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Short communication

Synthetic soft drink wastewater suitability for the production of volatile fatty acids

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ABSTRACT

In this study, the feasibility of acidogenic fermentation in synthetic soft drink wastewater (SDW) was evaluated. Batch experiments were conducted using a mixed microbial culture (anaerobic sludge inoculum) without any external sources of inhibition for methanogenic bacteria and with no pH control. Three food-to-microorganism (F/M) ratios (1.6, 4.0 and 6.4 gCOD/gVSS) and different initial alkalinity values (in the range 1.0–2.5 $gCaCO_3$/L) were tested. After six days of fermentation, accumulation of volatile fatty acids (VFA) was observed in all assays, indicating that SDW can serve as a substrate for VFA production. After 21 days of fermentation, the maximum degree of acidification achieved was 70.3 ± 0.4%, and it was obtained at an F/M of 4.0 gCOD/gVSS and an initial alkalinity of 2.0 $gCaCO_3$/L. The predominant VFA generated in all mixtures were butyrate and acetate. Additionally, a substantial percentage of propionate (approximately 10%) was observed when operating at pH values close to 7. In this study, the F/M ratio was the critical parameter for maintaining acidogenic conditions by inhibiting methanogenic bacteria (and other VFA consuming bacteria).

1. Introduction

Soft drink production generates effluents that have a moderate to high organic strength, most of which is easily biodegradable. The COD of soft drink wastewater (SDW) depends mainly on the specific production process and is usually in the range of 1–10 gO_2/L [1–6]. In some cases, higher COD values (up to 30 gO_2/L or more) are observed [7,8].

Anaerobic technologies for biogas production have been successfully used for SDW treatment [7–9]. However, due to its high carbohydrate content [10,11], SDW may also be a suitable substrate for acidogenic fermentation, i.e., the conversion of organic compounds into volatile fatty acids (VFA) through the hydrolytic and acidogenic stages of the anaerobic process [12,13]. The VFA so produced constitutes an important bulk chemical for other biological processes [14], such as biohydrogenation [15], the microbial production of polyhydroxyalkanoates (PHA) [16], the production of bio-alcohol [17], denitrification [18] and enhanced biological phosphorus removal [19].

Among the VFA valorization options, the use of VFA mixtures as substrate for the microbial production of PHA can be considered one of the most valuable because it is an efficient way of recovering resources from wastewater [20,21]. The use of organic residues as a carbon source for PHA production can also reduce costs by up to 50% [22]. However, for this purpose, attention must be paid to the composition of the VFA because it affects both the efficiency of PHA production and the characteristics of the final polymers [23–25]. The latter can be predicted to some extent by determining the ratio between odd and even VFA [16].

This paper presents the results of batch experiments on acidogenic fermentation of a synthetic SDW, whose suitability for the production of VFA has never been tested, to the best of our knowledge. Different food-to-microorganism (F/M) ratios and initial alkalinity values were tested and the role of pH was also investigated. The performance of the fermentation process was evaluated mainly in terms of degree of acidification and VFA composition.

2. Materials and methods

2.1. Wastewater

The synthetic SDW was prepared by mixing two different beverages, cola and a multi-fruit juice, and diluting the

Abbreviations: COD, chemical oxygen demand; DA, degree of acidification (%); DOC, dissolved organic carbon; F/M, food-to-microorganism ratio (gCOD/gVSS); HAc, acetic acid; HCa, caproic acid; HPr, propionic acid; i-HBu, iso-butyric acid; i-HVa, iso-valeric acid; n-HBu, n-butyric acid; n-HVa, n-valeric acid; PHA, polyhydroxyalkanoates; sCOD, soluble part of the chemical oxygen demand; SDW, soft drink wastewater; TN, total nitrogen; TVFA, total volatile fatty acids (gCOD/L); TSS, total suspended solids; VFA, volatile fatty acids; VSS, volatile suspended solids.

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mixture with tap water [9–11]. To simulate different organic loads, three different blends (SDW_1, SDW_2 and SDW_3) were prepared using different dilution rates (93%, 83% and 73%, respectively) and the volumetric ratio of cola to juice was 1:2. The characteristics of the three blends were in the following ranges of values: 4.7–18.8 gCOD/L, 93% of which is soluble; 1.7–6.7 gDOC/L; 0.14–0.55 gTSS/L; a VSS/TSS ratio of 0.95; less than 1 mgN/L; pH in the range 5.5–6.5; 0.02–0.08 gTVFA/L as COD.

2.2. Experimental conditions

Seven anaerobic batch assays (R1-R7) plus one blank assay were run in triplicate in 320 mL glass vessels (140 mL working volume) for 21 days. Mesophilic anaerobic sludge inoculum, taken from a full-scale municipal wastewater treatment plant, was added at a concentration of 2.5 ± 0.3 gVSS/L, which is within the range of values reported in previous studies on batch fermentation of wastewater [26–29]. To evaluate the suitability of SDW for acidogenic fermentation, no external sources of inhibition for VFA consuming bacteria were added. The strategy used to promote acidogenesis and inhibit methanogenesis consisted of applying high organic loads. A buffer ($NaHCO_3$) was added to promote pH autoregulation. Different F/M ratios and initial alkalinity values were tested (Table 1) and the initial pH was approximately the same in all assays (5.98 ± 0.05). Inorganic nutrients were also added, as described by Van Lier et al. [30]. Vessels were kept in a temperature-controlled cabinet, where temperature was set at 32 ± 1 °C. Mixing was ensured by a magnetic stirring system. Analyses of VFA, COD and pH were performed at day 0, 4, 6, 8, 15 and 21. To prevent exposure to air, nitrogen sparging was applied during the sealing procedure and after each sampling.

2.3. Analytical methods

Analyses of COD (total and soluble), DOC, TSS, VSS, pH, alkalinity and TN were performed according to Standard Methods [31]. Quantification of VFA was carried out by gas chromatography, as described by Silva et al. [28].

2.4. Calculations

The total volatile fatty acids (TVFA) concentration was calculated as the sum of the individual VFA, expressed as COD-equivalents (gCOD/L). The net amount of TVFA produced was calculated by subtracting the initial TVFA concentration. The degree of acidification (DA) was defined as the percentage of the net TVFA on the initial COD fed and used to evaluate the success of the acidogenic fermentation process, composed of both hydrolysis and acidogenesis. The odd-to-even VFA ratio was defined as the sum of the odd-equivalent carboxylic acids (HPr, n-HVa), divided by the sum of the even-equivalent carboxylic acids (HAc, i-HBu, n-HBu, i-HVa, HCa) [28]. The designation of odd-equivalent and even-equivalent acids refers to the metabolism products derived from each acid during the PHA synthesis. Acids containing an even number of carbon atoms (HAc, i-HBu, n-HBu, HCa) result in the synthesis of hydroxybutyrate, whereas HPr and n-HVa led to the formation of hydroxyvalerate [16,24]. The isomer i-HVa, although has an odd number of carbon atoms, is firstly degraded to HAc [32] and, thus, in this study was considered an even-equivalent acid.

3. Results and discussion

3.1. Process feasibility

After 6 days, in all the vessels, a substantial percentage of the COD was transformed into VFA: the median DA from all the R1–R7 vessels was 46% and the lower quartile was 37%. This confirmed that SDW provides good fermentation. However, after day 6, the results were not consistent and two groups of vessels were observed, characterized by very different final COD removals. In the first group, composed of 12 vessels, low COD removal (between 16% and 44%) was obtained at the end of the experiment. In the remaining 9 vessels, the VFA produced in the first 6 days was consumed, and the final COD removals were very high (between 78% and 95%).

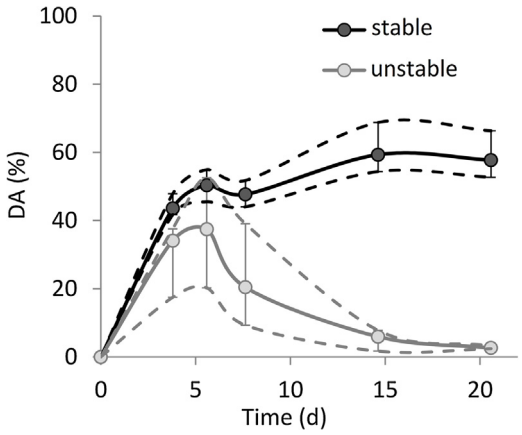

Fig. 1. Average evolution of DA (degree of acidification) over time for stable and unstable vessels. Median (continuous lines) and lower and upper quartiles (dotted lines) for each group of vessels are shown.

The vessels belonging to the first group were defined stable and the vessels belonging to the second group were defined unstable, because only the former group successfully maintained the acidogenic conditions (i.e., the inhibition of VFA consumption) until the end of the experiment. In other words, in stable vessels acidogenic fermentation occurred, whereas in unstable vessels a complete anaerobic process occurred. Fig. 1 presents the average evolution of the DA over time for stable and unstable vessels, respectively. The final results were consistent within each group, irrespective of F/M and initial alkalinity.

The F/M ratio had the greatest effect on the process, so it can be considered a critical parameter for maintaining acidogenic conditions. Most of the vessels (5 out of 6) fed with the lowest F/M ratio (1.6 gCOD/gVSS) were *unstable* and most of the vessels (11 out of 15) fed with higher F/M ratios (4.0–6.4 gCOD/VSS) were stable. High F/M ratios restricted methanogenic activity even at a slightly alkaline pH (up to 7.8), indicating that the main cause of inhibition was the high concentration of VFA generated. However, due to the complexity of the ecosystem involved in the anaerobic process, the application of high organic loads did not necessarily inhibit VFA consumption. Indeed, the triplicates for each assay were not homogeneous in terms of the maintenance of acidogenic conditions, as indicated by the number of stable vessels reported in Table 1. When VFA consumption was inhibited, the differences between the DA values in the triplicates were moderate (relative standard deviations between 1% and 19%).

To estimate the acidogenic potential of the SDW and to evaluate the composition of the VFA mixture produced, the data related to unstable vessels were excluded because they were not representative of an acidogenic fermentation process.

3.2. Acidogenic potential and VFA composition

The acidogenic potential of the SDW was evaluated based on TVFA production, regardless of the VFA composition. Due to the small amount of VFA produced in the blank assay (net TVFA of 0.2 ± 0.1 gCOD/L), the contribution of the substrate inherent to the inoculum to the net TVFA produced in assays R1–R7 was considered