MINISTÉRIO DA EDUCAÇÃO
Universidade Federal de Juiz de Fora
Faculdade de Engenharia
Programa de Pós-Graduação em Engenharia Civil

Fernanda Bento Rosa Gomes

Assessment of ozonation as a pretreatment to increase the methane production potential of dairy manure wastewater

Juiz de Fora
2023

Fernanda Bento Rosa Gomes

Assessment of ozonation as a pretreatment to increase the methane production potential of dairy manure wastewater

Dissertação apresentada ao Programa de Pós-Graduação em Engenharia Civil da Universidade Federal de Juiz de Fora como parte dos requisitos para obtenção do título de Mestre em Engenharia Civil. Área de concentração: Saneamento e Meio Ambiente.

Orientadora: Profa. Dra. Sue Ellen Costa Bottrel – UFJF
Coorientador: Prof. Dr. Emanuel Manfred Freire Brandt – UFJF

Juiz de Fora
2023

Ficha catalográfica elaborada através do programa de geração
automática da Biblioteca Universitária da UFJF,
com os dados fornecidos pelo(a) autor(a)

Bento Rosa Gomes, Fernanda.
Assessment of ozonation as a pretreatment to increase the methane production potential of dairy manure wastewater / Fernanda Bento Rosa Gomes. -- 2023.
89 p.

Orientadora: Sue Ellen Costa Bottrel
Coorientador: Emanuel Manfred Freire Brandt
Dissertação (mestrado acadêmico) - Universidade Federal de Juiz de Fora, Faculdade de Engenharia. Programa de Pós-Graduação em Engenharia Civil, 2023.

1. Balanço energético. 2. Biogás. 3. Digestão anaeróbia. . 4. Ozônio. I. Costa Bottrel, Sue Ellen , orient. II. Manfred Freire Brandt Emanuel, coorient. III. Título.

Fernanda Bento Rosa Gomes

Assessment of ozonation as a pretreatment to increase the methane production potential of dairy manure wastewater

Dissertação apresentada ao Programa de Pós-Graduação em Engenharia Civil da Universidade Federal de Juiz de Fora como parte dos requisitos para obtenção do título de Mestre em Engenharia Civil. Área de concentração: Saneamento e Meio Ambiente.

Aprovada em (dia) de (mês) de (ano)

BANCA EXAMINADORA

Profa. Dra. Sue Ellen Costa Bottrel – Orientadora
Universidade Federal de Juiz de Fora - UFJF

Prof. Dr. Emanuel Manfred Freire Brandt – Coorientador
Universidade Federal de Juiz de Fora - UFJF

Profa. Dra. Renata de Oliveira Pereira
Universidade Federal de Juiz de Fora - UFJF

Profa. Dra. Camila Costa Amorim
Universidade Federal de Minas Gerais – UFMG

Prof. Dr. Marcelo Henrique Otenio
Empresa Brasileira de Pesquisa Agropecuária – EMBRAPA

AGRADECIMENTOS

Ao meu esposo e melhor amigo, Wilson, pelo apoio incondicional durante toda a minha trajetória. A todas as professoras e professores do Programa de Pós-Graduação em Engenharia Civil e do Departamento de Engenharia Sanitária e Ambiental da Universidade Federal de Juiz de Fora que, de alguma forma, contribuíram para a minha formação. Em especial, aos professores e amigos Sue Ellen, Emanuel, Samuel, Renata e Edgard pelos ensinamentos, orientação, parceria, compreensão e amizade têm me proporcionado desde o início da minha formação acadêmica. Aos professores Camila Amorim e Marcelo Otenio, por aceitarem o convite para compor a banca examinadora desta dissertação e contribuírem para o aperfeiçoamento deste trabalho que desenvolvi com tanto carinho junto aos meus orientadores ao longo dos últimos anos. Ao Marcelo Otenio e à Embrapa Gado de Leite, pelo apoio durante a pesquisa. À Iramaia, do Laboratório de Qualidade Ambiental (LAQUA) e demais colegas que me apoiaram na execução dos ensaios desta pesquisa. Aos colegas do PEC/UFJF, da Engenharia Ambiental e Sanitária/UFJF e do DEMLURB.

RESUMO

O Brasil detém um dos rebanhos bovinos mais numerosos do mundo e, como consequência desse fato, a geração de águas residuárias da bovinocultura brasileira também se torna expressiva. Devido a sua constituição rica em compostos orgânicos complexos, como a lignina e a celulose, tem-se a hidrólise como a etapa limitante na digestão anaeróbia desse efluente. Nesse contexto, a ozonização tem sido amplamente estudada como uma alternativa para promover a solubilização da matéria orgânica recalcitrante e aumentar sua biodegradabilidade. Em vista disso, os objetivos desse estudo foram avaliar os efeitos da ozonização como pré-tratamento visando o incremento na produção de metano durante a digestão anaeróbia de efluente da bovinocultura leiteira e determinar condições ótimas para um sistema de tratamento constituído de ozonização e digestão anaeróbia, pautando-se ainda no balanço energético do sistema. Para tal, realizou-se uma revisão sistemática da literatura, na qual avaliou-se o incremento na produção de metano devido a ozonização e calculou-se o balanço energético de cada experimento. De posse dos resultados, realizou-se um estudo em escala de bancada, no qual se avaliou o efeito de diferentes doses de ozônio (20 mg O_3. g^{-1} VS, 40 mg O_3. g^{-1} VS, 100 mg O_3. g^{-1} VS e 180 mg O_3.g^{-1} VS) sobre a produção de metano de efluente da bovinocultura de leite em pH natural e sobre o balanço energético do sistema proposto. Na revisão da literatura, observou-se que a ozonização tende a ter um efeito positivo sobre o potencial de produção de metano de efluentes e resíduos. Verificou-se, no geral, que substratos de biodegradabilidade reduzida ou ainda experimentos em condições desfavoráveis à biodigestão apresentaram balanço energético positivo quando doses baixas de ozônio foram aplicadas. Na etapa experimental, foram observadas altas eficiências de transferência de massa de ozônio em todas as doses aplicadas. Embora a dose de 100 mg O_3. g^{-1} VS tenha aumentado significativamente o potencial de produção de metano do efluente da bovinocultura leiteira, o balanço energético foi negativo em todos os cenários estudados. Ainda assim, observou-se que a pré ozonização resultou em potenciais benefícios tais como um menor tempo de fase *lag* na digestão anaeróbia, indicando uma aceleração da hidrólise, bem como uma maior estabilização do efluente final.

Palavras-chave: Balanço energético. Biogás. Digestão anaeróbia. Ozônio.

ABSTRACT

Brazil has one of the largest cattle herds in the world. Consequently, cattle manure wastewater production is also substantial in the country. Due to the presence of hardly-biodegradable organic molecules such as lignin and cellulose, hydrolysis is the rate-limiting step of the anaerobic digestion of cattle manure wastewaters. Within this context, ozonation has been widely studied as an alternative to promote the solubilization of refractory organic compounds and improve their biodegradability. Given these facts, the major objectives of this work were to assess the effects of ozonation as a pretreatment for increase methane production in anaerobic digestion of dairy manure wastewater (DMW) and determine optimal conditions for a treatment system constituted of pre-ozonation followed by anaerobic digestion, based on the energy balance of the system. For this, a systematic literature review was carried out to assess the effect of ozone pretreatment on methane production potential of wastes/wastewaters and on process energy balance. Subsequently, a bench-scale study was performed to assess the effect of different ozone doses (20 mg O_3. g^{-1} VS, 40 mg O_3. g^{-1} VS, 100 mg O_3. g^{-1} VS e 180 mg O_3.g^{-1} VS) on the methane production potential of a DMW at natural pH and on the energy balance of the proposed system. The literature review evidenced that ozonation tends to have a positive effect on the methane production potential of wastes and wastewaters. In general, substrates with a very low degradability or experiments under conditions unfavorable to anaerobic digestion (e.g., acidic pH or low temperatures) had positive energy balances when low ozone doses were applied. High ozone mass transfer efficiencies were observed for all ozone doses applied in the experimental stage. The dose of 100 mg O_3. g^{-1} VS significantly increased the methane production potential of DMW. However, the energy balance was negative in all studied scenarios. Even so, ozone pretreatment resulted in potential benefits such the reduction in anaerobic digestion lag phase time, which indicates an acceleration in hydrolysis, and a high stabilization of the final effluent.

Keywords: Anaerobic digestion. Biogas. Energy balance. Ozone.

LISTA DE FIGURAS

LISTA DE TABELAS

LISTA DE ABREVIATURAS E SIGLAS

AB	abstract
ar	article
ARG	antibiotic resistance gene
BMP	biomethane production potential
CH	coffee husks
$CH_{4,OZ}$	methane production of ozonated feedstock
$CH_{4,N\text{-}OZ}$	methane production of non-ozonated feedstock
CHP	combined heat and power
COD	chemical oxygen demand
DMW	dairy manure wastewater
E_B	energy balance
e_{O3}	electrical energy required per mass of ozone generated
FW	food waste
HRT	hydraulic loading rate
ISR	inoculum to substrate ratio
k	kinetic constant
$M_{O3,applied}$	mass of ozone applied
$M_{O3,consumed}$	mass of ozone consumed
$M_{O3,off\ gas}$	mass of ozone in the off gas
na	not analyzed
NCV_{CH4}	net calorific value for methane
NH3-N	ammoniacal nitrogen
nr	not reported
η	electric efficiency conversion in combined heat and power engines
$O_{3,applied}$	applied ozone dose
$O_{3,consumed}$	consumed ozone dose
OM	organic matter;
OM_{ox}	oxidized organic matter
OM_{sol}	solubilized organic matter
ORL	organic loading rate

P	methane production potential
pH	hydrogenionic potential
PPCP	pharmaceutical and personal care products
re	review article
r_m	maximum methane production rate
rRNA	ribosomal ribonucleic acid
sCOD	soluble chemical oxygen demand
sCOD/COD	soluble to total chemical oxygen demand ratio
SMP	specific methane production
SRT	sludge retention time
SS	suspended solids
STP	standard temperature and pressure
T	temperature
TI	title
TITLE-ABS-KEY	title, abstract, or keywords
TKN	total Kjeldahl nitrogen
TS	total solids
TSS	total suspended solids
UASB	upflow anaerobic sludge blanket
UV	ultraviolet
VFA	volatile fatty acids
VS	volatile solids
VSS	volatile suspended solids
VS/TS	volatile to total solids ratio
VSS/TSS	volatile suspended solids to total suspended solids
WWTP	wastewater treatment plant
Y	biomass yield coefficient
λ	lag phase time
μ_{max}	maximum growth rate of biomass

SUMÁRIO

1 INTRODUCTION

Animal products are among the most economic-relevant commodities produced in the world (USDA 2021). In this framework, Brazil has the second largest cattle stock of the word (USDA, 2023), which is estimated at more than 218 million heads (IBGE, 2020). Animal feeding operations are responsible for producing large amounts of manure and wastewaters. It is estimated that confined dairy cattle can generate from 21 to 600 L of wastewater per animal per day, depending on the type of operation (MITO *et al.*, 2018). Besides that, wastes and wastewaters from livestock and animal feeding operations typically contain high concentrations of organic matter and nutrients in addition to having of several contaminants, such as pathogens and estrogens, which may pose critical risks to the environment and human health (FONT-PALMA, 2019; USDA, 2012).

Within this context, anaerobic digestion is a well-documented treatment technology applicable to several wastes and wastewaters (BRASIL, 2015; CHERNICHARO, 2019). Due to its economic and environmental benefits (e.g., low cost and potential for energy recovery) the use of anaerobic lagoons and digesters for manure/wastewater treatment has been expanding worldwide (AGGA *et al.*, 2022; FONT-PALMA, 2019; LOYON, 2018). However, the excess of particulate organic matter and complex organic compounds may make hydrolysis a limiting step of the anaerobic digestion of specific substrates (CHERNICHARO, 2019; PEI *et al.*, 2016; SILVESTRE *et al.*, 2015), including animal wastes and other lignocellulosic feedstocks (ADARME *et al.*, 2017; CHEN *et al.*, 2021), sludges from wastewater treatment plants (SILVESTRE *et al.*, 2015), and organic solid wastes (YUE *et al.*, 2020).

Given these facts, several pretreatment processes have been studied focusing on solubilization or partial oxidation of complex substrates, aiming to improve their degradation and increase biogas production. These approaches may involve both physicochemical and biological processes, such as milling, microwave, ultrasound, pre-oxidation (UV radiation, Fenton, ozonation, H_2O_2, etc.), enzymatic or fungal pretreatments (AI *et al.*, 2019; DOMAŃSKI *et al.*, 2017; PEI *et al.*, 2016; PEI *et al.*, 2015; TAHERZADEH; KARIMI, 2008; TIAN *et al.*, 2015).

From this point of view, ozonation has been widely studied as a pretreatment for anaerobic digestion of complex substrates (BAKHSHI *et al.*, 2018; BOUGRIER *et al.*, 2007; CHEN *et al.*, 2021; GOEL *et al.*, 2003; WEEMAES *et al.*, 2000b). The partial oxidation of organic matter through ozonation can promote solubilization and/or formation of more bioavailable organic compounds such as carboxylic acids, aldehydes, and ketones (VON SONNTAG; VON GUNTEN, 2012). Due to this, several studies have reported that pre-ozonation can increase methane production during anaerobic digestion (BAKHSHI *et al.*, 2018; CHEN *et al.*, 2021; DAS *et al.*, 2021; SETHUPATHY *et al.*, 2020; WENJING *et al.*, 2019).

Despite the potential benefits, the knowledge about the effects of ozone pretreatment on the anaerobic digestion of dairy manure wastewaters (DMW) is still limited. A recent study performed by Chen *et al.* (2021) has focused on the effect of ozone pretreatment on the fate of enteric indicator bacteria and antibiotic resistance genes before and after anaerobic digestion of DMW. Their work included pre-ozonation at applied ozone doses varying from 7.4 mg O_3. g^{-1} VS to 22 mg O_3. g^{-1} VS (pH not reported) increased the methane production potential of DMW by up to 11%. Furthermore, ozonation reduced the relative abundance of antibiotic resistance genes (copies of ARGs/copies 16S rRNA) but did not reduce their absolute concentration (log copies/L) in the digestate. Therefore, studies to assess the effect of ozonation on the anaerobic digestion of DMW and to investigate optimal conditions for ozone pretreatment to pre-oxidize and solubilize complex organic compounds in DMW to improve its methane production potential are still needed.

1.1 OBJECTIVES

The major aims of this work were to assess the effects of ozonation as a pretreatment to increase methane production potential of DMW and investigate the optimal conditions for a DMW treatment system constituted of pre-ozonation and anaerobic digestion.

1.2 SPECIFIC OBJECTIVES

The specific objectives of this work were:

i) To collect literature data regarding the experimental conditions and the corresponding results of previous studies, in order to assess the effect of ozone pretreatment experimental conditions (ozone dose, pH, and organic content) on methane production potential and energy balance (E_B) of earlier experiments with different feedstocks;

ii) To evaluate the effect of ozone dose on the concentration of total volatile solids (VS), chemical oxygen demand (COD), suspended solids (TSS), volatile suspended solids (VSS), and soluble chemical oxygen demand (sCOD) of ozonated DMW;

iii) To evaluate the effect of ozone dose on the kinetic parameters of anaerobic digestion (methane production potential – P, maximum methane production rate – r_m, and lag phase time – λ) of DMW;

iv) To evaluate the effect of ozone dose on the E_B of the proposed system (ozonation + anaerobic digestion), in order to investigate optimal conditions for ozone pretreatment.

1.3 HYPOTHESIS

There is an experimental condition in which the applied ozone dose promotes the oxidation of complex organic compounds present in DMW, improving the methane production potential of DMW in parallel to maintaining the process energetic sustainability

2 FUNDAMENTAL CONCEPTS AND LITERATURE REVIEW

2.1 CATTLE MANURE AND CATTLE MANURE WASTEWATERS: CHARACTERISTICS AND MANAGEMENT PRACTICES

The quantity and the characteristics of manure and manure wastewater generated on a cattle farm can vary in a wide range (Table 1), depending on several aspects, including the type of farm, the type of cattle raised in the farm (beef or dairy cattle), the number of heads, the type of feeding system (e. g., pasture or confinement), and the manure management practices (MITO *et al.*, 2018; VARMA *et al.*, 2021).

Within this context, the concentration of solids and other constituents in manure/wastewater collected in confined or semi-confined feeding systems strongly depends on manure collection and transport systems. Scraping-based systems usually generate concentrated manure to be treated and/or disposed. On the other hand, the quantity, the constitution, and the dilution degree of wastewaters generated in flushing systems depend on factors such as the quantity and the quality of water used (HARNER; MURPHY, 1997).

A review conducted by Mito *et al.* (2018) gathered information regarding wastewater generation by confined beef and dairy cattle. According to their work, confined beef cattle can generate 20.5 to 80 L wastewater. animal^{-1}. d^{-1}, whereas large amounts of wastewater (21 to 600 L wastewater. animal^{-1}. d^{-1}) can be generated by dairy cattle raised in confined feeding systems. On average, wastewater generation from beef and dairy cattle can be estimated at 38.9 ± 18.3 L wastewater. animal^{-1}. d^{-1} (number of observations = 11) and 85.0 ± 105.5 L wastewater. animal^{-1}. d^{-1} (number of observations = 31), respectively.

In comparison with domestic wastewaters, manure and manure wastewater usually have high concentrations of solids, COD, nitrogen, and alkalinity (Table 1). Manure and manure wastewaters are also known to be rich in hardly biodegradable molecules such as lignin, cellulose, and hemicellulose (KAFLE; CHEN, 2016). In addition, several studies report the presence of pathogens, antimicrobial resistance bacteria/genes, and estrogen-like endocrine disrupting chemicals compounds in these wastewaters (FILGUEIRAS *et al.*, 2022; NASCIMENTO *et al.*, 2020; PEREIRA *et al.*, 2021; RESENDE *et al.*, 2014a; RESENDE *et al.*, 2014b). These specificities evidence the importance of proper wastewater management in animal feeding operations.

Table 1 – Main characteristics of cattle manure, cattle manure wastewaters, and domestic wastewaters

Sample	TS ($g.L^{-1}$)	VS ($g.L^{-1}$)	VS/TS	TSS ($g.L^{-1}$)	VSS ($g.L^{-1}$)	VSS/TSS	COD ($g.L^{-1}$)	sCOD ($g.L^{-1}$)	NH_3-N ($mgN.L^{-1}$)	TKN ($mgN.L^{-1}$)	pH	Alkalinity ($mgCaCO_3.L^{-1}$)	Reference
Cattle manure	94.0 [a]	77.6 [a]	0.83	nr	nr	nr	nr	nr	nr	nr	6.4	4,200	(ABDELWAHAB *et al.*, 2020; 2021a; ABDELWAHAB *et al.*, 2021b)
	223.0 [a]	157.0 [a]	0.69	nr	nr	nr	nr	nr	nr	nr	nr	nr	(BI *et al.*, 2019)
	112.0 [a]	92.7 [a]	0.83	nr	nr	nr	134.2	nr	nr	nr	nr	nr	(LIU *et al.*, 2018)
	223.1	195.0	0.87	nr	nr	nr	nr	nr	1,902	nr	7.0	9,990	(ZHANG *et al.*, 2022)
Beef cattle manure	310.0 [a]	200.0 [a]	0.65	nr	nr	nr	nr	nr	1,100	nr	7.7	nr	(BUENDÍA *et al.*, 2009)
	298.0 [a]	246.0 [a]	0.83	nr	nr	nr	44.9	nr	6,400	29,700	7.1	nr	(WU-HAAN *et al.*, 2010)
Dairy manure	184.2 [a]	151.0 [a]	0.82	nr	nr	nr	nr	nr	nr	nr	6.2	nr	(SINGH *et al.*, 2022)
	169.1 [a]	102.5 [a]	0.61	nr	nr	nr	13.8	2.4	nr	1,005	8.2	3,580	(KAFLE; CHEN, 2016)
	8.7 [a]	5.4 [a]	0.62	nr	nr	nr	nr	nr	nr	nr	nr	nr	(PANDEY *et al.*, 2010)
	112.0 - 161.0 [a]	98.4 - 143.0 [a]	0.88-0.89	nr	nr	nr	nr	nr	nr	nr	6.2 - 6.9	6,910 - 9,100	(SAADY; MASSÉ, 2015)
	130.7	120.0	0.92	nr	nr	nr	nr	nr	1,300	nr	7.8	nr	(ZEB *et al.*, 2019)
Dairy manure wastewater	48.0 [a]	36.0 [a]	0.75	13.0	nr	nr	43.7	19.5	nr	nr	nr	nr	(CHEN *et al.*, 2021)
	44.8	35.3	0.79	nr	nr	nr	80.4	39.1	nr	nr	nr	nr	(WANG *et al.*, 2019)
	105.0 [a]	91.0 [a]	0.87	nr	nr	nr	29.2	nr	25,200	55,500	6.9	nr	(WU-HAAN *et al.*, 2010)
Domestic wastewater	0.7 – 1.4	0.4 – 0.7	0.65	0.2 – 0.5	0.2 – 0.4	0.90	0.5 – 0.8	nr	20 - 35	35 - 60	6.7 - 8.0	100 - 250	(VON SPERLING, 2018)

Legend: a: density assumed as 1.0 kg. L^{-1}; TS: total solids; VS: volatile solids; TSS: total suspended solids; VSS: volatile suspended solids; COD: chemical oxygen demand; sCOD: soluble chemical oxygen demand; NH_3-N: ammoniacal nitrogen; TKN: total Kjeldahl nitrogen.

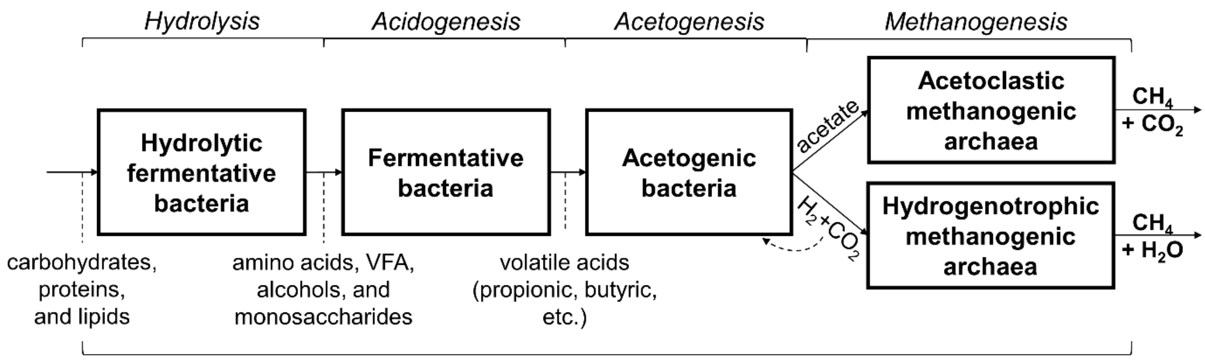
2.2 ANAEROBIC DIGESTION OF CATTLE MANURE AND CATTLE MANURE WASTEWATERS

2.2.1 Fundamentals

Anaerobic digestion is a consolidated technology that has been gaining attention due to its low cost and operational simplicity. This technology is applicable to several wastes and wastewaters, notably to those with high organic content, such as manure and livestock wastewaters (BRASIL, 2015; CHERNICHARO, 2019).

In this process, complex organic matter is sequentially converted to simple products by a microbial consortium (Figure 1). In summary, hydrolytic fermentative bacteria are responsible for excreting exoenzymes, which act in the hydrolysis of particulate complex organic matter, forming small and dissolved organic molecules (e. g., amino acids, volatile fatty acids - VFA, alcohols and monosaccharides) (CHERNICHARO, 2019). This stage is particularly critical in anaerobic digestion of cattle manure and cattle manure wastewaters, which are rich in hardly hydrolysable molecules (e. g., lignin, cellulose). Due to this fact, hydrolysis is considered the rate-limiting step of the anaerobic digestion of cattle manure and cattle manure wastewaters (LIEW *et al.*, 2020).

Figure 1 – Stages, products, and microbial consortium involved in anaerobic digestion



Legend: VFA: volatile fatty acids.
Adapted from Chernicharo (2019)

Subsequently, hydrolyzed products are converted by fermentative acidogenic bacteria and acetogenic bacteria to volatile acids (acidogenesis) and acetate, H_2 and CO_2 (acetogenesis) respectively (CHERNICHARO, 2019). It is worth to mention that the acidogenesis also deserves special attention due to the potential of accumulation of volatile fatty acids and hydrogen (AQUINO; CHERNICHARO, 2005). These processes can occur during organic shock loads as a result of the high growth rate of acidogenic bacteria (Table 2). The critical consequence of this accumulation is a high consumption of alkalinity and pH reduction, which may compromise the growth of methanogenic archaea (CHERNICHARO, 2019).

Table 2 – Kinetic parameters of the different stages of mesophilic anaerobic digestion

Micro-organism	**Substrate**	**Y (g VSS. g^{-1} COD)**	**μ_{max} (d^{-1})**
Fermentative bacteria	Carbohydrates	0.14 – 0.17	7.2 – 30.0
Fermentative bacteria	Long-chain fatty acids	0.04 – 0.11	0.09 – 0.55
Acetogenic bacteria	Short-chain fatty acids	0.025 – 0.047	0.13 – 1.20
Acetoclastic methanogenic archaea	Acetate	0.010 – 0.054	0.08 – 0.70
Hydrogenotrophic methanogenic archaea	$H_2 + CO_2$	0.017 – 0.045	0.05 – 4.07

Legend: Y: biomass yield coefficient; μ_{max}: maximum growth rate of biomass.
Adapted from Aquino and Chernicharo (2005)

Finally, acetate is oxidized into CH_4 and CO_2 by acetoclastic methanogenic archaea, whereas H_2 and CO_2 are converted into CH_4 and H_2O by hydrogenotrophic methanogenic archaea (CHERNICHARO, 2019).

2.2.2 Biogas and energy recovery

Biogas is the main product of organic matter degradation in anaerobic digestion. During this process, it is estimated that about 50 to 70% of the COD from the feedstocks is converted to biogas. Its typical composition includes about 50% to 70% CH_4, 30% to 40% CO_2 and trace amounts of water vapor, H_2S and other gases (CHERNICHARO, 2019).

Among these constituents, methane is highlighted due to both its global warming potential and its potential for energy recovery. According to the U. S. Environmental Protection Agency, the global warming potential of methane is about 27-30 times higher than CO_2 in a 100-year timescale (USEPA, 2022). Therefore, the management of gaseous emissions in anaerobic reactors is essential. This process usually involves flaring, a process in which methane is converted to CO_2 through combustion (KAMINSKI *et al.*, 2021; SANTOS *et al.*, 2021). However, the calorific value of methane also makes biogas a valuable source of renewable energy (CHERNICHARO *et al.*, 2017; SANTOS *et al.*, 2018). Methane can be recovered from biogas to produce thermal and electrical energy through boilers, gas engines, turbines, and combined heat and power (CHP) engines. Additionally, biogas can also be upgraded to biomethane and used as a vehicular fuel or as a substitute for the natural gas (BRASIL, 2015; SANTOS *et al.*, 2021).

2.2.3 Environmental requirements, control parameters, and earlier studies

The growth of the anaerobic microbial consortium and the efficiency of anaerobic treatment processes are directly affected by the environmental conditions. In this regard, carbon, nutrient, and micronutrient demands, pH, and alkalinity can be cited as among the main waste/wastewater characteristics that should be analyzed (CHERNICHARO, 2019).

It is expected that cattle manure and cattle manure wastewater (Table 1) meet the carbonaceous and nutritional requirements of anaerobic biota (CHERNICHARO, 2019). Furthermore, the typically high alkalinity observed in these feedstocks (Table 1) is expected to be sufficient to control pH during acidogenesis. According to Chernicharo (2019), the optimal pH for the growth of archaea ranges from 6.6 to 7.4, whereas methane production can be stable at a pH 6.0 to 8.0. As can be seen in Table 1, the values reported in cattle manure and cattle manure wastewater are typically in this range.

In addition, there are several operational control parameters that can influence the efficiency of anaerobic treatment processes, such as VFA concentration and reactor pH (as previously discussed), temperature, hydraulic retention time (HRT) and sludge retention time (SRT), organic loading rate (OLR), and inoculum to substrate ratio (ISR) (AKÇAKAYA, 2021; CHERNICHARO, 2019). In the case of temperature, it is known that the optimal growth temperature of anaerobic microbiota occurs in mesophilic (30 to 35ºC) and thermophilic (50 to 55ºC) conditions. Given this fact, experiments with cattle manure and cattle manure wastewater treatment are often maintained in mesophilic conditions, especially at 35ºC (Table 3).

Table 3 – Experimental conditions of batch anaerobic digestion tests applied to cattle manure and cattle manure wastewaters

Substrate	**$VS_{substrate}$ ($g.L^{-1}$)**	**Inoculum**	**ISR (gVS: gVS)**	**T (ºC)**	**SRT (d)**	**SMP**	**Reference**
Cattle manure (diluted 1:1)	77.6 (diluted)	Sludge from a digester treating cattle manure	1.0	33	28-30	18.6 mL CH_4. g^{-1} VS	(ABDELWAHAB *et al.*, 2020; 2021a; ABDELWAHAB *et al.*, 2021b)
Cow manure	157.0	Sludge from a digester treating manure + FW	0.7	37	30	111.0 mL CH_4. g^{-1} VS	(BI *et al.*, 2019)
Beef cattle manure	200.0	Anaerobic sludge from municipal WWTP	0.8	35	50	84.0 mL CH_4. g^{-1} VS	(BUENDÍA *et al.*, 2009)
Dairy manure wastewater	36.0	Anaerobic sludge from municipal WWTP	0.25	35	32	187.0 mL CH_4. g^{-1} VS	(CHEN *et al.*, 2021)
Dairy cattle manure	102.5	Sludge from a digester treating dairy manure	2.0	36.5	90	201.9 mL CH_4. g^{-1} VS	(KAFLE; CHEN, 2016)
Cattle slurry	92.7	Sludge from a digester treating agro-industrial wastes	2.0-3.3	37	27	259.1 mL CH_4. g^{-1} VS	(LIU *et al.*, 2018)
Dairy cattle manure (after solids separation)	5.4	Sludge from a digester treating potato starch waste	0.7, 1.0, 1.4, 1.8, 2.2, 2.5	35	50	nr	(PANDEY *et al.*, 2010)
Dairy cattle manure	98.4-143.0	Sludge from a digester treating dairy manure	0.6, 0.7, 0.8, 0.9, 1.0, 1.7	20	21	128.9 - 227.9 mL CH_4. g^{-1} VS	(SAADY; MASSÉ, 2015)
Dairy cattle manure	151.0	None	-	35	56	55.6 mL CH_4. g^{-1} VS	(SINGH *et al.*, 2022)
Dairy manure wastewater	35.3	Sludge from a digester treating dairy manure	4.3	37	30	351.0 mL CH_4. g^{-1} VS	(WANG *et al.*, 2019)
Beef cattle manure (diluted) Dairy manure wastewater (diluted) Dairy manure wastewater (after fibers separation and dilution)	246.0(undiluted) 91.0 (undiluted) 39.0 (undiluted)	Anaerobic sludge (source not reported)	1.0	35	30	nr	(WU-HAAN *et al.*, 2010)
Dairy manure wastewater	nr	Anaerobic sludge from municipal WWTP	0.25	35	60	nr	(ZAHER *et al.*, 2009)
Dairy manure	120.0	Anaerobic sludge from municipal WWTP	1.0	37	20	252.0 mL CH_4. g^{-1} VS	(ZEB *et al.*, 2017; ZEB *et al.*, 2019)
Cattle manure	195.0	Sludge from a digester treating pig manure	0.5	35	45	107.1	(ZHANG *et al.*, 2022)

Legend: VS: volatile solids; ISR: inoculum to substrate ratio; T: temperature; SRT: sludge retention time; SMP: specific methane production; FW: food waste; WWTP: wastewater treatment plant.

The HRT and SRT represent the time that the liquid fraction and the solid fraction (sludge) of a feedstock are maintained in the treatment system (CHERNICHARO, 2019). Conventional anaerobic treatment systems are characterized by the absence of solids retention mechanisms. Due to this fact, these systems often requires high HRTs to enable the microbial growth (CHERNICHARO, 2019). In parallel, high-rate anaerobic treatment processes, such as upflow anaerobic sludge blanket (UASB) reactors, are designed to promote the retention of the active microbiota in the reactor, which enables the operation at HRTs (CHERNICHARO, 2019).

Lab-scale batch anaerobic digestion tests usually represent conventional treatment systems in which the HRT is equal to the SRT. In these tests it is recommended to provide a SRT sufficiently high for the stabilization of the curve of methane production (DBFZ, 2022). As a result of the high suspended solids content and the presence of lignin, cellulose, and other hardly-biodegradable molecules, these feedstocks require high SRT (Table 3).

The ISR represents the proportion between the mass of active micro-organisms (inoculum) and the mass of substrate fed in the reactor (ZHANG *et al.*, 2020). The control of this parameter is particularly important to present operational problems such as nutritional deficiencies, which can occur at high ISRs and compromise the microbial growth, and VFA accumulation, which can occur at low ISRs and inhibit the growth of methanogens (LI *et al.*, 2022; SILVA *et al.*, 2020; ZHANG *et al.*, 2020). Due to these aspects, ISR is a key parameter for biomethane potential (BMP) tests (SILVA *et al.*, 2020)

There is no consensus regarding the optimal ISR for anaerobic digestion of cattle manure and cattle manure wastewater. A wide range of ISRs (0.25 to 4.3 g $VS_{inoculum}$: g $VS_{substrate}$) was reported in the consulted literature (Table 3). In this regard, Saady and Massé (2015) had the best results in terms of methane production potential at 0.9 and 1.0 g $VS_{inoculum}$: g $VS_{substrate}$.

Values reported for the specific methane production of these substrates also present a great variability (18.6 to 351.0 mL CH_4. g^{-1} VS), probably due to the variable composition of cattle manure and cattle manure wastewaters and the differences in the experimental conditions of each study. In general, the methane production potential reported for these feedstocks is maintained in the range described in *ProBiogas* guide (120 – 300 mL CH_4. g^{-1} VS) (BRASIL, 2015). In terms of stabilization of the final effluent, it is reported a reduction of about 5% in the volatile to total solids ratio (VS/TS) during anaerobic digestion of manure and wastewaters from cattle ranching (ABDELWAHAB *et al.*, 2020; 2021a; SINGH *et al.*, 2022).

2.3 PRETREATMENTS APPLICABLE TO IMPROVE ANAEROBIC DIGESTION

Different pretreatment techniques have been studied as alternatives to increase feedstocks bioavailability and improve anaerobic digestion (Table 4). These processes can increase the accessible surface area of particles and favor the hydrolytic activity, which frequently result in an improved organic matter degradation efficiency and an increased biogas yield (Table 4) (AKÇAKAYA, 2021; TAHERZADEH; KARIMI, 2008; ZHENG *et al.*, 2014). These effects also can reduce the required HRT, optimizing the process (AKÇAKAYA, 2021).

Increases in the soluble organic content and reduction of particle size are the main effects observed after the different pretreatment processes (Table 4). As can be seen in Table 4, physical pretreatments are the most commonly used processes (LI *et al.*, 2021).

Despite the fact that ozone pretreatment has been widely reported as a successfully alternative to improve anaerobic digestion of distinct feedstocks (BAKHSHI *et al.*, 2018; BESZÉDES *et al.*, 2009; CARBALLA *et al.*, 2007; CATENACCI *et al.*, 2022; CESARO; BELGIORNO, 2020; CHIAVOLA *et al.*, 2019; GOEL *et al.*, 2003; WEEMAES *et al.*, 2000a; YANG *et al.*, 2018), Table 4 evidences that published data about the effects of ozone pretreatment on the anaerobic digestion of cattle manure and cattle manure wastewaters are still limited.

Table 4 – Pretreatment processes used to improve the anaerobic digestion of cattle manure and their main effects

Pretreatment method	Pretreatment process	Substrate	Effect on substrate characteristics	Effect on anaerobic digestion	Reference
Physical	Shredding	Cattle manure	Particle size reduction	No significant effect	(COARITA FERNANDEZ *et al.*, 2020b)
	Shredding and mixing	Cattle manure	Particle size reduction Homogeneity	No significant effect	(COARITA FERNANDEZ *et al.*, 2020b)
	Shredding, mixing, and blending	Cattle manure	Particle size reduction	↑CH_4 (12%)	(COARITA FERNANDEZ *et al.*, 2020b)
	Milling	Digested cattle manure biofibers	Particle size reduction	↑CH_4 (8%)	(BRUNI *et al.*, 2010)
	Milling	Digested cattle manure biofibers	No significant effect on lignocellulosic content	↑CH_4 (9%)	(KHAN; AHRING, 2021)
	Milling	Cattle manure	Particle size reduction	↑CH_4 (15%)	(COARITA FERNANDEZ *et al.*, 2020a)
	Maceration	Cattle manure	Particle size reduction	↑CH_4 (16-20%)	(ANGELIDAKI; AHRING, 2000)
	Decompression explosion	Cattle manure	Particle size reduction	↑CH_4 (17%)	(ANGELIDAKI; AHRING, 2000)
	Mechanical pretreatment	Digested cattle manure biofibers	nr	↑CH_4 (0-45%)	(TSAPEKOS *et al.*, 2016)
	Solid-liquid separation	Cattle manure	*Solid phase:* Increased COD, TS, and VS *Liquid phase:* Reduced COD, TS, and VS	*Solid phase:* ↑CH_4 (67%) *Liquid phase:* ↑CH_4 (133%)	(NEGRAL *et al.*, 2017)
	Thermal pretreatment	Cattle manure	nr	↓CH_4 (7%)	(QIAO *et al.*, 2011)
	Thermal pretreatment	Cattle manure	nr	↑CH_4 (29%)	(CANO *et al.*, 2014)
	Thermal pretreatment	Digested cattle manure biofibers	Reduced cellulose and hemicellulose concentration	↑CH_4 (48%)	(KHAN; AHRING, 2021)
	Ultrasonic pretreatment	Dairy manure wastewater	Increased particle size No significant effect on sCOD and SS content	No significant effect	(CHEN *et al.*, 2021)
	Ultrasonic pretreatment	Cattle manure	Increases in the accessible surface of particles	↑CH_4 (59%)	(ORMAECHEA *et al.*, 2018)

Legend: VS: volatile solids; nr: not reported; sCOD: soluble chemical oxygen demand; SS: suspended solids.

Table 4 – (continued)

Pretreatment method	Pretreatment process	Substrate	Effect on substrate characteristics	Effect on anaerobic digestion	Reference
Chemical	Acid pretreatment (H_2SO_4)	Cattle manure	Reduced lignocellulosic content	↑CH_4 (117%)	(LI *et al.*, 2009)
	Acid pretreatment ($C_2H_4O_3$)	Cattle manure	Increased sCOD Reduced lignin concentration Increased cellulose and hemicellulose concentration	↑CH_4 (39%)	(RAMOS-SUÁREZ *et al.*, 2017)
	Alkaline pretreatment (CaO)	Cattle manure	Increased sCOD No significant effect on lignocellulosic content	↑CH_4 (26%)	(RAMOS-SUÁREZ *et al.*, 2017)
	Alkaline pretreatment (CaO)	Digested cattle manure biofibers	nr	↑CH_4 (59%)	(BRUNI *et al.*, 2010)
	Alkaline pretreatment (NaOH)	Cattle manure biofibers	nr	↑CH_4 (13-23%)	(ANGELIDAKI; AHRING, 2000)
	Alkaline pretreatment (NaOH)	Digested cattle manure biofibers	Reduced cellulose and hemicellulose concentration	↑CH_4 (11%)	(KHAN; AHRING, 2021)
	Alkaline pretreatment (NH_4OH)	Cattle manure biofibers	nr	↑CH_4 (0-23%)	(ANGELIDAKI; AHRING, 2000)
	Alkaline pretreatment (NaOH: KOH: $Ca(OH)_2$)	Cattle manure biofibers	nr	↑CH_4 (20%)	(ANGELIDAKI; AHRING, 2000)
	Acid ($C_2H_4O_3$) + Alkaline (CaO) pretreatment	Cattle manure	Increased sCOD Reduced lignin concentration Increased cellulose and hemicellulose concentration	↑CH_4 (141%)	(RAMOS-SUÁREZ *et al.*, 2017)
	Ozone pretreatment	Dairy manure wastewater	Particle size reduction Increased sCOD No significant effect on SS content	↑CH_4 (0-11%)	(CHEN *et al.*, 2021)
Biological	Enzymatic pretreatment	Digested cattle manure biofibers	nr	No significant effect	(BRUNI *et al.*, 2010)
	Partial aerobic pretreatment	Digested cattle manure biofibers	nr	No significant effect	(BRUNI *et al.*, 2010)
	Hemicellulose degrading bacterium B4	Cattle manure biofibers	nr	↑CH_4 (30%)	(ANGELIDAKI; AHRING, 2000)

Legend: VS: volatile solids; nr: not reported; sCOD: soluble chemical oxygen demand; SS: suspended solids.

Table 4 – (continued)

Pretreatment method	Pretreatment process	Substrate	Effect on substrate characteristics	Effect on anaerobic digestion	Reference
Combined (physical/ chemical/ biological) pretreatments	Catalyzed (H_3PO_4) steam explosion	Digested cattle manure biofibers	Increased VS content	↑CH_4 (6%)	(BRUNI *et al.*, 2010)
	Catalyzed (NaOH) steam explosion	Digested cattle manure biofibers	Mass loss Increased VS content	↑CH_4 (38%)	(BRUNI *et al.*, 2010)
	Catalyzed (NaOH) steam explosion + enzymatic pretreatment	Digested cattle manure biofibers	Mass loss Increased VS content	↑CH_4 (24%)	(BRUNI *et al.*, 2010)
	Catalyzed (H_3PO_4) steam explosion + enzymatic pretreatment	Digested cattle manure biofibers	Increased VS content	↑CH_4 (69%)	(BRUNI *et al.*, 2010)
	Thermal alkaline (NaOH) pretreatment	Digested cattle manure biofibers	nr	↑CH_4 (~0-420%)	(TSAPEKOS *et al.*, 2016)
	Thermal alkaline (NaOH) pretreatment	Digested cattle manure biofibers	Reduced cellulose and hemicellulose concentration	↑CH_4 (86-127%)	(KHAN; AHRING, 2021)
	Ultrasound/ozone pretreatment	Dairy manure wastewater	Particle size reduction Increased sCOD Reduced SS content	↑CH_4 (0-13%)	(CHEN *et al.*, 2021)

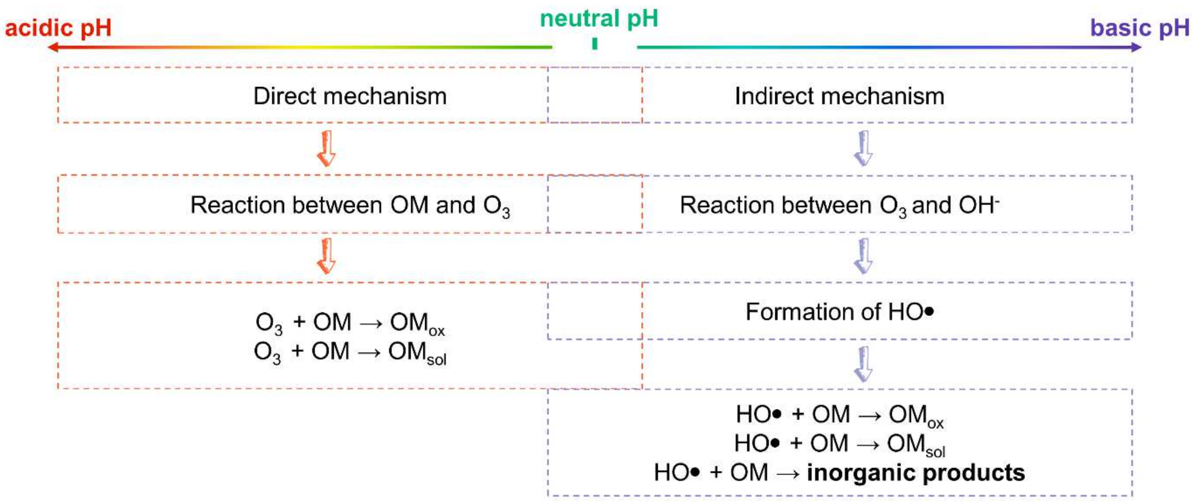
Legend: VS: volatile solids; nr: not reported; sCOD: soluble chemical oxygen demand; SS: suspended solids.

2.4 OZONATION AS A PRETREATMENT FOR IMPROVING ANAEROBIC DIGESTION

2.3.1 Fundamentals

The mechanisms of ozonation are known to be strongly influenced by pH and the presence of hydroxyl ions (HO^-) in solution. At pH<7 ozone reacts preferably with organic matter (direct mechanism), forming oxidized organic products or CO_2 (Figure 2) (VON GUNTEN, 2003; VON SONNTAG; VON GUNTEN, 2012).

Figure 2 – Ozone pretreatment reaction mechanisms



Legend: OM: organic matter; OM_{ox}: oxidized organic matter; OM_{sol}: solubilized organic matter.

On the other hand, under alkaline conditions, the main mechanism of reaction involves the interaction between ozone (O_3) and hydroxyl ions (HO^-) (indirect mechanism) forming oxidizers as hydroxyl radicals ($HO^\bullet$) and other radical species. As a result of their increased reduction potential, these radicals can completely oxidize organic matter easier than ozone (Figure 2) (VON GUNTEN, 2003; VON SONNTAG; VON GUNTEN, 2012). The Equations 1-6 show the reactions involved in the formation of $HO^\bullet$ and other radical species during ozonation as well as their respective kinetic constants (*k*) (VON GUNTEN, 2003; VON SONNTAG; VON GUNTEN, 2012).

$O_3 + OH^- \rightarrow HO_2^- + O_2$	$k = 70\ M^{-1}.\ s^{-1}$	Equation 1
$O_3 + HO_2^- \rightarrow HO^\bullet + O_2^{\bullet -} + O_2$	$k = 2.8 \times 10^6\ M^{-1}.\ s^{-1}$	Equation 2
$O_3 + O_2^{\bullet -} \rightarrow O_3^{\bullet -} + O_2$	$k = 1.6 \times 10^9\ M^{-1}.\ s^{-1}$	Equation 3
$O_3^{\bullet -} \rightleftharpoons O^{\bullet -} + O_2$	$k = 3.3 \times 10^9\ M^{-1}.\ s^{-1}$	Equation 4
$O^{\bullet -} + H_2O \rightarrow HO^\bullet + OH^-$	$k = 10^8\ M^{-1}.\ s^{-1}$	Equation 5
$HO^\bullet + O_3 \rightarrow HO_2^\bullet + O_2$	$k = 2.0 \times 10^9\ M^{-1}.\ s^{-1}$	Equation 6

Within this context, earlier studies suggest that mineralization of organic matter due to ozonation may reduce the organic fraction available for the anaerobic microbial consortium and decrease biogas production (CESARO; BELGIORNO, 2013; MARTIN *et al.*, 2002; YU *et al.*, 2014; YUE *et al.*, 2020).

2.3.2 Control parameters

The main control parameters of ozonation include those related with the ozone dose and the ozone mass transfer efficiency. These parameters include pH and alkalinity (which determine the reaction mechanisms), the ozone concentration in the feed gas, the gas flow rate, and ozonation time (which determine the applied ozone dose). Additionally, other important aspects that influence the ozone mass transfer efficiency can also be cited, such as the temperature (which is positively correlated with the solubility of ozone in aqueous phase), the wastewater constituents (organic and solids contents, size of the particles, presence of hydroxyl scavengers e. g., carbonates/bicarbonates), and the reactor design (TRAVAINI *et al.*, 2016; VON GUNTEN, 2003; VON SONNTAG; VON GUNTEN, 2012; ZHENG *et al.*, 2014).

2.3.3 Energy requirements

Table 5 shows typical values for the electrical energy required per kg of ozone produced reported in literature. As can be seen, these values ranged from 2.5 to 14.0 kW. h. kg^{-1} O_3 produced, with a median value of 7.5 kW.h. kg^{-1} O_3. Within this context, an important point that should be considered is that the use of a high ozone dose implies a high energy demand. Thus, it is fundamental to consider this demand when studying optimal ozone doses.

Table 5 – Electrical energy required per mass of ozone generated (e_{O3}) according previous studies

Reference	**Type of ozone generator**	**e_{O_3} (kW.h. kg^{-1} O_3)**
(ARIUNBAATAR *et al.*, 2014; CESARO; BELGIORNO, 2020)	Ambient air	2.5
(ADARME *et al.*, 2017; SANTOS *et al.*, 2018; TRAVAINI *et al.*, 2016)	Not reported	4.6
(DOMAŃSKI *et al.*, 2017)	Not reported	6.6
(ADARME *et al.*, 2017; AQUINO; PIRES, 2016)	Pure oxygen	7.0–8.0
(MARCELINO *et al.*, 2017)	Not reported	10.0
(BAKHSHI *et al.*, 2018; CHIAPPERO *et al.*, 2019; CHU *et al.*, 2009)	Pure oxygen	12.5
(ADARME *et al.*, 2017; AQUINO; PIRES, 2016)	Ambient air	14.0

3 MATERIAL AND METHODS

3.1 SYSTEMATIC LITERATURE REVIEW

3.1.1 Effect of ozone pretreatment on methane production potential and energy balance

This section was based on a systematic literature review, which aimed to collect studies that applied ozonation as a pretreatment for the anaerobic digestion of waste, and/or wastewaters. The search strategies and the consulted databases were described in Table 6.

Table 6 – Search strategies used in the databases consulted during the systematic literature review

Databases	Search terms	Search limits
Web of Science	TI=((ozone OR ozonation OR ozonolysis) AND ("anaerobic digestion" OR biodigestion OR biogas OR methane)) OR AB=((ozone OR ozonation OR ozonolysis) AND ("anaerobic digestion" OR biodigestion OR biogas OR methane))	Timespan: All years. Collections: WOS, DIIDW, KJD, RSCI, SCIELO.
Scopus	TITLE-ABS-KEY ((ozone OR ozonation OR ozonolysis) AND ("anaerobic digestion" OR biodigestion OR biogas OR methane)) AND (LIMIT-TO (DOCTYPE, "ar") OR LIMIT-TO (DOCTYPE, "re"))	Timespan: All years. Type of document: (*Review articles OR Research articles*)
ScienceDirect	Title, abstract or author-specified keywords ((ozone OR ozonation OR ozonolysis) AND ("anaerobic digestion" OR biodigestion OR biogas OR methane))	Timespan: All years.

Legend: AB: abstract; ar: research article; re: review article; TI: title; TITLE-ABS-KEY: title, abstract, or keywords.

Specific eligibility criteria were established according to the information required for each analysis performed, as described in Table 7: A total of 58 peer-reviewed research papers were selected in the screening process. In brief, data on the characterization of the feedstocks before and after ozonation and anaerobic digestion, applied ozone doses, pH of ozonation, and methane and/or biogas production were collected.

Table 7 – Eligibility criteria used for during the systematic literature review and their associated objectives

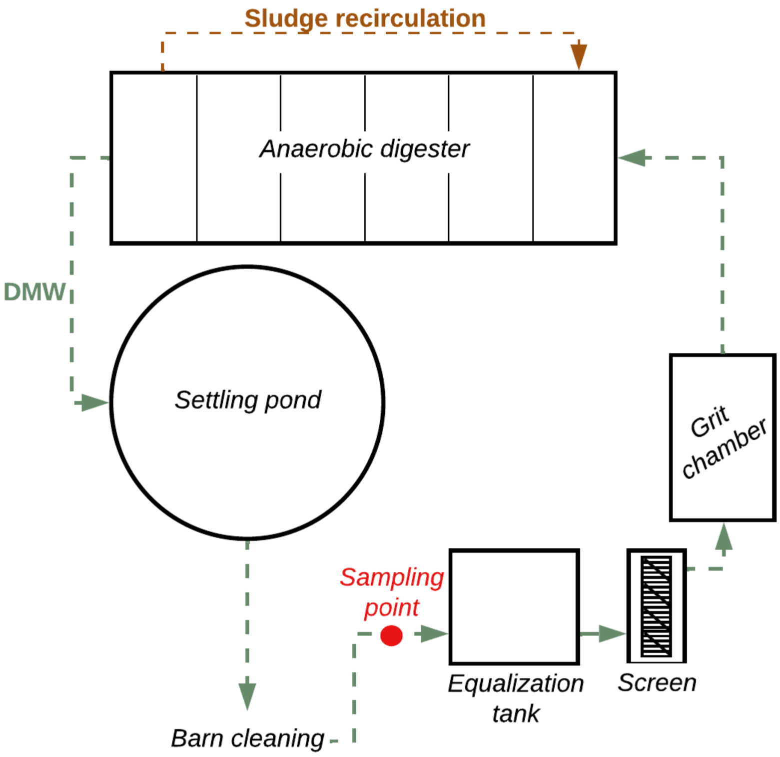
Eligibility criteria	Associated objective
Papers that provide the pH of ozonation, the concentrations of total and soluble chemical oxygen demand (COD and sCOD, respectively) before and after ozonation, and directly report or provide information for the calculation of the applied ozone dose in terms of the initial (non-ozonated) sCOD (mg O_3. g^{-1} sCOD).	To assess the effects of ozone pretreatment operating parameters (ozone dose, pH and concentration of soluble and total organic matter) on COD solubilization during ozone pretreatment
Papers that provide the pH of ozonation, the concentrations of volatile solids (VS) before and after ozonation, and directly report or provide information for the calculation of the volatile to total solids ratio (VS/TS) and applied ozone dose in terms of the initial VS content (mg O_3. g^{-1} VS).	To assess the effects of ozone dose, pH and concentration of total and volatile solids (TS and VS, respectively) on VS mineralization during ozone pretreatment.
Papers that report or provide information for the calculation of the applied ozone dose (mg O_3. g^{-1} VS or mg O_3. g^{-1} COD_0) and the specific methane production (mL CH_4. g^{-1} VS or mL CH_4. g^{-1} COD) in terms of the initial VS content (mg O_3. g^{-1} VS) or initial COD concentration.	To assess the effects of ozone pretreatment on methane production and process energy balance.
English or Spanish written peer-reviewed papers that describe studies on ozonation as pretreatment for anaerobic digestion of wastes or wastewaters.	Basic criterion.

3.2 EXPERIMENTAL INVESTIGATION

3.2.1 Substrate, inoculum, and digestate characterization

Fresh DMW was collected at a dairy farm (Embrapa Dairy Cattle) located in Coronel Pacheco, Brazil. The farm consists in a semi-confinement system in which the effluent from a DMW treatment plant is used as flush water to clean the free-stall barns. The wastewater generated by the manure flushing system is treated and recycled as schematized in Figure 3. About 50 $m^3.d^{-1}$ of recycled DMW is used in the flushing system. DMW is reused and recycled back to the treatment system for approximately 20 days during both dry and wet periods. After this cycle, the settling pond is emptied and filled with river water. As shown in Figure 3, DMW samples used in this work were collected in the inlet of the equalization tank.

Figure 3 – Scheme of the dairy manure wastewater (DMW) treatment and recycling plant and location of the sampling point



Anaerobic sludge from an UASB reactor located at a municipal wastewater treatment plant (União Indústria) from Juiz de Fora, Brazil, was used as inoculum in the BMP tests performed. Inoculum was analyzed in triplicate for TS and VS, whereas samples of raw, ozonated, and anaerobically digested DMW were analyzed in triplicate for TS, VS, TSS, VSS, COD, sCOD, pH and alkalinity, and in duplicate for N-NH_3 and TKN. The physicochemical characterization of DMW, inoculum, and digestate were carried out according to APHA/AWWA/WEF (2017). All the samples (substrate, inoculum, and digestate) were stored at 4 ºC prior to the experiments and analysis.

3.2.2 Ozone pretreatment

Ozone pretreatment of DMW was conducted using a bubble column reactor and an ambient air ozone generator (Hidrogeron, Brazil) at a gas flow rate of 1.0 L. min^{-1}, and an ozone production rate of 0.2 g O_3. h^{-1}.

Mass of ozone in the off gas ($M_{O_3,\text{off gas}}$) was determined by iodometric titration (SAWYER *et al.*, 1994) to quantify the consumed ozone dose ($O_{3,\text{consumed}}$) and assess the ozone mass transfer efficiency. In summary, the off gas passed through three traps placed in series containing a known volume of a potassium iodide (KI) solution (10 g. L^{-1}). During this process, residual ozone in the off gas oxidizes KI, leading to the formation of free iodine (I_2) (Equation 7). Thus, the concentration of I_2 in solution in each trap can be determined by titration with a solution of sodium thiosulfate ($Na_2S_2O_3$ 0.01N) (Equation 8).

Titration was performed in acidic media by adding a solution of sulfuric acid (1:5 H_2SO_4) in the KI solution. Soluble starch, which can be used as indicator of the presence iodine, and ammonium molybdate (NH_4OH), which can act as a catalyst in the reaction expressed in Equation 8, were also added in the KI solution immediately before titration. Titration was carried out in duplicate and the $M_{O_3,\text{off gas}}$ was determined as the sum of the masses of ozone captured in each of the three traps.

$$\mathbf{O_3 + 2KI + H_2SO_4 \rightleftharpoons I_2 + K_2SO_4 + 2H_2O} \qquad \text{Equation 7}$$

$$\mathbf{I_2 + 2Na_2S_2O_3 \rightleftharpoons Na_2S_4O_6 + 2NaI} \qquad \text{Equation 8}$$

After this procedure, the mass of ozone consumed ($M_{O_3,\text{consumed}}$) in the reaction with DMW was determined by Equation 9.

$$\mathbf{M_{O_3,consumed} = M_{O_3,applied} - M_{O_3,off\ gas}} \qquad \text{Equation 9}$$

Where $M_{O_3,\text{applied}}$ is the mass of ozone applied during ozonation.

The applied ($O_{3,\text{applied}}$) and consumed ozone ($O_{3,\text{consumed}}$) doses were normalized to the VS content of raw (non-ozonated) DMW (mg O_3. g^{-1} VS_{DMW}).

3.2.3 Anaerobic digestion

Specific methane production (SMP - mL CH_4.g^{-1} VS_{DMW}) of raw (non-ozonated) and ozonated DMW was assessed through batch anaerobic digestion tests. Experiments were set as triplicates in 1.0 L serum bottles. Reactors were maintained at continuous mixing under mesophilic conditions (35°C) until the stabilization of the curve of methane production (DBFZ, 2022). SMP was measured using a gas flow meter (Anaero Technology, UK) and was normalized to standard temperature and pressure (STP).

SMP of digesters fed with raw or ozonated DMW was compared at a 95% confidence level ($\alpha = 0.05$) by the Kruskal-Wallis' analysis of variance, which is appropriate for experimental data that do not fit the normal (Gaussian) distribution (SPERLING *et al.*, 2020). According to literature, the kinetic parameters of anaerobic digestion can be adequately estimated by the modified Gompertz model (BAKHSHI *et al.*, 2018; BI *et al.*, 2019; BUENDÍA *et al.*, 2009; WANG *et al.*, 2019; ZHANG *et al.*, 2022).

Thus, the experimental methane production curve was fitted to the modified Gompertz model (Equation 10) to estimate the methane production potential (P), the maximum methane production rate (r_m), and the lag phase time (λ). The parameters of the Gompertz model were estimated by least squares regression using the Levenberg-Marquardt algorithm in the IBM SPSS software.

$$\mathbf{SMP = P \cdot} \boldsymbol{exp}\left\{-\boldsymbol{exp}\left[\frac{\mathbf{r_m \cdot} \boldsymbol{e}}{\mathbf{P}}(\boldsymbol{\lambda} - \mathbf{t}) + \mathbf{1}\right]\right\} \qquad \text{Equation 10}$$

Where:

- SMP is the specific methane production (mL CH_4. g^{-1} VS_{DMW}) measured in the BMP test;
- P is the methane production potential (mL CH_4. g^{-1} VS_{DMW}) of DMW;
- r_m is the maximum methane production rate (mL CH_4. g^{-1} VS_{FMDW}. d^{-1}) of DMW;
- *e* is the Euler's constant (≈2.71828);
- λ is the lag phase time (d) of the anaerobic digestion of DMW; and
- t is the duration (d) of the BMP test.

3.2.4 Experimental setup

The work consisted in two phases: phase 1 and phase 2. The experimental apparatus used in the two phases was schematized in Figure 4.

Figure 4 – Experimental apparatus used for ozonation of dairy manure wastewater (DMW)

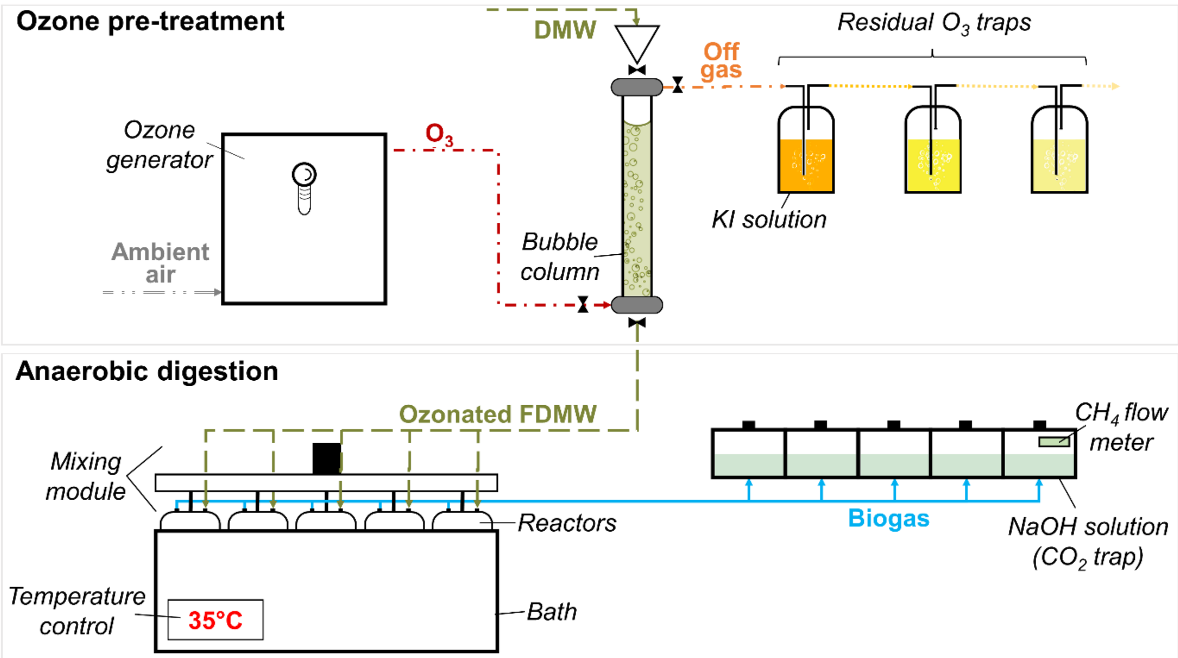
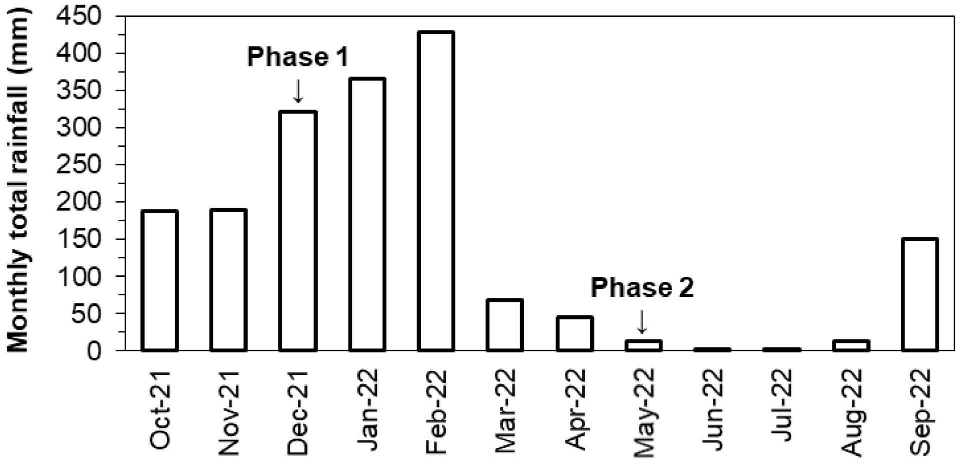


Figure 5 shows the monthly total rainfall data from the region of study during the hydrologic year of 2021-2022, which comprised the sampling campaigns performed for this work.

Figure 5 – Monthly total rainfall in the region of study during the sampling campaigns



Note: Data were collected from an automatic weather station located in Juiz de Fora, MG (latitude: -21.769965º, longitude: -43.364329º). Source: INMET (2023)

3.2.4.1 Phase 1

The phase 1 consisted in preliminary tests performed to support the subsequent tests (phase 1). As shown in Figure 5, inoculum and DMW samples for Phase 1 were collected in a wet period (December, 2021). Ozonation experiments were conducted in triplicate, using an applied ozone dose of 100 mg O_3. g^{-1} VS_{DMW} and at the natural pH of the collected DMW (pH = 8.46). The main characteristics of the inoculum collected in phase 1 were as follows: TS = 2.1 ± 0.6 % (w/w), VS = 0.9 ± 0.0% (w/w), and VS/TS = 0.44.

Batch anaerobic digestion tests of phase 1 were performed in triplicate. In this phase, a preliminary analysis of the effect of the ISR on the anaerobic digestion of raw DMW was performed. For this, the BMP of raw DMW was evaluated at ISRs of 0.8, 1.0, and 1.5 g VS. g^{-1} VS (SAADY; MASSÉ, 2015). The SMP at the different ISRs was compared at a 95% confidence level, using the Kruskal-Wallis test.

In parallel, a preliminary test on the effect of ozone pretreatment on anaerobic digestion was conducted. In this analysis, the BMP of ozonated DMW (100 mg O_3. g^{-1} VS_{DMW}) was investigated at an ISR of 1.5 g VS. g^{-1} VS. The ISR used for the tests with ozonated DMW was chosen to prevent possible VFA accumulation. The results were fitted using the Gompertz model and the parameters P, r_m, and λ obtained for raw and ozonated DMW were compared using the Mann-Whitney test at 95% confidence level. A summary of the experimental conditions of the BMP tests performed in phase 1 was shown in Table 8.

Table 8 – Experimental conditions of the biomethane potential (BMP) tests performed in the phase 1 of this study

Sample	**Ozone dose (mg O_3. g^{-1} VS_{DMW})**	**ISR (gVS: gVS)**	**T (ºC)**	**Number of replicates**
Inoculum	0	-	35	3
Raw DMW	0	0.8	35	3
Raw DMW	0	1.0	35	3
Raw DMW	0	1.5	35	3
Ozonated DMW	100	1.5	35	3

Legend: ISR: inoculum to substrate ratio; T: temperature.

3.2.4.2 Phase 2

Samples for the phase 2 (main test) were collected in a dry period (May, 2022). The main objected of this phase was to investigate the effect of the ozone dose on the methane production potential of DMW and on the E_B of each treatment condition. During this test, ozonation was carried out at the natural pH of DMW (pH = 8.34) at applied ozone doses of 20, 40, 100, and 180 mg O_3. g^{-1} VS_{DMW}. The doses were chosen based on the results of previous studies (see section 4.1). TS, VS, and the VS/TS ratio of the inoculum used in phase 2 were 9.8 ± 0.2% (w/w), 4.3 ± 0.1% (w/w), and 0.43, respectively. In accordance with previous studies and with the results of the phase 1, an ISR of 1.0 g $VS_{inoculum}$. g^{-1} VS of raw or ozonated DMW was provided to each reactor (ABDELWAHAB *et al.*, 2020; 2021a; ABDELWAHAB *et al.*, 2021b; SAADY; MASSÉ, 2015; WU-HAAN *et al.*, 2010; ZEB *et al.*, 2017; ZEB *et al.*, 2019). The results were fitted using the Gompertz model and the parameters P, r_m, and λ obtained for raw and ozonated samples were compared using the Kruskal-Wallis test at 95% confidence level. Table 9 shows the summary of the experimental conditions of the BMP test conducted in phase 2.

Table 9 – Experimental conditions of the biomethane potential (BMP) tests performed in the phase 2 of this study

Sample	**Ozone dose (mg O_3. g^{-1} VS_{DMW})**	**ISR (gVS: gVS)**	**T (ºC)**	**Number of replicates**
Inoculum	0	-	35	1
Raw DMW	0	1.0	35	3
Ozonated DMW	20	1.0	35	2
Ozonated DMW	40	1.0	35	3
Ozonated DMW	100	1.0	35	3
Ozonated DMW	180	1.0	35	3

Legend: ISR: inoculum to substrate ratio; T: temperature.

3.3 ENERGY BALANCE OF OZONE PRETREATMENT AND ANAEROBIC DIGESTION

A preliminary assessment on the energetic sustainability of ozone pretreatment and anaerobic digestion was carried out based on an analysis of energy balance (E_B) calculated for data from literature and for the experimental data obtained in this work. The E_B considered the electrical energy that can be potentially recovered from the additional methane produced due to ozonation ($E_{CH_4,increased}$ – kW. h. g^{-1} VS_{DMW}) and the energy requirement for ozone generation ($E_{O_3,required}$ – kW. h. g^{-1} VS_{DMW}) (Equation 11). For this, $E_{CH_4,increased}$ and $E_{O_3,required}$ were estimated by Equation 12 and Equation 13, respectively.

$$\mathbf{E_B = E_{CH_4,increased} - E_{CH_4,required}} \qquad \text{Equation 11}$$

$$\mathbf{E_{CH_4,increased} = \eta \cdot NCV_{CH4} \cdot (CH_{4,OZ} - CH_{4,N\text{-}OZ})} \qquad \text{Equation 12}$$

$$\mathbf{E_{O_3,required} = O_{3,applied} \cdot e_{O_3}} \qquad \text{Equation 13}$$

Where:

- η is the electric efficiency conversion (η = 30%) in combined heat and power engines (CHERNICHARO *et al.*, 2017);
- NCV_{CH4} (9.97×10^{-3} kW. h. L^{-1} CH_4) is the net calorific value for methane (CHERNICHARO *et al.*, 2017; SANTOS *et al.*, 2018);
- $CH_{4,OZ}$ (L CH_4. g^{-1} VS_{DMW}) is the methane production of ozonated feedstock;
- $CH_{4,N\text{-}OZ}$ (L CH_4. g^{-1} VS_{DMW}) is the methane production of non-ozonated feedstock (control group of the BMP test);
- $O_{3,applied}$ is the applied ozone dose; and
- e_{O_3} is the electrical energy required per gram of ozone produced, assumed as the minimum (2.5×10^{-3} kW. h. g^{-1} O_3), the median (7.5×10^{-3} kW. h. g^{-1} O_3) or the maximum (14×10^{-3} kW. h. g^{-1} O_3) value reported in earlier studies (Table 5).

Similarly, the E_B was also estimated as a percentage (Equation 12).

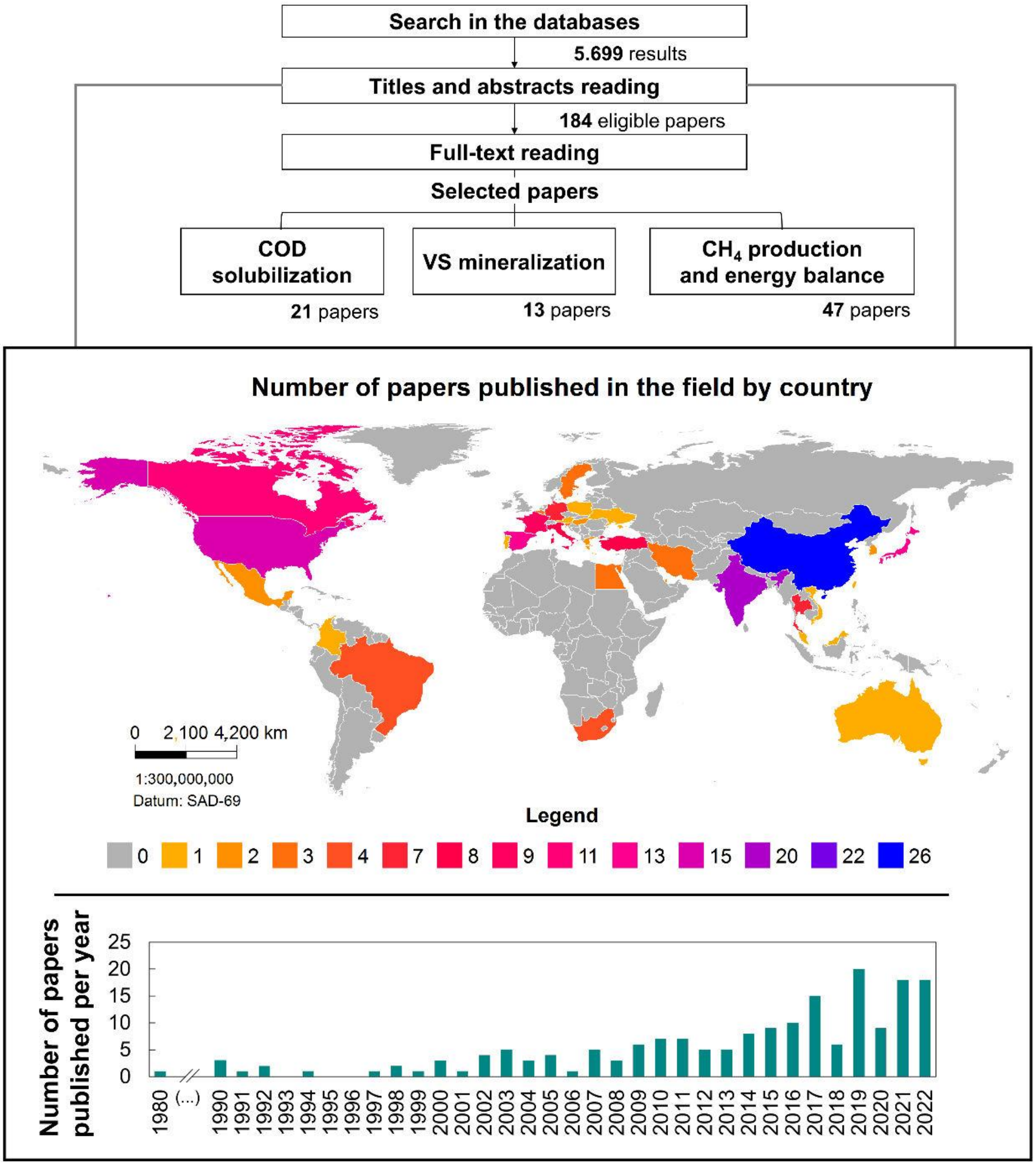
$$\mathbf{E_B\ (\%) = \frac{E_{CH_4,increased} - E_{CH_4,required}}{\eta \cdot NCV_{CH4} \cdot CH_{4,N\text{-}OZ} \cdot VS_{DMW}}} \qquad \text{Equation 12}$$

4 RESULTS AND DISCUSSION

4.1 EFFECT OF OZONATION ON METHANE PRODUCTION POTENTIAL AND ENERGY BALANCE: LITERATURE EXPERIMENTS

A general flowchart of the review results and the spatial distribution of the published studies in the field per year and country are depicted in Figure 6.

Figure 6 – Flowchart of the systematic literature review and spatio-temporal distribution of studies on ozonation as pretreatment for anaerobic digestion



Note: The spatial distribution comprises the countries of origin of the substrates studied (for original/research papers) and affiliation countries of the first authors (for the case of reviews). Legend: COD: chemical oxygen demand; VS: volatile solids.

Until September 2022, 184 research and review papers on ozonation as pretreatment for anaerobic digestion were published in journals indexed in the databases consulted. Figure 6 evidences a growing interest on the subject. These studies are distributed over 35 countries of origin and concentrated particularly in China (26 papers), India (20), USA (15), Japan (13), Spain (13), and Canada (11).

Methane production from ozonated substrates was higher than the production from non-ozonated in 77.8% of the experiments reported in the consulted literature (Tables 8-11). Ozone overdose was among the main aspects related to decreases in methane production potential (CESARO; BELGIORNO, 2013; MARTIN *et al.*, 2002; YU *et al.*, 2014; YUE *et al.*, 2020).

Analyses of sustainability and energetic efficiency are fundamental before implementing any pretreatment technology. Despite the high number of studies assessing ozonation as a pretreatment for anaerobic digestion (Tables 10-13), only a minor amount of studies have analyzed the energy balance (E_B) of the proposed treatment flowchart (ADARME *et al.*, 2017; AQUINO; PIRES, 2016; ARIUNBAATAR *et al.*, 2014; BAKHSHI *et al.*, 2018; BESZÉDES *et al.*, 2009; BRAGUGLIA *et al.*, 2012a; CHIAPPERO *et al.*, 2019; DOMAŃSKI *et al.*, 2017; KANNAH *et al.*, 2017; PACKYAM *et al.*, 2015; SALSABIL *et al.*, 2010; WENJING *et al.*, 2019; YUE *et al.*, 2020).

While a part of the studies concluded that the employment of a step of pre-ozonation may be energetically sustainable (ADARME *et al.*, 2017; BAKHSHI *et al.*, 2018; BESZÉDES *et al.*, 2009; CHIAPPERO *et al.*, 2019; DOMAŃSKI *et al.*, 2017; KANNAH *et al.*, 2017; SALSABIL *et al.*, 2010; WENJING *et al.*, 2019; YUE *et al.*, 2020), other reported that the biogas produced during the anaerobic digestion was insufficient to supply the electrical energy required for ozone generation (AQUINO; PIRES, 2016; ARIUNBAATAR *et al.*, 2014; BRAGUGLIA *et al.*, 2012a; PACKYAM *et al.*, 2015).

The procedure to calculate the E_B can vary depending on the assumptions that can be considered for this calculation, such as the energy required for ozone generation or the energy required in any additional step (e.g. heating, mixing, etc.). Thus, to enable a comparison between the E_B of the experiments of earlier studies, the E_B of previous studies was estimated considering the electrical energy that can be potentially recovered from the additional methane produced due to ozonation and the electrical energy required for ozone generation (see section 3.4).

Table 10 – Experimental conditions of previous studies on ozonation as pretreatment for anaerobic digestion of agro-industrial waste/wastewaters

Substrate characteristics				Ozonation conditions		Anaerobic digestion conditions			Effect of ozonation on anaerobic dgestion	Reference
Type of substrate	**VS ($g.L^{-1}$)**	**VS/TS**	**COD ($g.L^{-1}$)**	**pH**	**O_3 doses**	**Source of inoculum**	**ISR**	**T (ºC)**		
Dairy manure wastewater	36.0	0.75	43.7	nr	7 – 22[a] $mgO_3.g^{-1}VS$	Municipal WWTP	0.25[c]	35	↑CH_4 (0-11%) Max. SMP at 22$mgO_3.g^{-1}VS$	(CHEN *et al.*, 2021)
Cattle manure biofibers	691	0.76	nr	nr	0.7[a] $mgO_3.g^{-1}VS$	nr	nr	37	Nr	(AI *et al.*, 2019)
Coffee husks	nr	Nr	nr	3.0 - 11.0	7 - 81[a] $mgO_3.g^{-1}CH$	Municipal WWTP and bovine manure	1.4[d]	35	Max. SMP at 19$mgO_3.g^{-1}CH$	(SANTOS *et al.*, 2018)
Lawn grass	681	0.71	nr	nr	1,117[a] $mgO_3.g^{-1}VS$	Municipal WWTP	1.0[c]	37	↓CH_4	(YU *et al.*, 2014)
Rice straw	842	0.92	nr	nr	1[a] $mgO_3.g^{-1}VS$	Nr	nr	37	Nr	(AI *et al.*, 2019)
Catfish processing wastewater	nr	Nr	3.9	7.2	3[a] $mgO_3.g^{-1}COD$	Municipal WWTP	nr	35	↓CH_4	(ZAPPI *et al.*, 2019)
Olive oil mil effluent	nr	Nr	79.9	5.2	85[a] $mgO_3.g^{-1}COD$	Agroindustrial digester	nr	37	↓CH_4	(TSINTAVI *et al.*, 2013)
Palm oil mil effluent	nr	Nr	58.0	4.1	7[a] $mgO_3.g^{-1}COD$	Digester treating POME	nr	nr	↑CH_4 (1,957%)	(CHAIPRAPAT; LAKLAM, 2011)
	nr	Nr	3.0-40.0	4.6	10[a] $mgO_3.g^{-1}COD$	Digester treating food waste	nr	37	↓CH_4 (≥32$gCOD.L^{-1}$) ↑CH_4 (6-54%: 3-25$gCOD.L^{-1}$)	(TANIKKUL *et al.*, 2014)
Vinasse	nr	Nr	109	4.4	440[a] $mgO_3.g^{-1}COD$	Digester treating brewery wastewater	nr	35	↓CH_4	(MARTIN *et al.*, 2002)
Anaerobically digested distillery wastewater	26.8	0.91	37.0	7.5	104 - 209[a] $mgO_3.g^{-1}VS$	Digester treating distillery wastewater	nr	37	↑CH_4 (249-384%) Max. SMP at 139$mgO_3.g^{-1}VS$	(GUPTA *et al.*, 2015)
	25.5	0.48	37.0	7.7	180[a] $mgO_3.g^{-1}VS$	Nr	nr	37	↑CH_4 (150%)	(MALIK *et al.*, 2019)
Wood dust, sheep and cow dung, and treated wastewater	29.4 - 70.7	0.68 - 0.70	120 - 145	7.4 - 7.5	22 - 23[a] $mgO_3.g^{-1}VS$	Nr	2.0[c]	35	↓CH_4 (23$mgO_3.g^{-1}VS$) ↑CH_4 (16-34%: 86$mgO_3.g^{-1}$ VS)	(ALMOMANI *et al.*, 2019)

Legend: VS: volatile solids; TS: total solids; COD: chemical oxygen demand; ISR: inoculum to substrate ratio; T: temperature; nr: not reported; CH: coffee husks; WWTP: wastewater treatment plant; Max. SMP: maximum specific methane production; a: applied ozone dose; b: consumed ozone dose; c: g $VS_{inoculum}$:g $VS_{substrate}$; d: g $VSS_{inoculum}$: g $COD_{substrate}$.

Table 11 – Experimental conditions of previous studies on ozonation as pretreatment for anaerobic digestion of sludges from municipal wastewater treatment plants

Substrate characteristics					Ozonation conditions	Anaerobic digestion conditions			Effect of ozonation on methane production	Reference
Type of substrate	**VS ($g.L^{-1}$)**	**VS/TS**	**COD ($g.L^{-1}$)**	**pH**	**O_3 doses**	**Source of inoculum**	**ISR**	**T (ºC)**		
Dewatered sewage sludge	85.3	0.48	143	7.0	50 - 110[a] $mgO_3.g^{-1}VS$	Digester treating food waste	6.7[c]	37	↑CH_4 (53-83%) Max. SMP at 110$mgO_3.g^{-1}VS$	(WENJING *et al.*, 2019)
Primary sludge	nr	Nr	44.0	nr	50 - 210[b] $mgO_3.g^{-1}COD$	Municipal WWTP	-	35	↓CH_4	(CHACANA *et al.*, 2017a)
Mixed sludge	23.2	0.87	33.2	6.2	6 - 84[a] $mgO_3.g^{-1}VS$	Municipal WWTP	nr	35	↓CH_4 (≥84$mgO_3.g^{-1}VS$) ↑CH_4 (3%: 6$mgO_3.g^{-1}VS$)	(CHIAVOLA *et al.*, 2019)
	40.0-42.6	0.77 - 0.82	nr	5.2-5.6	24 - 162[a] $mgO_3.g^{-1}VS$	Municipal WWTP	2.5[c]	35	↓CH_4 (152$mgO_3.g^{-1}VS$) ↑CH_4 (7-14%) Max. SMP at 26$mgO_3.g^{-1}VS$	(DAVIDSSON *et al.*, 2013)
	13.0	0.78	16.8	5.9	46[a] $mgO_3.g^{-1}VS$	Nr	2.3[c]	35	↑CH_4 (18%)	(TIAN *et al.*, 2015)
	6.4	0.59	7.5	7.8	59 - 234[a] $mgO_3.g^{-1}VS$	Digester treating food waste	nr	35	↓CH_4 (234$mgO_3.g^{-1}VS$) ↑CH_4 (40-42%) Max. SMP at 59$mgO_3.g^{-1}VS$	(WEEMAES *et al.*, 2000b)
Mixed sludge spiked with PPCPs	45.0	0.62	70.0	5.7	28[a] $mgO_3.g^{-1}VS$	Nr	nr	37; 55	↑CH_4 (21%)	(CARBALLA *et al.*, 2007)
Anaerobically digested mixed sludge	9.3	0.6	16.7	8.3	168[b] $mgO_3.g^{-1}VS$	None	0	35	↑CH_4 (13%)	(BERNAL-MARTINEZ *et al.*, 2007)
Anaerobically digested mixed sludge	6.7	0.52	12.0	7.8	225[b] $mgO_3.g^{-1}VS$	None	0	35	↑CH_4 (71%)	(BERNAL-MARTINEZ *et al.*, 2005)
Anaerobically digested sludge	nr	Nr	15.0	nr	49 - 192[b] $mgO_3.g^{-1}COD$	None	0	35	↓CH_4 (192$mgO_3.g^{-1}COD$) ↑CH_4 (7-52%) Max. SMP at 86$mgO_3.g^{-1}$ COD	(CHACANA *et al.*, 2017a)
	nr	Nr	15.0	nr	50 - 210[b] $mgO_3.g^{-1}COD$	Municipal WWTP	nr	35	↑CH_4 (9-55%) Max. SMP at 140$mgO_3.g^{-1}COD$	(CHACANA *et al.*, 2017a)
	nr	Nr	nr	nr	19 - 201[b] $mgO_3.g^{-1}VS$	Nr	nr	nr	↑CH_4 (72-217%) Max. SMP at 115$mgO_3.g^{-1}$ VS	(SCHEMINSKI *et al.*, 2000)
Anaerobically digested waste activated sludge	19.7	0.65	32.7	7.8	31[a] $mgO_3.g^{-1}VS$	Municipal WWTP	nr	35	↑CH_4 (13%)	(KOBAYASHI *et al.*, 2009)

Table 11 – (continued)

Substrate characteristics					Ozonation conditions	Digestion conditions			Effect of ozonation on methane production	Reference
Type of substrate	**VS ($g.L^{-1}$)**	**VS/ TS**	**COD ($g.L^{-1}$)**	**pH**	**O_3 doses**	**Source of inoculum**	**ISR**	**T (ºC)**		
Waste activated sludge	8.6	0.80	8.3	7.2	63[a] $mgO_3.g^{-1}VS$	Municipal WWTP	0.5-2.0[c]	35	↑CH_4 (150%)	(CHENG; HONG, 2013)
	7.4	0.52	Nr	6.9	19[b] $mgO_3.g^{-1}VS$	Nr	nr	36	↑CH_4 (25%)	(ERDEN; FILIBELI, 2011)
	8.0	0.53	-	7.1	187[b] $mgO_3.g^{-1}VS$	Digester treating brewery wastewater	nr	37	↑CH_4 (55%)	(ERDEN; FILIBELI, 2019)
	10.3	0.90	12.1	6.7	4 - 60[a] $mgO_3.g^{-1}VS$	Municipal WWTP	nr	35	↑CH_4 (4-29%) Max. SMP at $4mgO_3.g^{-1}$ VS	(CHIAVOLA *et al.*, 2019)
	12.3	0.76	Nr	6.5	5 - 43[b] $mgO_3.g^{-1}VS$	None	0	35	nr	(DU *et al.*, 2020)
	9.9	-	14.1	7.5	20[a] $mgO_3.g^{-1}VS$	Municipal WWTP	nr	20	↑CH_4 (2,142-2,190%)	(BAKHSHI *et al.*, 2018)
	15.2	0.76	15.0	-	132 - 211[b] $mgO_3.g^{-1}VS$	Municipal WWTP and winery WWTP	2.0[d]	35	↑CH_4 (11-23%) Max. SMP at $211mgO_3.g^{-1}VS$	(BOUGRIER *et al.*, 2006)
	14.0	0.84	17.5	6.7	18 - 214[b] $mgO_3.g^{-1}VS$	Municipal WWTP and winery WWTP	2.0[d]	35-37	↑CH_4 (14-144%) Max. SMP at $179mgO_3.g^{-1}$ VS	(BOUGRIER *et al.*, 2007)
	25.3	0.76	37.8	6.2	247[a] $mgO_3.g^{-1}VS$	Municipal WWTP	nr	20	↑CH_4 (13%)	(CHIAPPERO *et al.*, 2019)
	21.0	0.72	-	7.0	55 - 129[a] $mgO_3.g^{-1}VS$	Municipal WWTP	1.0[c]	33	↓CH_4 (≥$129mgO_3.g^{-1}VS$) ↑CH_4 (5-21%) Max. SMP at $81mgO_3.g^{-1}VS$	(SILVESTRE *et al.*, 2015)
	5.7	0.48	9.8	6.9	52[a] $mgO_3.g^{-1}VS$	Digester treating slurry	nr	37	↑CH_4 (764%)	(KANNAH *et al.*, 2017)
	2.7	-	4.8	-	7 - 9[a] $mgO_3.g^{-1}VS$	Municipal WWTP	2.0[c]	nr	↓CH_4	(NILSSON *et al.*, 2019)
	9.6	0.51	-	7.4	195[a]; 176[b] $mgO_3.g^{-1}VS$	Municipal WWTP	nr	nr	↑CH_4 (32%)	(PEI *et al.*, 2016)
	nr	nr	Nr	nr	50 - 70[a];45 - 63[b] $mgO_3.g^{-1}TS$	Nr	nr	37	↓CH_4 ($50mgO_3.g^{-1}TS$) ↑CH_4 (14%: $50mgO_3.g^{-1}TS$)	(BRAGUGLIA *et al.*, 2012b)

Legend: VS: volatile solids; TS: total solids; COD: chemical oxygen demand; ISR: inoculum to substrate ratio; T: temperature nr: not reported; WWTP: wastewater treatment plant; mixed sludge: primary+secondary sludge; Max. SMP: maximum specific methane production; a: applied ozone dose; b: consumed ozone dose; c: $gVS_{inoculum}$:$gVS_{substrate}$; d: $gVSS_{inoculum}$: $gCOD_{substrate}$.

Table 12 – Experimental conditions of previous studies on ozonation as pretreatment for anaerobic digestion of sludges from wastewater treatment plants treating different substrates

Substrate characteristics				**Ozonation conditions**		**Anaerobic digestion conditions**			**Effect of ozonation on methane production**	**Reference**
Type of substrate	**VS ($g.L^{-1}$)**	**VS/TS**	**COD ($g.L^{-1}$)**	**pH**	**O_3 doses**	**Source of inoculum**	**ISR**	**T (ºC)**		
Dissolved air flotation float + waste activated sludge (oil refinery)	45.4	0.89	166	7.4	56[b] $mgO_3.g^{-1}VS$	Municipal WWTP	nr	37	↓CH_4 ↑CH_4 (3%)	(ROY *et al.*, 2016)
Mixed sludge (bakery wastewater)	8.0	0.85	14.6	7.1	16 - 51[a] $mgO_3.g^{-1}VS$	Nr	nr	nr	↑CH_4 (200-300%) Max. SMP at 51$mgO_3.g^{-1}VS$	(LIU *et al.*, 2001)
Mixed sludge (fishery WWTP)	111.3	0.64	54.1	nr	31[a] $mgO_3.g^{-1}VS$	Nr	nr	nr	↑CH_4 (54%)	(LE *et al.*, 2019)
Waste activated sludge (dairy WWTP)	5.6	0.44	10.0	6.9	0.1[a] $mgO_3.g^{-1}VS$	Bovine rumen fluid	nr	35	↑CH_4 (1,649%)	(PACKYAM *et al.*, 2015)
Sludge (canned maize production wastewater treatment)	nr	Nr	69.5	nr	77[a] $mgO_3.g^{-1}COD$	Municipal WWTP	nr	30	↑CH_4 (882-1,034%) Max. SMP at 154$mgO_3.g^{-1}$ COD	(BESZÉDES *et al.*, 2009)
Primary sludge spiked with resin acids (pulp and paper mills)	0.7	0.32	2.9	nr	211[a] $mgO_3.g^{-1}VS$	Municipal WWTP	2.0[d]	38	↑CH_4 (74%)	(DAS *et al.*, 2021)
Waste activated sludge (pulp and paper mills)	16.2	0.69	19.2	7.4	58[a] $mgO_3.g^{-1}VS$	Municipal WWTP	nr	35	↑CH_4 (357%)	(SETHUPATHY *et al.*, 2020)
Waste activated sludge (oil refinery)	3.1	0.77	5.0	7.2	87[a]; 65[b] $mgO_3.g^{-1}VS$	Municipal WWTP	5.0[c]	35	↑CH_4 (91%)	(HAAK *et al.*, 2016)
	18.3	0.87	26.0	7.2	65[b] $mgO_3.g^{-1}VS$	Municipal WWTP	nr	37	↑CH_4 (66%)	(ROY *et al.*, 2016)
Waste activated sludge (pharmaceutical)	5.3	0.37	6.4	nr	274[a];247[b] $mgO_3.g^{-1}VS$	Municipal WWTP	1.7[c]	35	↑CH_4 (4%)	(PEI *et al.*, 2015)
	14.9	0.83	nr	6.9	120[a]; 109[b] $mgO_3.g^{-1}VS$	Municipal WWTP	nr	nr	↑CH_4 (122%)	(PEI *et al.*, 2016)
Waste activated sludge (synthetic wastewater)	22.0	0.92	nr	2.0	16 - 54[a] $mgO_3.g^{-1}VS$	Municipal WWTP	nr	35	↓CH_4 (16$mgO_3.g^{-1}VS$) ↑CH_4 (98%: 54$mgO_3.g^{-1}VS$)	(GOEL *et al.*, 2003)
Waste activated sludge amended with metals (synthetic wastewater)	22.0	0.92	nr	2.0	54[a] $mgO_3.g^{-1}VS$	Municipal WWTP	nr	35	↑CH_4 (96%)	(GOEL *et al.*, 2003)

Legend: VS: volatile solids; TS: total solids ratio; COD: chemical oxygen demand; ISR: inoculum to substrate ratio; T: temperature; nr: not reported; WWTP: wastewater treatment plant; mixed sludge: primary+secondary sludge; Max. SMP: maximum specific methane production; a: applied ozone dose; b: consumed ozone dose; c: $gVS_{inoculum}$:$gVS_{substrate}$; d: $gVSS_{inoculum}$: $gCOD_{substrate}$.

Table 13 – Experimental conditions of previous studies on ozonation as pretreatment for anaerobic digestion of other substrates

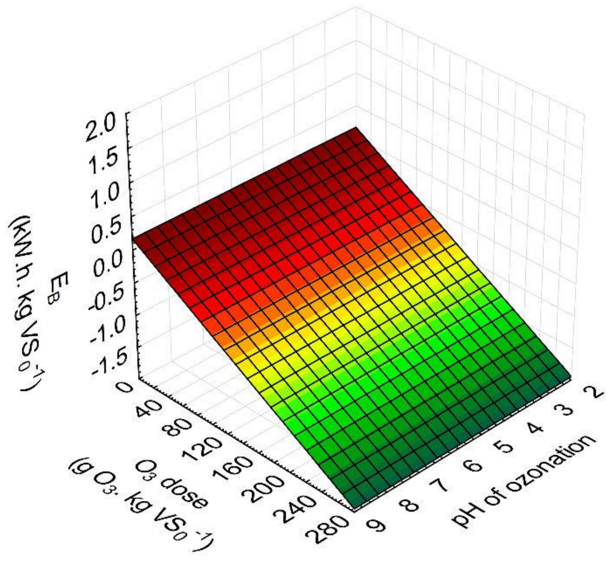
Substrate characteristics				**Ozonation conditions**		**Digestion conditions**			**Effect of ozonation on methane production**	**Reference**
Type of substrate	**VS ($g.L^{-1}$)**	**VS/TS**	**COD ($g.L^{-1}$)**	**pH**	**O_3 doses**	**Source of inoculum**	**ISR**	**T (ºC)**		
Organic solid waste	93.1-140	0.93	nr	nr	172[a] $mgO_3.g^{-1}VS$	Municipal WWTP	2.0[c]	35	↑CH_4 (14-94%)	(CESARO; BELGIORNO, 2020)
	77.8	0.89	nr	6.5	180 - 1,353[a] $mgO_3.g^{-1}VS$	Municipal WWTP	1.0[c]	35	↓CH_4 (≥ 451$mgO_3.g^{-1}VS$) ↑CH_4 (47%: 180$mgO_3.g^{-1}VS$)	(CESARO; BELGIORNO, 2013)
	78.8	0.73	nr	nr	20 - 800[a] $mgO_3.g^{-1}VS$	Digester treating wastes/wastewaters	2.0[c]	35	↓CH_4 (≥50$mgO_3.g^{-1}VS$) ↑CH_4 (3-6%: 20$mgO_3.g^{-1}VS$)	(YUE *et al.*, 2020)
	211	0.90	nr	nr	38 - 225[a] $mgO_3.g^{-1}VS$	Agroindustrial digester	2.0[c]	32-34	↓CH_4 (225$mgO_3.g^{-1}VS$) ↑CH_4 (6-9%) Max. SMP at 76$mgO_3.g^{-1}VS$	(ARIUNBAATAR *et al.*, 2014)
Organic solid waste with glycerol trioleate	131	0.84	nr	nr	20 - 800[a] $mgO_3.g^{-1}VS$	Digester treating wastes/wastewaters	2.0[c]	35	↓CH_4 (≥200$mgO_3.g^{-1}VS$) ↑CH_4 (5-21%) Max. SMP at 20$mgO_3.g^{-1}VS$	(YUE *et al.*, 2020)
Anaerobically digested organic solid waste	26.6	0.58	22.1 - 89.2	7.2 - 8.4	190 - 276[a] $mgO_3.g^{-1}VS$	Municipal WWTP	nr	nr	↑CH_4 (300-500%) Max. SMP at 190$mgO_3.g^{-1}VS$	(CESARO *et al.*, 2019)
Microalgae	7.2 - 14.6	Nr	nr	8.0	47 - 382[a] $mgO_3.g^{-1}VS$	Digester treating brewery wastewater	2.0[c]	35	↑CH_4 (6-66%) Max. SMP at 382$mgO_3.g^{-1}VS$	(CARDEÑA *et al.*, 2017)
Microalgae	nr	Nr	nr	nr	0.2 - 0.7[a] $mgO_3.g^{-1}TS$	Bovine rumen fluid	nr	35	↑CH_4 (4,714%: 0.5$mgO_3.g^{-1}TS$)	(TAMILARASAN *et al.*, 2019)
Tobacco waste + waste activated sludge (municipal WWTP)	nr	Nr	nr	nr	10 - 120[a] $mgO_3.g^{-1}TSS$	Municipal WWTP	nr	35	↑CH_4 (10-38%) Max. SMP at 120$mgO_3.g^{-1}TSS$	(LI *et al.*, 2017)
HLWW (microalgae)	nr	Nr	9.9	4.5	211[a] $mgO_3.g^{-1}COD$	Municipal WWTP	nr	37	↑CH_4 (85%)	(YANG *et al.*, 2018)
HLWW (swine manure)	nr	Nr	5.0-2.0	nr	105 - 420[a] $mgO_3.g^{-1}COD$	Digester treating HLWW	nr	37	↓CH_4 (420$mgO_3.g^{-1}COD$) ↑CH_4 (16-281%) Max. SMP at 105$mgO_3.g^{-1}COD$	(SI *et al.*, 2019)

Legend: VS: volatile solids; TS: total solids ratio; COD: chemical oxygen demand; ISR: inoculum to substrate ratio; T: temperature; nr: not reported; WWTP: wastewater treatment plant; mixed sludge: primary+secondary sludge; Max. SMP: maximum specific methane production; HLWW: hydrothermal liquefaction wastewater; a: applied ozone dose; b: consumed ozone dose; c: $gVS_{inoculum}$:$gVS_{substrate}$; d: $gVSS_{inoculum}$: $gCOD_{substrate}$

As can be seen in Figure 7, E_B tends to decrease with the increase of ozone doses as a result of the increase in energy consumption. Furthermore, data also suggest that ozone pretreatment at neutral to low pH may lead to slightly high E_B, probably due to low mineralization rates induced by the direct reaction between ozone and organic matter.

It is worth highlighting that the formation of radical species is favored in alkaline media (Equations 1-6). In addition, the reaction described in the Equation 1 is very fast ($k = 70\ M^{-1}.\ s^{-1}$). Due to its occurrence, ozone dose and gas flow rate are fundamental operational control parameters of ozone pretreatment. The occurrence of the reaction shown in Equation 1 in large scale may lead to an excessive consumption of both ozone and hydroxyl radicals, which can increase the ozone demand and the treatment costs. The referred reaction is favored when there are few organic molecules capable to react with hydroxyl radicals (e. g., highly diluted media) or in the absence of radical scavengers such as carbonates and bicarbonates (VON SONNTAG; VON GUNTEN, 2012). Taking into account the aforementioned conclusions, it appears that the indirect ozonation mechanism, which occurs at pH > 7, tends to promote less favorable results in terms of energy balance.

Figure 7 – Effect of ozone dose and pH of ozonation on the energy balance (E_B) of ozone pretreatment and anaerobic digestion according to data from previous studies



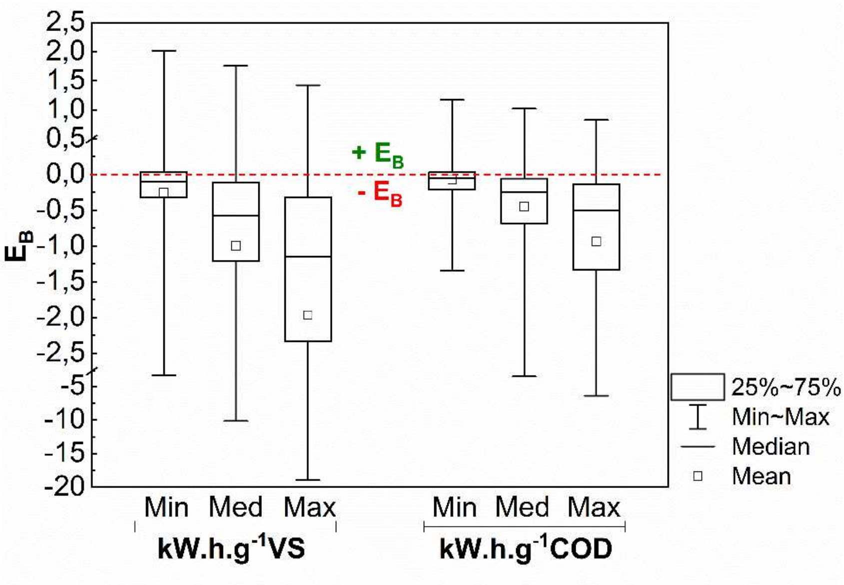
Only 27.6% (16 studies) of 58 consulted papers reported energy balance or cost-benefit analyses for the conditions studied (BAKHSHI *et al.*, 2018; BESZÉDES *et al.*, 2009; CARBALLA *et al.*, 2007; CATENACCI *et al.*, 2022; CESARO; BELGIORNO, 2020; CHIAPPERO *et al.*, 2019; CHIAVOLA *et al.*, 2019; GOEL *et al.*, 2003; KANNAH *et al.*, 2017; PACKYAM *et al.*, 2015; SETHUPATHY *et al.*, 2020; TIAN *et al.*, 2015; WEEMAES *et al.*, 2000a; WENJING *et al.*, 2019; YANG *et al.*, 2018; YUE *et al.*, 2020). In one of these studies, the authors concluded that the employment of ozone pretreatment was not sustainable energetically- and/ or cost-feasible (CHIAPPERO *et al.*, 2019), whereas 10 concluded that a pre-ozonation unit can lead to energetic and cost benefits (BAKHSHI *et al.*, 2018; BESZÉDES *et al.*, 2009; CARBALLA *et al.*, 2007; CATENACCI *et al.*, 2022; CESARO; BELGIORNO, 2020; CHIAVOLA *et al.*, 2019; GOEL *et al.*, 2003; WEEMAES *et al.*, 2000a; YANG *et al.*, 2018). Additionally, five studies suggested the feasibility of ozonation when combined with additional pretreatment processes (ultrasound, enzymatic hydrolysis, dispersion-induced treatment (KANNAH *et al.*, 2017; PACKYAM *et al.*, 2015; SETHUPATHY *et al.*, 2020; TIAN *et al.*, 2015; WENJING *et al.*, 2019; YUE *et al.*, 2020).

Figure 8 illustrates the variation of the energy balance (E_B) calculated for literature experimental data. Assuming a best-case scenario in which more efficient ozone generators are used (ozone demand equal to 2.5 kW. h. kg^{-1} O_3), about 34% of the literature experimental data had positive energy balances. On the other hand, only about 4% of the experimental data had a positive energy balance for a worst-case scenario in which a high amount of energy is required to supply the ozone generator (14 kW. h. kg^{-1} O_3).

These results evidence that the energetic sustainability of ozone pretreated anaerobic digestion is intrinsically dependent on the power efficiency of the ozone generator. Furthermore, as previously discussed, it is important to consider that the effect of ozonation on substrates composition and, consequently, on methane production and energy balance, is strongly influenced by several other control parameters, such as pH, alkalinity, the ozone concentration in the feed gas, gas flow rate, ozonation time, temperature, the wastewater constituents, and reactor design

(TRAVAINI *et al.*, 2016; VON GUNTEN, 2003; VON SONNTAG; VON GUNTEN, 2012; ZHENG *et al.*, 2014).

Figure 8 – Energy balance (E_B) of ozone pretreatment and anaerobic digestion in terms of the initial volatile solids (VS) content (kW. h. kg^{-1} VS) and of the initial chemical oxygen demand (COD) (kW. h. kg^{-1} COD)



Considering a median energy demand for ozone generation (7.5 kW. h. kg^{-1} O_3), only 11% of the experimental data had positive energy balances, which represents 15 of the 136 literature data collected for this work (Table 14). As slight increases in the methane production potential of DMW (up to 11%) were observed in the study of Chen *et al.* (2021), negative E_B were estimated for their experiments (-0.002 kW.h.L$^{-1}_{DMW}$ to -0.004 kW.h.L$^{-1}_{DMW}$).

Almomani *et al.* (2019) assessed ozone pretreatment for anaerobic co-digestion of agro-industrial wastes (wood dust, sheep and cow manure, and treated wastewater), with ozone doses varying between 21.6 and 23.2 mg O_3. g^{-1} VS and pH of 7.4 – 7.5 (Table 10). The scenario with the lowest ozone dose in terms of VS was the only one with a positive E_B (Table 14), even with VS/TS and sCOD/COD ratios slightly higher than the other experimental setups (Table 10).

Experiments using mixed sludge (primary + secondary sludge) from a wastewater treatment plant treating fishery wastewater had a positive E_B at the conditions shown in Table 14 (LE *et al.*, 2019). In addition to the relatively low ozone dose used, the raw substrate was characterized by a relatively low organic content (VS/TS = 0.64) which also was poorly soluble (sCOD/COD = 0.01). Thus, ozone pretreatment led to a great increase in the methane production potential of the feedstock (134.2 L $CH_4.kg^{-1}$ VS). The pH of ozonation was not reported in the referred work.

Table 14 – Conditions of experiments that had positive energy balances (E_B) at a median electrical energy demand (7.5 kW. h. kg^{-1} O_3) for ozone generation

Substrate	O_{3dose}	VS ($g.L^{-1}$)	VS/TS	COD ($g.L^{-1}$)	sCOD ($g.L^{-1}$)	pH	E_B (%)	Reference
Wood dust, sheep and cow dung, and treated wastewater	22[a] $mgO_3.g^{-1}VS$	49.6	0.73	130.0	30.0	7.5	6.2	(ALMOMANI *et al.*, 2019)
Palm oil mill effluent	10[a] $mgO_3.g^{-1}COD$	-	-	10.0	-	4.6	38.6	(TANIKKUL *et al.*, 2014)
	10[a] $mgO_3.g^{-1}COD$	-	-	15.0	-	4.6	40.2	(TANIKKUL *et al.*, 2014)
	7[a] $mgO_3.g^{-1}COD$	-	-	58.0		4.1	1,907	(CHAIPRAPAT; LAKLAM, 2011)
Organic solid waste with glycerol trioleate	20[a] $mgO_3.g^{-1}VS$	130.6	0.84	-	-	-	5.4	(YUE *et al.*, 2020)
	20[a] $mgO_3.g^{-1}VS$	130.6	0.84	-	-	-	11.9	(YUE *et al.*, 2020)
Mixed sludge (fishery WWTP)	31 $mgO_3.g^{-1}VS$	111.3	0.64	54.1	0.3	-	22.2	(LE *et al.*, 2019)
Sludge (canned maize production wastewaters treatment)	77 $mgO_3.g^{-1}COD$	-	-	69.5	-	-	182.1	(BESZÉDES *et al.*, 2009)
Waste activated sludge (pulp and paper mills wastewaters treatment)	58[a] $mgO_3.g^{-1}VS$	16.2	0.69	19.2	1.6	7.4	41.8	(SETHUPATHY *et al.*, 2020)
Waste activated sludge amended with metals (synthetic wastewater)	54[a] $mgO_3.g^{-1}VS$	22.0	0.92	-	-	2.0	5.1	(GOEL *et al.*, 2003)
Waste activated sludge (municipal WWTP)	52 $mgO_3.g^{-1}VS$	5.7	0.48	9.8	0.2	6.9	625.8	(KANNAH *et al.*, 2017)
	20 $mgO_3.g^{-1}VS$	9.9	-	14.1	-	7.5	587.1	(BAKHSHI *et al.*, 2018)
	20 $mgO_3.g^{-1}VS$	9.9	-	14.1	-	7.5	522.0	(BAKHSHI *et al.*, 2018)
	4 $mgO_3.g^{-1}VS$	10.3	0.90	12.1	0.9	6.7	20.9	(CHIAVOLA *et al.*, 2019)
Mixed sludge (municipal WWTP) spiked with PPCP	28 $mgO_3.g^{-1}VS$	45.0	0.62	70.0	6.0	5.7	0.6	(CARBALLA *et al.*, 2007)

VS: volatile solids; VS/TS: volatile to total solids ratio; COD: initial chemical oxygen demand; sCOD: initial soluble chemical oxygen demand; WWTP: wastewater treatment plant; PPCP: pharmaceutical and personal care products

Carballa *et al.* (2007) conducted experiments at the conditions reported in Table 11 and Table 14. In their work, a group of substrates was digested under mesophilic conditions (37°C) and other was digested under thermophilic conditions (55°C). Only the group of mesophilic anaerobic digestion had a slightly positive E_B, which was very close to zero (Table 14).

Tanikkul *et al.* (2014) applied ozone in palm oil mill effluent at different COD concentrations (3.0 – 40 g. L^{-1}) prior to anaerobic digestion (adjusting pH to 7 before digestion) (Table 10). With a low ozone dose (9.7 mg O_3. g^{-1} COD) and pH = 4.6 during ozonation (Table 14), experiments with palm oil mill effluents at COD concentrations of 10 and 15 g.L^{-1} had positive E_B comparatively with non-ozonated substrates digested at pH 7.0.

On the other hand, Chaiprapat and Laklam (2011) applied an ozone dose of 6.9 mg O_3. g^{-1} COD in a palm oil mill effluent with a COD concentration of 58 g. L^{-1} and pH = 4.2 (Table 10). Since pH was not adjusted prior digestion, a failure due to an accumulation of volatile fatty acids in the anaerobic reactor with non-ozonated substrate was noticed as a result of the critically low pH and alkalinity (CHAIPRAPAT; LAKLAM, 2011). Due to this, the methane production from the ozonated feedstock was far higher than the non-ozonated (Table 10), and the E_B of this experiment was estimated as 1,907% (Table 14).

Goel *at al.* (2003) tested the methanogenic potential of a waste activated sludge with a high volatile solids fraction (VS/TS = 0.92) obtained from a synthetic wastewater treatment plant. The effects of the presence of metals (Fe, Ni, and Co) in substrate were also assessed. Their experiments were carried out at ozone doses of 16.3 and 54.3 mg O_3. g^{-1} VS for the case of waste activated sludge and 54.3 mg O_3. g^{-1} VS for waste activated sludge amended with metals (pH of ozonation = 2.0) (Table 12). A positive E_B was computed only for the case of waste activated sludge amended with Fe, Ni, and Co (Table 14), in which the potential of energy recovery of methane produced due to ozonation was sufficiently high to supply the energy demand of ozonation.

Kannah *et al.* (2017) assessed ozonation as a pretreatment for anaerobic digestion of waste activated sludge from a municipal wastewater treatment plant. The substrate used in their experiments had one of the lowest volatile contents (VS/TS = 0.48) and soluble organic fraction (sCOD/COD = 0.02) between the waste activated sludges reported in the literature consulted (Table 11) and positive E_B were estimated for their experiments.

Furthermore, different approaches can also be used to assess the energy balance of pretreated digesters. Yue *et al.* (2020) assessed the energy conversion efficiency of pre-ozonated food waste and food waste amended with glycerol trioleate (a lipid commonly found in organic solid wastes). In their analysis, the authors compared the calorific values of the food waste and food waste with glycerol trioleate with the potential of energy recovery of methane and hydrogen produced through anaerobic digestion was greater than of substrates. One of their conclusions was that the potential of energy recovery of these substrates by anaerobic digestion was greater than by incineration. Furthermore, Yue *et al.* (2020) reported that ozone pretreatment at the lowest dose tested (20 mg O_3.g^{-1} VS) led to an enhancement in the potential of energy recovery of food waste amended with lipids. At the other ozone doses tested in their work (50 - 800 mg O_3. g^{-1} VS), methane and hydrogen production were lower than non-ozonated substrate.

According to Bakhshi *et al.* (2018), at 10ºC ambient temperature, ozonation at 20 mg O_3. g^{-1} VS followed by anaerobic digestion at 20ºC can be more energetically feasible when compared to non-ozonated anaerobic digestion operating at an optimal mesophilic temperature (35ºC).

Within this framework, Chiappero *et al.* (2019) also reported a positive E_B for ozone pretreatment and anaerobic digestion. The authors compared the total amount of methane produced during the anaerobic digestion of pre-ozonated waste activated sludge (municipal wastewater treatment plant) at 20ºC with the produced in anaerobic digestion of non-ozonated waste activated sludge at 35°C. They concluded that the first system (anaerobic digestion of pre-ozonated waste activated sludge at 20ºC) was more interesting from an energetic point of view than the second one, even using a high ozone dose of about 247.3 mg O_3. g^{-1} VS (pH = 6.2).

Further positive aspect of ozone pretreatment reported in literature is a potential reduction in disposal costs resulting from sludge stabilization. Packyam *et al.* (2015) and Salsabil *et al.* (2010) concluded that a reduction in operating costs of sewage sludge may be achieved as a result from a high total solids removal, characterizing the systems proposed in their studies as economically sustainable, even when the energy balance was negative (PACKYAM *et al.*, 2015). Without considering the potential of energy recovery, Chiavola *et al.* (2019) also reported a reduction of 14% in sewage sludge disposal costs.

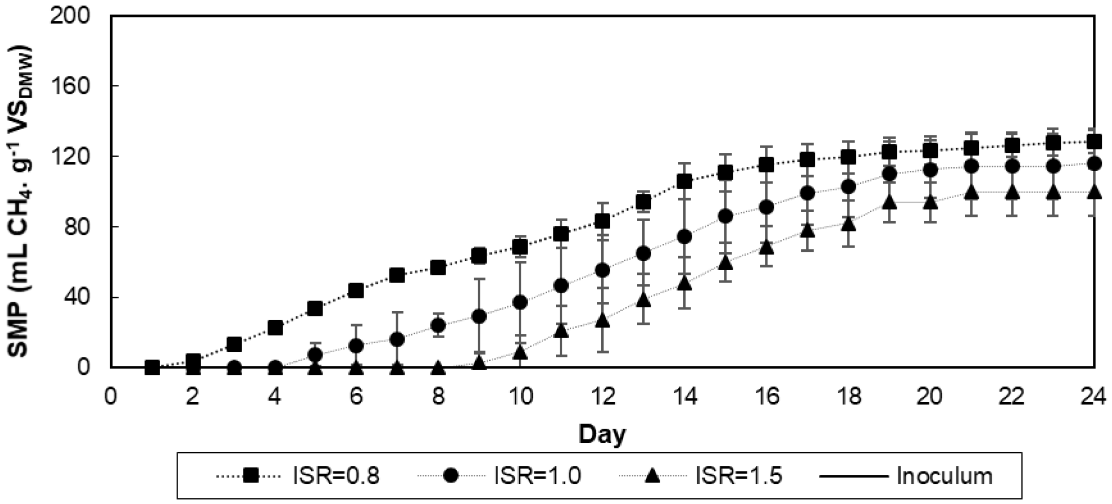
The results of previous researches indicate that ozone pretreatment can has technical and economic advantages along with a high versatility. However, this review evidence that the energetic feasibility of this process is strongly affected by the dose of ozone applied and the power efficiency of the ozone generator.

4.2 EXPERIMENTAL INVESTIGATION

4.2.1 Effect of ISR on the anaerobic digestion of DMW

The results of a preliminary analysis on the effect of the ISR on the anaerobic digestion of DMW are shown in Figure 9. The methane production potential was 128.7 ± 6.6 mL CH_4. g^{-1} VS_{DMW} for ISR=0.8 g VS. g^{-1} VS, 116.4 ± 19.5 mL CH_4. g^{-1} VS_{DMW} for ISR=1.0 g VS. g^{-1} VS, and 99.8 ± 13.4 mL CH_4. g^{-1} VS_{DMW} for ISR=1.5 g VS. g^{-1} VS. Despite this, the Kruskal-Wallis test indicated no statistically significant differences (p-value=0.1) between the SMP of the feedstock at the distinct ISRs tested.

Figure 9 – Effect of inoculum to substrate ratio (ISR) on the specific methane production (SMP) of dairy manure wastewater (DMW)



The performance of the reactors at relatively high organic loads (ISR=0.9 and ISR=1.0) may indicate the buffering capacity of DMW, which maintained growth conditions for archaea (AQUINO; CHERNICHARO, 2005; SAADY; MASSÉ, 2015).

Results of previous studies were not consistent to indicate the best condition for the anaerobic digestion of DMW, probably due to the variable composition of this feedstock. Saady and Massé (2015) tested the effect of diferent OLRs on the methane production potential of cattle manure. At an OLR of 6.0 mg COD. g^{-1} inoculum. d^{-1}, the ISRs tested (1.0 and 1.7 g VS. g^{-1} VS) resulted in no statistically significant effects in the SMP (225.7 mL CH_4. g^{-1} VS at ISR = 1.0 g VS. g^{-1} VS; 225.7 mL CH_4. g^{-1} VS at ISR = 1.7 g VS. g^{-1} VS; and 184.7 mL CH_4. g^{-1} VS at ISR = 1.0). No statistically significant differences were also registered at the other conditions tested (ORL = 7.0 mg COD. g^{-1} inoculum. d^{-1} and ISRs = 0.8, 0.9, and 1.0 g VS. g^{-1} VS; and ORL = 8.0 mg COD. g^{-1} inoculum. d^{-1} and ISRs = 0.6 and 0.7 g VS. g^{-1} VS). The best results were observed at 1.0 g VS. g^{-1} VS (225.7 mL CH_4. g^{-1} VS for ORL = 6.0 mg COD. g^{-1} inoculum. d^{-1}), at 0.9 g VS. g^{-1} VS (227.9 mL CH_4. g^{-1} VS for ORL = 7.0 mg COD. g^{-1} inoculum. d^{-1}), and at 0.7 g VS. g^{-1} VS (182.2 mL CH_4. g^{-1} VS for ORL = 8.0 mg COD. g^{-1} inoculum. d^{-1}) (SAADY; MASSÉ, 2015).

On the other hand, Pandey *et al.* (2010) and Shin *et al.* (2019), which analyzed the methane production potential of DMW and dairy manure at different ISRs, observed high methane yields in the experiments conducted under the high inoculum concentrations. In the study of Pandey *et al.* (2010), the results were as follows: 160.2 mL CH_4 (ISR = 2.5 g VS. g^{-1} VS); 117.6 mL CH_4 (ISR = 1.8 g VS. g^{-1} VS); 106.6 mL CH_4 (ISR = 1.4 g VS. g^{-1} VS); 93.96 mL CH_4 (ISR = 1.0 g VS. g^{-1} VS); 61.5 mL CH_4 (ISR = 0.7 g VS. g^{-1} VS); and 52.2 mL CH_4 (ISR = 2.2 g VS. g^{-1} VS). In addition, the results reported by Shin *et al.* (2019) were: 38.0 mL CH_4. g^{-1} VS (ISR = 2.0 g VS. g^{-1} VS); 35 mL CH_4. g^{-1} VS (ISR = 0.5 g VS. g^{-1} VS); 32.4 mL CH_4. g^{-1} VS (ISR = 1.0 g VS. g^{-1} VS); and 17.4 mL CH_4. g^{-1} VS (ISR = 0.25 g VS. g^{-1} VS). However, results of these studies can indicate that the responses of the ISR on the methane production were not linear. Given these uncertainties, the experiments of the phase 2 were performed at an ISR of 1.0 g VS. g^{-1} VS, even though the best results obtained in the preliminary analyses of this work were observed in the BMP test conducted at the ISR of 0.8 g VS. g^{-1} VS. This decision was also made because the wastewater collected in the phase 2 was notably more concentrated terms of TS and VS (250%) and COD (170%) than the collected in phase 1, and less concentrated in terms of alkalinity (-71%), as shown in the section 4.2.2.

4.2.2 Effect of ozonation on DMW characteristics

During the phase 1, ozone pretreatment led to the removal of 21.7% of COD and 18.5% of the VS concentration of DMW (Table 15). In parallel, it was observed a slight increase in the sCOD of DMW after ozonation, which can result in positive effects on anaerobic digestion of the feedstock. These results may be a consequence of the formation of $HO^{\bullet}$, which has high potential for organic matter mineralization in comparison to ozone (VON GUNTEN, 2003; VON SONNTAG; VON GUNTEN, 2012).

Table 15 – Characteristics of raw and ozonated dairy manure wastewater (DMW) in the phase 1

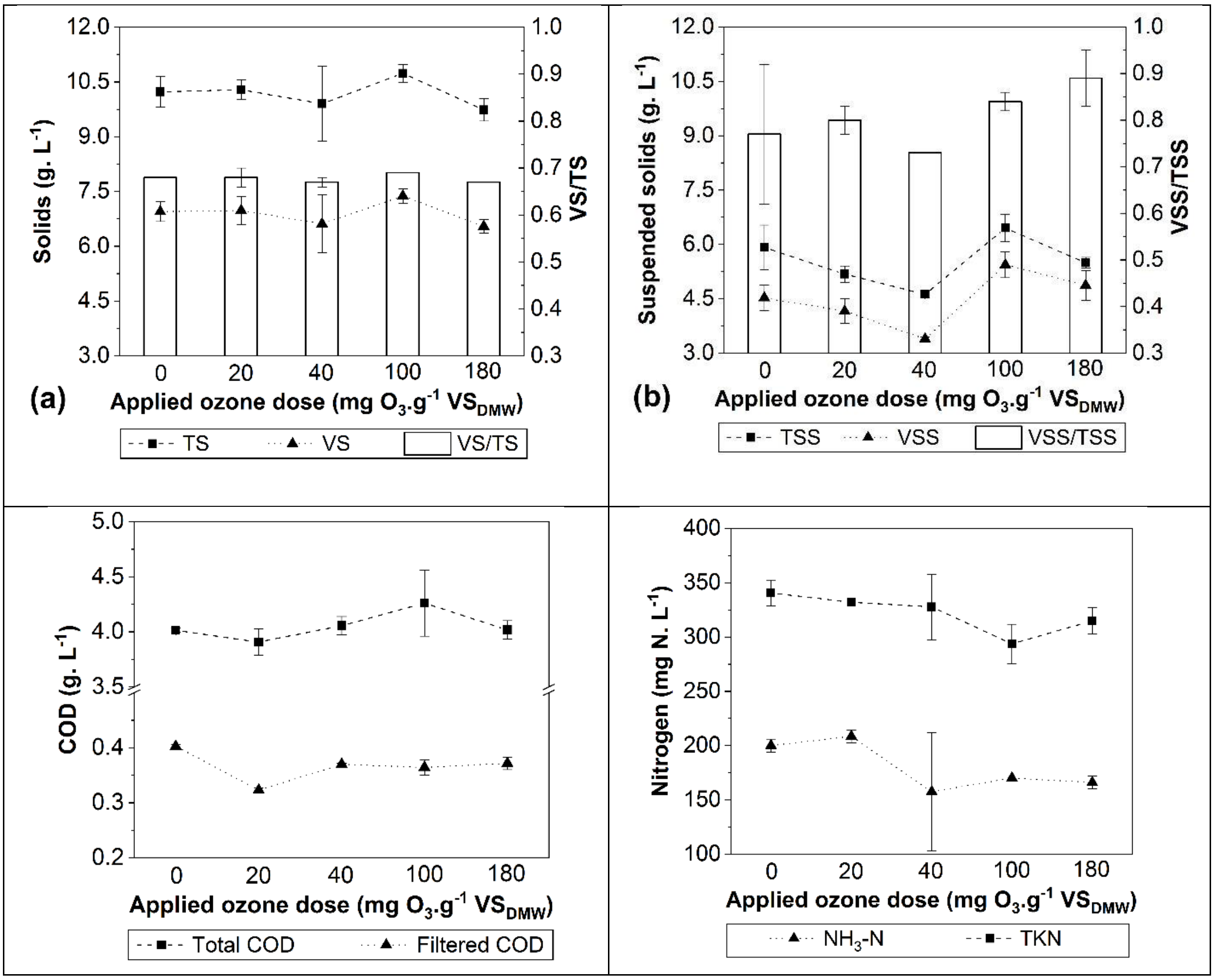
Applied ozone dose	**TS ($g. L^{-1}$)**	**VS ($g. L^{-1}$)**	**COD ($g. L^{-1}$)**	**sCOD ($g. L^{-1}$)**	**NH_4^+ ($mg N. L^{-1}$)**	**TKN ($mg N. L^{-1}$)**
0 mg O_3. g^{-1} VS_{DMW}	4.1 ± 0.2	2.7 ± 0.1	2.3 ± 0.7	0.49 ± 0.00	113 ± 3	160 ± 52
100 mg O_3. g^{-1} VS_{DMW}	3.4 ± 0.1	2.2 ± 0.1	1.8 ± 0.2	0.54 ± 0.02	88 ± 3	129 ± 4

Values were expressed as mean ± standard deviation of the replicates. Legend: TS: total solids; VS: volatile solids; COD: chemical oxygen demand; sCOD: soluble (filtered) chemical oxygen demand; NH_3-N: ammoniacal nitrogen; NTK: total Kjeldahl nitrogen.

Furthermore, as depicted in Figure 10, during the phase 2, ozone pretreatment also led to slight changes in the physicochemical properties of DMW. VS, VS/TS, and COD concentrations suggest little to no organic matter mineralization during the phase 2. It is worth highlighting that the samples collected for this phase were obtained in a dry period (Figure 5), which implied in a more complex and concentrated wastewater. Therefore, further physicochemical analyses are recommended for better investigating the effects of the pretreatment on substrate composition, such as total organic carbon, dissolved organic carbon, lignin, cellulose, and hemicellulose.

Previous studies reported different effects of ozone pretreatment on sCOD concentration (ALMOMANI *et al.*, 2019; BERNAL-MARTÍNEZ *et al.*, 2005; BOUGRIER *et al.*, 2007; CARBALLA *et al.*, 2007; CATENACCI *et al.*, 2022; CESARO *et al.*, 2019; CHENG; HONG, 2013; CHENG *et al.*, 2012; CHIAPPERO *et al.*, 2019; CHIAVOLA *et al.*, 2019; HAAK *et al.*, 2016; KAMESWARI *et al.*, 2014; LIU *et al.*, 2001; MARTÍN SANTOS *et al.*, 2003; PACKYAM *et al.*, 2015; ROY *et al.*, 2016; TIAN *et al.*, 2015; TSINTAVI *et al.*, 2013; XU *et al.*, 2010). In general, ozone pretreatment tends to increase sCOD with the applied ozone dose CHIAVOLA *et al.* (2019) and CHACANA *et al.* (2017a).

Figure 10 – Characteristics of raw and ozonated dairy manure wastewater (DMW) (phase 2) at different ozone doses (20, 40, 100, and 180 mg O3. g-1 VSDMW) a) Total solids (TS), volatile solids (VS), and VS/TS ratio; b) Total suspended solids (TSS), volatile suspended solids (VSS), and VSS/TSS ratio; c) Total and filtered chemical oxygen demand (COD); d) Total Kjeldahl nitrogen (TKN) and ammoniacal nitrogen (NH_3-N).

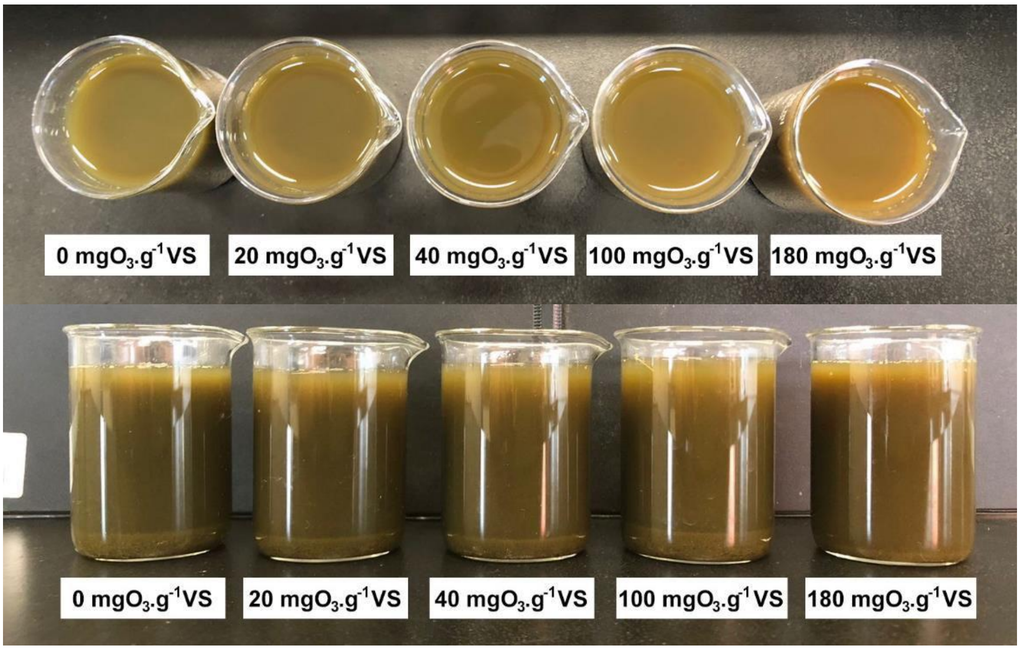


Values were expressed as mean ± standard deviation of the replicates. Legend: TS: total solids; VS: volatile solids; COD: chemical oxygen demand; sCOD: soluble (filtered) chemical oxygen demand; NH_3-N: ammoniacal nitrogen; NTK: total Kjeldahl nitrogen.

CHIAVOLA *et al.* (2019) also reported a linear relation between ozone dose and VS mineralization. Accordingly, rising ozone dose may also alter the total COD (CHACANA *et al.*, 2017a; CHACANA *et al.*, 2017b), not only through mineralization but also through partial oxidation (CHACANA *et al.*, 2017b), which may be related with the results of this work (Figure 10).

Figure 11 shows the visual aspects of raw and ozonated DMW. As can be seen, raw DMW had a greenish-brown color, which was becoming browner with the increase of the applied ozone dose.

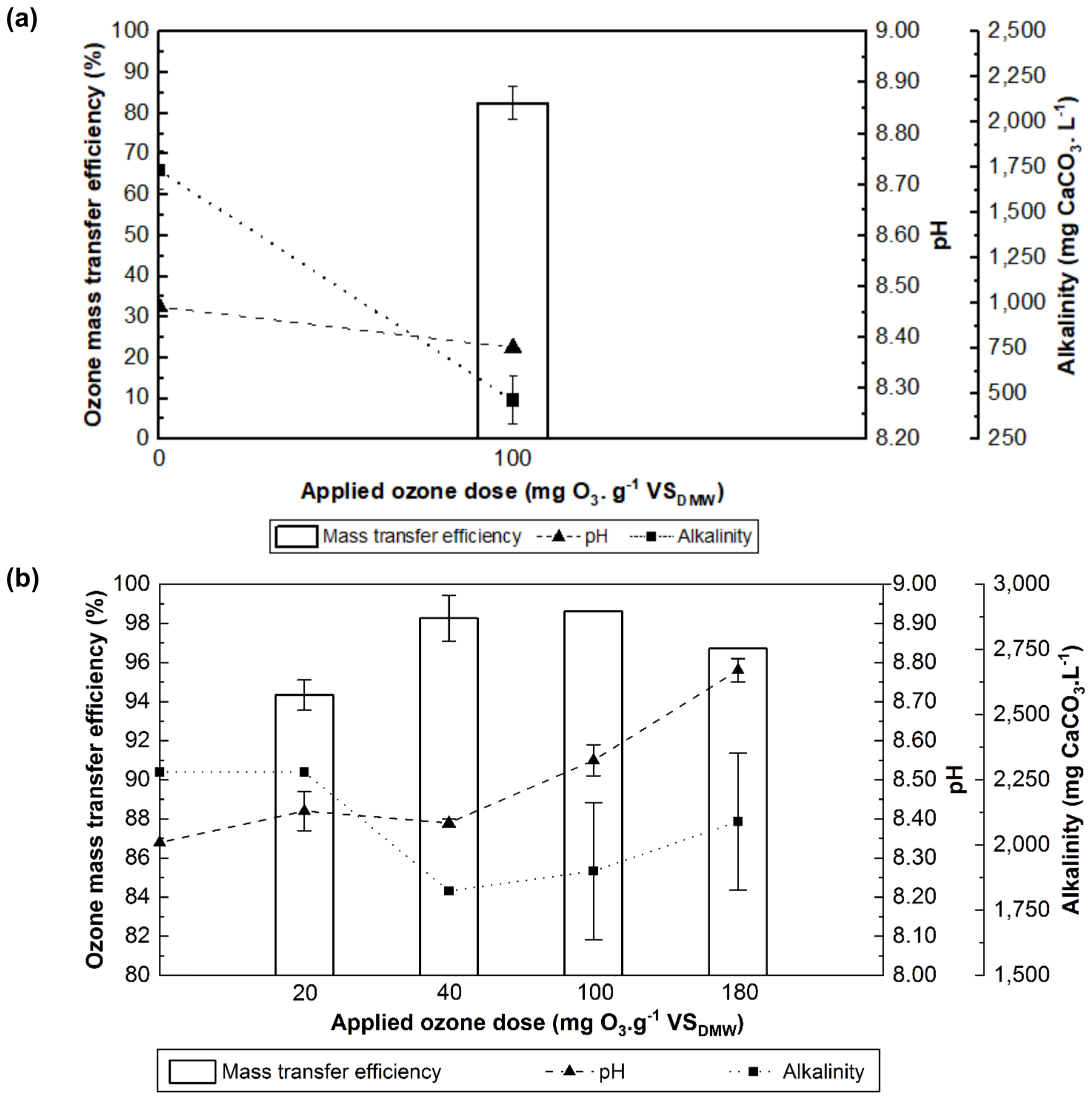
Figure 11 – Dairy manure wastewater (DMW) before and after ozonation at different ozone doses (phase 2)



The pH of DMW after ozonation varied from 8.46 to 8.38 in phase 1 (Figure 12a) and from 8.34 to 8.78 in phase 2 (Figure 12b). This low variation may be a result of the buffering capacity of the wastewater, which can be evidenced by the consumption of alkalinity during the process.This occurs mainly as a consequence of the neutralization of the acids produced by the oxidation of complex organic molecules during ozonation (VON SONNTAG; VON GUNTEN, 2012). It is worth mentioning that at this pH range, the indirect mechanism of ozonation is favored, which corroborates the reduction of VS and TS concentration as observed in phase 1 (Table 15; Figure 12).

Figure 12 – Ozone mass transfer efficiency and variation of pH and alkalinity at different applied ozone doses

a) Phase 1; b) Phase 2



Ozone mass transfer efficiency was 82.6% in the phase 1 (Figure 12a) and 94.3% to 98.6% (Figure 12b). With the increase of the ozone dose, slight increases in mean pH and alkalinity were also observed. Although it was not possible to confirm the occurrence of mineralization based on the analyses performed, it can be associated to the mineralization of the organic matter followed with CO_2 dissolution and consequent formation of bicarbonates. A formation of organic acids with pKa below the pH of the reaction media can also explain a potential increase in the alkalinity (VON SONNTAG; VON GUNTEN, 2012). It is worth highlighting that the complexity of the matrix analyzed can also affect the accuracy of the analyses of pH and alkalinity.

As expected, the ozone mass transfer efficiencies observed in phase 2 were higher than in phase 1, as a result of the increased organic matter concentration of DMW in phase 2 (Table 15; Figure 10) (VON SONNTAG; VON GUNTEN, 2012). The relatively high ozone mass transfer efficiencies evidenced the high reactivity between DMW and ozone. Previous studies conducted with bubble column reactors reported ozone mass transfer efficiencies of 68% for primary sludge (domestic wastewater, ozone doses: 10-220 mg $O_3.g^{-1}$ COD) (CHACANA *et al.*, 2017b), 73% for digested sludge (domestic wastewater, ozone doses: 50-210 mg O_3. g^{-1} COD) (CHACANA *et al.*, 2017b), 86% for mixed sludge (domestic wastewater, ozone doses: 59-234 mg O_3. g^{-1} VS) (WEEMAES *et al.*, 2000b), 90% for waste activated sludge (pharmaceutical wastewater, ozone dose: 274 mg O_3. g^{-1} VS) (PEI et al., 2015), and 21-39% for water (distilled water amended with 50 mg. L-1 of humic acids; ozone dose: 33.3-200 mg O_3. L^{-1}. h^{-1}) (YANG *et al.*, 2021).

4.2.3 Effect of ozonation on anaerobic digestion of DMW

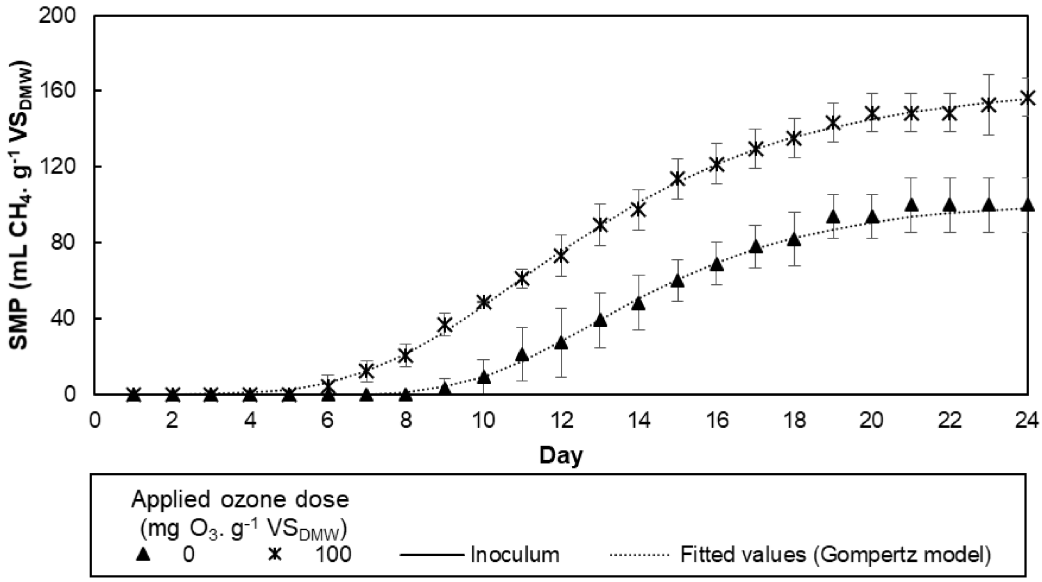
Ozonation at 100 mg O_3. g^{-1} VS_{DMW} significantly ($p<0.05$) increased the SMP of DMW in both phase 1 and phase 2. The dose of 100 mg O_3. g^{-1} VS_{DMW} increased the methane production potential (P) of DMW by 79.6% in phase 1 and 332% in phase 2 (Figure 13; Table 16). The pretreatment at low ozone doses (20 and 40 mg O_3. g^{-1} VS_{DMW}) had a slight effect in the methane production potential of DMW, as observed by Chen *et al.* (2021) (Table 10).

In addition, ozone pretreatment induced reductions in the lag phase time (λ) of anaerobic digestion and increases in the maximum methane production rate (r_m) (Table 16). In phase 1, the lag phase time was reduced to 12.8 to 10.9 days, which corresponds to a reduction of 15%. In parallel, maximum methane production rate was increased from 9.1 to 11.5 mL CH_4. g^{-1} VS. d, which corresponded to an improvement of 26% (Table 16).

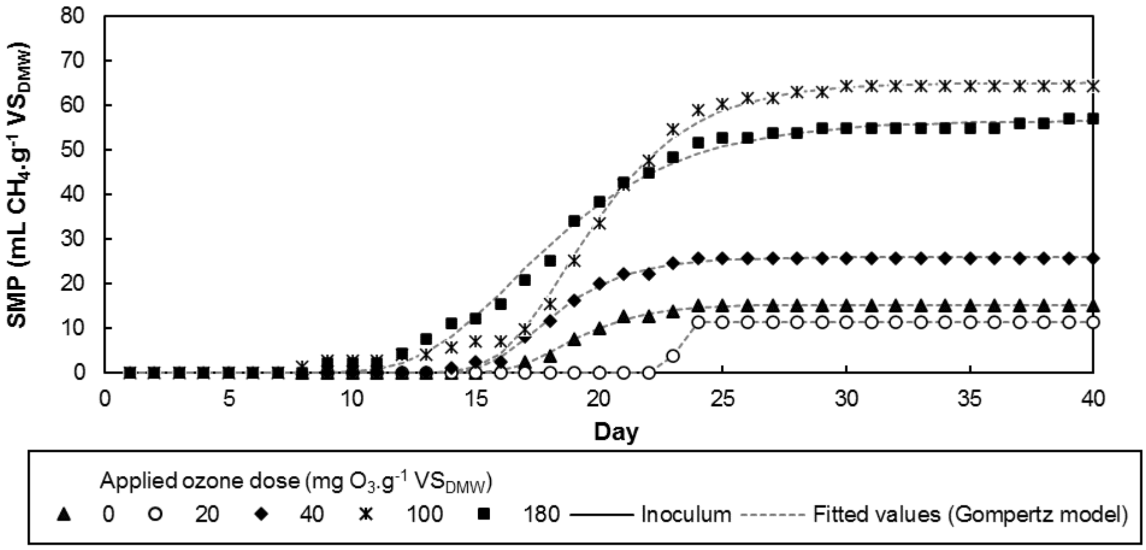
Figure 13 – Specific methane production (SMP) of raw and ozonated dairy manure wastewater (DMW)

a) Phase 1; b) Phase 2

(a)



(b)



In the best conditions tested in phase 2 (100 mg O_3. g^{-1} VS_{DMW}), the lag phase time decreased by 4% (from 17.2 to 15.7 days), whereas the maximum methane production rate was improved by 136% (from 3.9 to 9.2 mL CH_4. g^{-1} VS. d) (Table 16). It is worth mentioning that the reduction in the lag phase time indicate that ozone pretreatment produced a more bioavailable substrate, facilitating the hydrolysis and reducing the spend by the micro-organisms to acclimate and produce biogas (PACKYAM *et al.*, 2015). On the other hand, the maximum methane production rate indicate the maximum capacity of a system for COD removal, which suggest the maximum organic load that can be applied without unbalancing anaerobic process

(AQUINO; CHERNICHARO, 2005). Therefore, results suggest that the ozone pretreatment can enable optimum operating conditions at increased organic loads, which may enable an adequate treatment at a low-scale treatment plants.

Table 16 – Kinetic parameters of the anaerobic digestion of raw and ozonated dairy manure wastewater (DMW) predicted by modified Gompertz model

Parameter	**Raw DMW**	**20 mg O_3. g^{-1} VS_{DMW}**	**40 mg O_3. g^{-1} VS_{DMW}**	**100 mg O_3. g^{-1} VS_{DMW}**	**180 mg O_3. g^{-1} VS_{DMW}**
		Phase 1			
P (mL CH_4. g^{-1} VS)	101.4 ± 14.4^a	na	na	182.1 ± 10.1^b	na
λ (d)	12.8 ± 0.7^a	na	na	10.9 ± 1.3^a	na
r_m (mL CH_4. g^{-1} VS. d^{-1})	9.1 ± 0.8^a	na	na	11.5 ± 2.2^a	na
		Phase 2			
P (mL CH_4. g^{-1} VS)	15.3 ± 3.7^a	11.5*	25.9 ± 7.2^a	66.1 ± 33.4^b	56.5 ± 17.7^b
λ (d)	17.2 ± 1.5^a	22.9*	15.7 ± 1.1^a	16.5 ± 1.9^a	12.8 ± 2.2^a
r_m (mL CH_4. g^{-1} VS. d^{-1})	3.9 ± 1.5^a	10.7*	5.1 ± 2.0^a	9.2 ± 2.9^a	6.1 ± 1.8^a

Legend: P: methane production potential; λ: lag phase time; r_m: maximum methane production; na: not analyzed. * During the BMP tests, a leak was noticed in one of the two replicates tested for the dose of 20 mg O_3. g^{-1} VS_{DMW} and the results for this replicate could not be recorded. Note: the indexes *a* and *b* represent statistically significant differences at 95% confidence level. The statistical tests were performed separately for each different parameters (P, λ, and r_m) in each experimental phase.

Improvements in terms of the maximum methane production rate and the lag phase time induced by ozone pretreatment were also observed in previous studies. The lag phase time was reduced by 26% for distillery wastewater (139 mgO_3. g^{-1} VS) (GUPTA *et al.*, 2015). After ozonation, the maximum methane production rate was increased by 410% for distillery wastewater (139 mgO_3. g^{-1} VS) (GUPTA *et al.*, 2015), 19-22% for mixed sludge from sewage treatment plant (46-139 mgO_3. g^{-1} VS) (CHIAVOLA *et al.*, 2019; TIAN *et al.*, 2015), 37% for waste activated sludge (4 mgO_3. g^{-1} VS) (CHIAVOLA *et al.*, 2019), and 132% for hydrothermal liquefaction wastewater (211 mgO_3. g^{-1}COD) (YANG *et al.*, 2018).

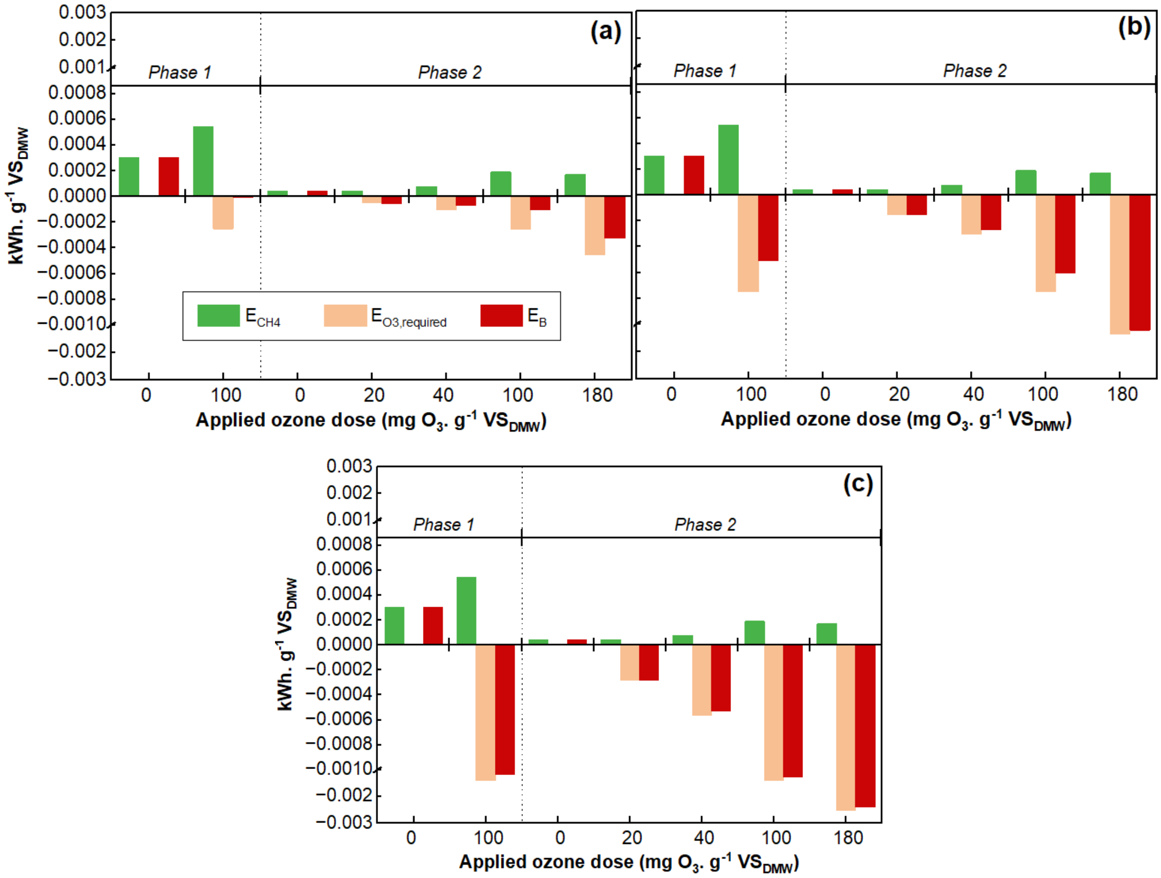
4.2.4 Energy balance

A preliminary assessment on the energetic sustainability of the experiments suggests negative energy balances in all scenarios, with deficits ranging from 0.009

to 1.16 kW.h. kg^{-1} VS_{DMW} in phase 1 and 0.052 to 2.39 kW.h. kg^{-1} VS_{DMW} in phase 2 (Figure 14). As shown in Figure 14, the energy balance was strongly influenced by the ozone dose and by the power efficiency assumed for the ozone generator.

Figure 14 – Energy balance of ozone pretreatment and anaerobic digestion of dairy manure wastewater (DMW) at different electrical energy demands for ozone generation

a) low = 2.5 kW. h. kg^{-1} O_3; b) median = 7.5 kW. h. kg^{-1} O_3; and c) high = 14 kW. h. kg^{-1} O_3



Legend: E_{CH4}: electrical energy that can be potentially recovered from the methane produced; $E_{O3,required}$: electrical energy required for ozone generation

In the most efficient scenario assumed, in which the electrical energy required for ozone generation was 2.5 kW.h. kg^{-1} O_3, the energy balance was near to zero (-8.6×10^{-6} kW.h. g^{-1} VS_{DMW}), indicating the potential of the pretreatment to has a positive energy balance at low ozone doses applied with efficient ozone generators in less concentrated DMW.

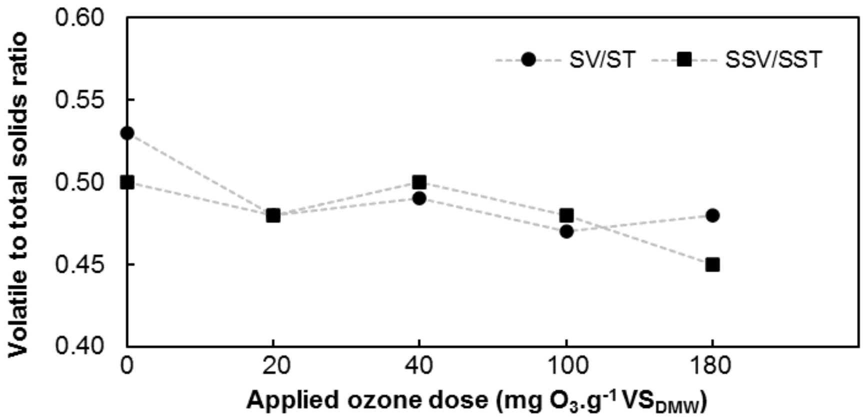
The low energy balance observed in phase 2 may be associated with the high concentration of other constituents that may harm process efficiency, such as a high presence of hydroxyl scavengers (VON SONNTAG; VON GUNTEN, 2012). This can be indicated by the high ozone mass transfer efficiencies (Figure 12) and by the low COD solubilization rates (Figure 10) observed the phase 2.

As discussed in item 4.1, the reduction of the working pH can change ozone pretreatment reaction mechanism, reducing the effects of the presence of hydroxyl scavengers and the potential for organic matter mineralization (VON GUNTEN, 2003; VON SONNTAG; VON GUNTEN, 2012).

4.2.5 Effect of ozonation on digestate characteristics

Results evidenced low concentrations of TS, TSS, and COD and high stabilization degrees in digestates from ozonated experiments as additional benefits of pre-ozonation (Table 17; Figure 15).

Figure 15 – Volatile to total solids ratio in digestates from batch anaerobic reactors fed with raw or ozonated dairy manure wastewater (DMW)



Ai *et al.* (2019) reported a reduction of 22% in the VS/TS ratio in the digestate of cattle manure biofibers submitted to ozone pretreatment. For sewage sludge, the VS/TS ratio was reduced by up 26% (BERNAL-MARTINEZ *et al.*, 2007; CARBALLA *et al.*, 2007; CHENG; HONG, 2013; CHIAPPERO *et al.*, 2019; GOEL *et al.*, 2003; LE *et al.*, 2019; PEI *et al.*, 2016; SILVESTRE *et al.*, 2015; WENJING *et al.*, 2019).

In addition, results suggest that ozonation did not compromise the potential for agricultural reuse of the digestate, maintaining its nitrogen load (Table 17). It is worth considering that ozone pretreatment may also be beneficial to reduce microbial risks associated with the DMW digestate reuse. Results of Chen *et al.* (2021) indicated that a potential of the process to increase the inactivation of enteric micro-organisms and reduce the relative abundance of ARGs.

Table 17 – Characteristics of digestates from batch anaerobic reactors fed with raw or ozonated dairy manure wastewater (DMW)

Parameter	**Raw DMW**	**20 mg O_3. g^{-1} VS_{DMW}**	**40 mg O_3. g^{-1} VS_{DMW}**	**100 mg O_3. g^{-1} VS_{DMW}**	**180 mg O_3. g^{-1} VS_{DMW}**
TS ($g.L^{-1}$)	25.0 ± 3.0	22.0 ± 1.0	21.7 ± 0.4	22.0 ± 0.4	19.0 ± 5.0
VS ($g.L^{-1}$)	13.4 ± 0.4	10.4 ± 0.4	10.7 ± 0.2	10.4 ± 0.2	9.0 ± 2.0
TSS ($g.L^{-1}$)	19.0 ± 2.0	18.5 ± 0.1	19.0 ± 1.0	16.0 ± 2.0	17.0 ± 2.0
VSS ($g.L^{-1}$)	9.0 ± 1.0	8.3 ± 0.07	9.4 ± 0.5	8.0 ± 1.0	7.3 ± 0.4
sCOD ($g.L^{-1}$)	0.43 ± 0.22	0.25 ± 0.02	0.38 ± 0.20	0.23 ± 0.02	0.40 ± 0.08
NH_3-N (mg $N.L^{-1}$)	311 ± 27.0	316 ± 4.0	303 ± 12.0	268 ± 24.0	334 ± 8.0
NTK (mg $N.L^{-1}$)	514 ± 72.0	551 ± 102	545 ± 143	646 ± 73.0	649 ± 61.0

Values were expressed as mean ± standard deviation of the replicates. Legend: TS: total solids; VS: volatile solids; COD: chemical oxygen demand; sCOD: soluble chemical oxygen demand; NH_3-N: ammoniacal nitrogen; NTK: total Kjeldahl nitrogen;

5 CONCLUSIONS

This work assessed the main effects of ozone pretreatment on anaerobic digestion of dairy manure wastewater (DMW) based on data from a systematic literature review and on experimental data from a bench scale study. Results indicate the influence of solubilization and mineralization of organic matter during ozonation on the energetic sustainability of anaerobic digestion of pre-ozonated substrates. Rising ozone doses and pH of ozonation may result not only in an increased COD solubilization, but also in a high energy consumption and a high volatile solids mineralization, which can negatively affect the energetic sustainability the processes. The experimental investigation indicated that the application of low doses and energetically efficient ozone generators are required for best results in terms of energy balance, particularly for the case of the less concentrated DMW. The best performance of the phase 1 may indicate the need for a preliminary treatment to reduce solids concentration in addition to the application of a low ozone dose. In parallel, ozone pretreatment led to the improvement of anaerobic digestion kinetic parameters, which may enable optimum full-scale operating conditions at increased organic loads and reduce costs. Results from the systematic literature review evidenced that ozonation can be more energetically feasible when the conventional anaerobic digestion is ineffective, particularly in cases of high restriction of readily available organic matter and non-ideal growth conditions for anaerobic micro-organisms. The application of ozone pre-treatment to oxidize substrates rich potential inhibitory compounds is also promising. Further efforts are required to reduce the electrical energy demand of commercially available ozone generators in order to improve the energetic sustainability of ozone pretreated anaerobic digestion. Importantly, in addition to the use of energetically efficient ozone generators, further studies should prioritize the application of low ozone doses and investigate the optimum working pH for ozonation, in order to solubilize part of the organic matter, increase the efficiency of methane production and maintain an energetically sustainable system. For further investigations on the effect of ozone pretreatment of complex matrices, such as DMW, it is also recommended to perform sensitive physicochemical analysis for key constituents as total organic carbon, lignin, cellulose, and hemicellulose.

REFERENCES

ABDELWAHAB, T. A. M.; MOHANTY, M. K.; SAHOO, P. K.; BEHERA, D. Impact of iron nanoparticles on biogas production and effluent chemical composition from anaerobic digestion of cattle manure. **Biomass Conversion and Biorefinery**, v. 12, n. 2022, p. 5583-5595, 2020.

ABDELWAHAB, T. A. M.; MOHANTY, M. K.; SAHOO, P. K.; BEHERA, D. Impact of nickel nanoparticles on biogas production from cattle manure. **Biomass Conversion and Biorefinery**, v. 13, n. 2023, p. 5205–5218, 2021a.

ABDELWAHAB, T. A. M.; MOHANTY, M. K.; SAHOO, P. K.; BEHERA, D.; FODAH, A. E. M. Cobalt nanoparticles to enhance anaerobic digestion of cow dung: focusing on kinetic models for biogas yield and effluent utilization. **Biomass Conversion and Biorefinery**, v. 13, n. 2023, p. 11657-11669, 2021b.

ADARME, O. F. H.; BAÊTA, B. E. L.; LIMA, D. R. S.; GURGEL, L. V. A.; AQUINO, S. F. Methane and hydrogen production from anaerobic digestion of soluble fraction obtained by sugarcane bagasse ozonation. **Industrial Crops and Products**, v. 109, p. 288-299, 2017.

AGGA, G. E.; COUCH, M.; PAREKH, R. R.; MAHMOUDI, F.; APPALA, K.; KASUMBA, J.; LOUGHRIN, J. H.; CONTE, E. D. Lagoon, Anaerobic Digestion, and Composting of Animal Manure Treatments Impact on Tetracycline Resistance Genes. **Antibiotics**, v. 11, n. 3, p. 391, 2022.

AI, P.; ZHANG, X.; DINAMARCA, C.; ELSAYED, M.; YU, L.; XI, J.; MEI, Z. Different effects of ozone and aqueous ammonia in a combined pretreatment method on rice straw and dairy manure fiber for enhancing biomethane production. **Bioresource Technology**, v. 282, p. 275-284, 2019.

AKÇAKAYA, M. **Effect of ozone pretreatment on biogas production and microbial community structure of two-stage anaerobic digester**. 2021. (M.S. - Master of Science), Middle East Technical University. 2021

ALMOMANI, F.; BHOSALE, R. R.; KHRAISHEH, M. A. M.; SHAWAQFAH, M. Enhancement of biogas production from agricultural wastes via pre-treatment with advanced oxidation processes. **Fuel**, v. 253, p. 964-974, 2019.

ANGELIDAKI, I.; AHRING, B. K. Methods for increasing the biogas potential from the recalcitrant organic matter contained in manure. **Water Sci Technol**, v. 41, n. 3, p. 189-194, 2000.

APHA/AWWA/WEF. **Standard Methods for the Examination of Water and Wastewater**. Washington: American Public Health Association, American Water Works Association, Water Environment Federation, 2017.

AQUINO, S.; PIRES, E. C. ASSESSMENT OF OZONE AS A PRETREATMENT TO IMPROVE ANAEROBIC DIGESTION OF VINASSE. **Brazilian Journal of Chemical Engineering**, v. 33, n. 2, p. 279-285, 2016.

AQUINO, S. F.; CHERNICHARO, C. A. L. Acúmulo de ácidos graxos voláteis (AGVs) em reatores anaeróbios sob estresse: causas e estratégias de controle. **Engenharia Sanitária e Ambiental**, v. 10, n. 2, p. 152-161, 2005.

ARIUNBAATAR, J.; PANICO, A.; FRUNZO, L.; ESPOSITO, G.; LENS, P. N. L.; PIROZZI, F. Enhanced anaerobic digestion of food waste by thermal and ozonation pretreatment methods. **Journal of Environmental Management**, v. 146, p. 142-149, 2014.

BAKHSHI, Z.; JAUFFUR, S.; FRIGON, D. Assessing energy benefits of operating anaerobic digesters at low temperature with solids pre-ozonation. **Renewable Energy**, v. 115, p. 1303-1311, 2018.

BERNAL-MARTINEZ, A.; CARRERE, H.; PATUREAU, D.; DELGENES, J. P. Combining anaerobic digestion and ozonation to remove PAH from urban sludge. **Process Biochemistry**, v. 40, n. 10, p. 3244-3250, 2005.

BERNAL-MARTINEZ, A.; CARRÈRE, H.; PATUREAU, D.; DELGENÈS, J. P. Ozone pre-treatment as improver of PAH removal during anaerobic digestion of urban sludge. **Chemosphere**, v. 68, n. 6, p. 1013-1019, 2007.

BERNAL-MARTÍNEZ, A.; CARRÈRE, H.; PATUREAU, D.; DELGENÈS, J. P. Combining anaerobic digestion and ozonation to remove PAH from urban sludge. **Process Biochemistry**, v. 40, n. 10, p. 3244-3250, 2005.

BESZÉDES, S.; KERTÉSZ, S.; LÁSZLÓ, Z.; SZABÓ, G.; HODÚR, C. Biogas production of ozone and/or microwave-pretreated canned maize production sludge. **Ozone: Science and Engineering**, v. 31, n. 3, p. 257-261, 2009.

BI, S.; HONG, X.; BAI, Y.; LIU, J.; YU, X.; FANG, S.; GAO, Y.; YAN, L.; WANG, Z.; WANG, Y.; WANG, W. Methane Production Dynamics of Co-Digestion of Cow Manure and Food Waste Under Mesophilic Condition. **Journal of Biobased Materials and Bioenergy**, v. 13, n. 2, p. 257-263, 2019.

BOUGRIER, C.; ALBASI, C.; DELGENÈS, J. P.; CARRÈRE, H. Effect of ultrasonic, thermal and ozone pre-treatments on waste activated sludge solubilisation and anaerobic biodegradability. **Chemical Engineering and Processing: Process Intensification**, v. 45, n. 8, p. 711-718, 2006.

BOUGRIER, C.; BATTIMELLI, A.; DELGENES, J. P.; CARRERE, H. Combined ozone pretreatment and anaerobic digestion for the reduction of biological sludge production in wastewater treatment. **Ozone: Science and Engineering**, v. 29, n. 3, p. 201-206, 2007.

BRAGUGLIA, C. M.; GIANICO, A.; MININNI, G. Comparison between ozone and ultrasound disintegration on sludge anaerobic digestion. **Journal of Environmental Management**, v. 95, n. SUPPL., p. S139-S143, 2012a.

BRAGUGLIA, C. M.; GIANICO, A.; MININNI, G. Comparison between ozone and ultrasound disintegration on sludge anaerobic digestion. **Journal of Environmental Management**, v. 95, p. S139-S143, 2012b.

BRASIL. **Tecnologias de digestão anaeróbia com relevância para o Brasil: substratos, digestores e uso de biogás. Brasília, DF**. Brasília, DF: Ministério das Cidades, 2015.

BRUNI, E.; JENSEN, A. P.; ANGELIDAKI, I. Comparative study of mechanical, hydrothermal, chemical and enzymatic treatments of digested biofibers to improve biogas production. **Bioresource Technology**, v. 101, n. 22, p. 8713-8717, 2010.

BUENDÍA, I. M.; FERNÁNDEZ, F. J.; VILLASEÑOR, J.; RODRÍGUEZ, L. Feasibility of anaerobic co-digestion as a treatment option of meat industry wastes. **Bioresource Technology**, v. 100, n. 6, p. 1903-1909, 2009.

CANO, R.; NIELFA, A.; FDZ-POLANCO, M. Thermal hydrolysis integration in the anaerobic digestion process of different solid wastes: Energy and economic feasibility study. **Bioresource Technology**, v. 168, p. 14-22, 2014.

CARBALLA, M.; MANTEROLA, G.; LARREA, L.; TERNES, T.; OMIL, F.; LEMA, J. M. Influence of ozone pre-treatment on sludge anaerobic digestion: Removal of pharmaceutical and personal care products. **Chemosphere**, v. 67, n. 7, p. 1444-1452, 2007.

CARDEÑA, R.; MORENO, G.; BAKONYI, P.; BUITRÓN, G. Enhancement of methane production from various microalgae cultures via novel ozonation pretreatment. **Chemical Engineering Journal**, v. 307, p. 948-954, 2017.

CATENACCI, A.; PERONI, M.; GIEVERS, F.; MAINARDIS, M.; PASINETTI, E.; MALPEI, F. Integration of sludge ozonation with anaerobic digestion: From batch testing to scenario analysis with energy, economic and environmental assessment. **Resources, Conservation and Recycling**, v. 186, p. 106539, 2022.

CESARO, A.; BELGIORNO, V. Sonolysis and ozonation as pretreatment for anaerobic digestion of solid organic waste. **Ultrasonics Sonochemistry**, v. 20, n. 3, p. 931-936, 2013.

CESARO, A.; BELGIORNO, V. Ozone pretreatment for the anaerobic digestion of organic solid waste. **Detritus**, v. 12, p. 51-56, 2020.

CESARO, A.; BELGIORNO, V.; SICILIANO, A.; GUIDA, M. The sustainable recovery of the organic fraction of municipal solid waste by integrated ozonation and anaerobic digestion. **Resources, Conservation and Recycling**, v. 141, p. 390-397, 2019.

CHACANA, J.; ALIZADEH, S.; LABELLE, M. A.; LAPORTE, A.; HAWARI, J.; BARBEAU, B.; COMEAU, Y. Effect of ozonation on anaerobic digestion sludge activity and viability. **Chemosphere**, v. 176, p. 405-411, 2017a.

CHACANA, J.; LABELLE, M.-A.; LAPORTE, A.; GADBOIS, A.; BARBEAU, B.; COMEAU, Y. Ozonation of Primary Sludge and Digested Sludge to Increase Methane Production in a Chemically Enhanced Primary Treatment Facility. **Ozone: Science & Engineering**, v. 39, n. 3, p. 148-158, 2017b.

CHAIPRAPAT, S.; LAKLAM, T. Enhancing digestion efficiency of POME in anaerobic sequencing batch reactor with ozonation pretreatment and cycle time reduction. **Bioresource Technology**, v. 102, n. 5, p. 4061-4068, 2011.

CHEN, X.; TANG, R.; WANG, Y.; YUAN, S.; WANG, W.; ALI, I. M.; HU, Z.-H. Effect of ultrasonic and ozone pretreatment on the fate of enteric indicator bacteria and antibiotic resistance genes, and anaerobic digestion of dairy wastewater. **Bioresource Technology**, v. 320, p. 124356, 2021.

CHENG, C. J.; HONG, P. K. A. Anaerobic digestion of activated sludge after pressure-assisted ozonation. **Bioresource Technology**, v. 142, p. 69-76, 2013.

CHENG, C. J.; HONG, P. K. A.; LIN, C. F. Improved solubilization of activated sludge by ozonation in pressure cycles. **Chemosphere**, v. 87, n. 6, p. 637-643, 2012.

CHERNICHARO, C. A. L. **Reatores anaeróbios**. Belo Horizonte: Editora UFMG, 2019.

CHERNICHARO, C. A. L.; BRANDT, E. M. F.; BRESSANI-RIBEIRO, T.; MELO, V. R.; BIANCHETTI, F. J.; MOTAFILHO, C. R.; MCADAM, E. Development of a tool for improving the management of gaseous emissions in UASB-based sewage treatment plants. **Water Practice and Technology**, v. 12, n. 4, p. 917-926, 2017.

CHIAPPERO, M.; DEMICHELIS, F.; LIN, X.; LIU, C.; FRIGON, D.; FIORE, S. Investigation of pre-treatments improving low-temperature anaerobic digestion of waste activated sludge. **Process Safety and Environmental Protection**, v. 131, p. 28-37, 2019.

CHIAVOLA, A.; D'AMATO, E.; BONI, M. R. Effects of low-dosage ozone pre-treatment on the anaerobic digestion of secondary and mixed sludge. **Environmental Science and Pollution Research**, v. 26, n. 35, p. 35957-35967, 2019.

CHU, L.; YAN, S.; XING, X. H.; SUN, X.; JURCIK, B. Progress and perspectives of sludge ozonation as a powerful pretreatment method for minimization of excess sludge production. **Water Research**, v. 43, n. 7, p. 1811-1822, 2009.

COARITA FERNANDEZ, H.; AMAYA RAMIREZ, D.; TEIXEIRA FRANCO, R.; BUFFIÈRE, P.; BAYARD, R. Methods for the Evaluation of Industrial Mechanical Pretreatments before Anaerobic Digesters. **Molecules**, v. 25, n. 4, p. 860, 2020a.

COARITA FERNANDEZ, H.; TEIXEIRA FRANCO, R.; BAYARD, R.; BUFFIERE, P. Mechanical Pre-treatments Evaluation of Cattle Manure Before Anaerobic Digestion. **Waste and Biomass Valorization**, v. 11, n. 10, p. 5175-5184, 2020b.

DAS, S.; DAGNEW, M.; RAY, M. B. Anaerobic digestibility of resin acids in primary sludge: Effect of ozone pretreatment. **Chemosphere**, v. 262, p. 128316, 2021.

DAVIDSSON, A.; ERIKSSON, E.; FICK, J. Ozonation and Thermal Pre-Treatment of Municipal Sewage Sludge-Implications for Toxicity and Methane Potential. **Journal of Residuals Science & Technology**, v. 10, n. 2, p. 85-91, 2013.

DBFZ. **Standard BMP Methods**. 2022. Disponível em: https://www.dbfz.de/en/projects/bmp. Acesso em: 10 de maio de 2022.

DOMAŃSKI, J.; MARCHUT-MIKOŁAJCZYK, O.; POLEWCZYK, A.; JANUSZEWICZ, B. Ozonolysis of straw from Secale cereale L. for anaerobic digestion. **Bioresource Technology**, v. 245, p. 394-400, 2017.

DU, H.; WU, Y.; WU, H.; LI, F. Effect of ozone pretreatment on characteristics of dissolved organic matter formed in aerobic and anaerobic digestion of waste-activated sludge. **Environmental Science and Pollution Research**, v. 28, p. 2779–2790, 2020.

ERDEN, G.; FILIBELI, A. Ozone oxidation of biological sludge: Effects on disintegration, anaerobic biodegradability, and filterability. **Environmental Progress and Sustainable Energy**, v. 30, n. 3, p. 377-383, 2011.

ERDEN, G.; FILIBELI, A. Disintegration of biological sludge. A comparison between ozone oxidation and ultrasonic pretreatment. **Environment Protection Engineering**, v. 45, n. 3, p. 55-67, 2019.

FILGUEIRAS, M. V.; PIRES, N. O.; NETO, J. M.; COSTALONGA, L. G.; SANTOS, J. P. E. D.; LEITE, M. A.; CORRÊA, L. M.; OTÊNIO, M. H.; BOTTREL, S. E. C.; PEREIRA, R. D. O. Avaliação da atividade estrogênica em efluente da pecuária leiteira: Fase sólida e líquida. **Revista Eletrônica De Gestão E Tecnologias Ambientais**, v. 9, n. 3, p. 191-202, 2022.

FONT-PALMA, C. Methods for the Treatment of Cattle Manure—A Review. **Journal of Carbon Research**, v. 5, n. 2, p. 27, 2019.

GOEL, R.; TOKUTOMI, T.; YASUI, H. Anaerobic digestion of excess activated sludge with ozone pretreatment. **Water Science and Technology**, v. 47, n. 12, p. 207-214, 2003.

GUPTA, S.; SHARMA, A.; SARATCHANDRA, T.; MALIK, S.; WAINDESKAR, V.; MUDLIAR, S. Effect of Ozone Pretreatment on Biodegradability Enhancement and Biogas Generation Potential From Biomethanated Distillery Effluent. **Ozone: Science and Engineering**, v. 37, n. 5, p. 411-419, 2015.

HAAK, L.; ROY, R.; PAGILLA, K. Toxicity and biogas production potential of refinery waste sludge for anaerobic digestion. **Chemosphere**, v. 144, p. 1170-1176, 2016.

HARNER, J. P.; MURPHY, J. P. Flushing manure systems for dairy facilities. **Kansas Agricultural Experiment Station Research Reports**, v. 0, n. 2, p. 19-23, 1997.

IBGE. **Pesquisa Pecuária Municipal**. 2020. Acesso em: mai. 2022.

INMET. **Dados históricos anuais**. 2023. Disponível em: https://portal.inmet.gov.br/dadoshistoricos. Acesso em: oct. 2023.

KAFLE, G. K.; CHEN, L. Comparison on batch anaerobic digestion of five different livestock manures and prediction of biochemical methane potential (BMP) using different statistical models. **Waste Management**, v. 48, p. 492-502, 2016.

KAMESWARI, K. S. B.; KALYANARAMAN, C.; THANASEKARAN, K. Evaluation of various pre-treatment processes on tannery sludge for enhancement of soluble chemical oxygen demand. **Clean Technologies and Environmental Policy**, v. 16, n. 2, p. 369-376, 2014.

KAMINSKI, G.; POSSETTI, G. R. C.; MANNICH, M.; SILVA, F. O. D. M. E.; RIETOW, J. C.; AISSE, M. M.; PUJATTI, J. F. Avanços nas ferramentas e técnicas para estimativa de produção e tratamento de biogás em ETEs com reatores anaeróbios Nota Técnica 5 – Combustão direta de biogás em queimadores. **Cadernos Técnicos Engenharia Sanitária e Ambiental**, v. 1, n. 1, p. 59-73, 2021.

KANNAH, R. Y.; KAVITHA, S.; BANU, J. R.; KARTHIKEYAN, O. P.; SIVASHANMUGHAM, P. Dispersion induced ozone pretreatment of waste activated biosolids: Arriving biomethanation modelling parameters, energetic and cost assessment. **Bioresource Technology**, v. 244, p. 679-687, 2017.

KHAN, M. U.; AHRING, B. K. Improving the biogas yield of manure: Effect of pretreatment on anaerobic digestion of the recalcitrant fraction of manure. **Bioresource Technology**, v. 321, p. 124427, 2021.

KOBAYASHI, T.; LI, Y. Y.; HARADA, H.; YASUI, H.; NOIKE, T. Upgrading of the anaerobic digestion of waste activated sludge by combining temperature-phased anaerobic digestion and intermediate ozonation. **Water Science and Technology**, v. 59, n. 1, p. 185-193, 2009.

LE, T. M.; VO, P. T.; DO, T. A.; TRAN, L. T.; TRUONG, H. T.; LE, T. T. X.; CHEN, Y. H.; CHANG, C. C.; CHANG, C. Y.; TRAN, Q. T.; THANH, T.; VAN DO, M. Effect of assisted ultrasonication and ozone pretreatments on sludge characteristics and yield of biogas production. **Processes**, v. 7, n. 10, 2019.

LI, R.; CHEN, S.; LI, X.; SAIFULLAH LAR, J.; HE, Y.; ZHU, B. Anaerobic Codigestion of Kitchen Waste with Cattle Manure for Biogas Production. **Energy & Fuels**, v. 23, n. 4, p. 2225-2228, 2009.

LI, X.; XU, X.; HUANG, S.; ZHOU, Y.; JIA, H. An efficient method to improve the production of methane from anaerobic digestion of waste activated sludge. **Water Science and Technology**, v. 76, n. 7-8, p. 2075-2084, 2017.

LI, Y.; CHEN, Z.; PENG, Y.; HUANG, W.; LIU, J.; MIRONOV, V.; ZHANG, S. Deeper insights into the effects of substrate to inoculum ratio selection on the relationship of kinetic parameters, microbial communities, and key metabolic pathways during the anaerobic digestion of food waste. **Water Research**, v. 217, p. 118440, 2022.

LI, Y.; ZHAO, J.; KROONEMAN, J.; EUVERINK, G. J. W. Strategies to boost anaerobic digestion performance of cow manure: Laboratory achievements and their full-scale application potential. **Science of The Total Environment**, v. 755, p. 142940, 2021.

LIEW, Y. X.; CHAN, Y. J.; MANICKAM, S.; CHONG, M. F.; CHONG, S.; TIONG, T. J.; LIM, J. W.; PAN, G.-T. Enzymatic pretreatment to enhance anaerobic bioconversion of high strength wastewater to biogas: A review. **Science of The Total Environment**, v. 713, p. 136373, 2020.

LIU, J. C.; LEE, C. H.; LAI, J. Y.; WANG, K. C.; HSU, Y. C.; CHANG, B. V. Extracellular polymers of ozonized waste activated sludge. **Water Science and Technology**, v. 44, n. 10, p. 137-142, 2001.

LIU, X.; SOULI, I.; CHAMAA, M.-A.; LENDORMI, T.; SABOURIN, C.; LEMEE, Y.; BOY, V.; CHAIRA, N.; FERCHICHI, A.; MORANCAIS, P.; LANOISELLE, J.-L. Effect of thermal pretreatment at 70 degrees C for one hour (EU hygienization conditions) of various organic wastes on methane production under mesophilic anaerobic digestion. **Aims Environmental Science**, v. 5, n. 2, p. 117-128, 2018.

LOYON, L. Overview of Animal Manure Management for Beef, Pig, and Poultry Farms in France. **Frontiers in Sustainable Food Systems**, v. 2, p. 36, 2018.

MARCELINO, R. B. P.; LEÃO, M. M. D.; LAGO, R. M.; AMORIM, C. C. Multistage ozone and biological treatment system for real wastewater containing antibiotics. **Journal of Environmental Management**, v. 195, p. 110-116, 2017.

MARTIN, M. A.; RAPOSO, F.; BORJA, R.; MARTIN, A. Kinetic study of the anaerobic digestion of vinasse pretreated with ozone, ozone plus ultraviolet light, and ozone plus ultraviolet light in the presence of titanium dioxide. **Process Biochemistry**, v. 37, n. 7, p. 699-706, 2002.

MARTÍN SANTOS, M. A.; FERNÁNDEZ BOCANEGRA, J. L.; MARTÍN MARTÍN, A.; GARCÍA GARCÍA, I. Ozonation of vinasse in acid and alkaline media. **Journal of Chemical Technology and Biotechnology**, v. 78, n. 11, p. 1121-1127, 2003.

MITO, J. Y. D. L.; KERKHOFF, S.; SILVA, J. L. G.; VENDRAME, M. G.; STEINMETZ, R. L. R.; KUNZ, A. **Metodologia para estimar o potencial de biogás**

e biometano a partir de plantéis suínos e bovinos no Brasil. Concórdia, SC: CIBiogás/Embrapa Suínos e Aves, 2018.

NASCIMENTO, A. D. M.; DE PAULA, V. R.; OLIVEIRA DIAS, E. H.; CARNEIRO, J. C.; OTENIO, M. H. Quantitative microbial risk assessment of occupational and public risks associated with bioaerosols generated during the application of dairy cattle wastewater as biofertilizer. **Science of the Total Environment**, v. 745, p. 140711, 2020.

NEGRAL, L.; CASTRILLÓN, L.; MARAÑÓN, E.; FERNÁNDEZ-NAVA, Y.; ORMAECHEA, P. Inverted phase fermentation as a pretreatment for anaerobic digestion of cattle manure and sewage sludge. **Journal of Environmental Management**, v. 203, p. 741-744, 2017.

NILSSON, F.; DAVIDSSON, Å.; FALÅS, P.; BENGTSSON, S.; BESTER, K.; JÖNSSON, K. Impact of activated sludge ozonation on filamentous bacteria viability and possible added benefits. **Environmental Technology**, v. 40, n. 20, p. 2601-2607, 2019.

ORMAECHEA, P.; CASTRILLÓN, L.; SUÁREZ-PEÑA, B.; MEGIDO, L.; FERNÁNDEZ-NAVA, Y.; NEGRAL, L.; MARAÑÓN, E.; RODRÍGUEZ-IGLESIAS, J. Enhancement of biogas production from cattle manure pretreated and/or co-digested at pilot-plant scale. Characterization by SEM. **Renewable Energy**, v. 126, p. 897-904, 2018.

PACKYAM, G. S.; KAVITHA, S.; KUMAR, S. A.; KALIAPPAN, S.; YEOM, I. T.; BANU, J. R. Effect of sonically induced deflocculation on the efficiency of ozone mediated partial sludge disintegration for improved production of biogas. **Ultrasonics Sonochemistry**, v. 26, p. 241-248, 2015.

PANDEY, P. K.; NDEGWA, P. M.; ALLDREDGE, J. R.; PITTS, M.; SOUPIR, M. L. Modeling effects of granules on the start-up of anaerobic digestion of dairy wastewater with Langmuir and extended Freundlich equations. **Bioprocess and Biosystems Engineering**, v. 33, n. 7, p. 833-845, 2010.

PEI, J.; YAO, H.; WANG, H.; REN, J.; YU, X. Comparison of ozone and thermal hydrolysis combined with anaerobic digestion for municipal and pharmaceutical

UNIVERSIDADE FEDERAL DE JUIZ DE FORA

PRÓ-REITORIA DE PÓS-GRADUAÇÃO E PESQUISA

ATA DE DEFESA DE TRABALHO DE CONCLUSÃO DE PÓS-GRADUAÇÃO *STRICTO SENSU*

PROGRAMA DE PÓS-GRADUAÇÃO EM ENGENHARIA CIVIL

Nº PROPP: 739.24112023.40-M

Nº PPG: 42

AVALIAÇÃO DA BANCA EXAMINADORA

Tendo o(a) senhor(a) Presidente declarado aberta a sessão, mediante o prévio exame do referido trabalho por parte de cada membro da Banca, o(a) discente procedeu à apresentação de seu Trabalho de Conclusão de Curso de Pós-graduação *Stricto sensu* e foi submetido(a) à arguição pela Banca Examinadora que, em seguida, deliberou sobre o seguinte resultado:

(x) APROVADO (Conceito A)

( ) APROVADO CONDICIONALMENTE (Conceito B), mediante o atendimento das alterações sugeridas pela Banca Examinadora, constantes do campo Observações desta Ata.

( ) REPROVADO (Conceito C), conforme parecer circunstanciado, registrado no campo Observações desta Ata e/ou em documento anexo, elaborado pela Banca Examinadora

Novo título da Dissertação/Tese (só preencher no caso de mudança de título):

Observações da Banca Examinadora caso:

- O discente for Aprovado Condicionalmente
- Necessidade de anotações gerais sobre a dissertação/tese e sobre a defesa, as quais a banca julgue pertinentes.

Nada mais havendo a tratar, o(a) senhor(a) Presidente declarou encerrada a sessão de Defesa, sendo a presente Ata lavrada e assinada pelos(as) senhores(as) membros da Banca Examinadora e pelo(a) discente, atestando ciência do que nela consta.

INFORMAÇÕES

- Para fazer jus ao título de mestre(a)/doutor(a), a versão final da dissertação/tese, considerada Aprovada, devidamente conferida pela Secretaria do Programa de Pós-graduação, deverá ser tramitada para a PROPP, em Processo de Homologação de Dissertação/Tese, dentro do prazo de 90 dias a partir da data da defesa. Após a entrega dos dois exemplares definitivos, o processo deverá receber homologação e, então, ser encaminhado à CDARA.

- Esta Ata de Defesa é um documento padronizado pela Pró-Reitoria de Pós-Graduação e Pesquisa. Observações excepcionais feitas pela Branca Examinadora poderão ser registradas no campo disponível acima ou em documento anexo, desde que assinadas pelo(a) Presidente(a).

- Esta Ata de Defesa somente poderá ser utilizada como comprovante de titulação se apresentada junto á Certidão da Coordenadoria de Assuntos e Registros Acadêmicos da UFJF (CDARA) atestando que o processo de confecção e registro do diploma está em andamento.

BANCA EXAMINADORA

Profa. Dra. Sue Ellen Costa Bottrel - Orientador(a) e Presidente da Banca

Universidade Federal de Juiz de Fora (UFJF)

Prof. Dr. Emanuel Manfred Freire Brandt - Coorientador

Universidade Federal de Juiz de Fora (UFJF)

Profa. Dra. Camila Costa Amorim Amaral - Membro Titular Externo

Universidade Federal de Minas Gerais (UFMG)

Prof. Dr. Marcelo Henrique Otênio - Membro Titular Externo

(Embrapa)

Profa. Dra. Renata de Oliveira Pereira - Membro Titular Interno

Universidade Federal de Juiz de Fora (UFJF

Juiz de Fora, 28 / 11 / 2023.

Documento assinado eletronicamente por **Marcelo Henrique Otenio**, **Usuário Externo**, em 28/11/2023, às 14:59, conforme horário oficial de Brasília, com fundamento no § 3º do art. 4º do Decreto nº 10.543, de 13 de novembro de 2020.

Documento assinado eletronicamente por **Fernanda Bento Rosa Gomes**, **Usuário Externo**, em 28/11/2023, às 17:15, conforme horário oficial de Brasília, com fundamento no § 3º do art. 4º do Decreto nº 10.543, de 13 de novembro de 2020.

Documento assinado eletronicamente por **Renata de Oliveira Pereira**, **Professor(a)**, em 29/11/2023, às 09:54, conforme horário oficial de Brasília, com fundamento no § 3º do art. 4º do Decreto nº 10.543, de 13 de novembro de 2020.

Documento assinado eletronicamente por **Emanuel Manfred Freire Brandt**, **Professor(a)**, em 29/11/2023, às 14:15, conforme horário oficial de Brasília, com fundamento no § 3º do art. 4º do Decreto nº 10.543, de 13 de novembro de 2020.

Documento assinado eletronicamente por **Camila Costa de Amorim Amaral**, **Usuário Externo**, em 29/11/2023, às 14:49, conforme horário oficial de Brasília, com fundamento no § 3º do art. 4º do Decreto nº 10.543, de 13 de novembro de 2020.

Documento assinado eletronicamente por **Sue Ellen Costa Bottrel**, **Professor(a)**, em 29/11/2023, às 16:38, conforme horário oficial de Brasília, com fundamento no § 3º do art. 4º do Decreto nº 10.543, de 13 de novembro de 2020.

A autenticidade deste documento pode ser conferida no Portal do SEI-Ufjf (www2.ufjf.br/SEI) através do ícone Conferência de Documentos, informando o código verificador **1598062** e o código CRC **129063B7**.