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Research Article

ANAEROBIC TREATABILITY STUDIES OF SUGAR INDUSTRY WASTEWATER IN BATCH REACTOR

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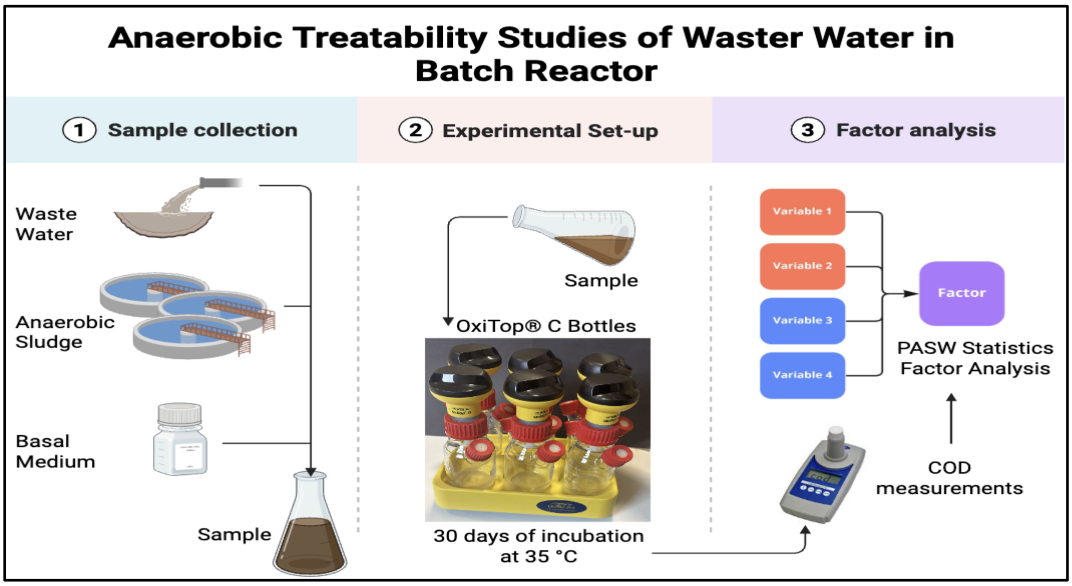
ABSTRACT: This study investigates the effectiveness of different anaerobic sludge inoculum in the removal of organic matter from sugar industry wastewater with varying initial COD concentrations. The inoculum was obtained from anaerobic treatment plants in Burdur, Eskişehir, and Kastamonu, and were tested in batch reactors under three different wastewater concentrations: 6.360 g/L, 12.310 g/L, and 21.735 g/L of COD. The treatment efficiency was assessed through the COD removal percentage. The results of the variance analysis showed that both inoculum location and wastewater concentration had a significant impact on COD removal, with an R^2 value of 0.980, indicating a highly robust model. The Tukey HSD test revealed significant differences in organic matter removal efficiency between the inocula from Kastamonu, Burdur, and Eskişehir, with Kastamonu demonstrating the highest efficiency. Additionally, higher wastewater concentration resulted in lower organic matter removal efficiencies, especially when the COD concentration increased from 6.360 g/L to 12.310 g/L. The interaction between inoculum location and wastewater concentration was also found to be significant, emphasizing the importance of both factors in optimizing anaerobic treatment processes. This study highlights the need for selecting appropriate inoculum and adjusting treatment conditions based on wastewater characteristics for improved anaerobic wastewater treatment performance.

Keywords: *Anaerobic Treatability, Wastewater, Sugar industry, Batch Reactor, Factor Analysis*



1. INTRODUCTION

The sugar industry is one of the largest agro-based industries, producing significant amounts of wastewater with high organic content. This wastewater primarily originates from various stages of sugarcane and sugar beet processing, such as washing, extraction, clarification, and crystallization. Sugar industry wastewater is characterized by high concentrations of carbohydrates, proteins, fats, oils, suspended solids, and nutrients, with elevated levels of COD and biochemical oxygen demand (BOD) (Beltrán et al., 2021). If discharged untreated, this wastewater can lead to severe environmental issues, including oxygen depletion in aquatic ecosystems, eutrophication, and toxicity to aquatic life. The high organic load also promotes microbial growth, resulting in the rapid deterioration of water quality and unpleasant odors (Simate et al., 2011). Conventional aerobic treatment methods, such as activated sludge systems, oxidation ponds, and trickling filters, have been widely used due to their ability to remove organic pollutants efficiently. However, these methods have significant drawbacks, including high energy consumption, large space requirements, and excessive sludge production (Simate et al., 2011). The need for sustainable and cost-effective treatment solutions has led to increased interest in anaerobic treatment technologies. Anaerobic wastewater treatment processes are widely recognized for their ability to effectively degrade organic pollutants, especially in wastewater with high organic load. These processes are advantageous due to their lower energy requirements, reduced sludge production, and ability to handle high-strength wastewater (Zhang et al., 2019; Wang et al., 2021). Among the various anaerobic treatment systems, the use of active sludge inocula plays a crucial role in determining the overall treatment efficiency. Active sludge, consisting of a diverse microbial community, is responsible for the degradation of organic pollutants, including COD and BOD (Liu et al., 2018). The performance of anaerobic treatment systems can be influenced by various factors, such as the inoculum's microbial composition, the wastewater characteristics, and the operational conditions. Previous studies have highlighted the importance of inoculum origin, noting that microbial communities from different regions or treatment plants may exhibit distinct abilities to degrade organic pollutants (Hussain et al., 2020; Yang et al., 2022). This variability in inoculum performance is often attributed to the microbial diversity, the presence of specialized strains, and the adaptation to local environmental conditions. Additionally, the concentration of organic matter in wastewater, commonly represented by COD levels, plays a significant role in the efficiency of anaerobic treatment processes. Higher COD concentrations often challenge the microbial community's capacity to effectively degrade the pollutants, resulting in reduced treatment efficiency (Zhou et al., 2020). Therefore, understanding the interaction between inoculum type and wastewater density is essential for optimizing anaerobic treatment systems for different wastewater characteristics. The primary objective of this study is to investigate the influence of inoculum type and wastewater density on the anaerobic COD removal efficiency. In this study, three different inocula, obtained from anaerobic treatment plants in Burdur, Eskişehir, and Kastamonu, were used to treat wastewater samples with varying COD concentrations (6.360 g/L, 12.310 g/L, and 21.735 g/L). The results of this research provide insights into the optimal selection of inoculum types and the management of wastewater characteristics for improved anaerobic treatment performance.

2. MATERIALS AND METHODS

2.1. Sugar Industry Wastewater

This study investigates the anaerobic treatment of wastewater obtained from the Eskişehir Sugar Factory using batch reactors. The wastewater sample was collected from the facility and immediately stored at 4°C to maintain its physicochemical stability. The initial pH of the sample was measured at 6.2 and was subsequently adjusted to 7.0 to optimize conditions for anaerobic microbial activity. The COD of the wastewater was determined to be 21.735 g/L.

2.2. Anaerobic Sludge

Three distinct anaerobic sludge samples were utilized as inoculum, each sourced from the anaerobic treatment units of sugar factories located in Burdur, Eskişehir, and Kastamonu. These inoculums were selected to represent a diverse microbial consortium, potentially exhibiting varying efficiencies in the degradation of organic matter. Prior to use, the sludge samples were homogenized through mixing and subsequently filtered using a 1 mm pore-size filter to remove larger particulates. Key physicochemical parameters relevant to anaerobic treatment efficiency, including pH, Total Suspended Solids (TSS), Total Solids (TS), Total Volatile Solids (TVS), and Volatile Suspended Solids (VSS), were analyzed and are presented in Table 3.1.

2.3. Basal Medium

The basal medium used in the experiments was formulated to support anaerobic microbial growth by providing essential micro- and macronutrients. The composition of the basal medium included NH_4Cl (1,200 mg/L), $MgSO_4 \cdot 7H_2O$ (400 mg/L), KCl (400 mg/L), $Na_2S \cdot 9H_2O$ (300 mg/L), $CaCl_2 \cdot 2H_2O$ (50 mg/L), $(NH_4)_2HPO_4$ (80 mg/L), $FeCl_2 \cdot 4H_2O$ (40 mg/L), $CoCl_2 \cdot 6H_2O$ (10 mg/L), KI (10 mg/L), $MnCl_2 \cdot 4H_2O$ (0.5 mg/L), $CuCl_2 \cdot 2H_2O$ (0.5 mg/L), $ZnCl_2$ (0.5 mg/L), $AlCl_3 \cdot 6H_2O$ (0.5 mg/L), $NaMoO_4 \cdot 2H_2O$ (0.5 mg/L), H_3BO_3 (0.5 mg/L), $NiCl_2 \cdot 6H_2O$ (0.5 mg/L), $NaWO_4 \cdot 2H_2O$ (0.5 mg/L), Na_2SeO_3 (0.5 mg/L), and cysteine

(10 mg/L) (Uygun et al., 2025; Ersen et al., 2021). This medium was essential for maintaining optimal conditions for microbial metabolism during the anaerobic treatment process.

2.3. Experimental Setup

The optimization of anaerobic treatment for sugar industry wastewater was examined through batch experiments designed using a statistical approach. Factorial experimental designs are particularly effective when multiple variables need to be assessed for their combined effects on a response variable (Akcal et al., 2011). In this study, the Biochemical Methane Potential (BMP) analysis system was employed, using 200 mL Oxitop C bottles (OxiTop® Control AN12, WTW, Weilheim, Germany) equipped with mixing mechanisms for each batch test (Tezcan et al., 2018; Malay et al., 2018). A full factorial design was implemented, comprising 9 distinct experimental setups. The experiments were designed to investigate two main factors: the initial wastewater concentrations and the type of inoculum used. The wastewater concentrations tested were 6.360 g/L, 12.310 g/L, and 21.735 g/L, with a total volume of 200 mL per batch. The inoculum types tested included: (a) Eskişehir Sugar Factory Anaerobic Sludge, (b) Burdur Sugar Factory Anaerobic Sludge, and (c) Kastamonu Sugar Factory Anaerobic Sludge. In all experimental conditions, a mineral medium containing essential nutrients required for the growth of anaerobic microorganisms was added. pH stability was maintained by the addition of $NaHCO_3$, while dissolved oxygen was removed using $Na_2S \cdot 9H_2O$. The pH was carefully adjusted to 7.0 ± 0.1 to ensure optimal conditions for microbial activity. The nitrogen gas was delivered for 3 to 4 minutes after the Oxitop bottles were closed, and thus oxygen in medium was removed. All experiments were conducted at 35°C over a 30-day incubation period (Iscen et al., 2024; Comoglu et al., 2016; Iscen et al., 2008). At the end of the experiment, the COD removal efficiency was quantified. Each experimental setup was performed in duplicate to ensure reliability. Statistical analyses were conducted using the SPSS 18 (PASW Statistics) software to interpret the data.

2.4. Analytical Methods

COD, TS, TSS, TVS, VSS were determined by standard methods (Uygun et al., 2025; Ersen et al., 2021; APHA, 1992). These parameters were used to assess the effectiveness of each inoculum in reducing organic load in the wastewater.

2.5. Data Analysis

The performance of each anaerobic sludge inoculum was analyzed by comparing the organic matter removal efficiencies across different experimental conditions. The removal efficiency was determined by calculating the percentage of COD removed in each setup. Statistical analysis was performed using Analysis of Variance (ANOVA) to evaluate the impact of two main factors: the inoculum source (from Burdur, Eskişehir, and Kastamonu) and the initial COD concentrations (6.360 g/L, 12.310 g/L, and 21.735 g/L). The Tukey Honestly Significant Difference (HSD) test was applied for multiple comparisons to identify significant differences in organic matter removal efficiency between the inocula. The interaction between inoculum source and wastewater concentration was also considered to assess their combined effect on treatment efficiency. All data analyses were conducted using SPSS 23 (PASW Statistics) software.

3. RESULTS

3.1. Inoculums Features

Some properties of anaerobic sludges used in batch reactor studies are given in Table 3.1. The pH values of the sludge samples varied between 6.83 and 8.6, with Eskişehir Sugar Factory sludge exhibiting the highest pH (8.6), while Kastamonu Sugar Factory sludge had the lowest (6.83). TS concentrations ranged from 50.09 g/L (Kastamonu) to 88.578 g/L (Burdur), indicating differences in the solid content of the sludge samples. Similarly, TSS values showed variation, with the highest concentration observed in Burdur (68.025 g/L) and the lowest in Kastamonu (30.35 g/L). Regarding organic content, TVS and VSS measurements highlighted notable differences among the samples. The Burdur sludge exhibited the highest TVS (24.56 g/L) and VSS (25.945 g/L), indicating a higher proportion of biodegradable organic matter compared to Eskişehir and Kastamonu sludges. In contrast, the Kastamonu sludge had the lowest organic content, with VSS values of 13.2375 g/L.

Table 3.1. Physicochemical characteristics of anaerobic sludge inoculum sourced from sugar factory treatment units in Burdur, Eskisehir, and Kastamonu.

Parameter	Eskişehir Sugar Factory Anaerobic Sludge	Burdur Sugar Factory Anaerobic Sludge	Kastamonu Sugar Factory Anaerobic Sludge
pH	8.6	7.15	6.83
TS (g/L)	78.39	88.578	50.09
TSS (g/L)	67.29	68.025	30.35
TVS (g/L)	9.664	24.56	12.068
VSS (g/L)	18.78	25.945	13.2375

3.2. Batch Reactor Studies

The full factorial experiment setup of sugar industry wastewater established in batch studies and the average COD removal rates obtained as a result of each experiment are given in Table 3.2. Two main factors were investigated: the initial wastewater concentration and the type of inoculum used. Wastewater concentrations were set at three levels: 6.360 g/L, 12.310 g/L, and 21.735 g/L of initial COD. For the inoculum, anaerobic sludge samples were sourced from three different regions: Eskişehir, Burdur, and Kastamonu, each assigned corresponding coded values (-1, 0, and 1) to facilitate factorial analysis. The results demonstrate that COD removal efficiency varied depending on both factors. Across all experiments, high COD removal efficiencies were achieved, generally exceeding 90%. Notably, the highest COD removal (96.96%) was observed in Experiment 9, which used the highest wastewater concentration (21.735

g/L) and Kastamonu inoculum (coded as 1). Conversely, the lowest removal efficiency (83.37%) was recorded in Experiment 2, corresponding to the lowest wastewater concentration and Burdur inoculum (coded as 0). These results indicate that both the initial concentration of wastewater and the source of inoculum significantly influenced the COD removal performance in batch reactor conditions. Specifically, the Kastamonu inoculum consistently showed superior performance across varying concentrations, suggesting that the microbial community from this source may possess a higher capacity for organic matter degradation in sugar industry wastewater.

Table 3.2. Full factorial experimental setup for sugar industry wastewater batch studies and the corresponding average COD removal rates.

Experiments	**Wastewater Concentration**		**Inoculum Type**		**COD Removal (%)**
	Real	**Code**	**Real**	**Code**	
1	6.360 g/L	-1	Eskişehir	-1	93.55
2	6.360 g/L	-1	Burdur	0	83.37
3	6.360 g/L	-1	Kastamonu	1	94.54
4	12.310 g/L	0	Eskişehir	-1	95.84
5	12.310 g/L	0	Burdur	0	91.17
6	12.310 g/L	0	Kastamonu	1	96.06
7	21.735 g/L	1	Eskişehir	-1	95.13
8	21.735 g/L	1	Burdur	0	94.54
9	21.735 g/L	1	Kastamonu	1	96.96

3.3. Factor Analysis

3.3.1. Variance Analysis

Based on the results presented in Table 3.2., variance analysis was conducted to evaluate the individual effects of factors as well as their interactions. The experimental design results were analyzed using SPSS 23 (PASW Statistics) software. Table 3.3. presents the variance analysis for COD removal.

Table 3.3. ANOVA results for COD removal, assessing the individual and interaction effects of experimental factors.

Tests of Between-Subjects Effects

Dependent Variable: COD Removal

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	273.663[a]	8	34.208	55.270	0.000
Intercept	157233.095	1	157233.095	254041.264	0.000
Inoculum Source	131.023	2	65.511	105.847	0.000
Wastewater Concentration	83.893	2	41.947	67.773	0.000
Inoculum Source * Wastewater Concentration (Interaction)	58.747	4	14.687	23.729	0.000
Error	5.570	9	0.619		
Total	157512.329	18			
Corrected Total	279.234	17			

a. $R^2 = 0.980$ (Adjusted $R^2 = 0.962$)

The variance analysis results revealed that the model, which includes both the inoculum location and wastewater concentration as factors, has a statistically significant effect on the COD removal percentage. The overall model was found to significantly explain the variation in the dependent variable, with an F-value of 55.270 and a p-value of 0.000 ($p < 0.05$), confirming that the model is statistically significant. The fixed term (Intercept) in the model was also found to be statistically significant ($F = 254.041$, $p = 0.000$), suggesting that the baseline removal percentage is a crucial factor in the model. Furthermore, both the inoculum location and wastewater density were identified as significant factors influencing the COD removal percentage.

The inoculum source had a significant impact on COD removal, with an F-value of 105.847 and a p-value of 0.000. This indicates that the origin of the active sludge plays a crucial role in the efficiency of COD removal in the anaerobic treatment process.

Wastewater concentration (initial COD concentration) also significantly affected the COD removal percentage, with an F-value of 67.773 and a p-value of 0.000. This suggests that different concentrations of organic matter in the wastewater influence the overall treatment efficiency.

The interaction between the inoculum location and wastewater density was found to be statistically significant ($F = 23.729$, $p = 0.000$). This indicates that the effect of inoculum type on COD removal varies depending on the density of the wastewater.

The model's explanatory power was very high, with an R^2 value of 0.980 and an adjusted R^2 of 0.962. This shows that 98% of the variation in the COD removal percentage can be explained by the model, indicating that the model is highly robust and reliable in explaining the observed outcomes.

3.3.2. Tukey HSD Test (Multiple Comparisons)

To further understand the source of the significant differences observed in the ANOVA results, a Tukey HSD test was performed for multiple comparisons.

Inoculum Location Comparisons:

According to statistical data, the comparison of inoculum sources in COD removal was given in Table 3.4. The comparison between Kastamonu and Burdur showed a significant difference in COD removal (6.1616%), with a p-value of 0.000, suggesting that the inoculum from Kastamonu was more efficient in COD removal compared to Burdur. The comparison between Kastamonu and Eskişehir showed a small difference of 1.0116%, which was not statistically significant ($p = 0.120$). This indicates that the inoculum from Kastamonu and Eskişehir had a similar performance in removing COD. The comparison between Burdur and Eskişehir showed a significant difference of -5.1500% ($p = 0.000$), with Burdur's inoculum showing a higher COD removal efficiency than Eskişehir's.

Table 3.4. Multiple comparisons of COD removal based on inoculum source using Tukey HSD test. Significant differences at the 0.05 level are marked with (*). The table presents mean differences, standard errors, significance values, and 95% confidence intervals for pairwise comparisons.

Multiple Comparisons						
Dependent Variable: COD Removal						
Tukey HSD						
Inoculum Source (I)	Inoculum Source (J)	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Kastamonu	Burdur	6.1616*	0.45421	0.000	4.8935	7.4298
	Eskişehir	1.0116	0.45421	0.120	-0.2565	2.2798
Burdur	Kastamonu	-6.1616*	0.45421	0.000	-7.4298	-4.8935
	Eskişehir	-5.1500*	0.45421	0.000	-6.4181	-3.8818
Eskişehir	Kastamonu	-1.0116	0.45421	0.120	-2.2798	0.2565
	Burdur	5.1500*	0.45421	0.000	3.8818	6.4181
Based on observed means. The error term is Mean Square(Error) = 0.619.						
*. The mean difference is significant at the 0.05 level.						

Wastewater Concentration Comparisons:

According to statistical data, the comparison of wastewater concentrations in COD removal was given in Table 3.5. The comparison between 6.360 g/L, 12.310 g/L wastewater concentration showed a significant difference of -3.8668% (p = 0.000), indicating that higher COD concentrations (12.310 g/L) resulted in a lower COD removal efficiency. The comparison between 6.360 g/L and 21.735 g/L wastewater concentration showed an even greater significant difference of -5.0574% (p = 0.000), suggesting that increasing the COD concentration further reduces the COD removal efficiency. The comparison between 12.310 g/L and 21.735 g/L wastewater concentration showed a smaller, non-significant difference of -1.1906% (p = 0.065), indicating that the impact of further increasing the COD concentration on COD removal was not as pronounced as the initial increases from 6.360 to 12.310 g/L.

*Table 3.5. Results of Tukey HSD multiple comparisons test for COD removal efficiency at different wastewater concentrations. The table displays mean differences, standard errors, significance levels, and 95% confidence intervals for pairwise comparisons. The error term is Mean Square(Error) = 0.619.**

Multiple Comparisons						
Dependent Variable: COD Removal						
Tukey HSD						
Wastewater concentration (I)		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
6.360	12.310	-3.8668*	0.45421	0.000	-5.1349	-2.5986
	21.735	-5.0574*	0.45421	0.000	-6.3256	-3.7892
12.310	6.360	3.8668*	0.45421	0.000	2.5986	5.1349
	21.735	-1.1906	0.45421	0.065	-2.4588	0.0775
21.735	6.360	5.0574*	0.45421	0.000	3.7892	6.3256
	12.310	1.1906	0.45421	0.065	-0.0775	2.4588
Based on observed means. The error term is Mean Square(Error) = 0.619						
*. The mean difference is significant at the 0.05 level.						

According to the results of the variance analysis (ANOVA) and the Tukey HSD test, both the Inoculum Source and Wastewater Concentration factors have statistically significant effects on the COD Removal Efficiency. In particular, the differences between Kastamonu and Burdur are statistically significant.

Moreover, the interaction between Inoculum Source and Wastewater Concentration is also statistically significant, indicating that these factors exert a combined and interactive influence on COD removal. Multiple comparison analysis revealed that there are significant differences between certain pairs of groups. For instance, while the differences between Kastamonu and Burdur are significant, the differences between Kastamonu and Eskişehir are not statistically significant. The corresponding profile plot illustrating these findings is presented in Figure 3.1.

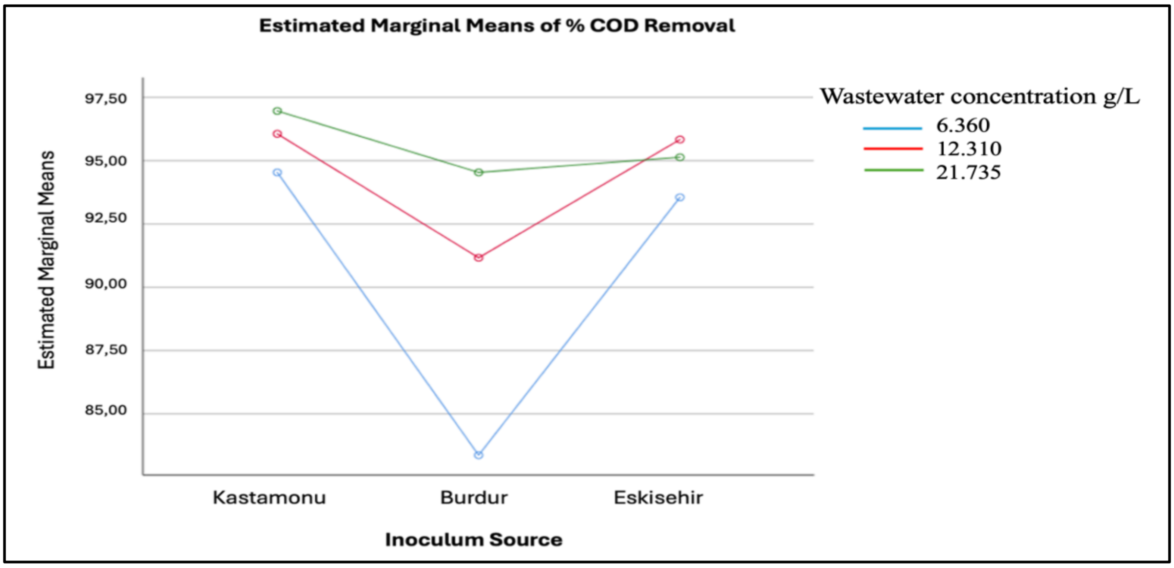


Figure 3.1. Effect of wastewater concentration and inoculum source on COD removal efficiency.

After examining Figure 3.1, it was observed that the highest COD removal occurred with a wastewater concentration of 21.735 g/L and the use of Kastamonu anaerobic sludge as the inoculum.

4. Discussion and Conclusion

The findings of this study emphasize the significant role of both inoculum location and wastewater density in determining the efficiency of COD removal in anaerobic treatment systems. The high R^2 value (0.980) from the variance analysis provides strong statistical support for the robustness of our model, indicating that the observed variations in COD removal are largely explained by the factors investigated.

The superior performance of the inoculum sourced from Kastamonu aligns with previous research highlighting the impact of microbial community composition on anaerobic digestion efficiency (Harirchi, 2022; Zhang et al., 2019; De Vrieze, 2016). The significant difference in COD removal between Kastamonu, Burdur, and Eskişehir highlights that the microbial communities present in the inoculum are crucial to the treatment process. Kastamonu's inoculum demonstrated the highest COD removal efficiency, suggesting that its microbial community was more adept at degrading organic pollutants compared to those from Burdur and Eskişehir. This could be due to the microbial diversity or specific strains present in the Kastamonu inoculum, which were better suited to the conditions of the wastewater in this study. Further investigation through techniques such as 16S rRNA gene sequencing for metagenomic analysis could elucidate the specific microbial populations responsible for the observed differences in COD removal efficiency (Krohn, 2022). Wastewater concentration, represented by initial COD concentrations, was also found to significantly influence treatment performance. As the initial COD concentration increased, the percentage of COD removed generally decreased. This is a common observation in anaerobic digestion, likely due to the increased load of organic matter, which may eventually exceed the capacity of the microbial community to efficiently degrade it under the given conditions. The finding of decreasing COD removal efficiency at higher organic loads in our study aligns with Parra-Orobio's work on food waste anaerobic digestion, suggesting a common limitation in microbial processing capacity (Parra-Orobio, 2021). The significant interaction between inoculum location and wastewater concentration suggests that the effect of the inoculum type on COD removal varies depending on the density of the wastewater. This implies that certain inoculum may perform better with lower COD concentrations, while others may be more effective with higher COD levels. Thus, understanding this interaction is essential for selecting the most appropriate inoculum for a given wastewater composition.

Our study elucidates the established understanding that both the choice of anaerobic inoculum and the operational conditions, particularly organic loading reflected by initial COD concentration, are critical determinants of treatment efficacy in anaerobic wastewater systems. The high R^2 value of our model (0.980) further substantiates this, confirming that these factors account for a significant portion of the variation in COD removal performance. This is also supported by the ANOVA and Tukey HSD test results, which revealed statistically significant differences in treatment efficiency based on both inoculum origin and initial COD levels. The practical implications of these findings for sugar industry wastewater management are significant. Strategic selection of inoculum, potentially informed by screening and characterization of microbial communities from various sources, offers a pathway to substantially improve COD removal. Moreover, the necessity of tailoring organic loading rates to the chosen inoculum to avoid inhibitory effects and maximize treatment performance is clearly highlighted. To further refine these optimization strategies, future research should prioritize the identification and functional characterization of the key microbial populations responsible for the observed performance variations across different inoculum. Exploring inoculum enrichment or adaptation strategies targeted towards the specific characteristics of high-strength industrial wastewaters, such as those from the sugar industry, holds promise for enhancing treatment outcomes.

Acknowledgement

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Conflict of Interest

No conflict of interest declared.

Data Availability Statement

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Authors Contribution

Berberler, Suleyman: Formal analysis, investigation, methodology, project administration, resources, software, validation, review & editing

Kocak, Kubra: Writing – review & editing

Altin Yavuz, Arzu: Formal analysis, software, statistics, writing – review & editing

Filik Iscen, Cansu: Methodology, project administration, supervision, writing – review & editing

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