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Unlocking biomethane and biohydrogen potential of lipid-rich wastewater using anaerobic granular sludge

RUSSO A.*, **OLIVA A.**, **CESARO A.**

Department of Civil, Architectural and Environmental Engineering, University of Naples Federico II, Via Claudio 21, 80125, Naples, Italy (E-mail: annalisa.russo2@unina.it; armando.oliva@unina,.it; alessandra.cesaro@unina.it)

*corresponding author: Annalisa Russo
e-mail: annalisa.russo2@unina.it

Abstract The recovery of biofuels from lipid-rich industrial waste, such as confectionery wastewater, offers a promising path toward sustainable energy. This study investigates confectionery wastewater's methane and hydrogen production potential using anaerobic granular sludge (AnGS) under varying inoculum-to-substrate ratios (ISRs). Methane yields were evaluated at ISRs of 3, 4, and 5, with the highest specific production (313.5 ± 4.3 mL CH_4/g COD_{inf}) observed with an ISR of 5. Methane production followed first-order kinetics with no lag phase, indicating rapid microbial activity and uninhibited degradation of long-chain and volatile fatty acids, particularly caproic acid. Hydrogen production was assessed at ISRs of 0.25, 0.5, and 1. No hydrogen was observed with the ISR set at 0.25, likely due to excessive VFA accumulation (mainly lactic acid) and a significant pH drop (i.e., down to 3.1 ± 0.1). At ISR of 1, a specific hydrogen yield of 44.7 ± 7.2 mL H_2/g COD_{inf} was achieved in two days. AnGS demonstrated strong resilience to lipid-induced inhibition, ensuring stable methane production and manageable VFA levels. These results highlight AnGS as a robust inoculum for treating high-lipid waste, offering new insights into optimising biofuel recovery from industrial wastewater.

Keywords: confectionery wastewater, lipid-rich waste treatment, anaerobic granular sludge, methane and hydrogen production, biofuel recovery

1. Introduction

The growing interest in renewable energy recovery from waste streams has drawn attention to confectionery wastewater, which is produced in large volumes from the process line cleaning operations and, as a consequence, contains high levels of sugars, lipids, and dyes. Its high lipid content makes it suitable for anaerobic digestion (AD), but the formation of long-chain fatty acids (LCFAs) during hydrolysis can limit the process potential. Under mesophilic conditions, LCFAs often remain solid and may adsorb onto microbial cells, limiting substrate uptake and microbial activity (Elsamadony et al., 2021). To address these challenges, several pretreatment strategies have been explored, such as saponification and enzymatic hydrolysis. The use of anaerobic granular sludge (AnGS) within the biological process has emerged as a promising alternative to the substrate pretreatment due to its robust structure and ability to retain high microbial biomass (Lim et al., 2014). Although AnGS has shown potential for handling complex substrates, its application in lipid-rich waste treatment remains poorly investigated. This research aims to evaluate the effectiveness of AnGS as inoculum in both anaerobic digestion and dark fermentation, focusing on its ability to mitigate lipid- and LCFA-induced inhibition.

2. Material and methods

The confectionery wastewater used in this study was obtained from an Italian company and was subjected to physicochemical characterisation before use. To assess its biomethane and biohydrogen potential, batch assays were conducted in 250 mL serum bottles with a working volume of 125 mL. Control tests were included to evaluate the endogenous methane and hydrogen production of the inoculum. Anaerobic conditions were established by flushing the bottle headspace with Argon gas. The bottles were incubated in a thermostatic water bath maintained at 37 ± 2 °C and were regularly agitated. Various inoculum-to-substrate ratios (ISRs) were tested, as reported in Table 1. For biohydrogen potential tests, AnGS was thermally pretreated at 105 °C for 1 hour to inhibit methanogenic activity. Biogas volume and composition were measured using the manometric method and a mass spectrometer, according to the procedure described by Oliva et al. (2022). Specific methane and hydrogen production were calculated as mL CH_4/g COD_{inf} and mL H_2/g COD_{inf}, respectively. The liquid phase was sampled to monitor the evolution of volatile fatty acids (VFAs) using high-performance liquid chromatography (HPLC), as detailed by Moscariello et al. (2024).

To evaluate the model fitting, the experimental data obtained from the batch tests were compared with a modified Gompertz model and a first-order kinetic model.

Table 1. Experimental conditions tested in the present study

Anaerobic process	ISR [g tCOD substrate/ g VS inoculum]	ID test
Anaerobic digestion	3:1	3-ISR_AD
	4:1	4-ISR_AD
	5:1	5-ISR_AD
Dark fermentation	1:4	0.25-ISR_DF
	1:2	0.5-ISR_DF
	1:1	1-ISR_DF

3. Results and discussion

The highest specific methane production, i.e., 313.5 ± 4.3 mL CH_4/g COD_{inf}, was achieved under the 5-ISR_AD condition, as shown in Figure 1a. The methane production curve followed first-order kinetics with no observable lag phase, indicating rapid microbial activity. In terms of specific hydrogen production, no hydrogen was detected under the 0.25-ISR_DF condition (Figure 1b), likely due to the higher substrate volume and the associated lipid-induced inhibition (Elsamadony et al., 2021). Conversely, under the 1-ISR_DF condition, a specific hydrogen yield of 44.7 ± 7.2 mL H_2/g COD_{inf} was achieved within two days, suggesting an absence of inhibitory effects (Figure 2b). In the anaerobic digestion process, the degradation of VFAs proceeded without inhibition, with caproic acid identified as a key intermediate. The presence of caproic acid (approximately 700 mg/L) during the AD process may indicate the absence of LCFAs accumulation, as this compound is an intermediate in the β-oxidation pathway (Weber, 2024). This hypothesis is further supported by the complete consumption of caproic acid by the end of the process, indicating its effective conversion to biogas. In contrast, during the dark fermentation process under the 0.25-ISR_DF condition, the accumulation of VFAs - predominantly lactic acid - resulted in a marked pH decline to 3.1 ± 0.1. On the other hand, under the 0.5-ISR_DF and 1-ISR_DF conditions, no lactic acid accumulation was observed and, starting from day 6, caproic acid began to accumulate, likely due to chain elongation. Under these conditions, the pH remained relatively stable at 4.7 ± 0.2.

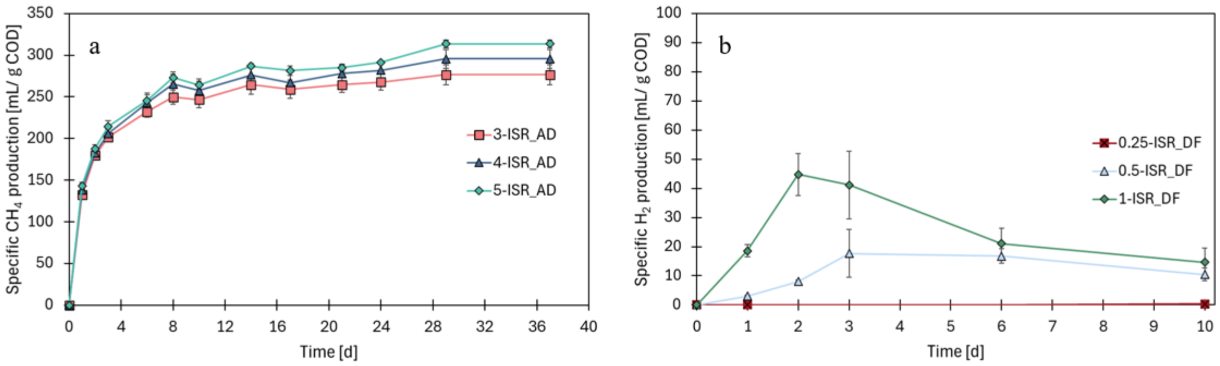

Figure 1. Specific methane (a) and hydrogen (b) production

4. Conclusions

This study demonstrates the effectiveness of AnGS for treating lipid-rich confectionery wastewater via anaerobic digestion and dark fermentation. The highest methane yield (313.5 ± 4.3 mL CH_4/g COD_{inf}) was achieved under the 5-ISR_AD condition, with no lag phase. In dark fermentation, the highest hydrogen production was obtained at the 1-ISR_DF condition (44.7 ± 7.2 mL H_2/g COD_{inf}), while lipid inhibition likely suppressed H_2 production at lower ISRs. AnGS showed strong resistance to lipid-related inhibition, enabling efficient biofuel production. These results highlight its potential as a promising inoculum for optimizing energy recovery from complex industrial wastewaters.

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