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Valorization of expired dairy products by anaerobic digestion

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DANIELI FERNANDA CANAVER MARIN

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To my dear husband Antonio.

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"The only source of knowledge is experience."

Albert Einstein.

ABSTRACT

The dairy industry is an important sector of the food industry, producing 928 million tons of milk in 2021. However, this sector is responsible for generating a high amount of waste, such as expired dairy products (EDP). Currently, the main destination of EDP are their disposal in landfills, causing environmental problems due to their high organic matter content. In this sense, this study proposed to evaluate strategies for using and valorizing EDP, transforming them into resources for bioproducts generation from anaerobic digestion (AD). As the first strategy, an autochthonous mixed culture was obtained from EDP using anaerobic batch reactors with *All Purpose Tween* (APT) culture medium as substrate. The *Lactobacillus* genus was identified with the highest relative abundance in the obtained culture with 78.4%. Subsequently, the autochthonous mixed culture was evaluated in anaerobic reactors using different substrates (xylose, arabinose, fructose, maltose, glucose, rhamnose, lactose, sucrose, EDP, and banana processing wastewater (BPW)). The mixed autochthonous culture grew on all tested substrates, with high carbohydrate removal (57.6-97.0%), producing lactic acid (LA) as the predominant soluble metabolite (2.40-7.85 g L^{-1}). The highest LA yield was obtained from BPW with 0.98 g g^{-1}. As the second strategy, it was evaluated the co-digestion of EDP with synthetic sewage (SS) in single-stage anaerobic reactors. Five assays were performed with the following percentages of EDP in the substrate mixture (1) 0%, (2) 5.0%, (3) 7.5%, (4) 10.0%, and (5) 15, 0% (v/v). Higher COD removal (89.2%) and higher CH_4 production (6583.6 NmL L^{-1}) were verified in Assay 4 (10.0% EDP). Higher CH_4 yield were obtained in Assay 3 (7.5% EDP) with 332.6 NmL gVS_{add}^{-1}. Acetoclastic (*Methanosaeta*) and hydrogenotrophic (*Methanolinea, Methanoregula,* and *Methanobacterium*) archaea were identified in assays 2, 3, and 4 (5.0% to 10.0% EDP), which probably contributed to the high efficiency of organic matter removal and high methane production. Assay 5 (15.0% EDP) was inhibited due to the accumulation of volatile acids in the anaerobic reactors. As the last strategy, the co-digestion of EDP with SS was evaluated from two-stage anaerobic systems. In the first stage (acidogenic reactors), three assays were assembled with the following substrate composition (v/v): (1) 10% EDP + 90% SS, (2) 15% EDP + 85% SS, and (3) 20% EDP + 80% SS. The liquid effluents generated at the end of the acidogenic stage of assays 1, 2, and 3 were used as substrates in the second stage (methanogenic reactors), corresponding to tests 4, 5, and 6, respectively. In the first stage, higher carbohydrate removal (92.3%) and higher hydrogen production (1309.3 NmL L_r^{-1}) were obtained in Assay 3 (20% EDP + 80% SS). In the second stage, higher COD removal (94.7%) and methane production (6813.4 NmL L_r^{-1}) were verified in Assay 5 (15% EDP + 85% acidified SS). Assay 6 (20% EDP + 80% acidified SS) was inhibited due to the accumulation of volatile fatty acids (11427.9 mg L^{-1}), mainly acetic acid (8455.6 mg L^{-1}). Thus, the experimental approaches of the studies carried out offer promising opportunities for EDP valorization through its application in biological processes. In addition, the use of expired dairy waste can contribute to its recovery by providing bioproducts and biofuels obtained from renewable sources.

Keywords: Expired dairy products; Anaerobic digestion; Methane; Hydrogen; Lactic acid.

RESUMO

A indústria de laticínios é um importante setor da indústria de alimentos com produção de 928 milhões de toneladas de leite em 2021. Entretanto, esse setor é responsável pela geração de uma elevada quantidade de resíduos, como os produtos lácteos vencidos (PLV). Atualmente, a principal destinação dos PLV é a sua disposição em aterros sanitários, causando problemas ambientais devido à sua elevada carga orgânica. Neste sentido, esse estudo propôs avaliar estratégias de utilização e valorização de PLV transformando-os em recursos para a geração de bioprodutos a partir do processo de digestão anaeróbia (DA). Como primeira estratégia uma cultura autóctone mista foi obtida a partir dos PLV usando reatores anaeróbios em batelada alimentados com meio de cultura *All Purpose Tween* (APT) como substrato. O gênero *Lactobacillus* foi identificado com maior abundância relativa na cultura obtida com 78.4%. Posteriormente, a cultura mista autóctone foi avaliada em reatores anaeróbios utilizando diferentes substratos (xilose, arabinose, frutose, maltose, glicose, ramnose, lactose, sacarose, PLV, e água residuária do processamento da banana (ARPB)). A cultura autóctone mista cresceu em todos os substratos testados, com elevadas remoções de carboidratos (57.6-97.0%) e produção predominante de ácido lático (AL) como metabólito solúvel (2,40-7,85 g L^{-1}). O maior rendimento de produção de AL foi obtido a partir da ARPB com 0,98 g g^{-1}. Como segunda estratégia, foi avaliada a co-digestão dos PLV com esgoto sintético (ES) em reatores anaeróbios em único estágio. Cinco ensaios foram realizados com as seguintes porcentagens de PLV na mistura de substratos (1) 0%, (2) 5,0%, (3) 7,5%, (4) 10,0%, e (5) 15,0% (v/v). A remoção mais elevada de DQO (89,2%) e maior produção de CH_4 (6583,6 NmL L^{-1}) foram verificadas no ensaio 4 (10,0% PLV). O rendimento mais elevado de CH_4 foi obtido no ensaio 3 (7,5% PLV) com 332.6 NmL gVS_{add}^{-1}. Archaeas acetoclásticas (*Methanosaeta*) e hidrogenotróficas (*Methanolinea, Methanoregula e Methanobacterium*) foram identificadas nos ensaios 2, 3 e 4 (5,0% a 10,0% PLV), o que provavelmente contribuiu para a eficiência elevada de remoção de matéria orgânica e alta produção de metano. O Ensaio 5 (15.0% PLV) foi inibido devido ao acúmulo de ácidos voláteis nos reatores anaeróbios. Como última estratégia, a co-digestão de PLV com ES foi avaliada a partir de sistemas anaeróbios em dois estágios. No primeiro estágio (reatores acidogênicos), foram montados três ensaios com a seguinte composição de substrato (v/v): (1) 10% PLV + 90% ES, (2) 15% PLV + 85% ES, e (3) 20% PLV + 80% ES. Os efluentes líquidos gerados ao final da etapa acidogênica dos ensaios 1, 2 e 3 foram utilizados como substratos no segundo estágio (reatores metanogênicos), correspondendo aos ensaios 4, 5 e 6, respectivamente. No primeiro estágio, a remoção mais elevada de carboidrato (92,3%) e maior produção de hidrogênio (1309.3 NmL L_r^{-1}) foram obtidas no ensaio 3 (20% PLV + 80% ES). No segundo estágio, a remoção mais elevada de DQO (94.7%) e produção de metano (6813.4 NmL L_r^{-1}) foram verificadas no ensaio 5 (15% PLV + 85% ES acidificado). O ensaio 6 (20% PLV + 80% ES acidificado) foi inibido devido ao acúmulo de ácidos graxos voláteis (11427,9 mg L^{-1}), principalmente ácido acético (8455,6 mg L^{-1}). Assim, as abordagens experimentais dos estudos realizados oferecem oportunidades promissoras para a valorização do PLV através da sua aplicação em processos biológicos. Além disso, a utilização de resíduos lácteos vencidos pode contribuir na sua valorização com o fornecimento de bioprodutos e biocombustíveis obtidos a partir de fontes renováveis.

Palavras-chave: Produtos lácteos vencidos; Digestão anaeróbia; Metano; Hidrogênio; Ácido lático.

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LIST OF ABBREVIATIONS AND ACRONYMS

AcoD - Anaerobic co-digestion

AD – Anaerobic digestion

APT - All Purpose Tween

ASBR - Anaerobic Sequential Batch Reactor

BPM - Biochemical potential of methane

BPW - Banana processing wastewater

CFU - Colony-forming unit

CG - Crude glycerol

COD – Chemistry Oxygen Demand

CSTR - Continuously stirred tank reactors

CW – Cheese whey

DF – Dark fermentation

DP – Dairy products

EDP – Expired dairy products

EGSB - Expanded granular sludge bed

EoL-DPs - End-of-Life Dairy Products

FOG - Fats, oils, and greases

FW – Food waste

GC - Gas chromatograph

HPB - Hydrogen-producing bacteria

HPLC - High-Performance Liquid Chromatography

HRT - Hydraulic retention time

LA – Lactic acid

LAB - Lactic acid bacteria

LCFA - Long-chain fatty acids

MRS - De Men, Rogrosa and Sharpe medium

NmL – Volume (mL) normalized by modified Gompertz equation

OLR - Organic loading rate

OUT - Operational taxonomic unit

PBS - Phosphate buffer saline solution

SAD – Single-stage anaerobic digestion

SS - Synthetic sewage

tC - total carbohydrates

TCD - Thermal conductivity detector

tCOD - total Chemistry Oxygen Demand

TS – Total solids

TSAD – Two-stage anaerobic digestion

UASB - Upflow anaerobic sludge blanket

VFA – Volatile fatty acids

VS – Volatile solids

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1 GENERAL INTRODUCTION

The dairy sector is one of the most important in the food industry, representing a relevant role in the world economy, with global production of 928 million tons in 2021 (FAO, 2021). Furthermore, the sector is projected to expand by 2025, particularly in the fresh dairy sector. (OECD; FAO, 2016).

However, dairy products are highly perishable, with a short shelf life (FANTOZZI et al., 2015). After expiration, these products are considered unfit for consumption, resulting in significant waste (SAKARIKA et al., 2020). The current management of this waste uses a small part for animal feed, and most of it is destined for landfill (ADGHIM et al., 2020; KOPSAHELIS et al., 2018).

Although dairy waste does not contain toxic substances, its disposal in landfills is harmful to the environment since it has a high organic matter content, easily biodegradable by microorganisms responsible for the production of greenhouse gases (DAT et al., 2020). Several studies demonstrate the adverse effects caused by the disposal of food waste in landfills and reinforce the urgency of converting these wastes into resources using sustainable techniques and processes (BRUNKLAUS B, REX E, CARLSSON E, 2018; OLIVEIRA; LAGO; DAL' MAGRO, 2021; VERZOLA, 2018).

Anaerobic digestion (AD) has been considered a suitable and efficient method for waste treatment. This biological process has great potential for waste valorization, turning it into raw material for biogas generation, which can be applied as a renewable energy source. AD also produces other value-added bioproducts, such as organic acids and alcohols, and reduces organic matter in waste, preventing environmental degradation (HUNTER; BLANCO; BORRION, 2021). Additionally, strategies such as anaerobic co-digestion and two-stage systems can also be used to improve AD efficiency, supporting the synergy and stability of the biological process (XU et al., 2018).

Expired dairy products (EDP) are composed of carbohydrates (57.5%), lipids (28.4%), and protein (14.0%) (SLOPIECKA et al., 2022), which makes them an excellent substrate for anaerobic digestion, as they can be easily degraded. Furthermore, EDP are the source of fermentative microorganisms, such as lactic acid bacteria, which can also be applied to obtaining products of biotechnological interest, such as lactic acid and ethanol (ESKIN; SHAHIDI, 2013; NOBLECOURT et al., 2018).

Many studies present the biotechnological potential of using other residues from the dairy industry (e.g., dairy wastewater and cheese whey) have been developed. However, few researches demonstrate the application of EDP with the same purpose. In this sense, the main objective of this work is to propose different strategies for using EDP by anaerobic digestion to valorize this waste, reducing its inappropriate disposal.

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GENERAL CONCLUSIONS

This study proposed valorization and recovery strategies for expired dairy products (EDP) from anaerobic digestion. The main information obtained from the experimental studies of this thesis (Chapters II-V) is summarized below:

i. A new autochthonous mixed culture was obtained from expired dairy products. This autochthonous mixed culture was able to consume and grow from different substrates such as glucose, sucrose, lactose, maltose, fructose, xylose, arabinose, rhamnose, EDP, and wastewater from banana processing (BPW). In addition, the autochthonous mixed culture favored the pathway for producing lactic acid (LA). Good results of LA production yields were observed from using sugars as the only substrate, but mainly from waste such as BPW and EDP. The new autochthonous mixed culture proved to be a promising alternative in bioprocesses contributing to generating value-added products from different carbon sources.

ii. EDP co-digested with synthetic sewage (SS) was an efficient substrate in single-stage anaerobic digestion. Assays with a concentration of up to 20 g COD L^{-1} (mixture of 10% EDP + 90% SS) effectively removed the organic matter from substrates and presented high yields in methane production. Nevertheless, concentrations above 20 g COD L^{-1} caused an accumulation of volatile fatty acids in the reactors with inhibition of methanogenic microorganisms and consequent failure of the biological process. Therefore, the co-digestion of EDP + SS is a viable alternative for recovering these residues in biological processes. However, further studies need to be conducted so that a higher concentration of EDP can be used in the anaerobic digestion.

iii. Co-digestion of EDP with SS was effective in two-stage anaerobic digestion. In the first stage, high values of carbohydrate removal were obtained with good yields of hydrogen production for all evaluated concentrations (20 – 40 g COD L^{-1}). In the second stage, efficient organic matter removals with high methane production were observed only for tests up to 30 g COD L^{-1}. At concentrations above 30 g COD L^{-1}, there was the accumulation of volatile fatty acids, with high concentrations of acetic acid. The accumulation of acids caused kinetic limitation of the acetoclastic microorganisms and the consequent failure in the biological process.

Therefore, the strategies tested in this study bring new prospects for EDP valorization from their application in biological processes, contributing to mitigating the environmental impacts caused by their improper disposal. In addition, EDP utilization can contribute to its valorization by providing bioproducts and biofuels from renewable sources.

RECOMMENDATIONS FOR FUTURE RESEARCH

1. Apply the evaluated parameters in a continuous process and scale-up reactors;
2. Evaluate the manure as a co-substrate for the anaerobic digestion of expired dairy products. The manure has a natural buffer capacity, which can contribute to avoiding the reactors' rapid acidification;
3. Evaluate pre-treatment using enzymatic hydrolysis to minimize the lipid's adverse effects (lag phase increasing and accumulation of long-chain fatty acids) in anaerobic digestion process.