in natural, wastewater, and treated water samples.

2.6.2 Density and turbidity

Density was determined using a 25 mL pycnometer previously brought to constant weight. Measurements were performed at 20 °C according to Equation 5.

$$\delta = \frac{(m_3 - m_1) \bullet \delta_{H_2O}}{m_2 - m_1} \tag{5}$$

where m_3 is the mass of the pycnometer with water (g), m_2 is the mass of the pycnometer with sample (g), m_1 is the mass of the empty pycnometer (g), and δ_{H_2O} is the density of water (g mL^{-1}).

Turbidity was measured using a HACH 2100P turbidimeter according to Mexican standard NMX-AA-038-SCFI-2001.

2.6.3 Settleable solids, total solids and total volatile solids

Settleable solids were determined according to Mexican standard NMX-AA-004-SCFI-2013. Total solids and total volatile solids were quantified following the methodology described in Mexican standard NMX-AA-034-SCFI-2015.

2.6.4 Fats and oils

Fats and oils were determined according to Mexican standard NMX-AA-005-SCFI-2013 for recoverable fats and oils in natural and wastewater samples.

2.6.5 Biochemical oxygen demand and chemical oxygen demand

Biochemical oxygen demand (BOD_5) was determined according to Mexican standard NMX-AA-028-SCFI-2021. While the chemical oxygen demand (COD) was determined according to Mexican standard NMX-AA-030/1-SCFI-2012 using the open reflux method.

2.6.6 Macronutrients, major minerals, trace elements and toxic heavy metals

The characterization of macronutrients (total nitrogen, P), major minerals (K, Na, Ca, Mg, S), trace elements (Fe, Cu, Zn, Mn, Br) and toxic heavy metals (Pb, Cr, Cd) in influent and effluent samples was carried out at the Soil Science Department of the Central University Laboratory of the Autonomous University of Chapingo, Mexico.

Total nitrogen was determined by Kjeldahl steam distillation after diacid digestion. Total phosphorus was quantified by molybdovanadate photocolorimetry. Sodium (Na) and potassium (K) were determined by flame emission spectrophotometry, whereas calcium (Ca), magnesium (Mg), iron (Fe), copper (Cu), zinc (Zn), and manganese (Mn) were quantified by atomic absorption spectrophotometry.

Sulfur (S) was determined by barium chloride photocolorimetry and boron (B) by the azomethine-H method. Lead (Pb), chromium (Cr), and cadmium (Cd) were quantified by atomic absorption spectrophotometry after acid digestion.

2.7 Statistical analysis

The results of the physicochemical properties of the influent and effluent were analyzed using ANOVA with a 95% confidence level, followed by Tukey's test to define significant differences between means.

3 Results and discussion

3.1 Physicochemical characterization of acid whey and its potential environmental impact

Tables 1 and 2 show the physicochemical properties and chemical composition of the acid whey (influent) samples studied. The acid whey generated by the artisanal cheese factory under study exhibited a high organic and mineral load, reflecting the environmental risks associated with its direct discharge into natural water bodies (Obayomi *et al.*, 2026). Figure 1 shows the discharge channel contaminated with untreated acid whey in Libres, Puebla, Mexico, where the continuous disposal of this agri-food waste has led to the deterioration of local environmental conditions. The yellowish-green color and the visible turbidity of the wastewater are consistent with the high concentration of suspended and dissolved organic compounds characteristic of dairy wastes.

The wastewater under study presented a density value higher than the acid whey from a dairy plant from Santiago de Anaya, Hidalgo, Mexico (1.026 g/cm^3) (Callejas-Hernández *et al.*, 2012a); and an acidic pH (4.86 ± 0.06), which is characteristic of acid whey generated during artisanal cheese production processes involving lactic fermentation (Mazorra-Manzano and Moreno-Hernández, 2019; Vargas-Díaz *et al.*, 2023). Acidification of receiving environments may negatively affect microbial communities, nutrient availability, and aquatic ecosystems, particularly under conditions of continuous discharge (Jóźwiakowski *et al.*, 2026; Obayomi *et al.*, 2026).

The acid whey also exhibited elevated concentrations of total solids (62.30 ± 0.77 g L^{-1}) and total volatile solids (47.25 ± 0.10 g L^{-1}), indicating a substantial organic fraction associated with lactose residues, proteins, lipids, and suspended particles (Miranda, 2019; Obayomi *et al.*, 2026). In whey, volatile solids represent between 80 and 95% of total solids (Callejas-Hernández *et al.*, 2012a). In the present study, a 75.84% of the total reported solids correspond to volatile solids. Likewise, turbidity values reached 896.50 ± 7.34 NTU, while settleable solids were 9.51 ± 0.57 mL L^{-1}, confirming the presence of suspended organic material capable of generating sediment accumulation and oxygen depletion in receiving water bodies (Callejas-Hernández *et al.*, 2012a; Muvdi-Nova *et al.*, 2021; Obayomi *et al.*, 2026).

The most relevant characteristics of the acid whey was its extremely high organic load (Azkarahman *et al.*, 2025; Obayomi *et al.*, 2026). Chemical oxygen demand (COD) and biochemical oxygen demand (BOD_5) reached values of 76,002.12 ± 4,675.49 mg L^{-1} and 39,917.74 ± 888.85 mg L^{-1}, respectively (Table 1). The high standard deviation in the data may be associated with the natural variability of the wastewater; specifically, in this work, 3 different batches were evaluated over a period of two months. Regardless, the concentrations of COD and BOD_5 greatly exceed the limits commonly established for wastewater discharge (COD Permissible Limit in NOM-001-SEMARNAT-2021: 60 to 210 mg L^{-1}, depending on the receptor bodies). The elevated BOD_5/COD relationship suggests that a large fraction of the organic matter can be biologically degraded,

Table 1. Physicochemical properties of the influent and effluent of the subsurface horizontal flow wetland prototype.

Variable	Unit	Influent[a]	Effluent[b]	Removal (%)
pH	----	4.86 ± 0.06[b]	7.10 ± 0.14[a]	
Density	g cm^{-3}	1.07 ± 0.00[a]	1.06 ± 0.03[a]	
Turbidity	NTU	896.50 ± 7.34[a]	252.00 ± 1.73[b]	71.89
Settleable Solids (SS)	mL L^{-1}	9.51 ± 0.57[a]	3.13 ± 0.25[b]	67.09
Total Solids (TS)	g L^{-1}	62.30 ± 0.77[a]	15.78 ± 0.67[b]	74.67
Total Volatile Solids (TVS)	g L^{-1}	47.25 ± 0.10[a]	10.18 ± 0.79[b]	78.46
Fats and oils	mg L^{-1}	1,206.00 ± 0.64[a]	14.50 ± 0.00[b]	98.80
COD	mg L^{-1}	76,002.12 ± 4,675.49[a]	2,003.62 ± 19.75[b]	97.36
BOD_5	mg L^{-1}	39,917.74 ± 888.85[a]	1,140.98 ± 77.51[b]	97.14
Color	----	Yellowish-green	Yellowish	

The reported data are the mean and its standard deviation (n = 3). In the same row, the means that do not share a superscript letter are significantly different from each other ($p < 0.05$).
[a]Three batches were analyzed (days 1, 60 and 120).
[b]One batch was analyzed with three replicates on day 131.

Table 2. Macronutrients, major minerals, trace elements and toxic heavy metals in the influent and effluent of the subsurface horizontal flow wetland prototype.

Classification	Variable	Influent (mg L^{-1})[a]	Effluent (mg L^{-1})[b]
Macronutrient	Total N	522.40 ± 53.17	632.70
	Total P	113.88 ± 52.43	58.26
Major mineral	K	765.05 ± 339.34	2,015.00
	Na	647.50 ± 109.60	1,265.00
	Ca	588.05 ± 89.02	247.60
	Mg	285.35 ± 2.90	128.10
	S	243.55 ± 46.10	180.30
Trace element	Fe	27.55 ± 1.13	15.20
	Cu	0.78 ± 0.11	0.66
	Zn	7.00 ± 0.28	4.29
	Mn	3.79 ± 0.43	2.70
	Br	41.36 ± 17.57	38.87
Toxic heavy metal	Pb	ND	ND
	Cr	ND	ND
	Cd	ND	ND

ND: not detected.
[a]Three batches were sent to an external laboratory for analysis (days 1, 60 and 120). The reported data are the mean and its standard deviation (n = 3).
[b]One batch was sent to an external laboratory for analysis (day 131).

Figure 1. Discharge channel contaminated with acid whey in Libres, Puebla, Mexico.

which supports the potential application of biological treatment systems such as constructed wetlands (Kadlec and Wallace, 2009; Obayomi *et al.*, 2026).

Fats and oils also presented high concentrations (1,206.00 ± 0.64 mg L^{-1}), higher than that reported by Torres-Martínez and Romero-León (2020) (400 mg L^{-1}) and lower than the value obtained by Mazorra-Manzano and Moreno-Hernández (2019) (5,000 mg L^{-1}). The value obtained in the present study was higher than the Permissible Limit in NOM-001-SEMARNAT-2021 (15 to 21 mg L^{-1}, depending on the receptor bodies). The fat content of whey depends on the type of cheese, the type of milk, and the cheesemaking process used by artisanal cheese producers. Fats and oils content may contribute to the formation of surface films, oxygen transfer limitations, and clogging phenomena within natural and engineered systems (Kaur *et al.*, 2026). The incorporation of a grease trap prior to the wetland was therefore considered necessary to reduce the hydraulic and operational impacts associated with lipid accumulation.

In addition to the high organic load, the acid whey contained considerable concentrations of macronutrients and dissolved minerals (Table 2), particularly total nitrogen, total phosphorus, potassium, sodium, and calcium. These components may contribute to eutrophication processes when wastewater is discharged without treatment, promoting excessive microbial and algal growth in receiving environments. However, the nutrient-rich composition of acid whey also suggests the possibility of nutrient and mineral recovery and partial reutilization when appropriate treatment technologies are implemented (Azkarahman *et al.*, 2025; Kravtsov *et al.*, 2026; Obayomi *et al.*, 2026).

The environmental implications associated with the direct disposal of untreated acid whey are especially relevant in rural agroindustrial regions, where artisanal cheese production commonly lacks adequate wastewater treatment infrastructure. Under these conditions, the implementation of low-cost and low-energy treatment alternatives such as subsurface horizontal flow wetlands may represent a feasible strategy for reducing the organic pollution load and mitigating the environmental impact associated with artisanal dairy production.

3.2 *Design criteria, scaling approach, and construction of the subsurface horizontal flow wetland prototype*

The implementation of a subsurface horizontal flow constructed wetland was proposed as an economically and environmentally sustainable alternative for the treatment of acid whey generated by artisanal cheese production in Libres, Puebla, Mexico. Prior to the experimental design, a topographic survey in an artisanal cheese factory was conducted to identify the available land area for the potential installation of a full-scale treatment system under real operating conditions. Figure 2.a shows the evaluated area and the spatial conditions considered during the preliminary planning stage of the proposed wetland system.

The estimated wetland area was calculated using the simplified Kickuth model based on first-order organic matter removal kinetics (Equation 1). A design flow rate of 25 m^3 day^{-1}, an initial influent BOD_5 concentration of 39,917.74 mg L^{-1} (Table 1), and a target effluent BOD_5 concentration of 60 mg L^{-1} were considered as design criteria in accordance with the expected wastewater generation of the artisanal cheese factory. The projected area obtained from the model (approximately 1083 m^2) indicated that the treatment system could be physically implemented within the available land area identified during the topographic assessment (Figure 2.a, red lines). Consequently, a subsurface horizontal flow wetland (Figure 2.b and 2.c) as a treatment strategy for acid whey generated under artisanal production conditions was proposed.

Subsequently, a laboratory-scale wetland prototype was constructed to evaluate the hydraulic behavior and pollutant removal capacity of the projected full-scale system under controlled experimental conditions. Figure 3 shows the laboratory-scale wetland. The prototype constructed had internal dimensions of 2.40 m × 1.20 m and an operating depth of 0.60 m, resulting in a total treatment area of 2.88 m^2. The dimensions of the wetland surface area were determined according to Equation 3.

The wetland prototype was longitudinally divided into three functional sections (A, B, and C) with proportional distributions of 25:50:25 according to the projected wetland (Figure 2.c). Coarse gravel was incorporated into the inlet and outlet zones to improve hydraulic distribution and reduce the risk of clogging caused by the elevated concentration of suspended solids and fats present in the acid whey. In the central section, a mixture of sand and red tezontle was incorporated as filtering media to promote solid retention, microbial attachment, and root development. Red tezontle was included due to its high porosity and surface area, properties that favor water retention, microbial colonization, and oxygen transfer within the wetland matrix.

The macrophyte selected for the experimental system was *Phragmites australis* due to its adaptability to wastewater exposure and its extensive application in constructed wetland systems. The cuttings were collected from zones previously exposed to wastewater, thereby facilitating their acclimation to the experimental conditions and the high organic load associated with acid whey treatment.

To reduce the concentration of fats and settleable solids before entering the wetland, a gravity grease trap consisting of three consecutive compartments was integrated into the treatment system. Figure 4 presents the final experimental wetland configuration (Figure 4.a) and the pretreatment unit (Figure 4.b). The grease trap promoted the progressive separation of floating fats and sedimentable material, thereby reducing hydraulic obstruction risks and improving the operational stability of the wetland during prolonged treatment periods.

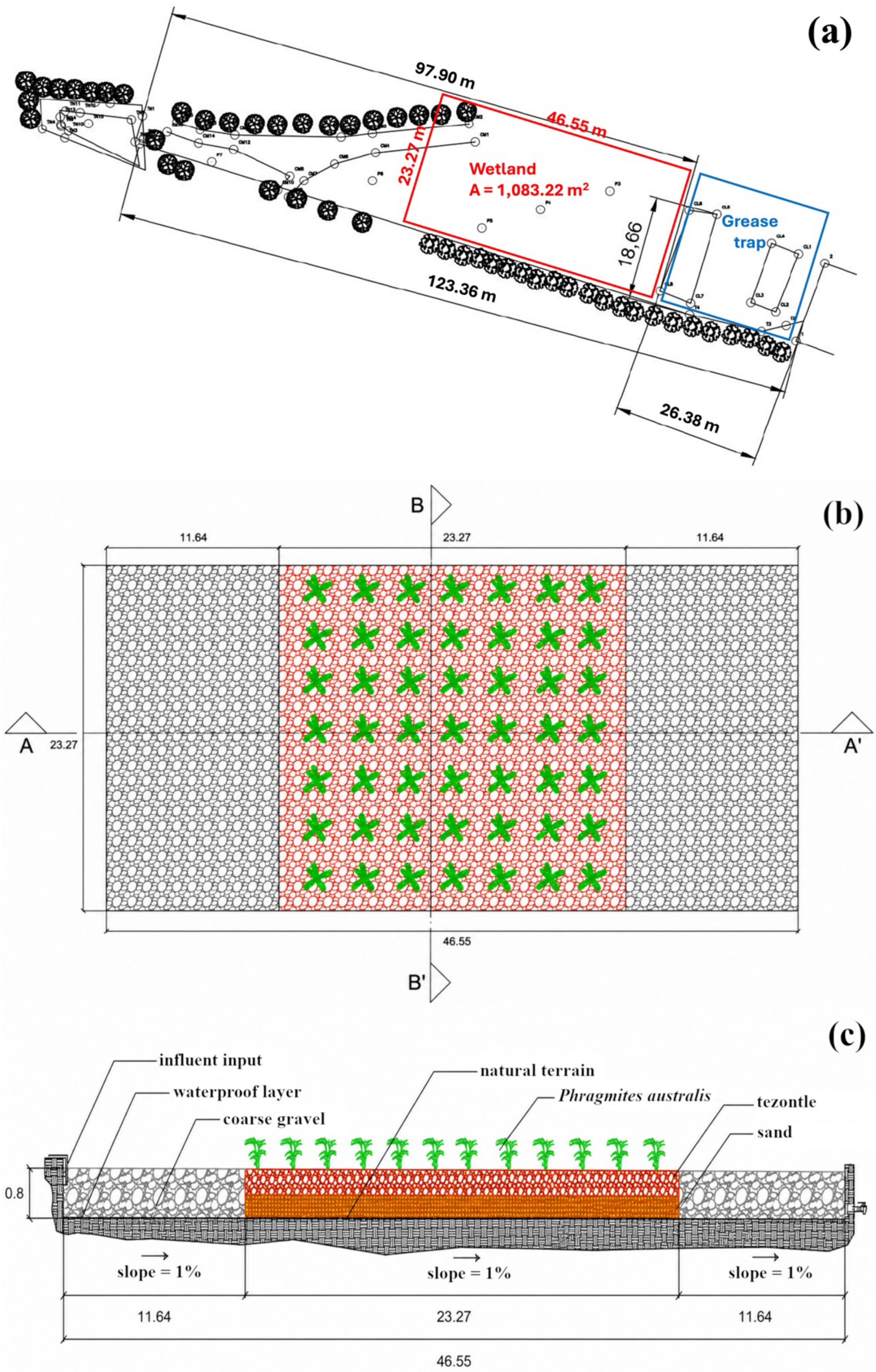

Figure 2. Land available for subsurface horizontal flow wetland installation (a), plan view (b) and longitudinal section A-A' of the projected full-scale wetland.

Figure 3. Subsurface horizontal flow wetland prototype. a) Acrylic box divided in three sections to load the substrates, b) coarse gravel at the influent inlet and effluent outlet, c) wetland with tezontle and *Phragmites australis* cuttings (Day 1), and wetland with *Phragmites australis* ready to start the discharge of acid whey (Day 30).

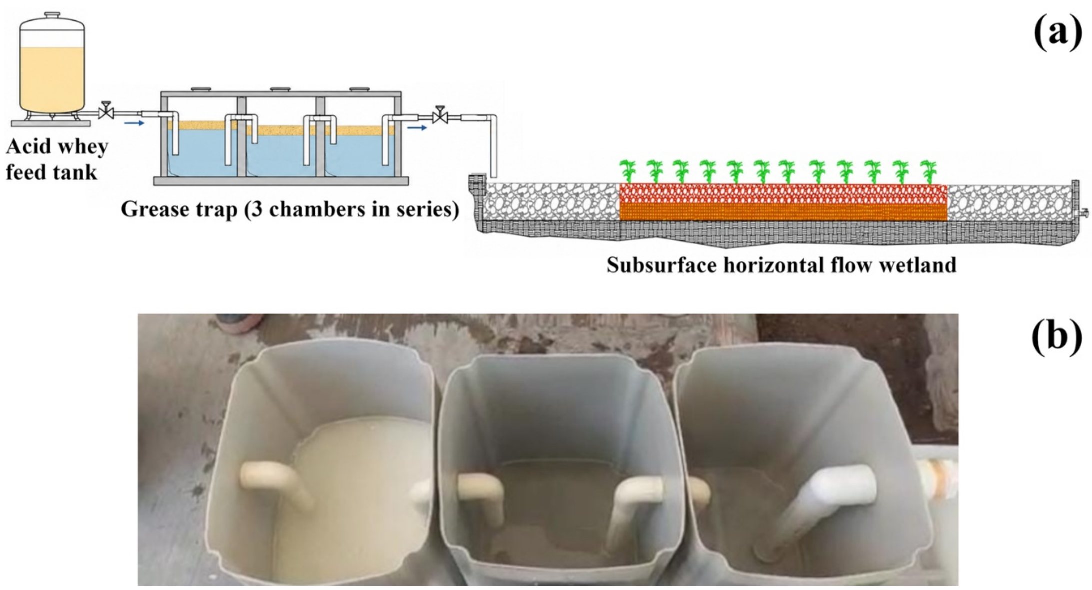

Figure 4. Subsurface horizontal flow wetland diagram (a) with the incorporation of the grease trap (b) for the pre-treatment of acid whey.

During the first 125 days of operation, the wetland prototype was fed with 51.5 L of acid whey every five days in order to promote the acclimation of the macrophytes and the stabilization of biological processes within the system. Subsequently, from day 126 to day 131, the feeding regime was adjusted to 51.5 L day^{-1} to satisfy the hydraulic loading criterion established by the scaling relationship ($Q/A \cong 0.02$)

(Equation 2). This operational modification allowed preserve the surface hydraulic loading conditions between the projected system and the experimental prototype.

3.3 Treatment performance and pollutant removal efficiency of the subsurface horizontal flow wetland prototype

The treatment performance of the subsurface horizontal flow wetland prototype was evaluated through the comparison of physicochemical parameters measured in the influent and effluent acid whey samples after 131 days of operation (Table 1). Overall, the system demonstrated a substantial reduction in organic load, suspended material, and fats, indicating that the combined action of sedimentation, filtration, adsorption, and biological degradation contributed significantly to wastewater treatment.

One of the most relevant results was the reduction in chemical oxygen demand (COD), which decreased from 76,002.12 ± 4,675.49 mg L^{-1} in the influent to 2,003.62 ± 19.75 mg L^{-1} in the effluent, corresponding to a removal efficiency of 97.36%. Similarly, biochemical oxygen demand (BOD_5) decreased from 39,917.74 ± 888.85 mg L^{-1} to 1,140.98 ± 77.51 mg L^{-1}, achieving a removal efficiency of 97.14% (Table 1). These results suggest that the wetland prototype was capable of reducing a considerable fraction of the biodegradable organic matter present in the acid whey.

Similar trends of high COD (80%) and BOD_5 (97%) removal values were obtained by Mahmoudi *et al.* (2024), but it is important to highlight that the authors studied the efficiency of three consecutive horizontal subsurface flow wetland for the treatment of dairy wastewater in Tunisia, using a mixture of common reed (*Phragmites australis*), cattails (*Typha latifolia*) and papyrus (*Cyperus papyrus*). Moreover, a dilute whey (20% in water) with lower COD and BOD_5 concentrations was treated.

The high removal of organic matter in the present study may be associated with several simultaneous mechanisms occurring within the treatment system. The pretreatment stage provided by the grease trap likely contributed to the reduction of suspended fats and particulate material before entering the wetland. Subsequently, the filtering media promoted physical retention of solids and favored the establishment of microbial biofilms responsible for the degradation of dissolved organic compounds (Kadlec and Wallace, 2009; Kaur *et al.*, 2026). In addition, the presence of *Phragmites australis* may have enhanced oxygen transfer within the rhizosphere, stimulating aerobic and facultative microbial processes involved in organic matter stabilization (Kadlec and Wallace, 2009; Kaur *et al.*, 2026; Zhou *et al.*, 2024).

Despite the significant reductions observed for COD and BOD_5, the final concentrations in the effluent remained considerably high compared to permissible limits commonly established for wastewater discharges into receiving bodies (NOM-001-SEMARNAT-2021). This behavior indicates that although the wetland prototype effectively reduced the organic pollution load, additional treatment stages or process optimization strategies would still be required for large-scale implementation under continuous operating conditions. The elevated residual organic load may be associated with the extremely high initial concentration of biodegradable compounds characteristic of acid whey generated during artisanal cheese production.

The wetland prototype also showed considerable efficiency in the removal of fats and oils, reducing their concentration from 1,206.00 ± 0.64 mg L^{-1} to 14.50 ± 0.00 mg L^{-1}, corresponding to a 98.80% removal efficiency (Table 1). This reduction may be attributed to the combined effect of the grease trap, sedimentation processes, filtration through the substrate matrix, and microbial degradation of lipid compounds (Jóźwiakowski *et al.*, 2026; Kadlec and Wallace, 2009; Kaur *et al.*, 2026). The decrease in fats and oils is particularly relevant because lipid accumulation is one of the principal factors associated with clogging and hydraulic instability in constructed wetlands treating dairy wastewater (Kaur *et al.*, 2026).

Similarly, total solids and total volatile solids were reduced by 74.67% and 78.46%, respectively (Table 1). The reduction of volatile solids suggests that part of the biodegradable organic fraction was biologically transformed during the treatment process. In contrast, settleable solids showed a lower removal efficiency (67.09%), indicating that a fraction of suspended particulate matter remained in the effluent even after filtration through the substrate matrix.

The pH of the treated effluent increased from 4.86 ± 0.06 to 7.10 ± 0.14 after treatment (Table 1), indicating a substantial reduction in acidity during the operational period. This behavior may be associated with microbial degradation of organic acids, buffering effects generated by the substrate materials, and physicochemical stabilization processes occurring within the wetland (Kadlec and Wallace, 2009; Kaur *et al.*, 2026; Mahmoudi *et al.*, 2024). The increase toward neutral pH conditions is environmentally relevant because acidic discharges may negatively affect soil quality, aquatic ecosystems, and microbial communities in receiving environments (Azkarahman *et al.*, 2025; Obayomi *et al.*, 2026; Sultana *et al.*, 2014).

Turbidity also decreased considerably from 896.50 ± 7.34 NTU to 252.00 ± 1.73 NTU, reflecting the capacity of the filtering media to retain suspended particulate material.

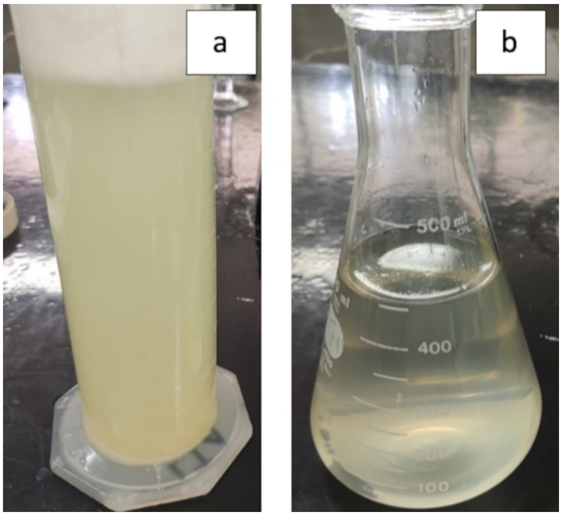

Figure 5. Physical appearance of the influent (a) and effluent (b) of the subsurface horizontal flow wetland prototype.

The physical appearance of the influent and effluent is shown in Figure 5. The combined use of gravel, sand, and red tezontle likely contributed to improving filtration efficiency and promoting sediment accumulation within the wetland matrix.

Regarding nutrients and dissolved minerals (Table 2), the behavior of nitrogen compounds suggested a complex transformation process within the wetland. Total nitrogen value increased in the effluent, which may be associated with the mineralization of nitrogen-containing organic compounds present in the acid whey, leading to the release of soluble nitrogen species during biological degradation (Kadlec and Wallace, 2009; Zhou *et al.*, 2024). Additionally, the high organic loading rate and the limited oxygen availability characteristic of horizontal subsurface flow wetlands may have restricted complete nitrification-denitrification processes (Zhou *et al.*, 2024). Similar behavior has been reported in wetlands treating highly concentrated agroindustrial effluents with elevated biodegradable organic loads (Kadlec and Wallace, 2009).

In contrast, total phosphorus concentration decreased after treatment, suggesting partial retention within the substrate matrix and assimilation by the macrophytes and microbial biomass (Nocetti *et al.*, 2020). The concentrations of several dissolved minerals and trace elements also decreased in the effluent, indicating that adsorption, sedimentation, and plant uptake mechanisms may have contributed to their partial removal during treatment (Mahmoudi *et al.*, 2024; Maine *et al.*, 2007).

However, some dissolved minerals such as sodium and potassium exhibited increased concentrations in the effluent. This behavior may be related to partial solubilization of the substrate matrix, concentration effects associated with evapotranspiration, or the release of soluble ions during organic matter degradation processes within the wetland (Kadlec and Wallace, 2009).

The concentrations of toxic heavy metals such as Pb, Cr, and Cd remained low (not detected) in both influent and effluent samples (Table 2), suggesting that the artisanal cheese production process did not represent a significant source of metallic contamination.

Overall, the results obtained demonstrate that the subsurface horizontal flow wetland prototype represents a promising preliminary treatment alternative for reducing the environmental impact associated with acid whey generated by artisanal cheese production. However, the persistence of high residual organic concentrations and the limited nitrogen removal capacity indicate that complementary treatment stages, longer hydraulic retention times, or hybrid wetland configurations may be necessary to improve treatment efficiency under full-scale operating conditions.

Summary

The acid whey generated by artisanal cheese production in Libres, Puebla, Mexico, exhibited a high organic and nutrient load, characterized by elevated concentrations of COD, BOD_5, total solids, fats and oils, and dissolved minerals. These characteristics confirm the significant environmental risk associated with the direct discharge of untreated acid whey into natural ecosystems, particularly in rural agro-industrial regions lacking wastewater treatment infrastructure.

Topographical assessment and the application of the simplified Kickuth model demonstrated the technical feasibility of implementing a subsurface horizontal flow wetland as a treatment alternative under the whey flow rate of a cheese production unit, and its physicochemical properties. Furthermore, the laboratory-scale wetland prototype allowed for the evaluation of the hydraulic behavior and contaminant removal capacity of the projected treatment system under controlled conditions.

The wetland prototype achieved substantial reductions in organic pollution indicators, including COD, BOD_5, fats and oils, turbidity, total solids, and volatile solids. These results suggest that the combined action of sedimentation, filtration, adsorption, microbial degradation, and plant-related processes significantly contributed to the stabilization of the acid whey. In particular, the incorporation of a grease trap and the use of porous filter materials, such as red tezontle (volcanic rock), improved the system's operational performance and reduced the potential risk of clogging.

Despite the high removal efficiencies observed for organic matter, the treated effluent still exhibited residual contaminant concentrations exceeding commonly accepted levels for wastewater discharge. Furthermore, the nitrogen dynamics within the wetland suggested that limited mineralization and nitrification-denitrification processes may have restricted complete nutrient removal under the evaluated operating conditions.

Conclusions

In general terms, the results obtained indicate that subsurface horizontal flow constructed wetlands represent an alternative for the preliminary treatment of acid whey generated by artisanal cheese production. However, further optimization strategies, including longer hydraulic retention times, hybrid wetland configurations, substrate types, or complementary post-treatment stages, are still required to improve nutrient removal and achieve effluent quality suitable for large-scale discharge or reuse applications. Furthermore, more extensive operational studies and a long-term evaluation under real environmental conditions are needed as future lines of research.

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AI tool use statement

AI-based tools were used for grammar correction and style enhancement. No AI systems were used to create, analyze, or interpret the scientific content of this manuscript. The experimental design, data analysis, discussions, and conclusions were developed exclusively by the authors.

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