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# REUSE OF EXPIRED DAIRY PRODUCTS AND SEWAGE IN THE PRODUCTION OF BIOGAS BY ANAEROBIC DIGESTION

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**Keywords:**
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## ABSTRACT

Dairy industry is a prominent sector of the agro-industry with global production of 906 million tonnes of milk. Several dairy products are wasted due their high perishability. Anaerobic digestion is a sustainable alternative for reducing this waste aiming biogas production. This study evaluated the co-digestion of expired dairy products (EDP) with synthetic sewage from two assays using two types of dairy waste, separately: Assay I (EDP mixture of 80% of milk + 15% of yogurt + 5% of milk cream) and Assay II (industrial raw mixture of EDP). Both assays were composed of 20 g COD $L^{-1}$ (Chemical Oxygen Demand per Liter) using anaerobic batch reactors (1.0 L) assembled in duplicates with 0.5 L headspace ($N_2$) and working volume of 0.5 L with initial pH 8.2, at 37 °C, in a static mode. The cumulative $CH_4$ productions were 2722.5 NmL in 31 days of operation (Assay I) and 3140.0 NmL in 25 days of operation (Assay II). Equivalent $CH_4$ yields were obtained for the both assays with ~330 $NmLCH_4$ g $COD_{rem}^{-1}$. Carbohydrates and COD removals were 96.8% and 98.0%; 87.3% and 89.4%, respectively. The co-digestion of EDP with sewage was effective to $CH_4$ production with efficient organic matter removal. These results encourage new strategies for reuse of expired dairy products by the use of anaerobic digestion.

**Palavras-chave:**
Produtos lácteos vencidos
Digestão Anaeróbia
Logística Reversa
Metano
Biogás

## REAPROVEITAMENTO DE PRODUTOS LÁCTEOS VENCIDOS E ESGOTO NA PRODUÇÃO DE BIOGÁS POR DIGESTÃO ANAERÓBIA

## RESUMO

A indústria de laticínios é um setor de destaque da agroindústria com produção global de 906 milhões de toneladas de leite. Vários produtos lácteos são descartados devido à sua alta perecibilidade. A digestão anaeróbia é uma alternativa sustentável para a redução desses resíduos visando a produção de biogás. Este estudo avaliou a codigestão de produtos lácteos vencidos (PLV) com esgoto sintético a partir de dois ensaios, testando dois tipos de resíduos lácteos separadamente: Ensaio I (Mistura de PLV com 80% de leite + 15% iogurte + 5% de creme de leite) e Ensaio II (Mistura industrial bruta de PLV). Ambos os ensaios foram compostos por 20 g DQO $L^{-1}$ (Demanda Química de Oxigênio por Litro) usando reatores anaeróbios em batelada (1,0 L) montados em duplicata com 0,5 L *headspace* ($N_2$), volume de trabalho de 0,5 L, com pH inicial de 8,2, a 37 °C, em modo estático. As produções cumulativas de $CH_4$ foram 2722,5 NmL em 31 dias de operação (Ensaio I) e 3140,0 NmL em 25 dias de operação (Ensaio II). Rendimentos equivalentes de $CH_4$ foram obtidos para ambos os ensaios com ~330 $NmLCH_4$ g $DQO_{rem}^{-1}$. As remoções de carboidratos e DQO foram 96.8% e 98.0%; 87.3% e 89.4%, respectivamente. A codigestão de PLV com esgoto foi efetiva para a produção de $CH_4$ com eficiente remoção de matéria orgânica. Esses resultados incentivam novas estratégias para recuperação de produtos lácteos vencidos por digestão anaeróbia.

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## INTRODUCTION

The dairy industry has stood out in the agro-industrial sector reaching the global production of 906 million tonnes of milk in 2020 (FAO, 2021). In the same year, Brazil set a record with the production of 35.4 million tonnes of milk (CNA, 2021). However, it has been reported that the annual dairy waste residue is around 4–11 million tonnes worldwide (USMANI et al., 2021).

Wastewater and cheese whey are known to be potential dairy industry residues (AHMAD et al., 2019). Furthermore, expired dairy products (EDP) are included in this context due to their high perishability and short shelf life. After the expiration date, they are considered unfit for consumption and they are usually disposed in landfills (SAKARIKA et al., 2020).

EDP contain large amounts of carbohydrates (45-50 g $L^{-1}$), proteins (6-30 g $L^{-1}$) and lipids (4-8 g $L^{-1}$) (STAVROPOULOS et al., 2016). Therefore, they are characterizing as organic matter rich-compounds (COD 121-191 g $L^{-1}$) (KOPSAHELIS et al., 2018; SAKARIKA et al., 2020; WU et al., 2011), which makes its disposal in landfills harmful to the environment. Furthermore, this practice is contrary to Brazil's National Solid Waste Policy instituted by the Law nº 12.305 of August 2nd, 2010, since this law aims reduce waste disposal from its reuse, recycling and treatment, as a way to minimize environmental impacts (VERZOLA, 2018).

The reverse logistic can be another way to overcome this problem, which is an alternative totally allied to the Brazilian's laws, where expired products can be sent to a deconditioning center, going through a de-packaged process with consequent separation of inert material (packaging) and organic material (expired dairy product) (NOBLECOURT et al., 2018). After that, the packaging can be recycled, while organic material can be used as a substrate in the anaerobic digestion (AD) process, instead of being disposed in landfill.

Reuse of dairy waste by anaerobic digestion (AD) has been a promising alternative for treating and valuing them (ADGHIM et al., 2020; WU et al., 2011), since AD is able to degrade organic matter to produce biogas, that is an emerging and essential biofuel for the world energy matrix (SAR et al., 2022). Sakarika et al. (2020) reported a maximum yield of 0.757 mol $H_2$ $mol^{-1}$ carbohydrates consumed and 318 mL $CH_4$ g $VS_{add}^{-1}$ using the co-digestion of expired dairy products with a mixture of agroindustrial wastes (pig manure, liquid cow manure, cheese whey, poultry wastes and slaughterhouse wastes) as substrates in a pilot-scale two-stage mesophilic (37ºC) anaerobic system.

Although AD has many advantages, its application for EDP treatment, which contain high amounts of carbohydrates, lipids and proteins, can result in the rapid formation of volatile fatty acids (VFAs), long chain fatty acids (LCFAs) and ammonia leading to methanogenisis supression with consequent inhibition of biogas formation (HASSAN; NELSON, 2012; XU et al., 2018).

Therefore, anaerobic co-digestion (ACoD) is a good option to overcome the monodigestion problem (LV et al., 2021). ACoD is frequently use to enhance anaerobic digestion and biogas production performance from substrates with complementary characteristics providing nutrient balance, synergistic effects of microrganisms and diluting harmful or excessive substances (XU et al., 2018).

Several industrial residues can be used in anaerobic co-digestion; however, it is important to evaluate the availability and costs of residues that will be used (SIDDIQUE; WAHID, 2018). In this scenario, sewage can be considered a good co-substrate for dairy waste, due to its high treatment requirements and low cost (ANA, 2017). Furthermore, sewage is basically composed of water (99.9%), acting as a good diluent (VON-SPERLING, 2014). Aditionally, it contains important nutrients, such as nitrogen and phosphorus, essential for the microorganisms growth in the biological process (MARTÍN-GONZÁLEZ et al., 2010). Therefore, feeding bioreactors with a mixture of both wastes is an exciting approach (ADAMES et al., 2021; MARTÍN-GONZÁLEZ et al., 2010).

Thus, the present study evaluated the co-digestion of expired dairy products with sewage in anaerobic batch reactors, operated with different mixtures of dairy wastes, in order to reuse them in the production of biogas.

## MATERIAL AND METHODS

### *Inoculum and substrates*

The methanogenic granular sludge was collected from a UASB (Upflow Anaerobic Sludge Blanket) reactor and it was applied to the treatment of poultry slaughterhouse wastewater (Tietê – SP –

Brazil). The inoculum was stored in plastic bottles and kept in refrigerator (Consul, model FFE24) at 4ºC until its utilization.

Dairy waste A consisted of a mixture of expired dairy products (80% of milk + 15% of yogurt + 5% of milk cream). These proportions were based on the study of KOPSAHELIS et al. (2018) and they were also similar to the proportion of expired products in the market that return to a dairy factory. The dairy waste A was composed of (g $L^{-1}$) Total Solids - TS (124.3); volatile solids - VS (116.8); total Chemical Oxygen Demand- tCOD (185.3); total carbohydrates - tC (52.3) and pH 6.2.

Dairy waste B consisted of a raw dairy waste composed of a mixture of expired dairy products containing milk, milk cream and yogurt collected from a dairy company (Descalvado – SP – Brazil). It was composed of (g $L^{-1}$) TS (150.9), VS (144.5), tCOD (297.2), tC (78.5) and pH 5.5.

Synthetic sewage was prepared according to methodology described by MARTÍN-GONZÁLEZ et al. (2010) (Table 1) with the same nutrient composition of domestic sewage. It was composed of (g $L^{-1}$) TS (0.3), VS (0.2), tCOD (0.4), tC (<0.01) and pH 6.8.

*Batch Reactors Setup*

Two assays were assembled (Assay I and Assay II) in duplicates of anaerobic batch reactors (1000 mL) and reaction medium (composed of substrates + inoculum) of 500mL. In both assays, the reactors were set up to get a substrates mixture with COD concentration of 20 g COD $L^{-1}$. Therefore, in Assay I, the substrates mixture was composed of 16% of Dairy Waste A + 84% of synthetic sewage, which corresponded to a total volume of 340 mL. In Assay II, the mixture was composed of 10% of Dairy Waste B + 90% of synthetic sewage, also corresponding to a total volume of 340 mL. An amount of 19 g VS $L^{-1}$ of inoculum (160 mL) and 5 g $L^{-1}$ buffer solution ($NaHCO_3$) were added in all reactors. The initial pH was adjusted to 8.2 with additions of 1M NaOH or 1M HCl. The headspace of the reactors (500mL) was filled with $N_2$ (99.9%) to create an anaerobic environment and they were capped with butyl rubber stoppers, wrapped and kept in a static mode, at mesophilic conditions (37 °C) using a dry oven (Solab, SL-101). Control reactors (without substrates addition) were operated to determine the background methane production from the inoculum. The methane production was calculated by subtracting the methane production of control reactors from that of the batch anaerobic reactors (ANGELIDAKI et al., 2009). The anaerobic reactors were operated until no biogas production was detected.

*Analytical Methods*

The tCOD, pH, TS and VS were analyzed according to "Standard Methods for the Examination of Water and Wastewater" (APHA, 1998).

**Table 1.** Composition of Synthetic Sewage

| Macronutrient solution | | Micronutrient solution | |
|---|---|---|---|
| Compound | [mg $L^{-1}$] | Compound | [mg $L^{-1}$] |
| Starch | 200 | $FeCl_3.4H_2O$ | 1000 |
| Ovoalbumine | 21.0 | $CoCl_2.6H_2O$ | 1000 |
| Sunflower oil | 13.1* | $MnCl_2.4H_2O$ | 250 |
| Urea | 13.0 | $CuCl_2.2H_2O$ | 15 |
| $KH_2PO_4$ | 5.26 | $ZnCl_2$ | 25 |
| $CaCl_2.2H_2O$ | 22.05 | $H_3BO_3$ | 25 |
| $MgSO_4.7H_2O$ | 0.43 | $(NH_4)_6Mo_7O_{24}.4H_2O$ | 45 |
| KCl | 21.3 | $NaSeO_3.H_2O$ | 50 |
| $NaHCO_3$ | 8.76 | $NiCl_2.6H_2O$ | 35 |
| Yeast extract | 100 | EDTA | 500 |
| Micronutrients | 1.0* | HCl 36% | 1.0* |
| | | Resarzurin | 250 |

* Amount expressed in mL $L^{-1}$

Total carbohydrate concentration was determined by colorimetric method using phenol-sulfuric acid. The measurements were performed using Nanocolor VIS II spectrophotometer (Macherey-Nagel) at the wavelength of 490 nm (DUBOIS et al., 1956; HERBERT, D.; PHILIPPS, O.S.; STRANG, 1971).

The biogas components ($H_2$, $N_2$, $CH_4$ and $CO_2$) were measured using a Shimadzu® gas chromatograph (GC-2014), equipped with a thermal conductivity detector (TCD) and Carboxen 1010 PLOT column. The column flow was 5.0 mL $min^{-1}$ with ultra-pure argon as the carrier gas. The injector and detector temperature were 220 °C and 230 °C, respectively. The column temperatures were: 120 °C (1 min), 40 °C/min up to 200 °C (3 min), 50 °C/min up to 230 °C (0.5 min), with total run time of 7.1 minutes (MAINTINGUER et al., 2008). The biogas volume was daily measured by water displacement method according to ZUO et al. (2020) methodology and the values were normalized to 273 K and 1013 hPa according to VDI 4630 (2006) methodology.

*Statistical analysis of experimental data*

Statistical analysis was performed using the average values obtained by ANOVA and Tukey's test at 5% significance level, considering a completely randomized design. The tests were applied to all $CH_4$ production parameters (cumulative production, maximum production rate and yield).

## RESULTS AND DISCUSSION

The bioreactors operation data are presented in Table 2. For both tests, the final pH values (8.0 and 7.9, respectively) were close to the initial ones (8.2) indicating that all acids formed during the anaerobic digestion were consumed.

In terms of removal of tC, COD and VS, both assays were succeeded with percentages above 85%. However, the Assay II obtained tC and COD removal percentages slightly higher (98.0% and 89.4%) than the Assay I (96.8% and 87.3%). However, VS removal was 0.6% higher for Assay I than for Assay II.

The removal of COD and VS obtained in this present study were higher than the results achieved by WU *et al.* (2011), which were 59.1% COD and 28% VS with the anaerobic co-digestion of milk waste with dairy manure using batch reactors (19.8 g COD $L^{-1}$) at 37 °C during 28 days.

The average volume of methane produced by the control reactors was 472.1 NmL and this value was discounted of methane volume produced in Assays I and II. Significant differences were observed for the parameters of cumulative $CH_4$ production and maximum $CH_4$ rate, demonstrating that the best results were achieved for Assay II (3140.0 NmL and 251.2 NmL $L_r^{-1}$ $d^{-1}$) than for Assay I (2722.5 NmL and 177.1 NmL $L_r^{-1}$ $d^{-1}$), respectively. In contrast, no significant differences were observed to $CH_4$ yield, which was close to 330 NmL $CH_4$ g $COD_{rem}^{-1}$ for both assays (Table 3).

The average of cumulative biogas volume produced is presented in Figure 1. A degradation pattern was identified in both assays, which is characterized by two exponential phases. In this pattern, methane was produced in the initial phase, followed by decreasing in its production, which can be considered an adaptation phase. Thereafter, a significant increase in $CH_4$ production was verified, even higher than that observed in the initial phase, until it reached the stationary phase.

The adaptation phase in Assay I was longer, remaining for 13 days, while for Assay II this period was approximately 5 days. The longer

**Table 2.** Bioreactors Operation Data and Removal Efficiency

| Parameters | Unit | Assay I (Dairy Waste A) | Assay II (Dairy Waste B) |
|---|---|---|---|
| Operation time | days | 31 | 25 |
| pH (initial – final) | – | 8.2 – 8.0 | 8.2 – 7.9 |
| Carbohydrates (tC) removal | % | 96.8 | 98.0 |
| COD removal | % | 87.3 | 89.4 |
| VS removal | % | 87.0 | 86.4 |

adaptation time in $CH_4$ production verified in Assay I may have contributed to a lower production rate observed.

The methane production pattern observed in this study had already been reported in other studies as a typical behavior of residues with high lipids content, such as those from dairy products. HANAKI; MATSUO; NAGASE (1981) evaluated the inhibitory effect of long-chain fatty acids (LCFAs) (product of fat hydrolysis) on the anaerobic digestion using batch experiments from synthetic substrates, such as powdered whole milk, sodium oleate and a fatty acids mixture. They verified that the accumulation of LCFAs were linked with an increase in lag phase and with a decrease of methane yield at beginning of reactors operation.

Similarly, SAGE; DAUFIN; GESAN-GUIZIOU (2008) studied the anaerobic degradation steps of milk fat evaluating the kinetics of fat degradation in comparison with other milk components (lactose, proteins). The experiments were conducted using different substrates (Thermized cream, anhydrous milk fat, commercial UHT skim milk) which were evaluated "in natura" and after alkaline hydrolyze. The reactors were assembled with 250mL of active volume fulfilled with 5 $kg_{VSS}/m^3$ of anaerobic biomass and substrate to biomass ratio of 0.1 to 10 $kg_{COD}/kg_{VSS,}$ at 35ºC, under agitation (60 rpm), for 70 days. The authors verified that the milk fat

**Table 3.** Average Values of Cumulative $CH_4$ production, maximum $CH_4$ production rate and $CH_4$ yield

| Analysis of variance | Cumulative $CH_4$ production (NmL) | | Maximum$CH_4$ Rate NmL $L_r^{-1}$ $d^{-1}$ | | $CH_4$ Yield ($NmLCH_4$ g $COD_{rem}^{-1}$) | |
|---|---|---|---|---|---|---|
| F treatments | 18.71 | * | 185.05 ** | | 0 | |
| C.V. (%) | 3.30 | | 3.50 | | 3.22 | |
| Tukey's Test (5%): | | | | | | |
| Assay I | 2722.50 | b | 177.07 | b | 330.40 | a |
| Assay II | 3140.04 | a | 251.20 | a | 330.53 | a |

Means followed by the same letter within the same row do not differ (p>0.05) by Tukey Test. ** Significant at 1% probability level (p<0.01); * Significant at 5% probability level (p<0.05). C.V.: coefficient of variation

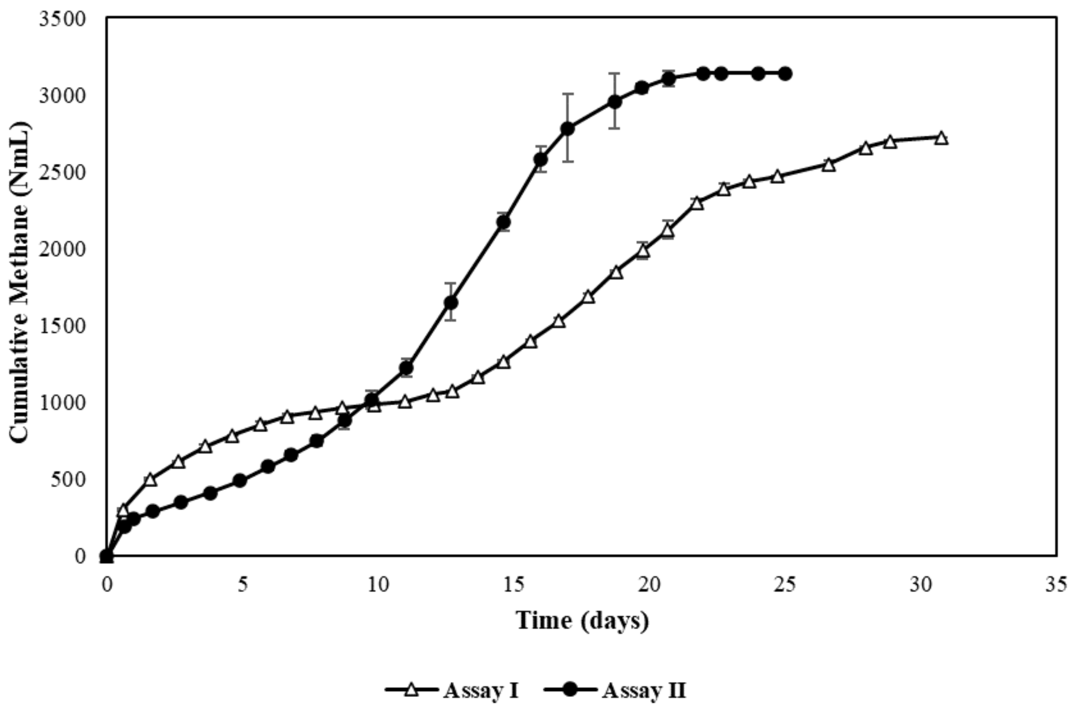


**Figure 1.** Cumulative Methane Production in Assay I (Dairy waste I) and Assay II (Dairy waste II)

was degraded after several days of lag phase and it occurred mainly due to unsaturated free fatty acids (FFA).

Therefore, lipids can be considered a limiting waste component in the AD due to its slow degradation caused by the accumulation LCFAs. This behavior leads to an increase in the adaptation phase and consequently a decrease in the initial methane production. This fact was verified by WU et al. (2011), who observed a decrease of $CH_4$ in biogas due to the increase of milk residue (from 66.5% for control to 63.9% for the assay with 19% of milk residue), which could be associated with more lipid content added to reactors. Despite that, no inhibition was observed and the authors obtained a methane yield of 324.1 mL $gCOD_{rem}^{-1}$.

The same phenomena was verified in the present study, in which despite a long adaptation phase mainly for Assay I, lipids degradation was not an inhibitory factor for none of the assays, since good results were achieved.

Sivakumar *et al.* (2012) the anaerobic digestion of 100% w/w of spoiled milk (200 g COD $L^{-1}$) using ASBR reactor fed daily for 30 days. The authors achieved lower values of the methane yield (220 mL $CH_4$ g $COD_{rem}^{-1}$) and of COD removal efficiency (92.8%) than the values got to Assay I and II.

Kopsahelis *et al.* (2018) investigated co-digestion of End-of-Life Dairy Products (~ 20% w/w) with agro-industrial wastes (composed of Pig Manure, Liquid Cow Manure, Cheese Whey, Poultry Wastes and Slaughterhouse Wastes) using mesophilic CSTR reactor with HRT of 37 days. For a methanogenic reactor (single-stage system), the maximum methane yield achieved was 230 mL $CH_4$ $gCOD_{rem}^{-1}$ with 71.9% of COD removal efficiency. These results were lower than those verified in the present study (with 330.4 and 330.5 NmL $CH_4$ g $COD_{rem}^{-1}$; and COD removal of 87.3% and 89.4% for Assay I and Assay II, respectively).

Thus, the results of this study demonstrated that the co-digestion of expired dairy products with synthetic sewage was efficient in the COD concentration proposed (20 g $L^{-1}$). Furthermore, satisfactory results of COD removal and methane production were obtained in both evaluated assays, especially when compared to previously studies with similar dairy wastes tested.

## CONCLUSIONS

- The anaerobic digestion of expired dairy products was effective, despite the longer adaptation phase in the initial methane production observed in both assays.
- The anaerobic co-digestion of expired dairy products with synthetic sewage was effective in the production of biogas.
- New approaches for reuse of dairy wastes by the use of anaerobic digestion are encouraged.
- Further studies should be undertaken in order to evaluate the effect of the increase of dairy wastes in the anaerobic digester performance.

## AUTHORSHIP CONTRIBUTION STATEMENT

**MARIN, D.F.C.:** Conceptualization, Investigation, Methodology, Writing – original draft, Writing – review & editing; **MENDOZA, L.M.:** Formal Analysis, Methodology; **CARVALHO JÚNIOR, R.P.:** Conceptualization, Methodology, Writing – review & editing; **MAINTINGUER, S.I.:** Methodology, Project administration, Supervision, Writing – review & editing.

## DECLARATION OF INTERESTS

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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