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Sodium bicarbonate as an acid-base modulator in anaerobic digestion of acidic candy wastewater

Bicarbonato de sodio como modulador ácido-base en la digestión anaeróbica de un agua residual ácida de la industria dulcera

J.M. Montes-González[1], [1], R. Beristain-Cardoso[1], G. González-Blanco[2]*

[1] Departamento de Recursos de la Tierra. Universidad Autónoma Metropolitana Unidad Lerma. Av. De las Garzas No 10, Colonia el Panteón, Municipio Lerma de Villada. C.P. *52005*.

[2] Instituto Interamericano de Tecnología y Ciencias del Agua (IITCA), UAEMex. Carretera Toluca-Atlacomulco, km 14.5, Estado de México.

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Abstract

This study investigates the role of sodium bicarbonate as an acid–base modulator during the anaerobic digestion of real wastewater from the candy industry (pH 4.2). Although pH correction is necessary for activating methanogenic pathways, quantitative guidance on bicarbonate requirements for highly acidic effluents remains limited. Batch assays were therefore conducted using metabolically stable UASB sludge to evaluate the effects of increasing $NaHCO_3$ concentrations (3–6 g/L) on COD removal, biochemical methane potential (BMP), buffering capacity (α-factor), and lag phase duration. In the absence of pH adjustment, COD removal was negligible, confirming the strong inhibition imposed by extreme acidity. The addition of 3 g$NaHCO_3$/L raised the initial pH to 6.54 and enabled 32.9% COD removal, while higher concentrations (4–6 g $NaHCO_3$/L) achieved 79.3–85.0% removal. Methane production declined with increasing alkalinity: BMP decreased from 68.8 to 36.0–43.9 mL CH_4/g COD consumed at 5–6 g $NaHCO_3$/L, likely due to elevated sodium levels (up to 1.64 g Na^+/L). The α-factor increased from 0.49 to 0.64, indicating enhanced buffering and process stability, while the lag phase shortened, suggesting sodium-stimulated metabolic activity. Overall, excessive buffering suppresses methanogenesis, underscoring the need for optimized neutralization to balance methane production and organic matter removal.

Keywords: buffering-capacity, sodium bicarbonate, acidic water, methane, inhibition.

Resumen

En este estudio se evalúa el bicarbonato de sodio como un modulador ácido–base en la digestión anaerobia de aguas residuales de la industria dulcera (con pH 4.2). En literatura hay escasa orientación cuantitativa sobre los requerimientos de bicarbonato para el tratamiento de efluentes ácidos. Se realizaron cultivos en lote con un lodo anaerobio estable proveniente de un reactor UASB para analizar el efecto de $NaHCO_3$ (3–6 g/L) en la remoción de DQO, el potencial bioquímico de metano (PBM), la capacidad amortiguadora (índice α) y la fase lag. Sin ajuste de pH no se observó remoción de DQO. La adición de 3 g $NaHCO_3$/L elevó el pH a 6.54 y permitió una remoción de la DQO de 32.9%, mientras que a concentraciones de 4–6 g $NaHCO_3$/L se alcanzaron eficiencias de remoción de la DQO de 79.3–85.0%. No obstante, el aumento de la alcalinidad redujo la producción de metano, con una disminución del PBM de 68.8 a 36.0–43.9 mL CH_4/g DQO consumida en 5–6 g $NaHCO_3$/L, probablemente por la elevada acumulación de sodio (de 1.64 g Na^+/L). El índice α aumentó de 0.49 a 0.64, lo que indicó un amortiguamiento del proceso, mientras la fase lag se redujo, lo que sugirió una estimulación de la actividad metabólica. En general, un amortiguamiento excesivo suprime la metanogénesis, lo que subraya la necesidad de una neutralización optimizada para equilibrar la producción de metano y la eliminación de materia orgánica.

Palabras clave: capacidad buffer, bicarbonato de sodio, agua ácida, metano, inhibición.

* *Corresponding author. E-mail*: gehovan25@yahoo.com.mx ;
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1 Introduction

Anaerobic digestion (AD) is a widely recognized and sustainable technology for the treatment of organic waste and the generation of renewable energy in the form of methane-rich biogas (Loreto-Muñoz *et al.*, 2024). This process offers significant environmental and economic benefits by reducing the organic load of wastewater while recovering energy (Gómez-Paredes *et al.*, 2020; Alvarado-Reyna *et al.*, 2024). However, the effectiveness of AD is highly dependent on the physicochemical characteristics of the influent, particularly pH, which directly affects microbial metabolism and the stability of the system.

Industrial wastewater originating from the food and confectionery sectors, such as those from the candy industry, often exhibit low pH values due to the presence of fermentable sugars and organic acids (Serrano-Meza *et al.*, 2020). These acidic conditions inhibit key microbial groups involved in AD, especially methanogenic archaea, which operate optimally within a narrow pH range of 6.5 to 7.6 (Beschkov and Angelov, 2025). As a result, acidified wastewaters require buffering agents to stabilize the pH and support the activity of the microbial consortia responsible for sequential metabolic stages such as acidogenesis and methanogenesis.

Sodium bicarbonate ($NaHCO_3$) is frequently used as a buffering compound in anaerobic systems due to its mild alkalinity, high solubility, and capacity to generate bicarbonate ions that help neutralize acidification. Based on operational observations in full-scale wastewater treatment plants, bicarbonate dosing is commonly performed without defined control strategies, resulting in increased chemical consumption and operating costs. Moreover, beyond its buffering capacity, $NaHCO_3$ introduces sodium ions (Na^+) into the system, which might exert both beneficial and adverse effects on process performance. For instance, at elevated concentrations, sodium can inhibit methanogenesis by disrupting membrane function and causing cellular dehydration (Lefebvre *et al*. 2005), whereas Xiao *et al*. (2022) observed complete inhibition of methanogenic activity at 9.1 g Na^+/L in a two-stage system.

In literature, there are more studies focused on evaluating the effect of bicarbonate on solid waste management than in wastewater treatment. Moreover, most of these studies are conducted using synthetic or laboratory-prepared wastewater. Therefore, the aim of this study is to evaluate the dual role of sodium bicarbonate in batch anaerobic digestion using wastewater from the candy industry, characterized by an initial pH of approximately 4.2. The study investigates the effect of different initial concentrations of $NaHCO_3$ (3–6 g/L) on COD removal, BMP, pH stabilization, alpha factor behavior, and lag phase duration. This approach seeks to provide new insights into the concentration-dependent effects of sodium bicarbonate and to inform optimal dosing strategies that enhance process performance while avoiding methanogenic inhibition.

2 Material and methods

Initially, an acrylic UASB reactor with an operating volume of 6.5 liters was used. The reactor was inoculated with anaerobic sludge collected from wastewater treatment plant. Once the UASB reactor reached a steady state, defined as a methane production coefficient of variation below 10% over at least five hydraulic retention times (HRTs), the biomass was used as the inoculum source for batch cultures. The mineral medium used in the UASB reactor had the following composition (in g/L): NH_4Cl (0.15), K_2HPO_4 (0.35), KH_2PO_4 (0.27), $NaHCO_3$ (1.5), $CaCl_2$ (0.01), and 0.5 mL/L of a trace element solution. The trace element composition was as follows (in mg/L): $FeSO_4$ (5000), EDTA (5000), $ZnSO_4 \cdot 7H_2O$ (430), $CoCl_2 \cdot 6H_2O$ (240), $MnCl_2$ (600), $CuSO_4 \cdot 5H_2O$ (250), $Na_2MoO_4 \cdot 2H_2O$ (220), $NiCl_2 \cdot 6H_2O$ (190), and $MgCl_2$ (500). The UASB reactor was operated with 1 g COD-glucose/L as energy and carbon source.

In the batch cultures, different initial concentrations of $NaHCO_3$ (3, 4, 5, and 6 g/L) were evaluated using real wastewater effluent from a candy industry. The effluent had been previously characterized at the wastewater treatment plant (WWTP), presenting 4000 ± 500 mg COD/L, 71 ± 20 mg TN/L, and 10 ± 7 mg TP/L. It was used in experimental work without dilution. The batch experiments were carried out in 120 mL nominal volume serum bottles with a working volume of 80 mL. The anaerobic sludge was washed twice with a physiological saline solution (9 g NaCl/L) to remove residual contaminants and impurities. The bottles were inoculated with 4 g VSS/L, 4000 mg COD/L, F/M = 1 g COD/g VSS and incubated in duplicate at 30 °C under constant agitation at 200 rpm in a temperature-controlled chamber. To ensure anaerobic conditions, helium was flushed through both the culture medium and headspace for 3 minutes to displace oxygen. Methane quantification was performed using liquid displacement in an inverted column containing a 5% sodium hydroxide solution, which absorbs CO_2 and allows only methane to be measured. The batch cultures evaluated the BMP (mL CH_4/g COD consumed), specific COD consumption rate (g COD/g VSS-d), COD removal efficiency (%), and the alpha factor. While BMP protocols often extend until gas production ceases, many published studies report valid BMP comparisons at fixed incubation times when comparing treatments under identical conditions (Filer *et al*., 2019). The objective was not to determine the absolute theoretical BMP, but to assess relative methane potential between treatments. Degradation rates were determined by nonlinear regression of the experimental data to the Gompertz model using OriginPro 8.0 (OriginLab Corp.). The specific equation employed is presented below:

$$y = Ae^{-e^{(-k(X-X_C))}} \tag{1}$$

A represents the saturation value, corresponding to the upper asymptote of the dataset (mg COD/L or mL CH_4). The parameter k (1/d) is the kinetic constant that governs the slope of the curve. The term x_c denotes the inflection point of the curve corresponding to the time. A and k were parameters obtained in the OriginPro program. By solving the function, the COD degradation rate was calculated as qCOD = A·k·0.368 (mg COD/L-d or mL CH_4/d). Statistical analysis was performed using Tukey's test for multiple comparisons at a significant level of 0.05, using Minitab 19 software.

3 Analytical methods

Soluble COD, volatile suspended solids (VSS), nitrogen and phosphorous total were determined according to Standard Methods (APHA, 2005). Methane was identified by gas chromatograph using a thermal conductivity detector (GOW-MAC Instrument 508) to corroborate its presence. pH was measured using a selective electrode (HANNA Instrument). The alpha factor was calculated as follows: a fixed sample volume of 50 mL was taken and titrated with 0.1 N HCl until reaching a pH of 5.75. The titrant volume at this point (V_1) corresponded to the bicarbonate alkalinity. The titration then continued until a pH of 4.3 was reached; this second titrant volume (V_2) corresponded to the alkalinity due to volatile fatty acids (Jenkins *et al*. 1983). The alpha factor was calculated using Equation 2:

$$\alpha = \frac{V_1}{V_1 + V_2} \tag{2}$$

4 Results and discussion

4.1 *Performance of the UASB reactor as inoculum source*

Initially, a UASB reactor was operated at hydraulic retention time (HRT) of 2 days at room temperature.

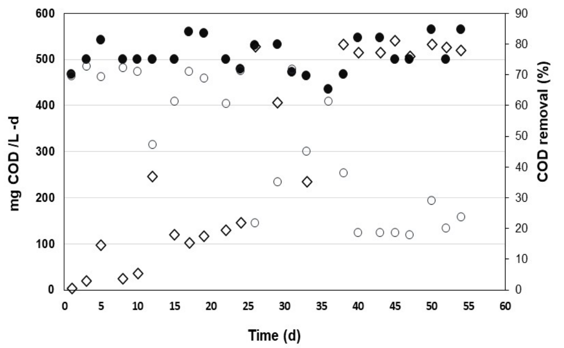

Figure 1. COD dynamics in the UASB reactor during continuous operation. (•) Influent; (o) Effluent; (◊) COD removal efficiency.

The goal was to obtain metabolically stable anaerobic sludge. This step is crucial to eliminate batch culture effects associated with the sludge's previous operational history, such as variability in organic loading rate (expressed as COD), shifts in microbial community structure, and other metabolic fluctuations that could compromise process reproducibility. Figure 1 shows the evolution of COD in the UASB reactor over 60-day operation period. The stationary state was reached on day 38, with a COD removal efficiency of 80 ± 5%. The effluent pH was 7.4 ± 3, and the alpha factor was 0.8 ± 2. Under these environmental conditions, the anaerobic sludge remained metabolically stable. Sludge samples were taken from the UASB reactor to perform batch cultures and evaluate the effect of alkalinity.

4.2 *Assessing the impact of alkalinity on anaerobic digestion using batch cultures*

This section analyzes and discusses the influence of the initial bicarbonate concentration on the kinetic and metabolic aspects of anaerobic digestion. Figure 2A shows the evolution of COD in a reaction period of 11 days. In the control study, COD values remained unchanged, due to a low initial pH of 4.2. Anaerobic digestion typically operates within the range of 6.5 to 7.5. At low pH, the metabolic pathways of anaerobic sludge are inhibited (Latif *et al*., 2017). At initial bicarbonate concentration of 3 g/L, COD consumption began, reaching a removal efficiency of 32.93 % (Figure 2B). This improvement is likely attributed to the initial pH increase, as the added alkalinity raised the pH to 6.54 (Table 1), a range more favorable for anaerobic activity. For instance, Rabbi *et al*. (2019) recommended an optimal pH range of 6.5–7.6 for methanogenic activity. Mao *et al*. (2015) suggested a slightly narrower range of 6.8–7.4. pH values above 8.0 can negatively affect anaerobic digestion, as they promote the formation of metabolic byproducts that exert an antagonistic effect on methanogenic

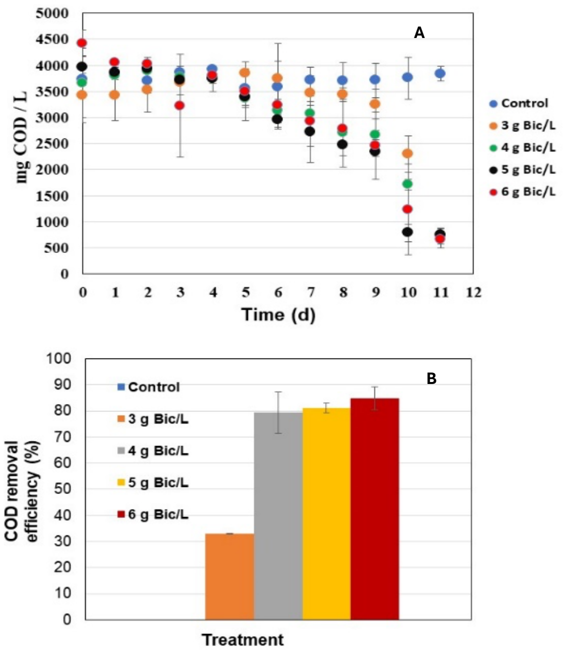

Figure 2. A) COD consumption profiles; **B)** COD removal efficiency under different alkalinity.

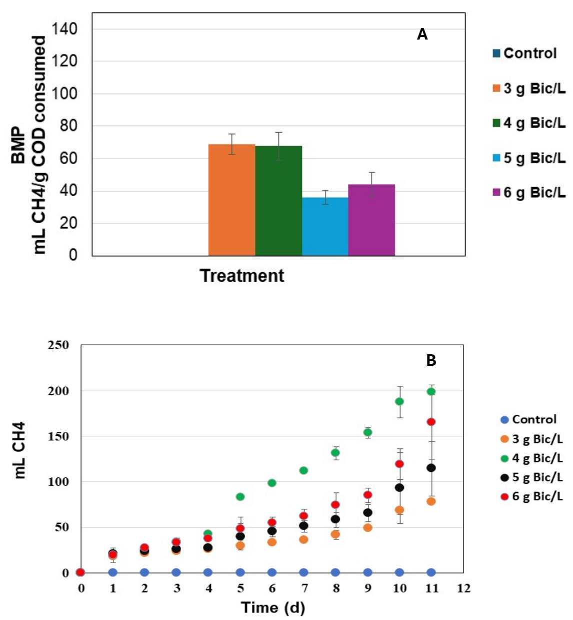

Figure 3. A) Biochemical methane potentials; **B)** methane production profiles under different alkalinity.

Table 1. Kinetic and physicochemical parameters obtained in batch cultures.

Initial $NaHCO_3$ concentration (g/L)	Initial pH	Final pH	mg COD/ g VSS-d	COD removal efficiency (%)	mL CH_4/ g COD removed	Lag phase (h)	Alpha factor Initial	Alpha factor Final
0 (control)	4.2	4.02	NA	NA	NA	NA	---	---
3	6.54	6.53	448.5 ± 75.66	32.93 ± 0.20	68.76 ± 6.3	6	0.49	0.45
4	6.79	6.99	460.42 ± 61.21	79.33 ± 8.00	67.71 ± 8.7	5	0.55	0.69
5	6.94	7.1	451.78 ± 70.20	81.13 ± 1.81	36.03 ± 4.2	4	0.62	0.72
6	7.1	7.14	463.75 ± 56.82	84.95 ± 4.41	43.93 ± 7.5	2	0.64	0.69

NA: Not activity

archaea (Cisneros *et al*., 2021). This condition disrupts the methanogenesis stage, leading to reduced biogas production and lower methane yields.

The addition of bicarbonate significantly influenced both the initial pH and the α. The alpha factor value is a key indicator of buffering capacity in anaerobic digesters, reflecting the balance between the accumulation of volatile fatty acids and the system's capacity to neutralize them through bicarbonate alkalinity. According to Pérez and Torres (2008), α values above 0.65 are typically indicative of a stable anaerobic digestion process. At 3 g/L of initial bicarbonate, the α was 0.49, which suggests limited buffering and a potentially unstable system. As the bicarbonate dose increased, the initial α rose to 0.55 (4 g/L), 0.62 (5 g/L), and 0.64 (6 g/L), approaching the stability threshold. This trend highlights the role of bicarbonate in improving the system's resistance to acidification during the initial phase of digestion. Moreover, in batch cultures supplemented with 4, 5, and 6 g/L of bicarbonate, the final α values increased to 0.69, 0.72, and 0.69, respectively. This progression indicates that, beyond the initial buffering effect, the digestion process itself contributed to the generation of alkalinity, most likely through CO_2 production. As CO_2 dissolves in the medium, it forms bicarbonate (HCO_3^-), which enhances the system's buffering capacity and thereby increases the α value. These results suggest a synergistic effect between the initial bicarbonate dose and the biogenic generation of bicarbonate during digestion, reinforcing the importance of an adequate alkalinity baseline to achieve and sustain process stability.

Additionally, another parameter affected by the increase in alkalinity was the lag phase, which is commonly associated with the adaptation period to environmental and cultivation conditions (Table 1). The experimental results revealed a notable trend: the adaptation (lag phase) shortened as alkalinity increased. This reduction in the lag phase might be associated with the presence of sodium ions, as sodium-substrate transport is a widely conserved mechanism for substrate uptake in living organisms (Henriquez *et al*., 2021). For example, Mei *et al*. (2025) reported that low levels of salinity can enhance methane production, since adequate

Na^+ concentrations support ATP generation and NADH oxidation, stimulating microbial activity. However, they also noted that high salinity conditions can damage salt-sensitive microorganisms, disrupt the microbial community structure, and inactivate key enzymes, ultimately hindering methanogenesis. Together, these findings suggest that sodium might contribute not only to substrate uptake but also to energy metabolism, potentially explaining the observed reduction in adaptation time under higher alkalinity conditions.

In summary, initial bicarbonate concentrations of 3 and 4 g/L positively influenced the biochemical methane potential, while higher concentrations of 5 and 6 g/L led to a noticeable decline in methane yield. This behavior may be associated with the dual role of sodium ions in anaerobic digestion. On one hand, low sodium levels are known to stimulate methanogenic activity by contributing to key cellular functions, such as osmotic balance and enzymatic activity (Lefebvre *et al*. 2005). On the other hand, excessive sodium concentrations can exert inhibitory effects, including cellular dehydration and disruption of membrane integrity. For example, Xiao *et al*. (2022) reported complete inhibition of methanogenesis at 9.1 g Na^+/L. In the present study, the maximum sodium concentration reached was 1.64 g Na^+/L (corresponding to 6 g/L of added bicarbonate). It is important to note that the intrinsic conductivity of the wastewater, which adds to the conductivity provided by sodium bicarbonate, is not being considered, as it was not measured. However, there is ultimately a cumulative effect. Although this value is well below reported inhibitory thresholds, it might have been sufficient to partially inhibit methanogenic archaea. Interestingly, while sodium appeared to enhance COD degradation, likely by stimulating acidogenic pathways, it simultaneously had a detrimental effect on methane production, highlighting a concentration-dependent dual effect on the anaerobic process.

Conclusions

These findings, obtained using real wastewater with high acidity, underscore the practical importance of optimizing sodium bicarbonate dosing in full-scale anaerobic treatment systems. The addition of bicarbonate had a dual impact: moderate concentrations (3–4 g/L) enhanced methane production and process stability, while higher doses (5–6 g/L) improved COD removal but inhibited methanogenesis. This behavior is attributed to the combined effects of pH buffering and increased sodium ion concentrations. Although sodium favored acidogenesis and shortened the adaptation phase, it exerted a negative effect on methanogenic activity even at sub-inhibitory levels. Overall, the study demonstrates that achieving a proper acid-base balance, beyond mere pH correction, is critical for maximizing both organic matter degradation and methane generation in the treatment of acidic industrial effluents.

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