



Development of a Molasses-Based Synthetic Wastewater for Simulating Sugar Industry Effluent: Physicochemical Characterization and Reproducibility Evaluation

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Key words	Abstract
Molasses wastewater, Synthetic wastewater, Sugar industry effluent, Reproducibility, Environmental realism, Nutrient balance.	The inherent variability of sugar industry wastewater constitutes a major challenge for the reproducibility of laboratory-scale experiments and the reliable evaluation of wastewater treatment technologies. This study presents the development of a molasses-based synthetic wastewater (MBSW) designed to provide a reproducible and representative model of sugar industry effluent under controlled experimental conditions. Unlike conventional synthetic formulations relying on simple carbon sources such as glucose, the proposed MBSW incorporates biodegradable organic matter and balanced nutrient concentrations to better reproduce the physicochemical characteristics of real agro-industrial wastewater. The synthetic wastewater was prepared by diluting raw molasses with distilled water and supplementing the mixture with nitrogen and phosphorus sources. Its physicochemical properties were characterized through measurements of pH, electrical conductivity, chemical oxygen demand (COD), biochemical oxygen demand (BOD ₅), total suspended solids (TSS), total nitrogen, and total phosphorus. The developed formulation exhibited a high biodegradable organic load, with COD values ranging from 4800 to 5050 mg/L and BOD ₅ values between 2700 and 2900 mg/L, yielding a BOD ₅ /COD ratio of approximately 0.57. Balanced nutrient concentrations, stable conductivity values, and low variability among replicate samples confirmed the reproducibility, physicochemical consistency, and suitability of the proposed MBSW for standardized biological wastewater treatment experiments and comparative research across laboratories worldwide.
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1. Introduction

Agro-industrial activities, particularly those related to sugar production, generate large volumes of wastewater characterized by high concentrations of organic matter, suspended solids, dissolved salts, and nutrients. The composition of these effluents varies considerably over time as a result of differences in processing operations, raw material quality, and seasonal fluctuations. Such variability constitutes a major challenge in environmental engineering, as it limits experimental reproducibility and hinders the reliable comparison of wastewater treatment technologies (Poddar and Sahu, 2017; Rawat et al., 2023). Recent studies have further emphasized that the heterogeneity of industrial wastewater remains a major obstacle to the standardization of laboratory investigations and the objective evaluation of treatment processes (Moussa et al., 2023; Zhang et al., 2022).

A wide range of treatment technologies has been investigated for sugar industry wastewater, including anaerobic digestion, activated sludge processes, physicochemical treatments, and nature-based systems (Gbadeyan et al., 2024). Although these approaches often achieve high pollutant removal efficiencies, their performance remains strongly influenced by the characteristics of the influent, particularly its organic load and nutrient composition (Rawat et al., 2023). Poddar and Sahu (2017) reported that fluctuations in wastewater quality significantly affect treatment efficiency, while Moussa et al. (2023) emphasized that wastewater composition strongly influences biological treatment efficiency and process stability. Consequently, variations in wastewater quality may alter microbial activity, substrate biodegradability, and process stability, thereby reducing the reproducibility and comparability of laboratory-scale investigations (Rawat et al., 2023; Zhang et al., 2022).

To address these limitations, synthetic wastewater formulations have been extensively employed in experimental studies because they provide controlled and reproducible operating conditions that facilitate process optimization and performance evaluation. Nevertheless, most conventional formulations rely on simplified carbon sources, such as glucose, acetate, or starch, which fail to reproduce the physicochemical complexity of real agro-industrial effluents (Sharma & Bhattacharya, 2021). Consequently, these synthetic media often provide only a limited representation of the interactions among organic matter, nutrients, dissolved minerals, and microbial communities that occur in actual wastewater systems. (Kato and Kansha, 2024). Improving the environmental realism of synthetic wastewater has therefore become an important requirement for increasing the reliability, reproducibility, and comparability of laboratory investigations (Rawat et al., 2023).

Molasses represents a promising alternative substrate for the development of synthetic wastewater. As a by-product of sugar manufacturing, it naturally contains fermentable sugars, organic compounds, minerals, and micronutrients that contribute to a more realistic simulation of sugar industry effluents. Compared with glucose-based formulations, molasses-based synthetic wastewater offers a more complex organic matrix capable of better reproducing the physicochemical characteristics of real agro-industrial wastewater. Furthermore, the use of molasses supports resource valorization and is consistent with the principles of sustainable waste management and the circular economy. Fito et al. (2019) and Gbadeyan et al. (2024) highlighted the environmental importance of valorizing sugar industry by-products within sustainable wastewater management strategies, while Gbadeyan et al. (2024) further emphasized their potential for sustainable resource recovery and circular bioeconomy applications.

Despite these advantages, studies devoted to the development of standardized and reproducible molasses-based synthetic wastewater remain scarce. In particular, limited attention has been paid to the physicochemical consistency of such formulations under controlled laboratory conditions. Existing studies have mainly focused on wastewater treatment technologies rather than on the formulation and validation of reproducible synthetic influents. For example, Macarie and Le Mer (2006) reviewed biological treatment processes for sugar mill wastewater, whereas Asaithambi and Matheswaran (2016) investigated the electrochemical treatment of simulated sugar industry effluents. Similarly, Nure et al. (2017) evaluated the treatment of molasses spent wash using activated carbon without specifically addressing the reproducibility of synthetic wastewater formulations. This lack of standardized synthetic wastewater models restricts the reproducibility of experimental investigations and complicates meaningful comparisons among biological wastewater treatment studies.

Accordingly, this study aims to develop a molasses-based synthetic wastewater (MBSW) that serves as a reproducible and representative model of sugar industry effluent. Specifically, the objectives are to (i) establish a

reproducible preparation protocol, (ii) characterize the physicochemical properties of the developed formulation, and (iii) evaluate its physicochemical consistency and reproducibility by comparison with values reported for real sugar industry wastewater in the literature.

2. Materials and Methods

2.1. Source and Preservation of Molasses

Raw molasses used in this study was collected from a local sugar processing facility in Cameroon. Immediately after collection, it was transferred into airtight polyethylene containers and stored at 4 °C to minimize physicochemical and microbiological alterations prior to use. All experimental analyses were conducted under controlled laboratory conditions at the Water Chemistry Laboratory of the École Nationale Supérieure des Sciences Agro-Industrielles (ENSAI), University of Ngaoundere, Cameroon.

The raw molasses exhibited a dark brown colour, an acidic pH (approximately 5.2), high viscosity, and a high organic content, characteristics commonly associated with sugar industry by-products. Preliminary characterization also revealed elevated conductivity and organic load, confirming its suitability as a substrate for synthetic wastewater preparation (Fito et al., 2019).

2.2. Preparation of Molasses-Based Synthetic Wastewater

Several preliminary dilution ratios (1:20, 1:40, 1:60, and 1:80, v/v) were evaluated to identify the formulation that best reproduced the physicochemical characteristics of sugar industry wastewater. Among the tested formulations, the 1:20 dilution ratio was selected because it produced COD and BOD₅ values that most closely matched those commonly reported for real sugar industry effluents (Poddar and Sahu, 2017).

The selected synthetic wastewater was prepared by diluting raw molasses with distilled water at a ratio of 1:20 (v/v). Nitrogen and phosphorus were supplied through the addition of ammonium chloride (NH₄Cl) and potassium dihydrogen phosphate (KH₂PO₄), respectively. Final concentrations of 150 mg/L NH₄Cl and 30 mg/L KH₂PO₄ were selected to provide nutrient conditions comparable to those commonly adopted in synthetic wastewater formulations reported in the literature.

The resulting mixture was homogenized by magnetic stirring for 15 min to ensure a uniform distribution of dissolved and suspended constituents. No pH adjustment was performed prior to physicochemical characterization in order to preserve the intrinsic properties of the molasses-based formulation.

Before analysis, all prepared samples were allowed to stabilize at room temperature for 12 h to ensure physicochemical equilibrium.

2.3. Physicochemical Characterization

Physicochemical analyses were performed in accordance with the *Standard Methods for the Examination of Water and Wastewater* (APHA et al., 2017). The following parameters were determined:

- pH was measured using a calibrated digital pH meter (accuracy ± 0.01);
- Electrical conductivity was measured using a conductivity meter calibrated at 25 °C;
- Chemical oxygen demand (COD) was determined using the closed reflux dichromate method;
- Biochemical oxygen demand (BOD₅) was determined using the respirometric method after five days of incubation at 20 °C;
- Total suspended solids (TSS) were quantified using the gravimetric filtration method;
- Total nitrogen (TN) was determined using the Kjeldahl digestion method;
- Total phosphorus (TP) was measured spectrophotometrically using the molybdenum blue method.

All analyses were performed in triplicate to assess the reproducibility and physicochemical consistency of the developed synthetic wastewater.

2.4. Statistical Analysis

Experimental results are presented as mean $\pm$ standard deviation (SD). Owing to the preliminary nature of the study and the limited number of replicates ($n = 3$), the data were analysed using descriptive statistics only. This approach

was considered sufficient to evaluate the variability and reproducibility of the proposed synthetic wastewater formulation.

3. Results

3.1. Physicochemical Characteristics of the Molasses-Based Synthetic Wastewater

The physicochemical characteristics of the developed molasses-based synthetic wastewater (MBSW) are summarized in Table 1. The results obtained from the three independent replicates demonstrate that the proposed formulation successfully reproduced the principal physicochemical features commonly associated with sugar industry wastewater. Moreover, the limited variability observed among replicate preparations confirms the consistency of the preparation protocol and the stability of the developed formulation.

Table 1. Physicochemical characteristics of the developed MBSW (n = 3).

Parameters	MBSW (This Study)	Literature Range
COD (mg/L)	4933	3000–6000
BOD ₅ (mg/L)	2800	1500–4000
TSS (mg/L)	847	400–1200
pH	5.5	4.5–7.0
Total Nitrogen (mg/L)	100	50–150
Total Phosphorus (mg/L)	19.7	10–50

The measured pH remained slightly acidic, varying between 5.4 and 5.6, with an average value of 5.50 ± 0.10 . Such a narrow range indicates excellent reproducibility of the formulation while preserving the acidic characteristics generally observed in molasses-derived effluents (Poddar and Sahu, 2017; Rawat et al., 2023). Electrical conductivity ranged from 2100 to 2300 $\mu\text{S}/\text{cm}$, reflecting the presence of dissolved ionic species originating from both the raw molasses and the nutrient supplements incorporated into the formulation.

COD values ranged from 4800 to 5050 mg/L, whereas BOD₅ values varied between 2700 and 2900 mg/L, yielding a BOD₅/COD ratio of approximately 0.57. This ratio indicates that a substantial fraction of the organic matter was readily biodegradable which is consistent with values reported for sugar industry wastewater (Fito et al., 2019; Moussa et al., 2023). The consistency of these measurements across the three independent preparations further demonstrates the reliability of the proposed formulation.

TSS concentrations ranged from 820 to 870 mg/L, while total nitrogen and total phosphorus averaged 100 ± 5 mg/L and 19.7 ± 1.5 mg/L, respectively. These nutrient concentrations provide conditions compatible with microbial growth and therefore support the use of the developed wastewater in biological treatment studies (Rawat et al., 2023; Kato and Kansha, 2024).

Taken together, these results demonstrate that the proposed MBSW combines biodegradable organic matter, suspended solids, dissolved minerals, and balanced nutrient concentrations, thereby reproducing the principal physicochemical characteristics of sugar industry wastewater.

3.2. Comparison with Literature Values

To evaluate the representativeness of the proposed formulation, its physicochemical characteristics were compared with values commonly reported for sugar industry wastewater in the literature. The corresponding comparison is presented in Table 2, while a graphical representation of the measured parameters relative to literature ranges is provided in Figure 1.

Table 2. Comparison between the developed MBSW and literature values.

Parameter	MBSW (This Study)	Literature Range
COD (mg/L)	4933	3000–6000
BOD ₅ (mg/L)	2800	1500–4000
TSS (mg/L)	847	400–1200
pH	5.5	4.5–7.0
Total Nitrogen (mg/L)	100	50–150
Total Phosphorus (mg/L)	19.7	10–50

The comparison indicates that all measured parameters, including COD, BOD₅, TSS, pH, total nitrogen, and total phosphorus, fall within the ranges generally reported for real sugar industry effluents (Poddar and Sahu, 2017; Fito et al., 2019; Rawat et al., 2023; Moussa et al., 2023). This close agreement demonstrates that the proposed formulation successfully reproduces the physicochemical characteristics of actual agro-industrial wastewater while maintaining controlled laboratory conditions.

As illustrated in Figure 1, the experimental values remain well within the ranges reported by previous studies. This agreement confirms not only the representativeness of the developed synthetic wastewater but also its potential as a reliable experimental matrix for laboratory-scale investigations involving both biological and physicochemical wastewater treatment processes.

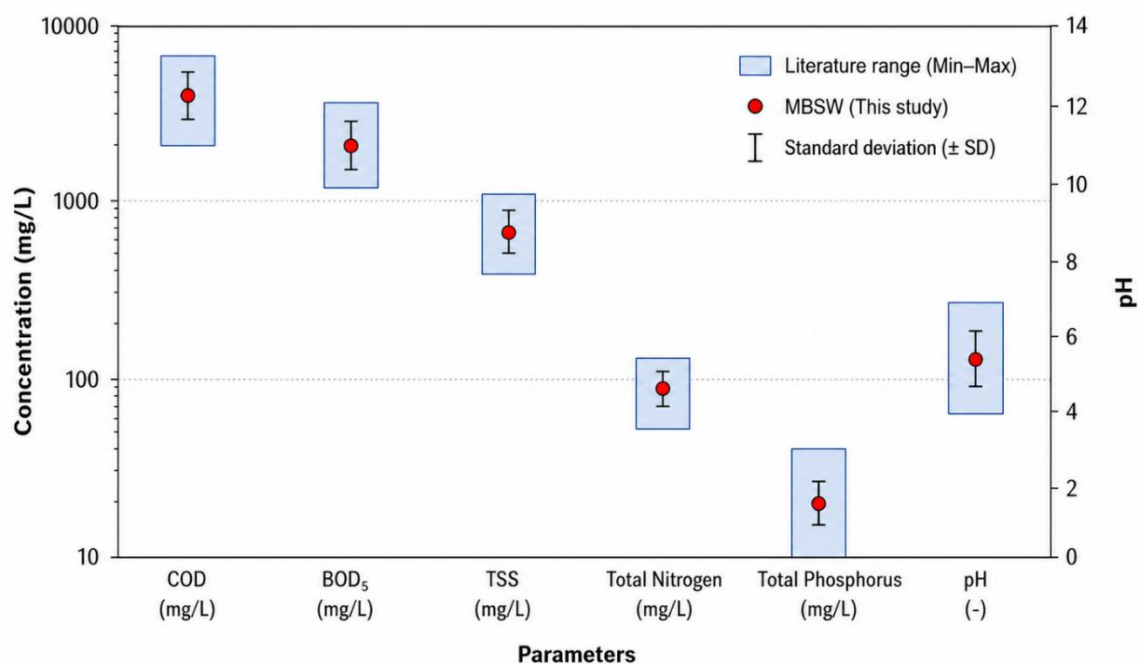


Figure 1. Comparison between the physicochemical characteristics of the developed MBSW and literature ranges reported for sugar industry wastewater. Error bars represent the variability reported in the literature.

3.3. Reproducibility Analysis

The reproducibility of the proposed formulation was assessed by examining the variability of all measured physicochemical parameters across the three independent preparations. The reproducibility of the two principal organic pollution indicators, COD and BOD₅, is illustrated in Figure 2.

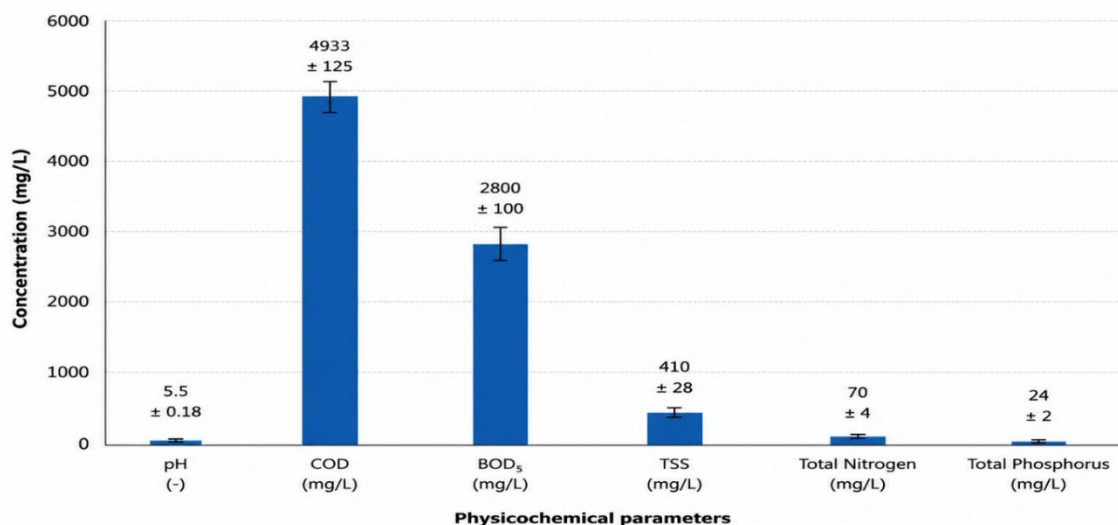


Figure 2. Reproducibility of the physicochemical parameters measured for the developed molasses-based synthetic wastewater (MBSW). Data are expressed as mean $\pm$ standard deviation from three independent replicates ($n = 3$).

Only minor variations were observed for pH, conductivity, COD, BOD₅, TSS, total nitrogen, and total phosphorus, indicating that the preparation protocol produced highly consistent wastewater samples.

The small standard deviations associated with COD and BOD₅ demonstrate that the biodegradable organic fraction remained remarkably stable from one preparation to another. Likewise, the limited variability observed for conductivity, suspended solids, and nutrient concentrations confirms that the proportions of dissolved minerals and particulate matter were consistently maintained throughout the preparation procedure.

Because nutrient availability strongly influences microbial activity and biological treatment efficiency, the reproducibility of nitrogen and phosphorus concentrations represents an important characteristic of the proposed formulation (Moussa et al., 2023; Rehman et al., 2024). Overall, these results demonstrate that the developed MBSW provides a stable and reproducible physicochemical matrix suitable for controlled laboratory investigations.

3.4. Integrated Physicochemical Profile

To provide a global overview of the developed formulation, the integrated physicochemical profile of the MBSW is presented in Figure 3.

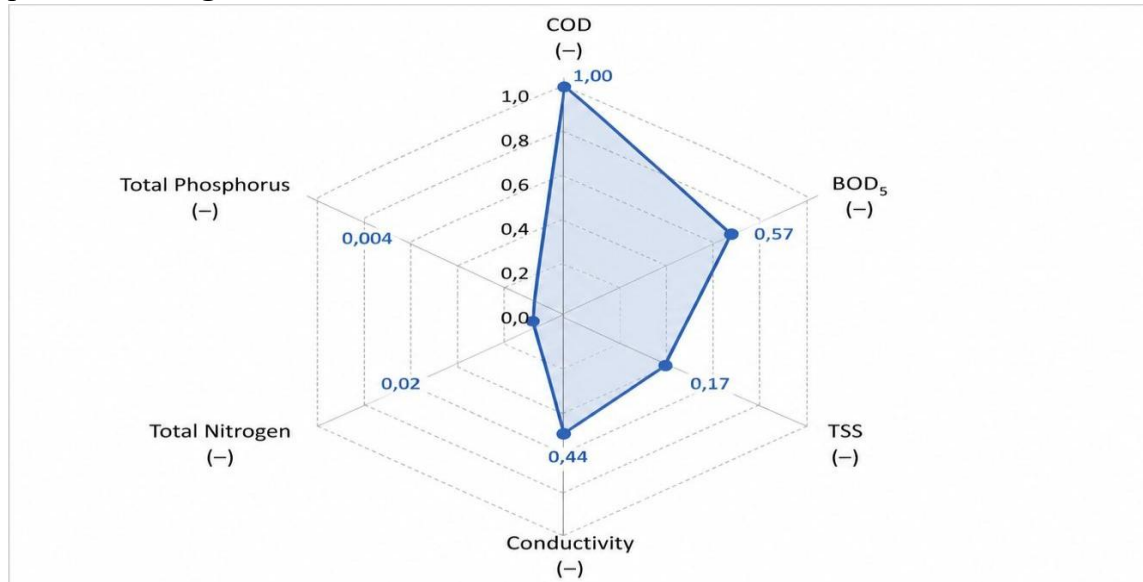


Figure 3. Normalized physicochemical profile of the developed molasses-based synthetic wastewater (MBSW). Physicochemical parameters were normalized using min–max normalization to facilitate comparison among variables expressed in different units and numerical scales.

To facilitate comparison among variables expressed in different units and numerical scales, all physicochemical parameters were normalized between 0 and 1 using min–max normalization.

The normalized profile highlights the balanced composition of the proposed formulation, combining a high biodegradable organic load with moderate suspended solids, dissolved mineral content, and adequate nutrient concentrations. COD and BOD₅ exhibited the highest normalized values, confirming the predominance of biodegradable organic matter (Fito et al., 2019; Gbadeyan et al., 2024). Conductivity and TSS also contributed substantially to the overall profile, reflecting the presence of dissolved ions and suspended particles typically encountered in molasses-derived wastewater.

In addition, nitrogen and phosphorus concentrations remained within ranges favourable for microbial growth, thereby ensuring adequate nutritional conditions for biological treatment processes (Rawat et al., 2023; Kato and Kansha, 2024). Collectively, these results demonstrate that the proposed formulation successfully reproduces the physicochemical complexity of sugar industry wastewater while maintaining excellent reproducibility under laboratory conditions.

5. Conclusion

This study developed a molasses-based synthetic wastewater (MBSW) capable of reproducing the principal physicochemical characteristics of sugar industry effluents under controlled laboratory conditions. The proposed formulation exhibited a high biodegradable organic load, balanced nutrient concentrations, dissolved mineral content, and suspended solids levels consistent with those commonly reported for real agro-industrial wastewater. The measured COD and BOD₅ concentrations, together with the BOD₅/COD ratio of approximately 0.57, confirmed the predominance of readily biodegradable organic matter within the developed formulation. Furthermore, the low variability observed among the three independent replicates demonstrated the reproducibility and physicochemical consistency of the preparation protocol, highlighting its suitability for laboratory-scale studies requiring stable and controlled experimental conditions.

Compared with conventional glucose-based synthetic wastewater, the proposed MBSW provides a more representative physicochemical matrix by preserving the intrinsic complexity of molasses, including fermentable sugars, dissolved minerals, and micronutrients. This enhanced environmental realism is expected to improve the reliability, reproducibility, and comparability of experimental investigations involving biological wastewater treatment processes.

Although the present work was limited to physicochemical characterization and reproducibility assessment, the developed formulation constitutes a reliable and cost-effective experimental matrix for future environmental engineering studies. Further investigations should evaluate its performance in biological treatment systems, including activated sludge processes, anaerobic digestion, and rhizoremediation technologies, in order to validate its applicability under operational conditions.

Overall, the proposed molasses-based synthetic wastewater represents a practical and reproducible alternative to conventional synthetic formulations and provides a robust and standardized experimental platform for future laboratory investigations on sugar industry wastewater treatment.

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Competing interests

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

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