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VALORIZATION OF ORANGE PEELS THROUGH TWO-STAGE MESOPHILIC ANAEROBIC REACTORS FOR BIOGAS PRODUCTION

VALORIZAÇÃO DE CASCAS DE LARANJA ATRAVÉS DE REATORES ANAERÓBIOS MESOFÍLICOS DE FASES SEPARADAS PARA PRODUÇÃO DE BIOGÁS

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Valorização de cascas de laranja através de reatores anaeróbios mesofílicos de fases separadas para produção de biogás

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RESUMO

O Brasil é líder mundial na produção de suco de laranja. O processamento industrial gera cerca de 50% dos resíduos, que podem ser processados para obter biogás, no entanto, a maioria deles não são reutilizados e pelo contrário são levados ao aterro sanitário. Com o objetivo de analisar quais são os processos biotecnológicos que podem gerar valor agregado nos resíduos e avaliar a situação atual do uso da casca de laranja para gerar biocombustíveis, uma análise bibliométrica da produção científica global de resíduos agroindustriais de laranja para produzir bioenergia por digestão anaeróbia e outras tecnologias foi realizada no presente estudo. O trabalho compreendeu o período de 1900 a 2019 e um total de 161 documentos foram selecionados. Os resultados mostram trabalhos reportando diferentes pré-tratamentos para obter o melhor rendimento de biogás em diversos tipos de digestão anaeróbia e outras tecnologias como fermentação para produção de etanol. Essas pesquisas foram feitas principalmente na Espanha, China e Estados Unidos. Assim, foram avaliadas as tendências da digestão anaeróbia dos resíduos agroindustriais da laranja, propondo uma solução para esses resíduos e subsidiando futuras tomadas de decisão nesse campo da ciência. Por outro lado, na parte experimental, o objetivo foi realizar uma digestão anaeróbia mesofílica em reatores do tipo fases separadas visando à obtenção de biogás e avaliar por meio de cálculos energéticos sua aplicação na indústria cítrica. Os resultados mostram que o biogás foi obtido em um sistema de 46 dias em escala de laboratório. A digestão em duas fases resultou em aumento da concentração de metano (60% em comparação com 48%) e volume (em 18%) em relação à digestão anaeróbia em uma fase (controle). As reduções na carga orgânica e na demanda química de oxigênio mostraram melhorias semelhantes e a imagem em microscopia eletrônica de barrido (SEM) dos sólidos dos reatores sugeriu uma quebra mais eficaz do digerido após a segunda fase do reator de duas fases em comparação com o controle. A quebra de compostos inibitórios, incluindo o D-limoneno, no reator acidogênico é uma explicação potencial do desempenho aprimorado. Em relação à energia, para o Estado de São Paulo, os resíduos anuais da indústria cítrica podem gerar $97{,}51 \times 10^3$ MWh / ano, que podem ser usados nas próprias instalações da indústria ou vendidos para a rede de energia. A mitigação potencial de gases de efeito estufa quando for usada para substituir a energia elétrica da matriz energética brasileira foi estimada em $9{,}05 \times 10^3$ tCO_2eq / ano.

Palavras chaves: *Resíduo Lignocelulósico, Digestão Anaeróbia*

ABSTRACT

Brazil is the world leader in orange juice production. Industrial processing generates about 50% of waste, which can be processed to obtain biogas; however, most of them are not reused and otherwise are taken to the landfill. In order to analyze which are the biotechnological processes that can generate added value in the residues and to evaluate the current situation of the use of orange peel to generate biofuels, a bibliometric analysis of the global scientific production of agro-industrial residues of orange to produce bioenergy by anaerobic digestion and other technologies was carried out in the present study. The work covered the period from 1900 to 2019 and a total of 161 documents were selected.The results show studies reporting different pretreatments to obtain the best biogas yield in different types of anaerobic digestion and other technologies as ethanol production by the fermentation of some orange peel sugars. These researches were done mainly in Spain, China and the United States. Thus, the trends of anaerobic digestion of orange agro-industrial residues were evaluated and possible solutions for these residues and future decision-making in this field of science were shown. On the other hand, in the experimental part, the objective was to perform an anaerobic mesophilic digestion in two stage anaerobic reactors in order to obtain biogas and to evaluate by energy calculations its application in the citrus industry. The results showed that the biogas was obtained in a 46-day laboratory scale system. Two-stage digestion resulted in increased methane concentration (60% compared to 48%) and volume (by 18%) compared to anaerobic digestion in one-stage (control). The reductions in organic load and chemical oxygen demand showed similar improvements and the Scanning Electron Microscope (SEM) image of digesters effluent suggested a more effective break in the feed after the second phase of the two-stage reactor compared to the control. The breakdown of inhibitory compounds, including D-limonene, in the acidogenic reactor is a potential explanation for the improved performance. In relation to energy, for São Paulo State. The potential electric power was estimated as $97.51x10^3$ MWh / year, which can be used in the industry's own facilities or sold to the power grid. The potential greenhouse gas mitigation when used to replace the electric energy of the Brazilian energy matrix was estimated at $9.05x10^3$ tCO2eq / year.

Keywords: *Lignocellulosic Residue, Anaerobic Digestion,*

SUMÁRIO

Capítulo 1 - Introdução geral, Justificativa, Objetivos e Estrutura da Tese

1.1.Introdução geral

Nos últimos anos a preocupação pelo aquecimento global, o esgotamento das fontes renováveis e o rápido crescimento das indústrias tem aumentado consideravelmente devido a diferentes consequências dessas atividades, entre elas, a alta quantidade de resíduos gerados que tem resultado em uma crescente contaminação ambiental (BUSSOLO DE SOUZA et al., 2018).

Segundo a FAO (KUCZMAN et al., 2018), a geração de resíduos alimentícios consiste em um dos principais fatores que geram contaminação em nível mundial. Estima-se que 1,3 bilhões de toneladas de resíduos alimentícios são gerados no mundo, a um custo de 680 bilhões de dólares por ano para seu tratamento. Estes resíduos são gerados nas etapas produtivas e são constituídos, em porcentagem, por 45% de frutas e vegetais, 26% de raízes e tubérculos, 18% de cereais e a porcentagem restante está repartida entre óleos, peixes, carnes e resíduos de restaurantes(XU et al., 2018). Os resíduos como as cascas de frutas procedentes das indústrias processadoras de sucos também são gerados em grandes quantidades, no entanto, a sua disposição final ainda ocorre através de aterros sanitários, onde a energia contida neste resíduo é desperdiçada e convertida em gases de efeito estufa, como o dióxido de carbono e o metano(DE CLERCQ et al., 2017). Por esse motivo, são muito importantes a gestão, valorização e a reciclagem dos resíduos com o objetivo de aproveitar as características de biodegradabilidade deles.

O resíduo alimentício é um substrato orgânico promissor devido à sua riqueza de nutrientes, alto potencial bioquímico de metano e alto conteúdo de matéria orgânica, que pode ser valorizado através da bioconversão a adubo e energia (biogás) (LI et al., 2016). A valorização do resíduo consiste em mitigar os impactos ambientais, produzir energia renovável e reduzir o volume de resíduos gerados, impulsionando tecnologias alternativas.

A digestão anaeróbia é uma tecnologia de tratamento e valorização de águas residuárias e resíduos orgânicos que se caracteriza por ser um processo biológico que converte a matéria orgânica em biogás como consequência do metabolismo de diferentes micro-organismos (KUMARI et al., 2018). Este processo divide-se em duas etapas: na primeira etapa a matéria orgânica é degradada em ácidos graxos voláteis através da solubilização, hidrólise, e acidogênese, e na segunda fase, chamada acetogênica e metanogênica o acetato, hidrogênio e dióxido de carbono são convertidos em metano (MAJHI; JASH, 2016).

Especificamente, o biogás gerado neste processo está composto por 50-70% de metano, 30-40% de dióxido de carbono e outros gases como hidrogênio e sulfuro de hidrogênio. Além disso, desse processo obtém-se como subproduto final um lodo ou melhor conhecido como biodigerido, rico em nutrientes que pode ser usado como adubo (XU et al., 2018). Os gases hidrogênio e metano são produtos importantes, pois tratam-se de gases combustíveis com alto potencial calorífico que permite a sua utilização como combustível e para geração de energia, ou seja, uma potencial fonte renovável de energia, que causa menor impacto ambiental.

Destaca-se que a digestão anaeróbia possui diversos tipos de reatores, e o reator tipo tanque agitado pode ser classificado em fase única ou fases separadas. Quando a reação ocorre em um único reator, todas as etapas do processo ocorrem ao mesmo tempo (etapas de hidrólise, acidogênica, acetogênica e metanogênica), já quando a reação ocorre em fases separadas, tem-se um reator para as etapas de hidrólise e acidogênese e outro para as etapas acetogênica e metanogênica. Essa última metodologia é mais eficiente, pois melhora a estabilidade do processo e a eficiência geral da degradação bioquímica dos resíduos orgânicos, com maior taxa de geração de metano e menor tempo de estabilização (MAJHI; JASH, 2016).

Neste contexto, este trabalho teve como finalidade fazer uma revisão bibliográfica usando como metodologia a bibliometria para analisar a valorização dos resíduos de casca de laranja procedentes da indústria de sucos e sua aplicação na área de biocombustíveis e para avaliar a situação atual dessa área de pesquisa ao nível mundial, assim como analisar o potencial da digestão anaeróbia em reatores de fases separadas visando controlar a atividade metabólica dos microrganismos para obter dois gases combustíveis, hidrogênio e metano. Assim mesmo, usar a data experimental obtida para avaliar o potencial de geração de energia elétrica para a indústria de suco de laranja do estado de São Paulo e a mitigação de gases de efeito estufa.

1.2. Objetivos

1.2.1. Objetivo Geral

O objetivo principal deste trabalho é valorizar o principal resíduo procedente da indústria de processamento de suco de laranja, as cascas, através da digestão anaeróbia mesofílica em reatores do tipo fases separadas visando à obtenção de biogás. Além disso, avaliar por meio de cálculos energéticos sua aplicação na indústria cítrica e realizar uma análise bibliométrica para analisar as mudanças que tem tido o uso da casca de laranja para produção de biocombustíveis ao longo do tempo.

1.2.2. Objetivos específicos

- Avaliar as tendências da digestão anaeróbica de resíduos agroindustriais de laranja.
- Verificar os principais usos da casca de laranja e tecnologias aplicadas para gerar produtos de alto valor agregado.
- Realizar a montagem de reatores anaeróbios de única fase (reator controle) e de fases separadas (dois reatores);
- Avaliar a produção em volume e composição em porcentagem no biogás da produção de hidrogênio e metano em todos os reatores;
- Realizar a comparação de eficiência de redução de carga orgânica entre os reatores;
- Estimar o potencial de geração de energia elétrica do metano produzido;
- Calcular o potencial de mitigação de gases de efeito estufa usando a energia elétrica previamente estimada.

1.3.Estrutura da tese

A tese está dividida em capítulos. Os capítulos que apresentam a revisão bibliográfica e os resultados experimentais correspondem a artigos que estão sendo revisados por revistas científicas de alto impacto.

O capítulo 1 está composto pela introdução, objetivos e estrutura da tese, o qual permite inserir ao leitor ao tema principal da tese e apresentar a sua importância, começando pela contextualização que o mundo precisa de novas estratégias e tecnologias para mitigar os impactos ambientais causados pelos resíduos de diferentes indústrias alimentícias e, além disso, aproveitar as ótimas características que eles possuem para gerar subprodutos de maior valor agregado. Por conseguinte, detalhou-se o caso específico do Brasil, maior produtor de suco de laranja, e sua alta produção de resíduos que precisam ser valorizados e reutilizados de forma sustentável. Em seguida, foi explicada a alternativa utilizada neste trabalho de usar a digestão anaeróbia para tratar os resíduos e produzir biogás, baseada na técnica de reatores em fases separadas para ter um maior controle nas condições do reator e consequentemente um melhor comportamento dos microrganismos.

O capítulo 2 baseia-se no estudo bibliométrico para analisar a valorização dos resíduos de casca de laranja procedentes da indústria de sucos e sua aplicação na área de biocombustíveis, assim como analisar o potencial da digestão anaeróbia usando como principal matéria prima as cascas de laranja. Além disso, mostra e discute os principais trabalhos encontrados na literatura em relação às diferentes variáveis utilizadas, processos desenvolvidos e produtos obtidos. Este estudo em forma de artigo de revisão foi submetido à Biofuels, Bioproducts & Biorefining com o título "*BIOENERGY PRODUCTION FROM ORANGE INDUSTRIAL WASTE: A BIBLIOMETRIC CASE STUDY*"

O capítulo 3 faz referência ao processo experimental utilizando reatores mesofílicos de fases separadas comparados a um reator controle. Além disso, apresenta as análises físico-químicas feitas para avaliar o andamento do processo e os resultados obtidos. Entre as principais análises feitas foram os sólidos totais, demanda química de oxigênio e composição do biogás para controle do processo e caracterização do produto. Os principais resultados mostram alta porcentagem de metano na composição do biogás em 46 dias de experimento ao mesmo tempo em que reduz a carga orgânica principalmente nos reatores metanogênico e controle. Este capítulo consiste em um artigo que foi submetido na revista Journal of Environmental Chemical Engineering intitulado "*TWO-STAGE ANAEROBIC DIGESTION*

OF ORANGE PEEL WITHOUT PRE-TREATMENT: EXPERIMENTAL EVALUATION AND APPLICATION TO SÃO PAULO STATE".

O Capítulo 4 apresenta uma discussão geral dos principais resultados obtidos neste trabalho, tanto na revisão bibliométrica quanto ao trabalho experimental, enquanto o capítulo 5 apresenta as conclusões gerais e sugestões para trabalhos futuros, onde são resumidos os principais resultados oriundos do desenvolvimento do projeto apresentado nessa tese e ainda são apresentadas algumas sugestões de pesquisas futuras. No Capítulo 6, é mostrado o memorial do período de mestrado, ou seja, todos os trabalhos acadêmicos realizados paralelamente ao desenvolvimento desta tese e à apresentação de pôster do trabalho.

Finalmente, o capítulo 7 contém uma lista com as referências utilizadas no capítulo 1 da tese devido que as referências de cada artigo estão nos capítulos 2 e 3.

Capítulo 2 - Casca de laranja para produção de biocombustíveis: Estudo de caso bibliométrico

Bioenergy production from orange industrial waste: A bibliometric case study

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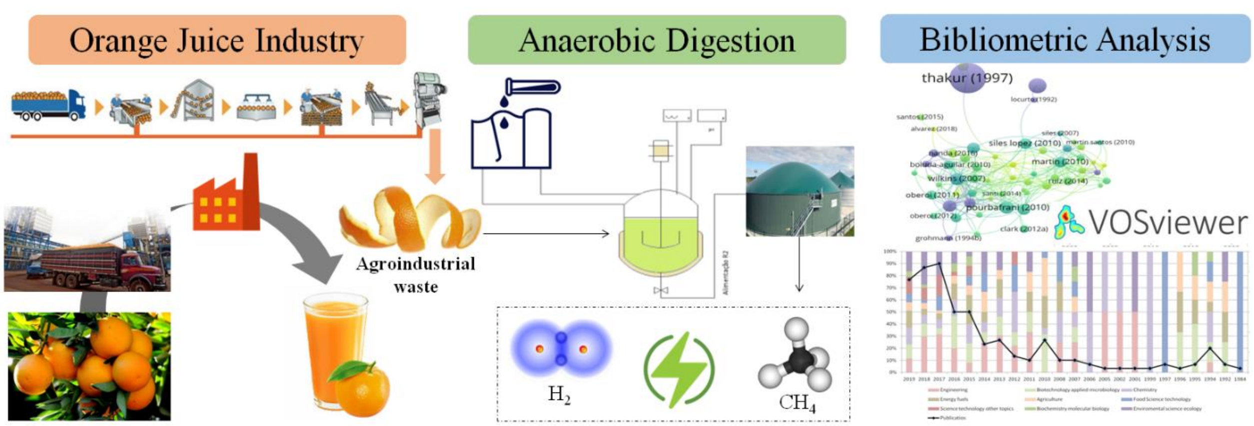
Graphical Abstract



Paper 1: Foi submetido na revista Biofuels, Bioproducts & Biorefining.

Abstract

The present study focuses on a bibliometric analysis of the global scientific production related to bioenergy recovery from orange industrial waste or by-products. Moreover, the potential dynamics of the bibliometric trend was used to observe the top-cited papers, international collaborative networks, top countries, and journals. The timespan comprised the period from 1900 to 2019, and the Web of Science© database was used. A total of 161 documents (research and review papers) were selected to carry out the investigation. Two main clusters of literature have been highlighted, respectively related to the top-cited papers authorship and authorship collaboration network. Based on observations, recent publications in the scientific field use different pretreatments to achieve the best biogas yield by many configurations of the anaerobic digestion system. The data obtained indicated that the countries with most publications are Spain, China, and the United States, and the journals with higher impact factors related to environmental assessments were responsible for the higher number of publications. In a biorefinery concept, orange peel could be submitted to different pretreatments to produce bioethanol and methane, as environmentally friendly fuels, and other coproducts likewise D-Limonene and pectine. Therefore, this study presents the trends of the bioenergy production from orange industrial by-products aiming to support further research on energy recovery from the orange industry solid waste and to subsidize future decision-making in this field of science.

Keywords: *Orange waste; Bibliometric analysis; Pretreatment; Anaerobic digestion; Bioenergy.*

Article Highlights

- Bibliometric tools show the evolution of orange industrial waste use for biofuels from 1900 to 2019;
- The research areas, source titles, organizations, and countries were analyzed for energy recovery from orange solid by-products.
- Orange solid waste could be a promising substrate to produce bioenergy through anaerobic digestion;

2.1. Introduction

Brazil is the largest producer of oranges, followed by the USA and China. The national 2018 production reached 16,677,091 tons (IBGE 2019). In the world, orange juice industrial processing presents a high amount of by-products or wastes generated in the form of peel, seed, and bagasse (Bussolo de Souza et al. 2018). Solid orange waste constitutes approximately 50–60 % of the processed fruit, and this fraction is composed of peels (50–60%), internal tissue (30–35%), and seeds (<1%) (Crawshaw 2003). Annually, the citrus industry produces 70 Mt of solid by-products being the major contributor to food waste (Ozturk et al. 2019). Industrial orange solid by-products are usually intended for animal feed, landfills, and incineration. However, it is necessary to improve waste management from an environmental perspective, to produce new industrial biomaterials with higher aggregated value (Negro et al. 2017; Xu et al. 2018).

According to Senit et al. (2019), orange peel waste is composed of free sugars (35 %), pectin (19 %), lignin (7 %), hemicellulose (14 %), cellulose (19 %), and ashes (3.7 %). The chemical composition of orange peel includes 3.5 % of ash, 1.95 % of lipids, 5.8 % of crude protein, 2.3 % of lactic acid, 2 % of acetic acid, 0.06 % of isobutyric acid, 0.73 % of calcium, and a pH of 3.64 (Lopez et al. 2010). Notwithstanding, due to the complex composition of orange industrial waste, it is necessary to use a pretreatment to reduce the content of antioxidant compounds, D-limonene, and others (Calabro et al. 2018).

Food waste will be a promising substrate to produce biogas due to its high energy content, large quantity, and ample availability (Xu et al. 2018). However, during the fermentation of organic waste, only 10–20% of the energy contained in the substrate is converted into hydrogen and carbon dioxide (Silva et al. 2018). The use of pretreatments in organic waste can be considered an innovative technique to increase energy recovery. Su et al. (2016) investigated biological pretreatment using single strains and two strain combinations to an anaerobic digestion (AD) reactor to assess biogas yield. They found a biogas yield of 15 ml/ gVS without pretreatment. Otherwise, the best biogas yield of pretreatment with single strains was 59 ml/ gVS. With two strains combination, the highest biogas yield was 150 ml/ gVS proving that the biogas yield can be successfully improved with a pretreatment.

Moreover, AD is considered one of the most promising techniques to produce biofuels because of its easy implementation, versatility, low investment, and well-consolidated status (Zema et al. 2018b; Xu et al. 2018; Rosas-Mendoza et al. 2020). Biotransformation of organic waste into biogas can be considered a rousing alternative with many benefits, minimizing the impact of inappropriate waste deposition in the ecosystem (Silva et al. 2018). In general, under developing countries do not present good management policies to avoid residues burning, open-air, and landfill disposal, which are unsuitable for decreasing environmental impacts, such as greenhouse gas emissions (Rosas-Mendoza et al. 2018; Behzad Satari and Karimi 2018). The development of new renewable energy sources could be an essential strategy to minimize environmental, social, and economic problems; and to support waste management and treatment policymaking (Rosas-Mendoza et al. 2020)

Due to the high amount of scientific documents in the literature related to the use of orange industrial by-products, it is necessary to apply a systematic review to choose the best conditions to produce biofuels and to understand how research has evolved over the years. Bibliometric analysis is one of the several possibilities to quantify the academic output and to measure scientific inquiry using the data available in the scientific database (Ball 2018). Bibliometric mapping allows the quick and easy visualization of the indicators presented as clusters and networks (Gonçalves et al. 2019). Moreover, this technique provides an overview of the institutions, research groups, countries, the impact of the journals, and research trends (Kamdem et al. 2019).

Therefore, this work aimed to conduct a bibliometric review of orange industrial by-products in the last 119 years (1900–2019), using the Web of Science© database. A systematic search was done to identify the studies regarding orange peel destination to bioenergy production, especially biogas, to analyze the trends on that topic. Moreover, research areas, organizations, top cited papers, authors and countries were analyzed to evaluate the international network, and the main research areas on the subject here discussed.

2.2.Materials and Methods

2.2.1. Data collection

The data collection was focused on scientific publications; because of that, several bibliographic references were related to the leading technologies used to convert orange peel into methane and/or hydrogen, especially by the anaerobic digestion. Besides, it was necessary to gather data related to the influence of pretreatments. Due to an orange peel's complex lignocellulosic composition, pretreatment is required to facilitate its use in several applications (Lopez et al. 2010; Martin et al. 2010; Calabro & Panzera, 2017). Therefore, keywords selected for the collection were: "orange peel", "orange waste", "anaerobic digestion", "biogas", "methane", "hydrogen" and "pretreatment" and for doing the research at Web of Science (WoS) the Boolean operators OR and AND were used.

The bibliometric study was done on the 27th of October 2019 using (WoS), a scientific database that gathers information about scientific studies and specifically, Web of Science Core Collection database was used. The research was done for the period between 1900 and 2019. In WoS, the keywords were credited, and the research was done in the "topic" field, which means that the selected keywords are included in the title, abstract, author's keyword, and keywords plus.

With these parameters, 177 documents were found, and the further manual selection was made to exclude records out of the scope here focused. Finally, 161 research and review papers were obtained to carry out the investigation. These types of documents were selected because they contain complete research result. A global illustration of the methodology can be observed in **Figure 1**.

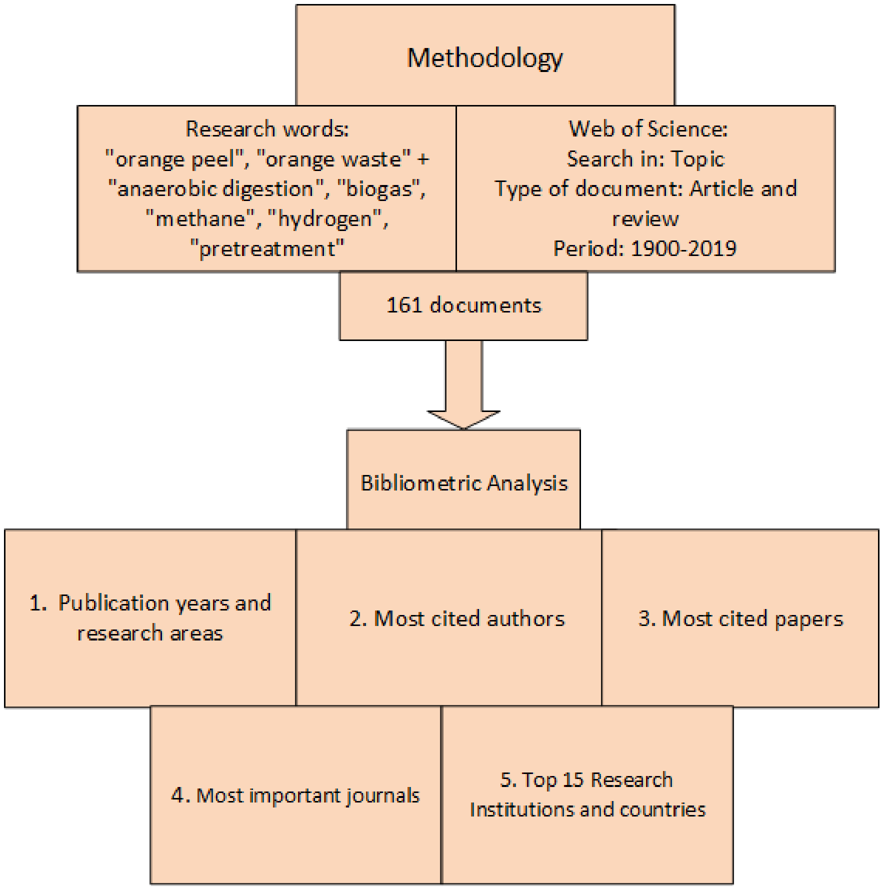


Figure 1. Synthesis of the bibliometric methodology employed.

2.2.2. Bibliometric analysis

All analyses were performed using the "Analyzing Results" tool provided by WoS with MS Excel support. WoS database was applied to obtain the scientific information for the research and to analyze the different features like:

i) Research areas to show which knowledge field is involved in the study;

ii) Source titles to know which are the leading journals that publish documents related to the subject, and

iii) Organizations and countries to determine the primary sources and regions interested in orange peels to produce biogas.

Additionally, further analysis was performed with Microsoft Excel® to build graphs and tables to gather WoS results. After this, the principal authors and the connections between them were performed with VosViewer® software. For this, the software was set to the maximum number of authors per document (25), and the minimum amount of records per author (1). The most cited papers analysis was done to a minimum number of citations per document of ten (10) and the documents were ordered by the number of citations. Lastly, it was possible to identify the documents that have received more attention by the scientific community.

2.3.Results and discussion

2.3.1. Publications and research areas

The research was done for the years between 1900 and 2019. Notwithstanding, the first document related to orange peel and its relationship with hydrogen, methane, and different pretreatments appeared only in 1984, as can be observed in Figure 2. The aforementioned document showed that orange peel could be a suitable substrate for anaerobic digestion when the feeding of oil is less than 0.075 grams per liter of digestion liquor per day, reaching a mean yield of 50 % of methane (Lane 1984).

Even if the core theme was connected to bioenergy fuels, a newsworthy aspect of that article is its research area classification as food science technology. Likewise, it is notable that the first article about AD presented in the WoS database is from 1928 (Neave and Buswell 1928). After 56 years, the scientific community decided to use orange peel as a substrate for the new development of AD technology.

The next articles were published only eight years later. One of them, related to the research areas of environmental science and energy fuels, studied methane production by orange peel pressing liquid anaerobic fermentation (Martinez Andreu et al. 1992). Another one was associated with agriculture and biotechnology applied microbiology research areas, being focused on flavonoids recovery after an acidic pretreatment of the peel (Locurto et al. 1992).

In 1994, an increase in publications was observed, along with a higher diversification in research areas: 5 articles were related to the orange peel fermentation process for citric acid (Aravantinoszafiris et al. 1994) and ethanol production (Grohmann et al. 1994a). Besides, one study defined in vivo anti-inflammatory and analgesic activities of hesperidin – an important flavanone present on Citrus sp. – obtained after an acidic pretreatment (Galati et al. 1994). Lastly, mass spectra analyses of various polymethoxylated flavones were assessed to determine its structure (Berahia et al. 1994).

Later, the published articles remained between 1–3 per year until 2010, when the total publications reached a higher number of papers (7) related to methane production by anaerobic digestion, adopting pretreatments to remove D-Limonene (Martin et al. 2010). At the time, other documents reported ethanol production from orange peels (Boluda-Aguilar et al. 2010; Oberoi et al. 2010); process design and economic analysis of a biorefinery for the treatment of citrus wastes by hydrolysis in diluted sulfuric acid and further processing to obtain limonene, ethanol, and biogas (Lohrasbi et al. 2010); an anaerobic digestion kinetic model for the wastewater derived from pressed orange peels (M. D. M. Santos et al. 2010); production of ethanol, biogas, pectin, and limonene from citrus wastes by an integrated process composed by hydrolysis, fermentation and anaerobic digestion (Pourbafrani et al. 2010); and finally, an enzymatic pretreatment was applied to orange peel to produce an environmentally friendly cotton fabric (Aly et al. 2010).

In 2011, the published articles declined to 3. However, after this year, the number of publications increased again from 5 papers in 2012 to 15 in 2015, which were focused on different pretreatment techniques, as follow:

i. polyvinylidene fluoride (PVDF) membrane-encapsulated cells by batch and semi-continuous fermentation for hydrogen and methane production (Akinbomi et al. 2015);

ii. dip pretreatment to evaluate the coating effects of orange and pomegranate peel extracts combined with chitosan nanoparticles on the quality of silver carp fillets during refrigerated storage (Zarei et al. 2015);

iii. use of lyophilization and oven-dry as orange peel pretreatment to obtain flavonoids (Molina-Calle et al. 2015);

iv. orange peel pyrolysis to evaluate its potential as solid biofuels and its heavy metals biosorption capacity (C. M. Santos et al. 2015);

v. steam explosion and sulfuric-acid soaking was investigated to enhance soluble dietary fiber yield and functionality (Wang et al. 2015);

vi. orange peel catalytic gasification with K2O-Ni/silica aiming hydrogen production (Vargas et al. 2015); and

vii. two papers related to biofuels production, one on bioethanol through a fermentation (Choi et al. 2015) and the other on anaerobic digestion to biogas production using hexane as a pretreatment to eliminate limonene consequently improving methane yield (Wikandari et al. 2015).

In the following years, a continuously rising trend was observed with 26 articles published until the 3rd of November 2019, where the majority themes remained to present meaningful participation of pretreatments to obtain different products to fulfill several industrial applications.

On the other hand, a different behavior was found in the research areas. During the early years, from 1984 to 1996, the most prolific areas were agriculture, energy fuels, biotechnology, and microbiology. In the next ten years, the publications were in a minimal number as well as the research areas. In 2001, for the first time, the engineering area was addressed in a study of the effectiveness of orange peel in adsorbing acid violet 17 from aqueous solutions as a function of the agitation time, adsorbent dosage, initial dye concentration and pH (Sivaraj et al. 2001). Since then, this area started to be relevant, and currently, it is the most important one, figuring a share of 19.2 % in the 161 documents.

Additionally, the diversification of the research areas occurred mainly since 2007, when only two articles were published, and somehow, both were related to 6 of the nine research areas identified in WoS. The documents were related to the effects of D-limonene concentration, enzyme loading, and pH on ethanol production from simultaneous saccharification and fermentation of citrus peel waste by Saccharomyces cerevisiae (Wilkins et al. 2007). This theme is directly associated with microbiology, molecular biology, biotechnology, and engineering. Another article was related to AD research of pressed orange peels wastewater using a physicochemical treatment with aluminum sulfate as a flocculant and a solution of sulfuric acid to pH reduction (Siles et al. 2007), which is related to agriculture, chemistry, food science, and technology.

Currently, although the articles are associated with several research areas, the most important after engineering (19.2 %) are biotechnology/applied microbiology (13.91 %), environmental science ecology (13.59 %), chemistry (12.30 %), and energy fuels (11.65 %).

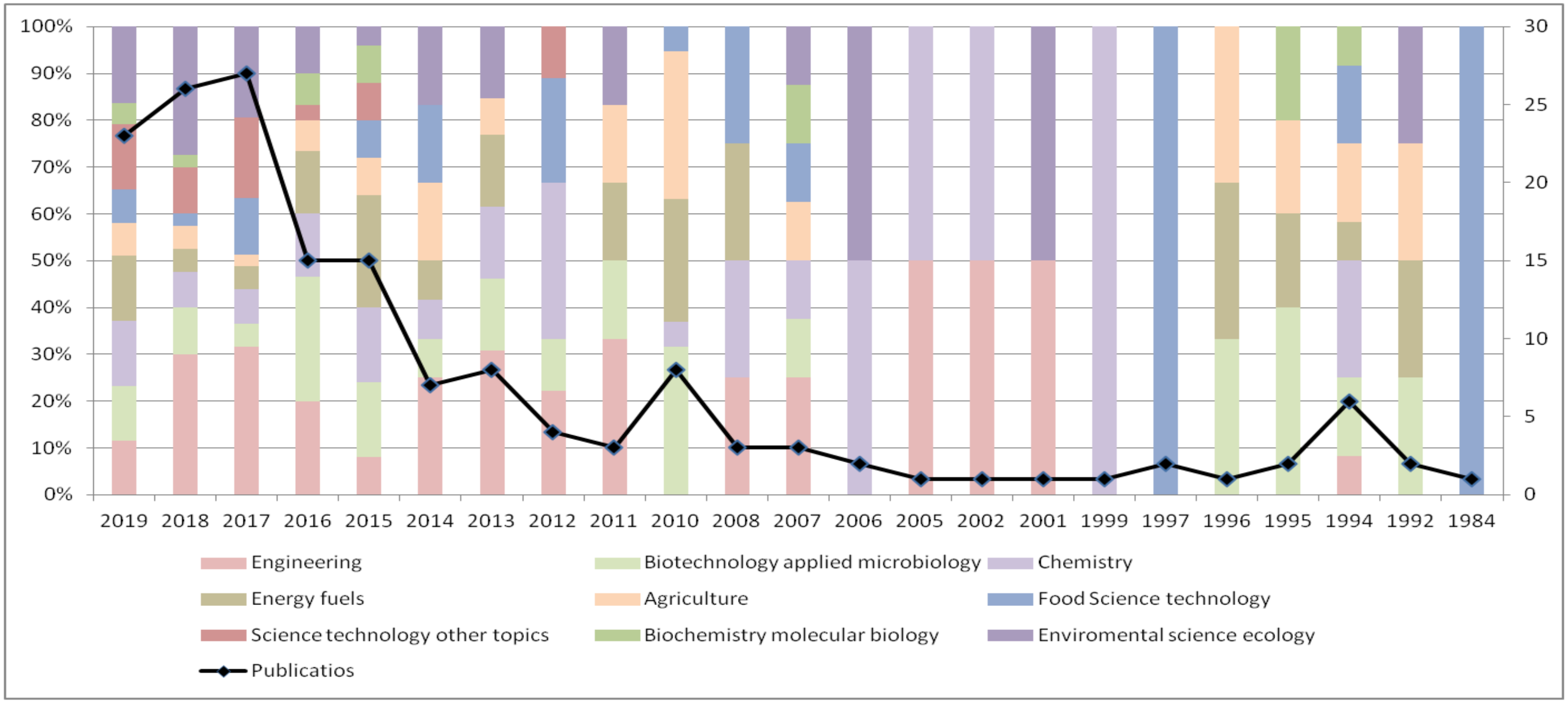


Figure 2. Evolution in publications during the period of 1900 to 2019 in different research areas.

2.3.2. Main articles and authors

Figure 3(a) shows that the most cited papers are the oldest ones due to their indexing time. Thakur et al. (1997) wrote the most cited article titled “Chemistry and uses of pectin - A review.”. As seen in **table 1**, this document has been cited 748 times and it was published by Critical Reviews in Food Science and Nutrition. This paper has been widely cited because it explains the applications of pectin in the food field through gel formation in the presence of Ca^{2+} ions or a solute at low pH. Pectin can be used in jams, jellies, frozen foods, and more recently in low-calories foods as a fat and/or sugar replacer. Also, in the pharmaceutical industry, it is used to reduce blood cholesterol levels and gastrointestinal disorders. Other applications of pectin include use in edible films, paper substitute, foams, and plasticizers.

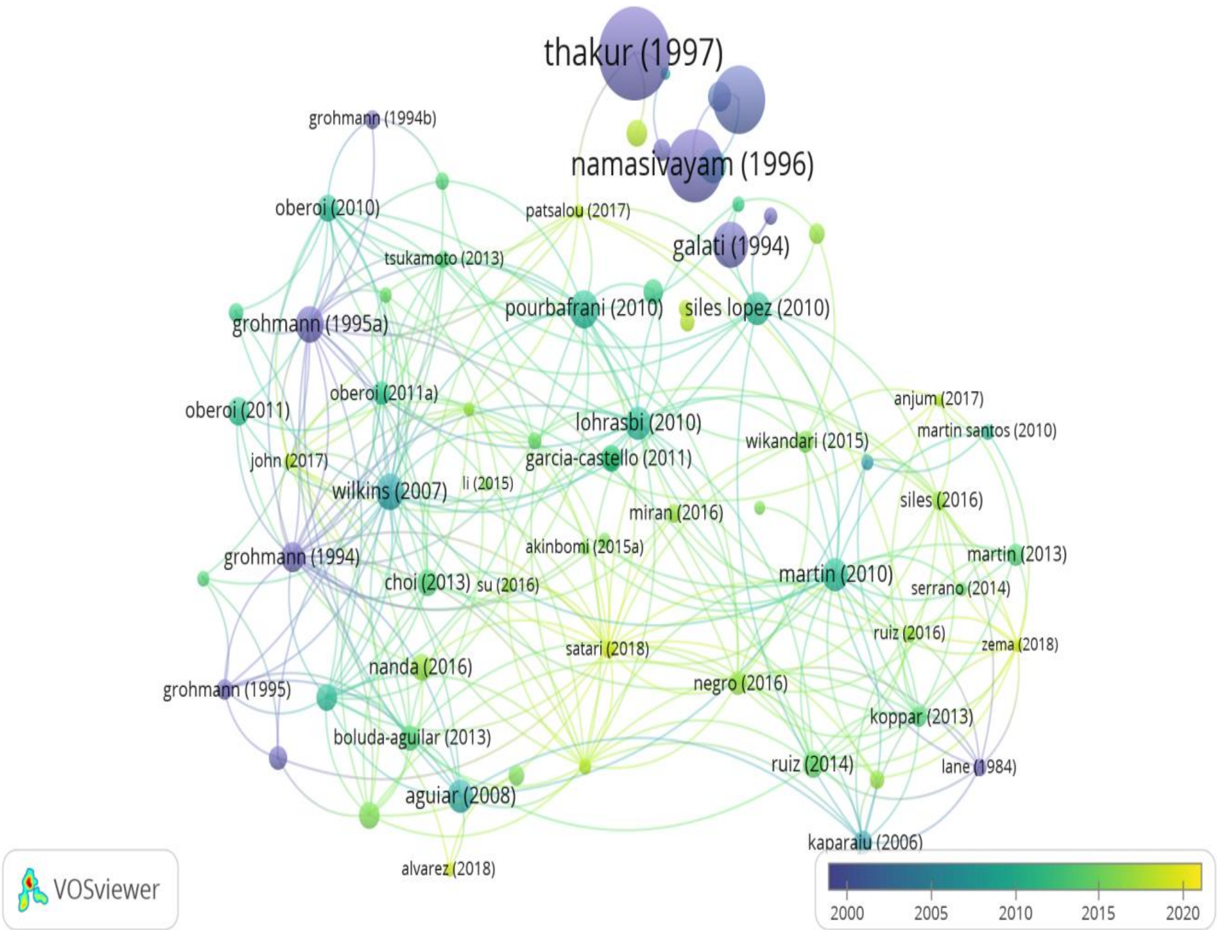


Figure 3. Illustration of the top-cited papersauthorship in the orange waste research field.

The second most cited paper is titled "Removal of dyes from aqueous solutions by cellulosic waste orange peel". **Table 1**. shows that it has been cited 435 times since it was published in 1996 by the Bioresource Technology. The authors Namasivayam et al. (1996) examined the adsorption of dyes as congo red, procion orange, and rhodamine-B using waste orange peel varying the different concentrations of dyes, adsorbent dosage, agitation time and pH. The results showed that waste orange peel had an adsorption capacity of 22.4, 1.3, and 3.22 mg/g for the dyes congo red, procion orange, and rhodamine-B, respectively, what figures an effective adsorbent for the removal of dyes in solutions of wastewater from industries.

The third most cited paper is "Orange peel as an adsorbent in the removal of Acid violet 17 (acid dye) from aqueous solutions" this paper was also written by Namasivayam, in the company of Sivaraj, R and Kadirvelu, K, it has been cited 392 times and it was published by Waste Management in 2001. The aim of the paper is almost the same as the previous one but using the orange peel waste as an absorbent to remove Acid violet 17. Orange peel was cut, dried, and powdered to be brought into contact with the dye. They found that for quantitative removal of Acid violet 17 from 50 ml of 10 mg/l, a maximum dosage of 600 mg is required, concluding that orange peel can be used successfully as an absorbent of that dye (Sivaraj et al. 2001).

"Biological effects of hesperidin, a citrus flavonoid with anti-inflammatory and analgesic activity", is a paper that presents 167 citations, most of them in pharmacology and pharmacy research area, because of that, this paper is available in Farmaco Journal. This event can be explained by hesperidin – an important flavanone of Citrus fruits - significant biological and health effects. To obtain the studied hesperidin, it was necessary to do an acid pretreatment in the solid residue of orange peel (Galati et al. 1994).

In 2010, the most cited paper about biofuels production using orange peel (108 citations) was titled "Production of biofuels, limonene, and pectin from citrus wastes" and written by Pourbafrani et al. (2010). This paper investigated the production of ethanol, biogas, pectin, and limonene from citrus wastes by an integrated process with a hydrolysis pretreatment in a dilute-acid process to remove pectin from the orange peel to obtain it as a by-product. The paper was published by Bioresource Technology and the findings show that 77.6 % of total pectin content could be recovered by solvent recovery. Besides, the limonene was effectively removed through hydrolysates flashing into an expansion tank. Also, sugars presented in the hydrolysates were converted into ethanol - ethanol yield of 0.43 g/g. Finally, the stillage and the remaining solid materials were used as raw materials for AD to produce biogas. The results were promising because it was possible to obtain a methane yield of 0.363 ± 0.02 l/g VS, ethanol yield of 0.43 ± 0.02 g/g, 77.6 % recovery of pectin content of citrus wastes and 99 % of the limonene content removed.

The top 5 most cited papers indicated that the initial research was mostly related to chemistry and focused on topics not explicitly dealing with energy recovery from waste. Notwithstanding, essential knowledge was provided to the understanding of orange peel characterization and applications.

The next publications' research field switched to biofuels production with different methods. Therefore, the sixth most cited paper refers to simultaneous saccharification and fermentation of citrus peel waste by *Saccharomyces cerevisiae* to produce ethanol, with 101 citations (**Table 1**) and it's available in Process Biochemistry. Wilkins et al. (2007) studied the effects of D-limonene concentration, enzyme loading, and pH in ethanol production by undergoing the orange peel in a steam explosion process. They reported an ethanol yield of 42 g/l with a final limonene concentration of 0.06 % v/v.

Table 1. Top 10 most cited papers

	Authors	Title	Journal	Publication year	Citations
1	Thakur, B. R., Singh, R. K., & Handa, A. K.	Chemistry and uses of pectin - A review	*Critical Reviews in Food Science and Nutrition*	1997	748
2	Namasivayam, C., Muniasamy, N., Gayatri, K., Rani, M., & Ranganathan, K.	Removal of dyes from aqueous solutions by cellulosic waste orange peel.	*Bioresource Technology*	1996	435
3	Sivaraj, R., Namasivayam, C., & Kadirvelu, K.	Orange peel as an adsorbent in the removal of Acid violet 17 (acid dye) from aqueous solutions.	*Waste Management*	2001	392
4	Galati, E. M., Monforte, M. T., Kirjavainen, S., Forestieri, A. M., & Tripodo, M. M.	Biological Effects Of Hesperidin, A Citrus Flavonoid .1. Antiinflammatory And Analgesic Activity	*Farmaco*	1994	167
5	Pourbafrani, M., Forgacs, G., Horvath, I. S., Niklasson, C., & Taherzadeh, M. J.	Production of biofuels, limonene and pectin from citrus wastes	*Bioresource Technology*	2010	108
6	Wilkins, M. R., Widmer, W. W., & Grohmann, K	Simultaneous saccharification and fermentation of citrus peel waste by Saccharomyces cerevisiae to produce ethanol.	*Process Biochemistry*	2007	101
7	Grohmann, K., Cameron, R. G., & Buslig, B. S.	Fractionation and pretreatment of orange peel by dilute acid hydrolysis.	*Bioresource Technology*	1995b	96
8	Martin, M. A., Siles, J. A., Chica, A. F., & Martin, A.	Biomethanization of orange peel waste.	*Bioresource Technology*	2010	84
9	Lohrasbi, M., Pourbafrani, M., Niklasson, C., & Taherzadeh, M. J.	Process design and economic analysis of a citrus waste biorefinery with biofuels and 84limonene as products.	*Bioresource Technology*	2010	84
10	Lopez, J. A. S., Li, Q., & Thompson, I. P.	Biorefinery of waste orange peel.	*Critical Reviews in Biotechnology*	2010	84

Another relevant study found the pretreatment of orange peel to be valuable. Grohmann et al. (1995b) published in Bioresource Technology a review of orange peel fractionation and pretreatment in dilute acid hydrolysis with sulfuric acid at 100, 120, and 140 °C, reaching 96 citations. However, only soluble sugars and sugars derived from the hemicellulose hydrolysis were efficiently released.

The last three papers to conclude the top 10 most cited, each had 84 citations: indicating new perspectives in the field. They focus on the biorefinery of orange peel and the biomethanization to obtain pectin, limonene, biogas, and ethanol. Lohrasbi et al. (2010) studied the process design and economic analysis of a biorefinery for the treatment of citrus wastes, concluding that the entire by-product is converted to valuable and environmentally friendly products, while significantly reducing the disposal of citrus wastes. Lopez et al. (2010) contributed with a review that includes a summary of the chemical composition of the substrate and an assessment of the range of applications in which the orange peel is deployed. Finally, Martin et al. (2010) studied the biomethanization of orange waste with a pre-treatment to extract D-limonene, which resulted in a removal of 70%. Regarding AD, they assessed laboratory and pilot scales under mesophilic and thermophilic conditions obtaining a methane yield coefficient of 0.27–0.29 LSTP CH_4/g of COD. Lopez´s article was published at Critical Reviews in Biotechnology and studies from Lohrasbi and Martin were published at Bioresource Technology, who authored 5 of the 10 most cited papers, which shows its importance in this field of study.

2.3.3. Authors' collaboration network

From **Figure 4,** it is observed that Namasivayam, from Bharathiar University in India, is the most cited author, accounting for 827 citations. This author developed two studies related to the removal of dyes from industrial wastewater, applying orange peel as adsorbent

(Namasivayam et al. 1996; Sivaraj et al. 2001). The first article, published in 1996, accounts for 435 citations while the other, from 2001, accounts for 392. In 1996, the research in this area was developed in collaboration with Saudi Arabia and the USA coauthors.

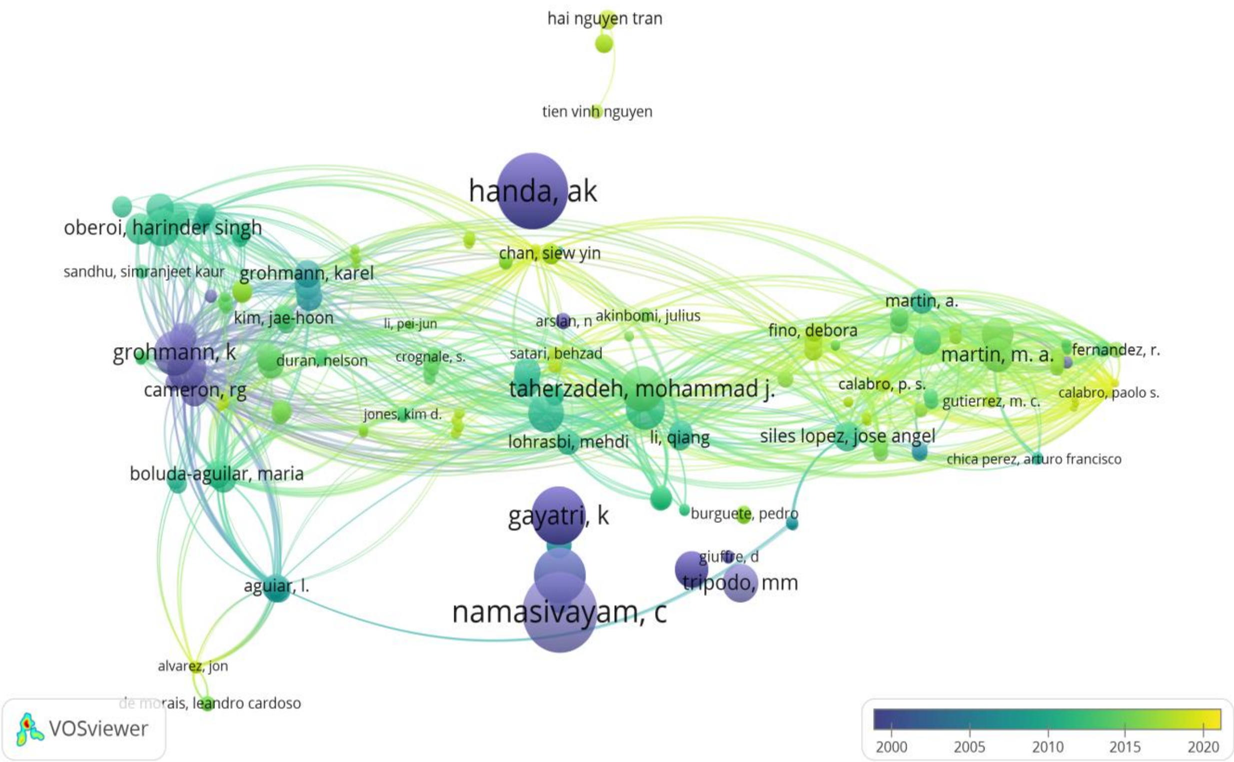


Figure 4. Illustration of the authors' collaboration network in the orange waste research field.

The second most-cited authors are Handa, A. K., Sing, R. K., and Thakur, B. R., in a review paper published in 1997. This paper focused on chemistry, structure, and industrial applications' use of pectin, which represents 23% of the orange peel composition (authors' own laboratory analysis) (Thakur et al. 1997). This study was done in cooperation with USA and India researchers.

Working with citrus peel waste Grohmann Karel, from the USA, was cited 356 times in six papers related to biochemistry, biotechnology, and energy research areas. Five of the documents are dated from the 1990s, and one was published in 2007. The major theme is related to the evaluation of citrus peel waste pretreatments, saccharification and fermentation for ethanol production (Grohmann et al. 1994a; Grohmann et al. 1994b; Grohmann and Bothast 1994; Grohmann et al. 1995b; Grohman et al. 2013; Grohmann et al. 1995a). Buslig, B.S. collaborated in 4 of the 6 Grohmann's articles. The core theme of their research is focused on pre-treatments based on different types of hydrolysis (recombinant Escherichia coli, dilute acid, enzymes) to produce ethanol by sugar fermentation (Grohmann et al. 1995a, 1995b; Grohmann et al. 1994a; Grohmann et al. 1994b).

Up to 2010, the most important scientific publications were conducted only by the USA and India authors' collaboration. However, from 2010 on, Taherzadeh M. J. from Swedish Centre for Resource Recovery, University of Boras (Sweden), also initiated research to evaluate biofuels production from orange peel. Since then, six papers were published with 265 citations (Pourbafrani et al. 2010; Lohrasbi et al. 2010; Akinbomi et al. 2015; Akinbomi and Taherzadeh 2015; Wikandari et al. 2015; B. Satari et al. 2017). In collaboration with Taherzadeh M. J., Niklasson (227 citations) studied the effect of the limonene in biogas yield from orange peel anaerobic digestion (Wikandari et al. 2015). In addition, in collaboration with Pourbafrani, M. from the University of Toronto (Canada), the process design and economic analysis of a citrus waste biorefinery development was addressed (Lohrasbi et al. 2010). It is notable that with only two papers from 2010, Pourbafrani, M. article presents 192 citations, which suggests that this research group produces high impact scientific papers.

Tripodo, M.M presents 188 citations on two studies from the 1990's related to flavonoids extraction from orange peel waste and hesperidin effects in human health (Locurto et al. 1992; Galati et al. 1994). With four documents published from 2010 to 2012, Oberoi, H. S. research deals with ethanol production from orange peels and other fruits waste (Oberoi et al. 2010; Oberoi et al. 2011a; Oberoi et al. 2011b; Oberoi et al. 2012). Martin, M.A., from the University of Cordoba (Spain), produced six articles from 2010 to 2018, all of them related to anaerobic digestion (AD), accounting for 155 citations. Calabro, P., from Universita Mediterranea di Reggio Calabria (Italy), developed seven works using orange peel in a short period - 2016 - 2019. Three of these papers were related to the conversion into biofuel by AD. On the other hand, just 56 citations were obtained due to the recent publication time.

Despite the low number of citations, both Martin, M. A, and Calabro, P. are the current leading researchers on orange peel AD. All of Martin's documents deal with orange peel, AD advances, and process improvements to obtain higher biogas yield using different pretreatments and one co-digestion study with glycerol (Martin et al. 2013; Martin et al. 2010; Serrano et al. 2014; Siles et al. 2016; Gil et al. 2018; Martin et al. 2018). Along with this, in only three years (from 2016 to 2019), Calabro, P. also focused on AD in seven published documents, including a review paper on citrus industry waste valorization (Zema et al. 2018a). His experimental papers aimed to evaluate the effect of different pretreatments including granular activated carbon combined with alkaline, zero Valens iron, ensiling, aeration, and thermal treatments to enhance methane production by focusing on D-limonene removal (Calabro et al. 2019a; Calabro et al. 2019b; Calabro et al. 2018; Calabro and Panzera 2017; Calabro et al. 2016; Zema et al. 2018b).

2.3.4. Most impact journals, institutions, and countries responsible for the researches

Evaluating the most important journals in the research field (**Table 2**), Bioresource Technology, Waste Management, and Journal of Cleaner Production were the journals with the most publications, totaling 30 papers, which correspond to 56 %, when comparing the 53 papers published in the ten most prestigious journals. Moreover, these journals present a higher impact factor, cite score, and h-index. The high publication on the cited journals is related to the scope and research area of the journals, all of them related to biomass, biological waste treatment, bioenergy, biotransformation, bioresource systems analysis, technologies associated with conversion or production, solid waste generation, treatment and disposal, and environmental assessments.

The number of publications ranked countries and institutions involved in the orange peel bibliometric analysis. **Figure 5(a)** shows that Spain, the most productive country, made up 19.37 % of the total publications, followed by China with 13.04 % and the United States with 12.4 %. These three countries produced almost 50% of the total publications of the 15 most important regions in the scientific field. Regarding the institutions involved in the research and publishing, University of Cordoba (UCO), Universita Mediterranea di Reggio Calabria (UNIRC), United States Department of Agriculture (USDA), and University of Boras (HB) developed the major part of the scientific works in the field (**Figure 5b**). The leading institution in this field is from Spain (UCO), with ten publications, where a research group called “RNM-271 Chemical Engineering” is lead by Martin, M. A., and Siles, J. A., evidencing their importance in the area The second most influential institution is UNIRC, a university placed among the best universities in Italy (Rankings, 2020). The leading researcher is Calabro, P. from the Department of Civil, Energy, Environmental, and Materials Engineering. Lastly, USDA has published six papers related to the recovery of pectin and ethanol production, and its primary researcher is Grohmann, K.

Table 2. The 10 most important journals, with its respective Impact Factor, Cite Score, and h-index.

Journal	Publications	Impact Factor	Cite Score	h-index
Bioresource Technology	16	6.669	7.08	251
Waste Management	8	5.431	6.15	127
Journal of Cleaner Production	6	6.395	7.32	150
Applied Biochemistry and Biotechnology	4	2.14	–	97
Journal of Agricultural and food Chemistry	4	3.571	3.80	262
Carbohydrate Polymers	3	6.044	6.12	172
Environmental Technology	3	2.800	3.16	65
Journal of Chemical Technology and Biotechnology	3	2.659	2.88	104
Journal of Environmental Management	3	4.865	5.32	146
Journal of Food Process Engineering	3	1.448	1.54	40

– data not available.

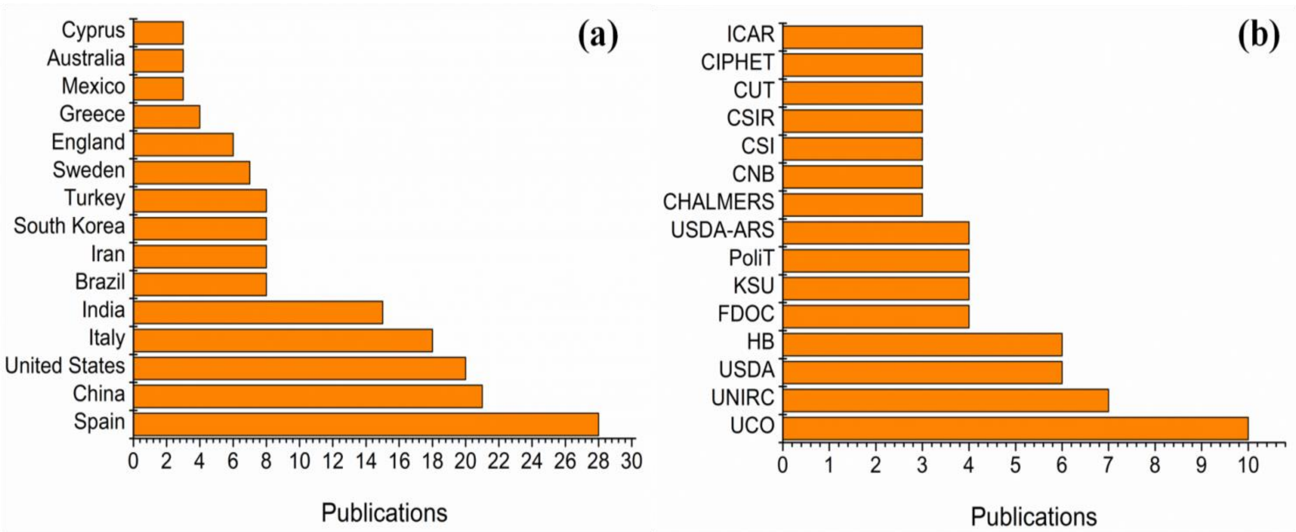


Figure 5. List of the countries and institutions by the number of publications in the research field of orange waste. (a) Top 15 countries; (b) Top 15 research institutions.

Legend: Universidad de Córdoba (UCO); Universita Mediterranea di Reggio Calabria (UNIRC); United States Department of Agriculture (USDA); University of Boras (HB); Florida Department of Citrus (FDOC); Kansas State University (KSU); Polytechnic University of Turin (PoliT); Citrus and Other Subtropical Products Research (USDA-ARS); Chalmers University of Technology (CHALMERS); Chonnam National University (CNB); Consejo Superior de Investigaciones científicas (CSI); Council of scientific industrial research (CSIR); Cyprus university of technology (CUT); Central Institute of Post Harvest Engineering and Technology (CIPHET); Indian Council of Agricultural Research (ICAR).

2.4.Concluding remarks

Due to the issue related to the orange industrial by-products proper disposal and destination, the adoption of emerging technologies should be encouraged to decrease environmental side effects and to revalue the significant amount of waste generating by the citrus industry (Bussolo de Souza et al. 2018). A global illustration of the problems caused by the disposal of orange waste and alternative routes can be observed in **Figure 6**.

The food industry demands a high amount of energy to feedstock processing. In the citrus industry, most of the energy is employed to process only 50 % of the whole fruit to produce juice (Crawshaw 2003). Consequently, the orange industrial solid waste represents costs, mandating the development of viable technologies to add value to this waste. As peel represents 50 – 60% of the waste, innovative processes should be considered for energy production. Specific peel pretreatments in a biorefinery concept show that it is possible to obtain biofuels as biogas and ethanol, and other high added value products, such as pectin and limonene (Oberoi et al. 2010; Pourbafrani et al., 2010; Rosas-Mendoza et al. 2018; Ozturk et al. 2019). Bioethanol can be used as transport fuel, an environmentally friendly substitution of fossil fuels (Guo et al. 2015), and the biogas could be burned in power units and converted into electricity and/or thermal energy (Kaparaju & Rintala, 2013). For biogas burning, electrical energy could be used by the own industry, reducing its global cost. In contrast, eventual energy surplus could be the grid, contributing to the reduction of non-renewable energy in the energy matrix.

There are several papers published since 1984 related to the state of the art of orange peel industrial applications, such as animal feed production, and pharmaceutics products. However, it is observed that only a few researches focus on orange peel pretreatment to improve bioenergy production. Research on orange peel to energy conversion is in constant evolution. The first research approach related to ethanol production decreased after the understanding of some limitations related to the scale-up and widespread adoption by the industry. In addition, the most expressive areas are related to Engineering, Biotechnology, Applied Microbiology, Ecology, Environmental Sciences, Chemistry, and Fuels Energetic. The most important journals are Bioresource Technology, Waste Management and Journal of Cleaner Production, accounting for 18,6% of the analyzed documents.

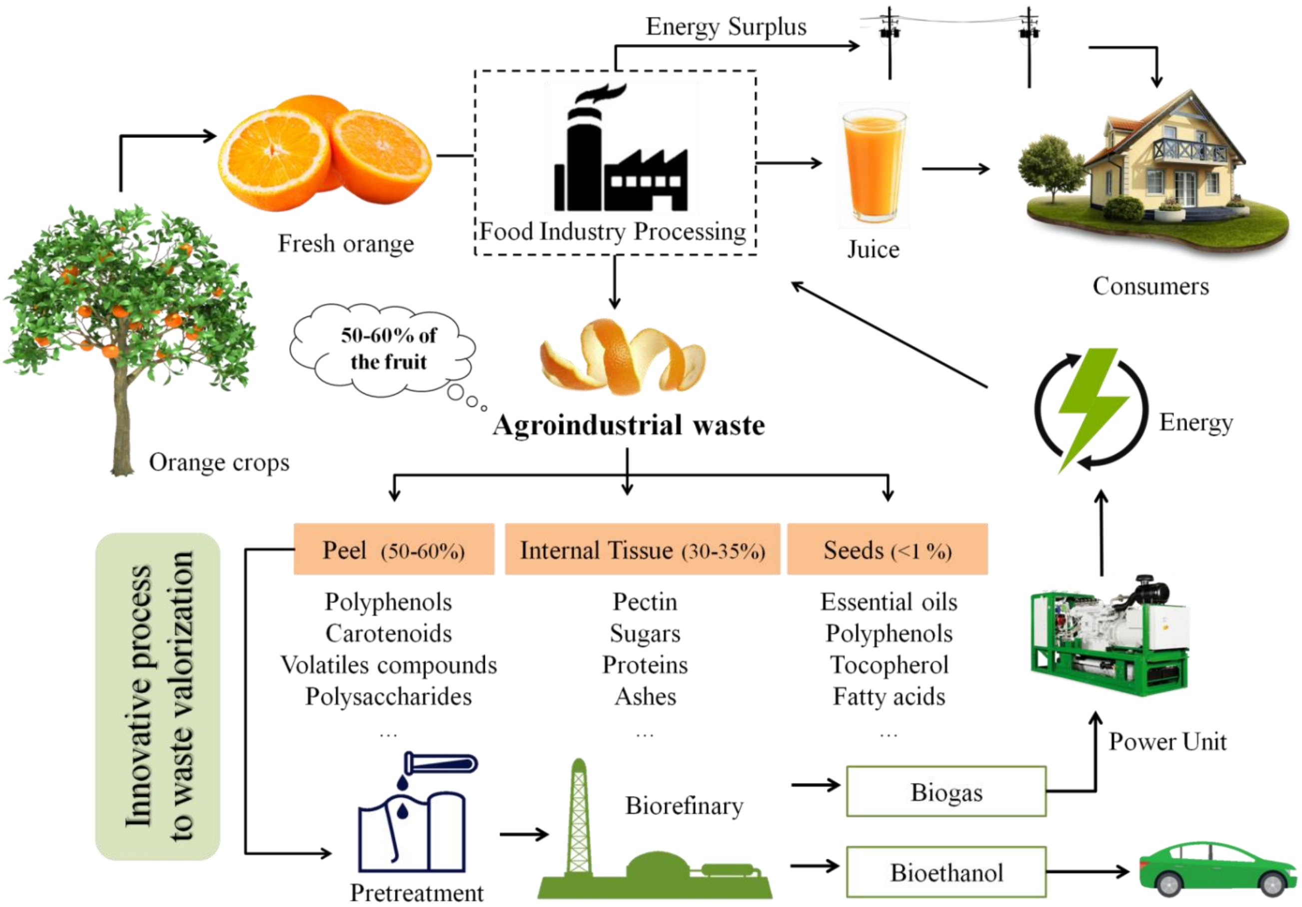


Figure 6. General discussion related to the scientific topic of orange agroindustrial waste generation by the citrus industry, and a possible solution proposed.

2.5.Conclusion

The bibliometric analysis indicated that research on orange industrial solid by-product or waste has grown in the last years. Considering the worldwide large orange industry, several biotechnological processes to solid waste energy recovery could contribute to a more environmentally friendly industrial activity. . Since the 1990's, several researches established theoretical solutions to the disposal of orange industrial waste, including its use to produce biofuels as ethanol and biogas among others applications like citric acid production, removal of dyes for the textile industry and flavonoids extraction for pharmaceutical purposes as anti-inflammatory and analgesic compounds. The most recent development in the field was related to AD, and several advances succeeded as possible routes to produce clean energy contributing to the economy decarbonisation. AD could be worldwide adopted as a strategy to support a more renewable energy generation, especially for developing countries.

Although the largest world production of orange is centered in Brazil, USA and China, research on the energy reuse of waste is based in Europe. The most important current leading researchers on orange peel AD are from the University of Cordoba, Spain led by Martin M.A, focusing on different pretreatments in orange peel to improve biogas yield. Another scientific group is based in the Universita Mediterranea di Reggio Calabria, Italy led by Calabro, P dedicated to evaluate the effect of different pretreatments to reduce D-limonene possible inhibitory effects on the anaerobic digestion.

Moreover, the scientific field proceeds with the discussion related to the use of waste products by the industry. It is known that innovative technologies are necessary to decrease industrial costs. The current concerns related to energy security and environmental aspects seem to influence the research field. Better communication of residues conversion technologies and scientific knowledge achievements should be considered by corporations, governments, and educational institutions as management, decision making, and public policy making tools. Finally, the bibliometric study was effective to evaluated the main collaborative networks and to facilitate the interpretation and diagnosis of research trends on orange industrial by-products what could subsidize future decision-making in this field of science, specially for under development countries, who responds for the major worldwide orange industry.

2.6.Acknowledgments

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2.7.Author contributions

All authors contributed to the study conception and design. Material preparation, data collection, analysis, first draft of the manuscript was performed by **Maria Paula Jiménez Castro**, **Luz Selene Buller,** and **William Gustavo Sganzerla**. **Tânia Forster-Carneiro** corrected and approved the final version of the manuscript.

2.8.Conflict of interest

The authors do not declare conflict of interest.

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Capítulo 3 - Produção de biogás a partir de resíduos da indústria cítrica: Uma estratégia para um desenvolvimento sustentável

TWO-STAGE ANAEROBIC DIGESTION OF ORANGE PEEL WITHOUT PRE-TREATMENT: EXPERIMENTAL EVALUATION AND APPLICATION TO SÃO PAULO STATE

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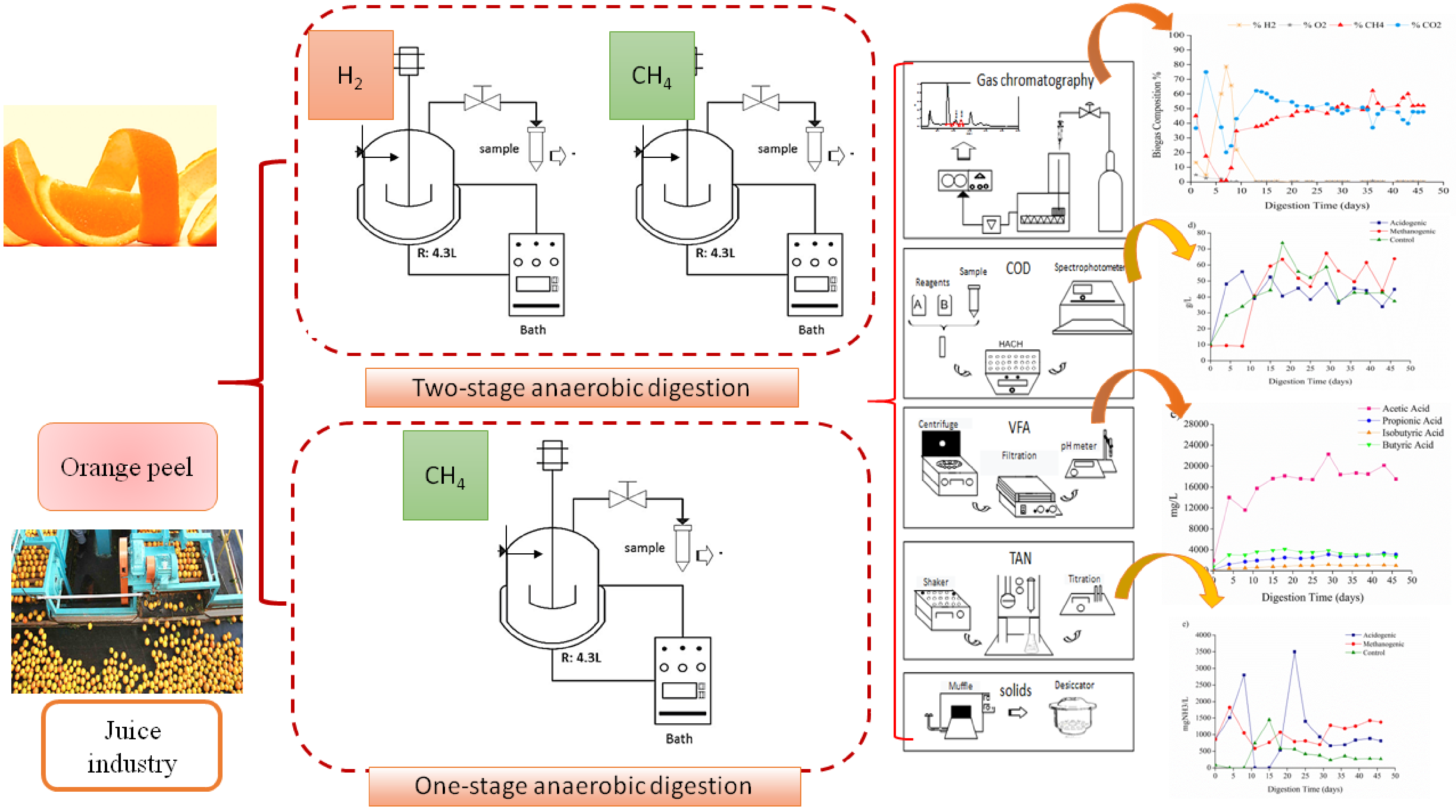
Graphical Abstract



Abstract

Orange juice production is an important industry worldwide, specifically in Brazil, which is the leading producer. Nevertheless, approximately 50% of the feed used for industrial production of orange juice is wasted, primarily as peels, a promising raw material for being used in the different biotechnological processes. This study evaluated the potential of two-stage anaerobic digestion (reactor of 4.3 L total volume) to valorize orange juice residues. The system replaced the use of other pretreatments and increased methane concentration (approximately 60% compared with about 50%) and volume (by 13%) relative to one-stage anaerobic digestion. The accumulated biogas yield was 0.79 L/g SVT and 0.49 L/g SVT for the methanogenic and control reactors, respectively. Also, the anaerobic reactors were able to start-up with high solids feed, which could be applied to the Upflow Anaerobic Sludge Blanket (UASB) reactors currently in operation in the citrus juice industry. The potential electric power generated was estimated as 97.5×10^3 MWh/year, which might be used on-site or sold back to the energy grid where it represents 0.2% of the total energy used by São Paulo State. The potential greenhouse gas mitigation from the substitution of electric energy from the Brazilian energy grid to a more renewable one from biogas burning was estimated to be 9.05×10^3 tCO_2eq/year. The results demonstrated that two-stage anaerobic digestion is a promising technology for the disposal of orange peel waste while producing a useful bioenergy product.

Keywords: *Lignocellulosic Residue, Anaerobic Digestion, Two-stage Reactor, Biogas, GHG mitigation*

3.1. Introduction

Rising energy costs, more stringent environmental regulations, and increased public awareness of climate change have pushed industries to pursue renewable means of energy production and to reuse residues to produce renewable fuels (Wood and Roelich, 2019). Brazil, a rapidly growing and industrializing country, is the largest exporter of orange juice in the world, and a consequence is the co-production of large amounts of solid residues in the form of peels, seeds, and bagasse (de Souza et al., 2018). A significant economic and environmental benefit can be realized by repurposing these by-products.

Currently, most fruit residues are used as animal feed or promising raw materials for the production of co-products (Luckstead et al., 2015) such as fertilizer, essential oils, pectin, ethanol, industrial enzymes, single-cell proteins, pollutant absorbents, paper pulp supplement, etc. (Martín et al., 2010; Qu et al., 2020; Sarkar et al.,2019; Esparza et al., 2020; Qu et al., 2019). Although some of these products are highly valuable, and they have limited markets that are not correctly matched to the quantities of orange juice residues currently available. Anaerobic digestion (AD) has potential as a robust technology to obtain energy using low-temperature microbial processes that convert organic matter into methane-rich biogas that can be used for heat or power generation. After many years of industrial-scale use (Kumaran et al., 2016), AD remains one of the most environmentally friendly technologies with effective bioenergy production. Moreover, despite the perceptions related to technological maturity, AD retains scope for further improvements by advancing reactor designs, microbial selection, etc. (Kamali et al., 2016).

Conversion of waste to energy using AD or other technology reduces the side effects associated with landfill disposal, including greenhouse gases (GHG) emissions, eutrophication, depletion of dissolved oxygen, and others (Kainthola et al., 2019). In addition to the biogas product, AD produces a nutrient-rich digestate or “effluent” that is useful for soil enrichment, meaning that – in principle – AD is a waste-free conversion technology.

AD can be classified as either liquid or wet for solid feed concentration less than 15%, while when termed “dry”, the process is fed with more than 15% (Momayez et al., 2019). Dry feeds have the advantage of better utilization of available reactor volume. On the other hand, water is essential to improve mass transfer and to accelerate the microbial growth. Dry AD is challenged by incomplete mass transfer as a result of the limited mixing when compared with wet AD (Adekunle and Okolie, 2015; Budiastuti et al., 2016; Kim et al., 2003; Kothari et al., 2014; Wang et al., 2014; Xu et al., 2018).

Although several types of reactors are used for AD, batch reactors and Continuously-fed Stirred Tank Reactors (CSTR) are the most common. AD can be further classified as one-stage or two-stage. In general, one-stage AD operational conditions are optimized to generate the highest CH_4 production and to produce only negligible amounts of hydrogen (H_2), while the two-stage configuration is used to achieve sequential production of H_2 and CH_4(Garcia et al., 2019; Zahedi et al., 2019). The two-stage configuration allows a higher control level since each reactor can be independently optimized, resulting in better pH control, better stability, increased CH_4 yield, and increased reduction of volatile solids (VS) (Náthia-Neves et al., 2018; Ren et al., 2018; Wang et al., 2018).

AD of orange peels (OP) could be applied at a large scale in the industry. However, the literature reported that OP presents a toxic compound, D-limonene (Ruiz and Flotats, 2014), a microbial toxin that can potentially inhibit AD. For that reason, previous studies of have evaluated several pre-treatments to remove or minimize the inhibitory effect of D-limonene prior to digestion. For example, Calabro et al. (2019) studied the effect of zero-valent iron and granular activated carbon, whereas Martín et al. (2010) performed steam distillation.

Previous research (Calabro et al. (2020) and Zema et al. (2018)) reported inhibition effects by this compound. Similarly, Koppar and Pullammanappallil (2013) studied anaerobic digestion of OP at thermophilic temperatures conditions and observed no toxicity due to limonene accumulation, although traces of bark oil were found in the wastewater sample. These authors did not analyze D-Limonene, and yet they obtained higher methane yield than Martin et al. (2010), who did report problems related to this compound.

Prior studies on AD of OP have focused on feed pre-treatment before digestion in a single reactor. However, OP pre-treatment has the disadvantages of increased process complexity and/or increased energy requirements (Serrano et al., 2014). Instead of the pre-treatment combined with the one-stage reactor, two-stage AD – with its aforementioned advantages and operational flexibility (Nasr et al., 2012)– has the potential to eliminate the pre-treatment step while providing acceptable biogas yields. In effect, the first reactor would operate as a pre-treatment to minimize D-limonene inhibition and ideally produce hydrogen, allowing the second reactor to be optimized for methane production.

The objective of this study was to evaluate the potential of two-stage AD to valorize orange juice solid residues without a pre-treatment step. Additionally, one-stage digestion was evaluated as a control reactor. The performance of the reactors was assessed by daily measurements of gas production and composition, solids loading, and other vital parameters such as pH. Moreover, to help understand the effect of AD on the substrate, the microstructure of the digesters effluent was analyzed by Scanning Electron Microscopy (SEM). Experimental biogas production data was used to evaluate the potential electric energy generation for the citrus industry of São Paulo State, which is the leader Brazilian orange juice producer and exporter, and the corresponding avoided GHG emissions for electric energy replacement from the Brazilian energy matrix was estimated. The results of this study could motivate further works on the solid residues digestion to support a significant adoption of this technology by the orange juice industry. Which could be applied to the UASB reactors currently in operation in the citrus juice industry.

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3.2. Materials and Methods

3.2.1. Inoculum and Substrate Characterization

OP was obtained from the juice manufacturer Citrus Juice, Itajobi City- São Paulo State (Brazil) - was grounded in a blender and stored at –14 ± 2 °C until use. The cellulose, hemicellulose, lignin, moisture, extractives, and an ash content of the OP were measured using methodologies recommended by the National Renewable Energy Laboratory (NREL) (Sluiter et al., 2008a; Sluiter et al., 2008b; Sluiter et al., 2005). Protein determination was made for orange peel by the Nitrogen-Kjeldahl method, and the protein content was calculated according to the following equation 1:

$$Extract\ of\ protein\ (\%) = \left(\frac{V_{HCl} x C_{HCl} x Fc_{HCl} x 0{,}014}{m_{amostra}}\right) x 100 x Fc_{protein} \qquad (1)$$

Where: V_{HCl} is the volume of chlorodric solution in the titration expenditure; C_{HCl} is the concentration of the solution used in titration; Fc_{HCl} is the correction factor of the level of the HCl solution used in titration; *m* is the mass of extract or raw material used in the analysis and *Fc* proteins, is the correction factor for conversion of the total nitrogen content present in the sample to proteins, which in this case corresponds to 6.25.

The methodology described by Lachos-Perez et al. (2018) was used for pectin quantification, with absorbance measurements made on a spectrophotometer (DR/4000, Hach).

Mesophilic anaerobic sludge was obtained from an Up-flow Anaerobic Sludge Blanket (UASB) reactor of a Brazilian brewery company (AmBev) and used as inoculum.

3.2.2. Experimental Design and Reactor Composition

Figure 1 provides schematics of the two-stage AD reactor that was the focus of this study and the one-stage reactor used as a control. In all cases, the reactor volume was 4.3 L, of which 60% contained digestate and 40% contained biogas.

Preliminary tests were performed to determine the optimal solids loading in the reactor. Solids loading must balance process intensity with mass transfer effects. The low mass density of OP made mixing AD reactors difficult with dry feeds, and the final decision was to fill reactors with a mixture consisting of wetted orange peel (35% v/v), inoculum (26% v/v), and water (39% v/v), resulting in a wet AD, i.e., 8-10% total solids (TS).

All the reactors were continuously operated under mesophilic conditions (35°C), and the temperature was kept constant by a thermostatic bath. The acidogenic reactor pH was maintained at 5-6 and the methanogenic reactor at 7-8 with the addition of 6N NaOH when necessary. Also, in two-stage reactors configuration, the acidogenic reactor was fed daily of 100 ml composed by 65 ml of water and 35ml of orange peels, and the methanogenic reactor was fed with 100 ml of the acidogenic reactor digestate. On the other hand, the control reactor was fed as the acidogenic one, and samples of all reactors were collected every three to four days for chemical analysis. The hydraulic retention time (HRT) was 25.8 days, and the organic loading rate (OLR) was 0.36 gCOD/(L.d) for the two-stage reactor and 0.40 gCOD/(L.d) for the control one-stage reactor.

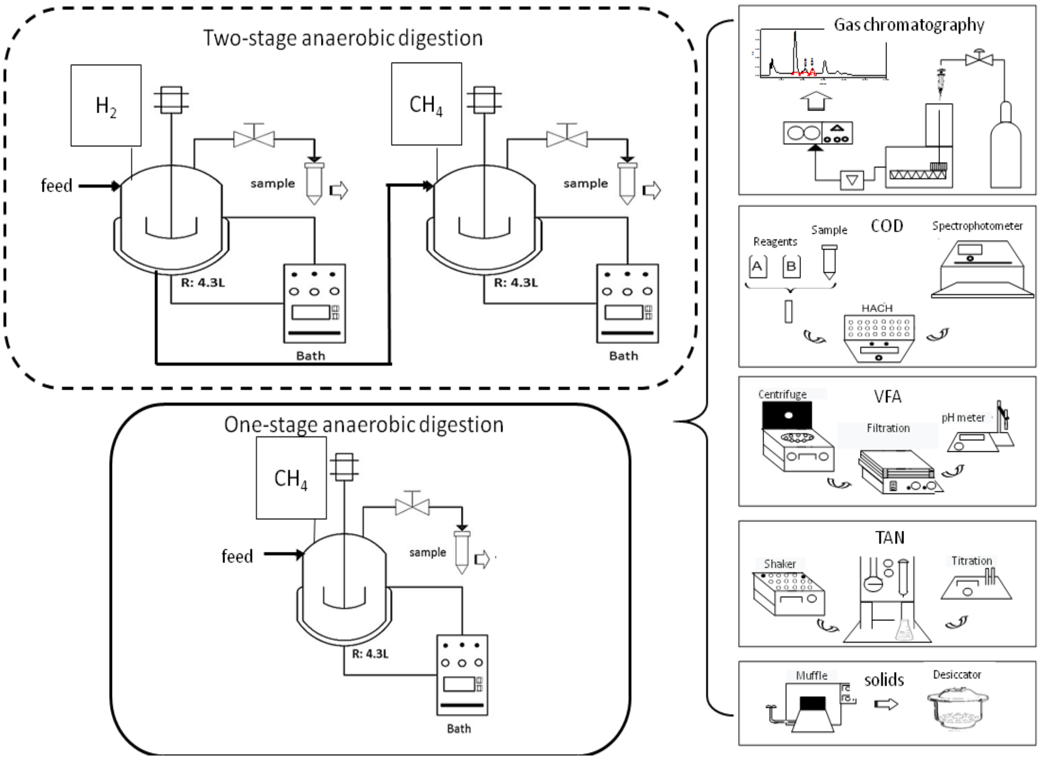


Figure 1. Experimental schematic and chemical analysis of the control of the process: R: 4.3L- Anaerobic reactor (V=4.3L total volume); Bath – thermostatic bath; Tedlar bag to H_2 and CH_4 sample.

3.2.3. Analytical Chemical Analysis of Control of Reactors

Samples were extracted on a daily basis and analyzed for pH (4500-H+ B), Total Solids(TS; 2540B), Total Volatile Solids (TVS; 2540E), Chemical Oxygen Demand (COD; 4520D), Total Alkalinity(2320B) and Total Ammonium Nitrogen (TAN) (45000NH3-C). Analyses were performed according to the Standard Methods for the Examination of Water and Wastewater (Apha, 1985).

For COD, alkalinity, and TAN, 5 g of sample were diluted with 50 mL of deionized water. This mixture was then placed in a shaker at 25°C and 200 rpm for one h. After the diluted sample was thoroughly mixed, the mixture was vacuum filtered to remove solid particles. All samples were analyzed in triplicate.

The pH was monitored every day and regulated every three or four days with the addition of NaOH (6 N) to maintain the reactor within ± 5-6 for acidogenic reactor and 7-8 for methane and control reactor units of the optimal pH.

For the determination of VFA, samples were diluted in deionized water and acidified with 3M phosphoric acid at a mixing rate of 1:20. VFA analysis was then performed using a gas chromatograph (GC) (Shimadzu®, model GC 2014) equipped with a capillary column (Agilent Technologies®, model DB-WAX, 30 m length, 0.25 mm internal diameter, and 0.25 μm film thickness). The operational parameters were: injection port and detector temperatures were both set to 250 °C; initial temperature of GC column was 100 °C (held for 1 min), and then increased by 8 $°C.min^{-1}$ until reaching 200 °C where it was held for 2 min; injected sample volume was 1.0 μL; nitrogen (N_2) - carrier gas and flow rate of 1.0 $mL.min^{-1}$ and pressure of 92.4 KPa. The quantification of acetic, propionic, isobutyric, and butyricacids, was performed using standard calibration curves, as is typical (Maciel-Silva et al., 2019).

3.2.4. Biogas Composition and Volume

Biogas samples were collected in Tedlar bags and composition analyzed daily using a gas chromatograph (GC-2014,Shimadzu) equipped with a thermal conductivity detector (TCD). Thecolumn was a Shincarbon ST 50/80 Mesh. The following chromatographic conditions were used: injection and detector temperatures were both kept at 200 °C; GC column initial temperature was 50.0 °C (held for 3 min); GC column temperature was increased by 5.0 $°C.min^{-1}$until reaching 180 °C where it was held for 5 min. The samplevolume injected was 0.5 mL. $N_{2,}$ carrier gas flow was 35 $mL.min^{-1}$ at 5 bar. Hydrogen (2 min), carbon dioxide (6 min), methane (15 min), and oxygen (23) were identified by retention time matching and concentrations determined by peak areas.

3.2.5. Scanning electron microscopy

The morphology of the orange peel particles extracted from the acidogenic, methanogenic, and control reactors were evaluated using Scanning Electron Microscopy (SEM). A minimum of 20 images was obtained for all samples. Representative images are shown here. Samples were coated with a nanometer-thick gold film using an SCD 050 sputter coater to improve their conductivity. SEM images were obtained in equipment with cannon FEG (Inspect F50), available at the Brazilian National Laboratory of Nanotechnology (LNNano), located in Campinas-SP, Brazil. Imaging was performed under a high vacuum < 1.3×10^{-2} Pa, at an acceleration of 4 kV.

3.2.6. Electricity generation (EG) and avoided GHG emissions

The potential power that could be generated by OP biogas produced for the entire São Paulo State was estimated using to Equation 2 (Campello et al., 2020: Cabral et al., 2017):

$$EG = Q_{biogas} * LCV_{CH_4} * C_m * \eta_e * \mathrm{CF} \qquad (2)$$

Where, *EG* is the potential of electricity generation (MWh/ton OP); Q_{biogas} is the volume of biogas (Nm^3 of biogas m^{-3}per ton OP), obtained according to the experimental data; LCV_{CH4} is the lower calorific value of methane (35.59MJ m^{-3}); C_m is the percentage of methane in biogas (%), according to the experimental data; η_e is the engine efficiency (%), assumed as 34% (Heywood, 1988) and *CF* is the conversion factor from MJ to MWh.

Equation (1) implicitly assumes that power generation was accomplished on site. Likewise, the integration of biogas collection followed by combustion was analyzed.

C_m values from experimental data, for control and two-stage reactors, were 42.22 % and 67.80 %, respectively. The experimental biogas volume, Q_{biogas}, for both control and two-stage reactors arrangements for their respective feeding rate, 0.019 m^3 for 1.649 kg of OP, and 0.026 m^3 for 1.432 kg of OP, respectively, was used to calculate the experimental EG per ton of OP. These biogas yields were assumed for the orange peels estimated production of São Paulo State in 2017. According to the National Association of Citrus Juice Exporters (CitrusBR, 2017), orange juice processing accounts for 86% of the *in nature* orange production for the same region, and about 50% of the orange used into the juice processing ends up as residues including peels, bagasse or seeds. The *in nature* orange production for São Paulo State, collected from Brazilian national statistics (IBGE, 2017), accounted for 13×10^6 tons in 2017. For the consequence of the amount destined to the juice industry and residues estimation, it corresponded to 5.74×10^6 ton of OP.

The avoided GHG emissions were obtained according to Equation 3, for the replacement of electricity derived from the national energy grid with more renewable bioenergy (dos Santos et al., 2018):

$$A_{GHG} = 0.0927 * EG \qquad (3)$$

Where *EG* is the electricity generated converted to MWh per ton orange peels. The emission factor for the grid was based on the annual mean (from January 2017 to December 2017), reported from official data of the Ministry of Science, Technology, Innovation, and Communication (MCTIC, 2019). The mean value was used to account for the energy supply seasonality that includes the influence of the dry season on the energy mix, i.e., during the dry rainfall period that relies more on hydroelectric generation (MME, 2018). After seasonal averaging, the resulting emission factor was 0.0927 ton of CO_{2eq} per MWh of electricity on an annual basis.

3.3. Results and Discussion

3.3.1. Characterization of raw materials

Table 1 summarizes the initial physicochemical composition of orange peel (OP) and inoculum. OP consists of 14.77% lignin, with roughly equal amounts soluble and insoluble and similar to data reported previously by Lachos-Perez et al. (2018). The relatively low lignin content of OP suggests that it may have potential as an AD substrate, which was further corroborated by its considerable organic matter content (measured as COD and TVS). In comparison with OP, lignocellulosic biomass contains greater amounts of lignin, which contributed toslow and incomplete degradation in the hydrolysis step, which is a rate-limiting step (Khan and Ahring, 2019).

The organic matter content is mostly composed of pectin and cellulose, both of which are suitable substrates for AD. Cellulose and pectin are polysaccharides that are readily decomposed through acidogenesis, primarily resulting in formation of VFA. However, high concentrations of carbohydrates can accelerate VFA concentration in the digester and decrease pH, which may compromise methanogenic activity and even cause colony collapse. The two-stage reactor can avoid this problem as entry into the second reactor, where methanogenesis takes place, can be controlled.

The nitrogen content of OP was 1.10 % of nitrogen (N) and its C/N ratio was 24:1 (Ángel Siles López et al., 2010), which is considered an optimal ratio for AD. Nitrogen is essential for protein synthesis, and it is primarily requiredas a nutrient by the microorganisms in AD (Adarme et al., 2019). . High N is not necessarily beneficial as ammonium released during the degradation of nitrogenous organic matter is a potent AD inhibitor AD(Solé-Bundó et al., 2019). Optimal nitrogen content increases biogas production due to reduced accumulation of ammonium and intermediate volatile compounds (Khalid et al., 2011).

A challenge with OP is acidity, a problem that was addressed – in part – by careful selection of the inoculum. The density (1001.49 kg/m^3) and TVS (8.03 %) of the inoculum used in this work were greater than reported byNáthia-Neves et al. (2018), 977 kg/$m^{3,}$ and 2.0 %, respectively. Similarly, Martín et al. (2010) used an inoclum pH of 7.22, while the pH of the inoculum used here was 7.53. The use of a dense inoculum with high TVS and pH can help the neutralization of orange peel acidity, helping maintain an appropriate pH for microbial growth.

Table 1. Physical and chemical composition of orange peels and inoculum.

Analytical Parameter	Orange Peel	Inoculum
pH	4.47	7.53
Density (kg/$\mathbf{m^3}$)	580.29	1.001.49
Total Solids (%)	21.01 ± 2.15	9.07 ± 0.59
Total Volatile Solids (%)	20.39 ± 1.95	8.03 ± 0.31
Total Fixed Solids (%)	0.62 ±0.17	1.49 ± 0.32
Chemical Oxygen Demand (g $\boldsymbol{O_2}$/L)	26.015 ± 7.5	0.57 ± 9.81
Ammonium nitrogen ($\boldsymbol{mgNH_3}$/L)	700 ± 0.01	1400 ± 0.01
Alkalinity ($\boldsymbol{mgCaCO_3}$/L)	2062 ± 15.3	14437.5 ± 0.21
Moisture (%)	71.84 ± 1.08	-
Ash (%)	3.19 ± 0.25	-
Total Nitrogen (%)	1.10 ± 0.03	-
Protein (%)	6.74 ± 0.20	-
Total Extractives (%)	5.56 ± 0.37	-
Total Lignin (%)	14.77 ± 1.75	-
Lignin Insoluble (%)	7.44 ± 1.50	-
Lignin Soluble (%)	7.30 ± 1.53	-
Hemicellulose (%)	12.08 ± 0.42	-
Cellulose (%)	21.23 ± 1.20	-
Pectin (%)	23.02 ± 1.40	-

3.3.2. Operational Performance

3.3.2.1. Solids concentration

Figure 2 presents operational data for the TS, TVS, and total fixed solids (TFS, defined as the difference between TS and TVS) measured for the one-stage control reactor and in the acidogenic and methanogenic stages of the two-stage system. The TS content of the control reactor decreases monotonically, from an initial value of 9,8% and reaching an apparent steady approximately 5% after 20 days. The behavior of the control is consistent with high initial microbial activity previously reported for single stage AD (Maciel-Silva et al., 2019). In contrast, the TS content of the acidogenic reactor remains relatively stable at a value of approximately 6-7% for the first 15 days of operation, at which point it drops precipitously. As discussed in the following section, no hydrogen evolution was observed at this point in the acidogenic reactor, and the decision was made to increase the OP content of the feed from 35% to 40%. After this corrective action, the TS content of the acidogenic reactor stabilized. The TS content of the methanogenic reactor was stable for the duration of the study.

TVS provides additional information. Here, the TVS of the acidogenic reactor decreases by 46% by day 43, the methanogenic reactor by 50% over the same time span, and the control by 72%. Náthia-Neves et al. (2018) reported an acidogenic reactor reduction of 64% and 51% for the methanogenic, and De Gioannis et al. (2017) obtained a final TVS removal efficiency of 34.1% in the acidogenic reactor and 53.3% in the methanogenic one. As will be shown later, the decreased TVS content cannot be ascribed to methane production, but it does provide an indication of organic carbon removal.

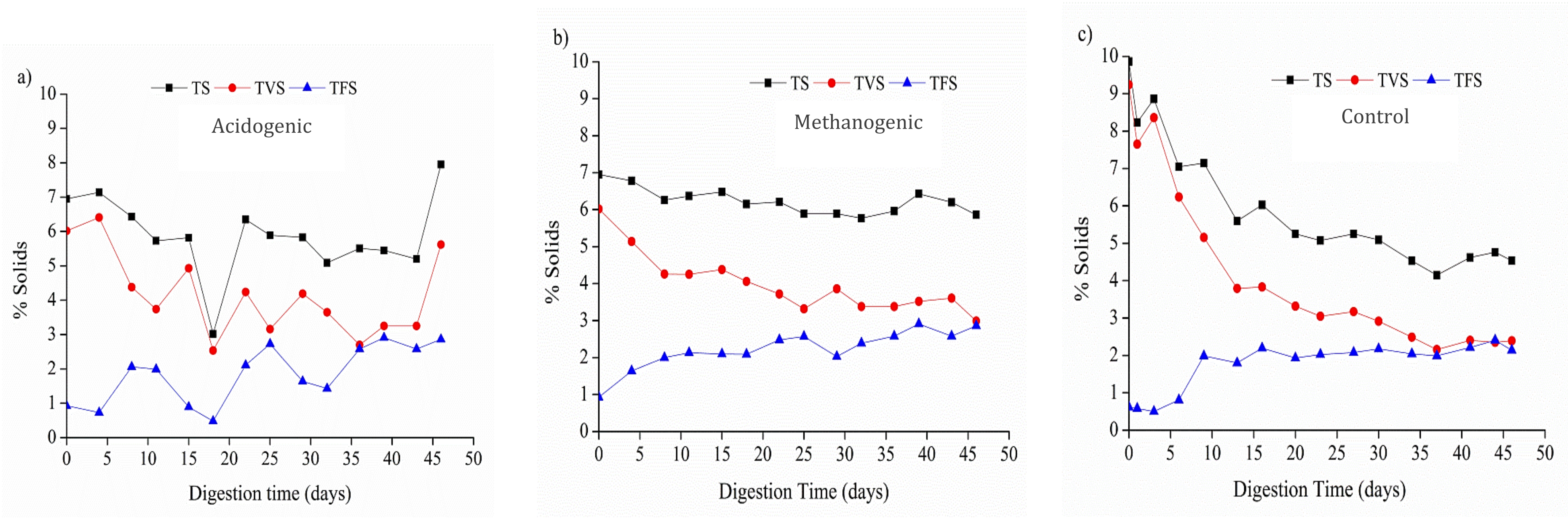


Figure 2. Evolution of solids concentration

3.3.2.2. Chemical oxygen demand and Total ammonium nitrogen

Figure 3a provides COD content measured for the three reactors. For all three reactors, the measured COD and TVS content are directly correlated with one another. In Figure2dshows that COD increased during the first few days of operation for the acidogenic and control reactors, consistent with microbial hydrolysis of the organic feed. After this initial period, COD stabilized. In contrast, the COD measured for the methanogenic reactor remained steady for the first 7 days, which can be attributed to the fact that the acidogenic reactor was fed from the raw waste (orange peel) while the methanogenic reactor was fed by material partially digested in the acidogenic reactor. After the induction period, the COD of the methanogenic reactor increased to a stable value of approximately 40 g/L.

The COD behavior shown in Figure 3a indicates increasing the hydraulic retention time would be necessary to increase organic matter removal. Silva et al. (2018), in experiments with food waste + inoculum (SS) + 1%glycerol and food waste + SS + 3% glycerol, found that COD removal did not exceed 20% during acidogenesis, whereas during methanogenesis it varied from 49 to 61%. Low COD reduction is therefore typical of acidogenesis stage, since during this stage organic compounds are mainly converted into soluble metabolic products (VFA and alcohols) that contribute to COD rather than gasesthat do not (Cooney et al., 2007).

Figure 3b provide ammonia measurements for the three reactors. Ammonia is generated during the biodegradation process of protein or other nitrogen-rich organic substrates (Zhang et al., 2014), and for this reason, ammonia content increases during the first days of AD for acidogenic and methanogenic reactors – as shown in Figure 3b. The ammonia content of the acidogenic reactor spiked at day 22, coincident with the aforementioned change in feed concentration. In contrast, ammonia content of the control reactor was negligible until day 10 at which point it increased rapidly and then gradually decreased for the remainder of the study. All of these observations are broadly consistent with stable operation of the various reactors resulting in conversion of feed proteins into soluble nitrogen, excluding the aforementioned brief excursions.

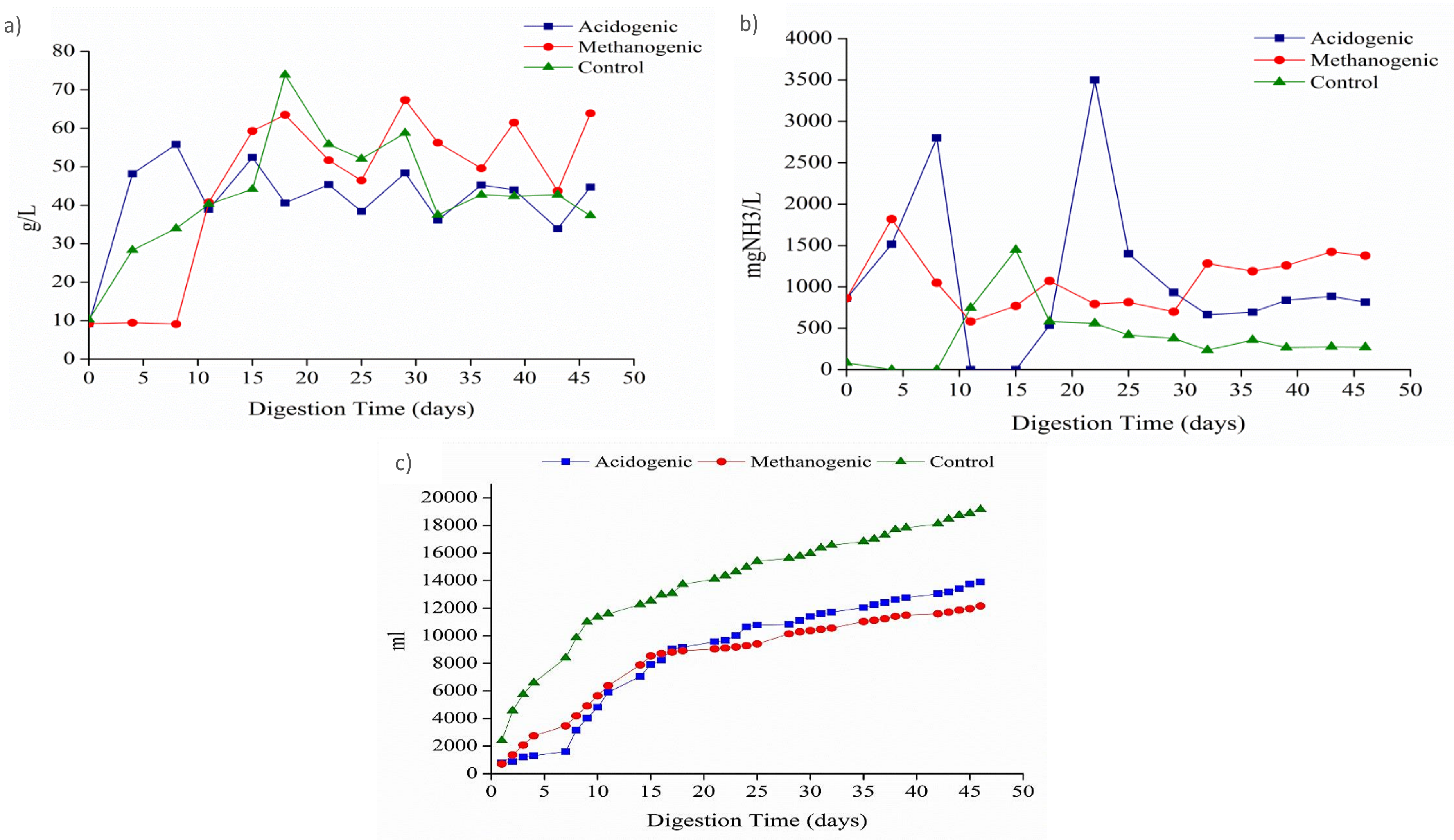


Figure 3. a) Chemical Oxygen Demand b) Ammonium Nitrogen and c) Accumulated volume of biogas.

3.3.2.3. pH, alkalinity and VFA

Digestate pH is an important parameter governing AD performance, affecting both microbial growth solubilization of organic matter (Feng et al., 2015). For this reason, operation at or near the optimal pH is required for both the acidogenicand methanogenic reactors in a two-stage AD system (Garoma and Pappaterra, 2018). As shown in Figure 4a, the pH of the acidogenic reactor was stable in the range from 5 to 6, except on day 22 when an aliquot of 6N NaOHwas added to increase the pH (Kyazze et al., 2007). However, recognizing potential inhibition effects, pH was immediately allowed to return to 5.

The alkalinity content steadily increased, which was caused by an accumulation of NaOH in the reactor. Since the reactors were connected in series, the saline solution was also conveyed to the second reactor, thus increasing its pH and total alkalinity(Baldi et al., 2019).For that reason, the alkalinity in the methanogenic reactor was greater than in the acidogenic one, and likewise the reactor pH was stable in the range from 7.0-8.0 after a short induction period.Consequently, pH was corrected with 6 N NaOH to ensureoptimal methanogenic conditions. After that, the alkalinity concentration remained between 10,000 and 22,000 mg$CaCO_3$/L, which are considered high values for this parameter.

ThepH fluctuations shown in Figure 4b might be attributed to production of VFAs, accumulation of which can decrease reactor pH (Liu et al., 2018). The main VFAs were acetic and propionic acids. Similarly, previous reports pointed out that VFA were significantly affected by the pH (Zhang et al., 2014). as shown in Figure 4, the acetic acid production in the acidogenic reactor was less than the other reactors. The reduced VFA production is related to the lower pH that favors the hydrolytic step in which the complex chemical compounds are not fully converted to VFAs.

In the control reactor, the dual action of the hydrolysis and acidogenic steps in the first 10 days led toacidic pH values in the range from 4.50 to 6.0. After this period, pH increased to 6.7-7.0, consistent with the methanogenesis. The alkalinity increased until a maximum peak on the day 30 of 15,000 mg$CaCO_3$/L, somewhat less than is observed for methanogenic reactor alkalinity.

In the methanogenic (e) and the control reactor, the organic acids were completely produced during the acetogenesis step in greater quantity (f) than in the acetogenic reactor and with main products as acetic and butyric acids.VFAs are required intermediatesin methane production;on the other hand, excessive accumulation can lead to a process failure (Ratanatamskul and Manpetch, 2016). The optimal VFA concentration range is difficult to know *a propri* since the optimal value depends on the substrate composition and reactor operating conditions. However, Figure 4e shows that the acetic acid concentration reaches a value of 23,000 mg/L on day 27, whichis enough to begin inhibiting methanogenesis. Accordingly, Figure 4bshows that the methane composition decreased on that day. In the control reactor, VFAs concentrations were also high as the acetic acid was between 1,000 and 14,000 mg/L during the first 10 days before decreasing.

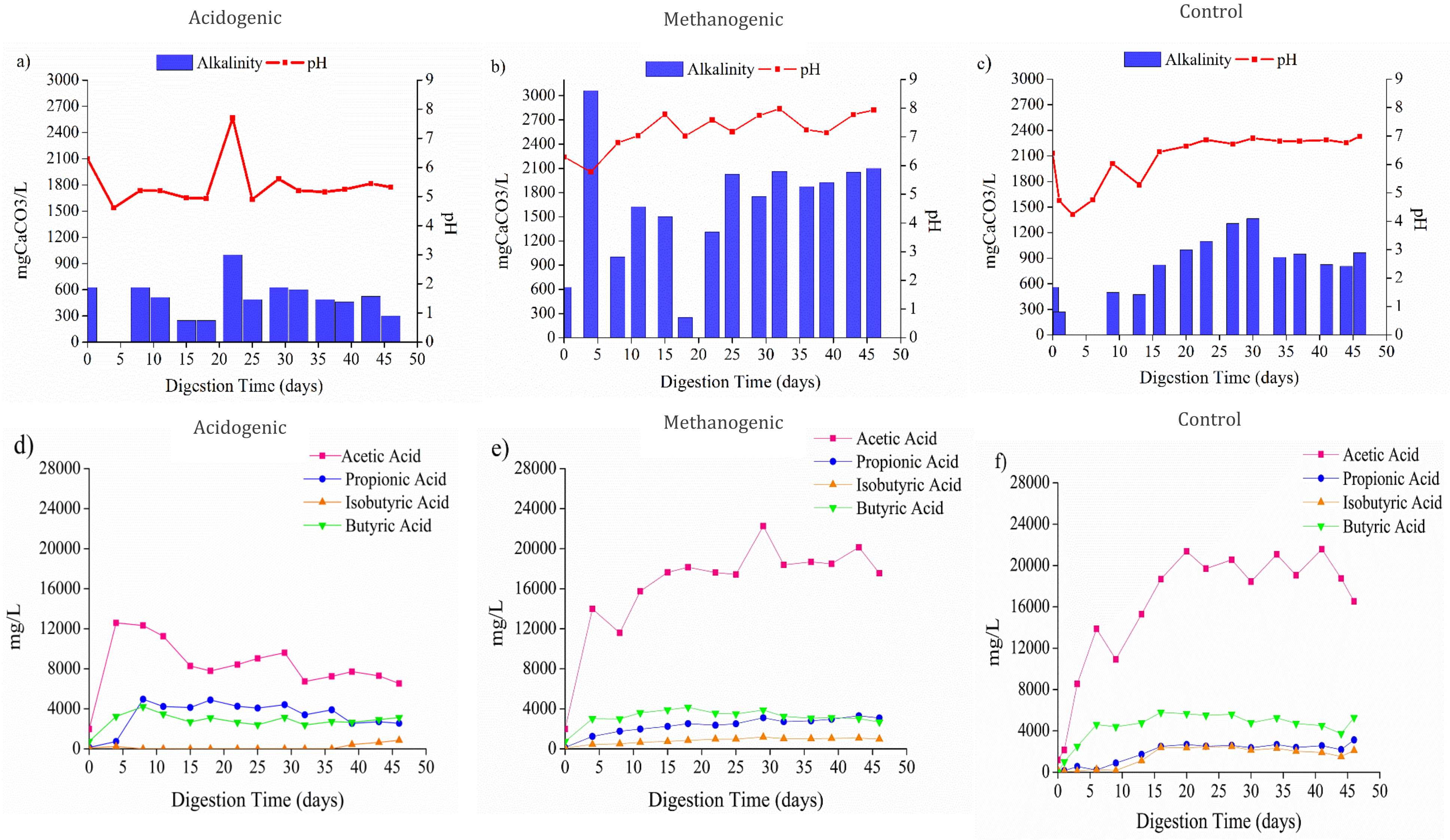


Figure 4. Evolution of pH-alkalinity and Production of volatile fatty acids.

3.3.2.4. Biogas composition

The primary anaerobic digestion performance metric is the biogas composition, which is presented in Figure 5. The hydrogen content of the acidogenic reactor biogas was variable and less than expected Its average value was approximately 5% with peaks at 35%. Only in the final days of the run did the hydrogen concentration begin to increase. The lack of hydrogen in the acidogenic reactor biogas can be attributed to the OP feed, as it contains lipids that are poor substrates for hydrogen production.

Peculiarly, while the acidogenic reactor failed to produce hydrogen in the expected quantities, the two-stage system outperformed the one-stage control reactor in terms of methane production. Here, the methanogenic reactor immediately began producing methane, with maximum concentrations approaching 70%. In comparison, the one-stage reactor required 8 days to begin producing methane, and the maximum concentration was only about 60% which was not sustained. In the final 20 days of the runs, the methane concentration in the methanogenic reactor of the two-stage system stabilized at approximately 60%, with only 40% carbon dioxide. In comparison, the methane and carbon dioxide concentrations were roughly equal at 50% in the biogas produced by the one-stage reactor in the same time period.

A methane yield of 0.79 L/gSVT and 0.49 L/gSVT was found for methanogenic reactor and control reactor respectively. Besides, a hydraulic retention time (HRT) of 25.7 for the three reactors. Similar results were reported byMartín et al. (2010) in their experiment of orange peel anaerobic digestion with a pretreatment by steam distillation in order to reduce d-limonene content, an HRT of 26 days generated a methane yield of 0.60 L/g SVT. However, they obtained 56-59% of methane composition.

These results are consistent with the acidogenic reactor serving as a type of pretreatment for OP digestion. The action of the acidogenic reactor seems to be transformation of one or more recalcitrant compounds or inhibitors into forms that are more amenable to methanogenesis. Limonene removal might be one of the effects, since limonene is known to inhibit AD.

Methane production is influenced by some of the operational parameters described previously, which may account for the fluctuations in biogas composition shown in Figure 5. For example, the high ammonia concentrationsobserved on some days and the NaOH used for pH regulation could have affected the process by inhibiting biogas production. Also, alkalinity can cause a shift from the H_2/butyrate fermentation pathway to the solvent/lactate fermentation pathway, resulting in a reduction of the activity of H_2-producing bacteria in the reactor (Lee et al., 2010).

Most previous studies describing OP digestionpretreated it to remove d-limonene compoundas it can have inhibiting properties (Martín et al., 2010; Ruiz et al., 2016; Ruiz and Flotats, 2014; Wikandari et al., 2015). This work showed that the use of two-stage anaerobic digestion and control of operating parameters can permit OP digestion without an explicit pretreatment step. This confers advantages in terms of cost and process simplicity.

Finally, Figure 3c shows the cumulative biogas volume. The control reactor produces the most gas, 20,000 ml, followed by the acidogenic (13,000 ml) and then the methanogenic reactor produced (10,000 ml). The sum of the acidogenic and methanogenic reactor gas volumes exceeds that of the control reactor. Other differences are explained by the greater production of gas in the first 10 days by the one-stage reactor, which is attributable to the use of a more highly activated inoculum in the control reactor.

The net effect of methane composition and biogas volume is that the two-stage reactor produces 20% more methane than the one-stage control reactor. This improvement is technologically significant and recommends further consideration of the two-stage approach for AD of OP.

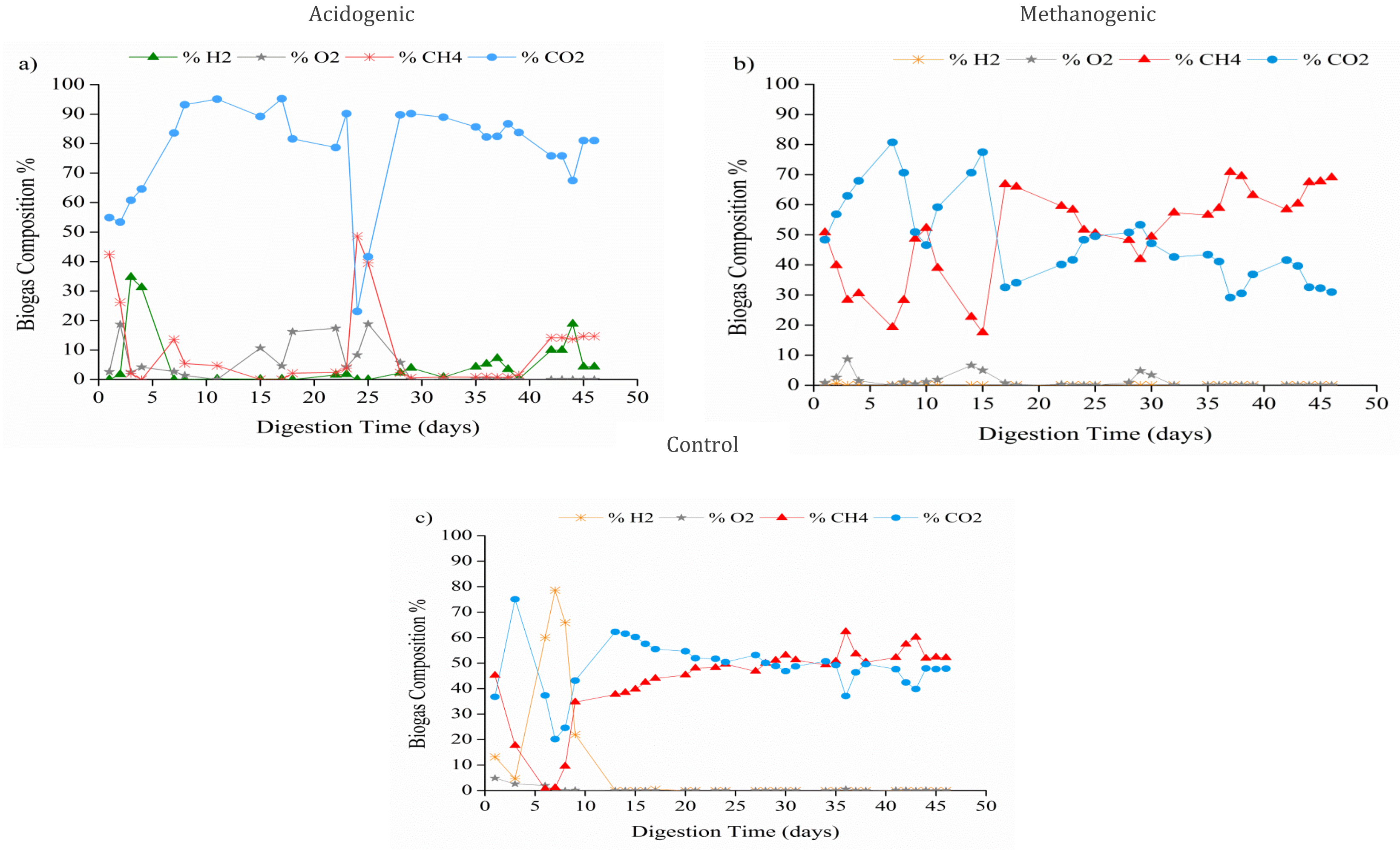


Figure 5. Composition of the biogas produced in a) Acidogenic reactor b) Methanogenic reactor c) Control reactor and d) accumulated volume of biogas.

3.3.3. SEM and morphological changes

For biomass feeds, AD preferentially converts hemicellulose and exposed cellulose, leaving behind inaccessible cellulose and lignin that retain fibrous morphologies. To understand the differences between one-stage and two-stage AD on OP, the digestate from these reactors were recovered and imaged using SEM.

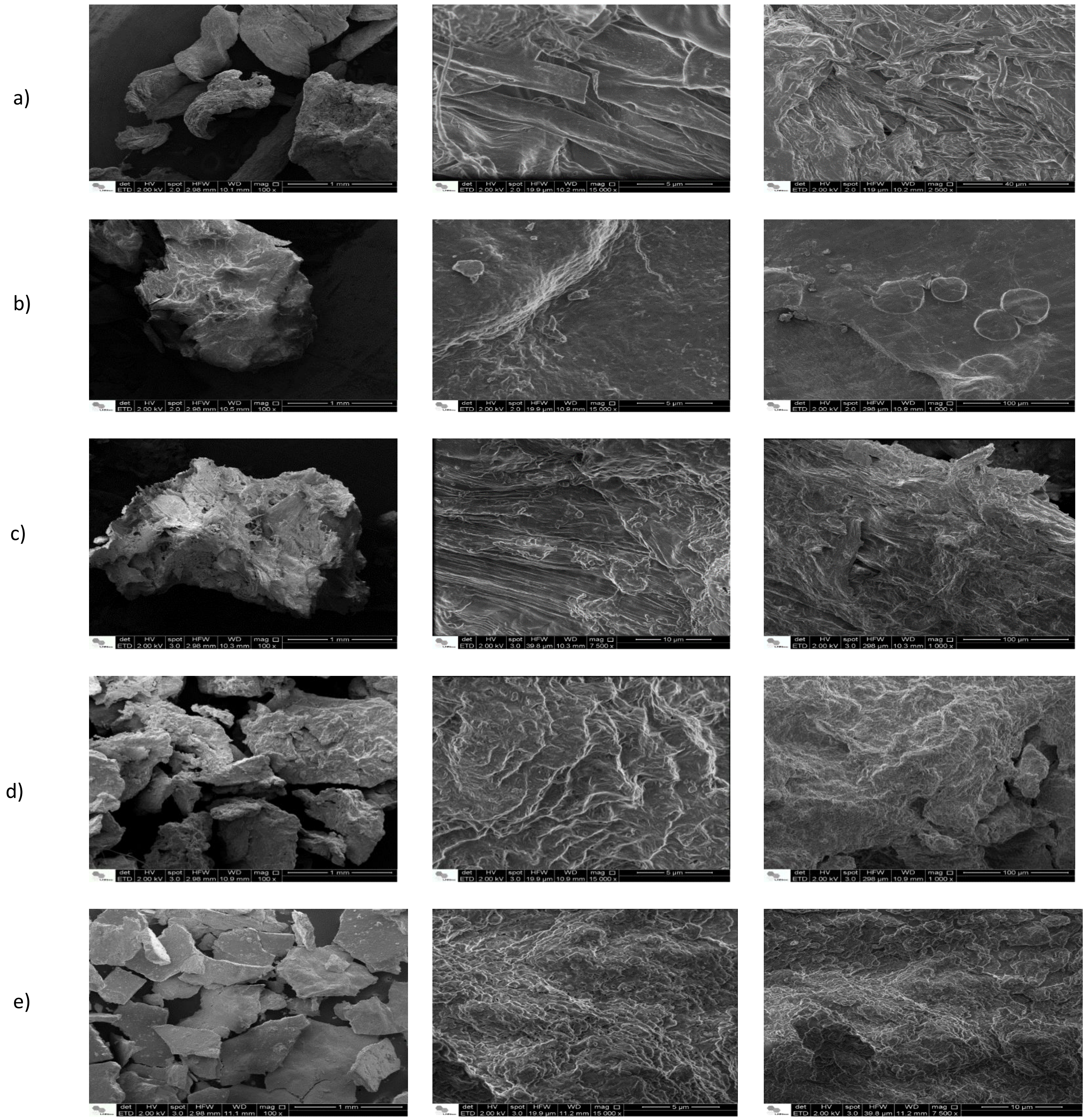
Figure 6 shows the SEM images of the raw material and the reactors effluents. Figure 6a shows the surface of the OP feed (i.e., without treatment) and its complex lignocellulosic structure. Irregular and heterogeneous porous structures are observed; besides channels and cavities, it may correspond to cellulose and lignin structures without a well-defined direction.

Figure 6b shows SEM images obtained from analysis of OP extracted from the initial stages of the one and two-stage reactor systems. These sampleshave formed a microbial sludge, with microorganisms visible in the imagesand an otherwise compact structure.

Figure 6c, d, and e show SEM images of the digestate solid recovered after the final AD day fromthe acidogenic, methanogenic, and control reactors, respectively. These images help visualize how the microbial community broke down the organic matter and converted it into simpler, dissolved materials, ideal for the AD. Especially, comparing images in Figure 5e with those in Figure 5c it indicates much greater OP disruption occurs after the second stage of the two-stage system compared with that observed in the singe-stage reactor. This observation is consistent with more robust digestion occurring in the two-stage AD than the one-stage control AD, thereby increasing the observed methane concentrations.

Figure 6. SEM images of the surface morphology of orange peels in-situ nature without pretreatment a) OP wast b) Day 0 c) Day 46 (Acidogenic reactor) d) Day 46 (Methanogenic reactor) e) Day 46 (Control reactor).

Magnitude (i) (100x) (ii) (15000x) and (iii) (1000x).



3.3.4. Electricity generation (EG) and avoided GHG emissions

Citrus juice production in São Paulo State (Brazil) is estimated to produce approximately 5.7×10^6 tons of OP on an annual basis. In turn, up to 97.5×10^3 MWh/year could be produced from these OP residues using the two-stage system. Table 2 summarizes results. These values represent approximately 0.2% of the annual power use in São Paulo State (GOVSP, 2019); alternatively, the power generated from OP biogas could be used on-site or sold back to the grid in the event of surplus.

The referred electric energy generation is equivalent to 0.0085 kWh/kg of *in nature* orange, accounting for 11% of the demand of the industrial orange processing which is estimated as 0.077 kWh/kg of *in nature* orange processed for Not-From-Concentrate (NFC) juice industry (Doublet G., 2013; Rosas-Mendoza et al., 2020).

In comparison with Rosas-Mendoza et al. (2020) results for the AD of orange industry effluents in a hybrid reactor, 2.15 kWh/ton of orange processed, the electric energy generation of 8.5 kWh/ton of processed orange, in a two-stage arrangement used in the present study is 395 % higher. This outstanding result derives precisely from the configuration applied in AD to maximize methane yield. The addition of solid waste in AD reactors, especially in the UASB ones already existing in some industrial facilities, still requires additional scale-up studies. Nevertheless, the results here presented reveal the viability of OP (solid residue) treatment by AD as an excellent opportunity for its destination.

From the environmental side, orange peels electric energy production would mitigate between 7.5×10^3 and 9.05×10^3 tCO_2eq/year, offsetting the emissions of the citrus industry of São Paulo State (Table 2). Currently, the Brazilian orange juice industry Global Warming Potential represents approximately 1 kg of CO_2eq per liter of juice (Doublet et al., 2013), or 437 ton CO_2eq/ ton of in nature orange processed. Thereby, as a strategy for reducing both carbon footprint san environmental side effects associated with GHG emissions, as well as saving costs from the electric energy obtained from the national grid, could be achieved using the two-stage AD as a robust, reliable technology.

Brazil is facing the challenge of cleaning its energy matrix due to a new state policy launched in 2017 – RenovaBio (MME, 2017) that aims to foster nation-wide environmental, economic, and social sustainability associated with the reinforcement and dissemination of biofuel production. AD is a relatively mature technology that is not currently widespread in Brazil. Accordingly, adoption of AD should be encouraged in Brazil and other agricultural nations seeking to reduce their environmental footprints, while growing their economies.

.

Table 2. Potential annual electricity generation (EG) and avoided GHG emissions (A_{GHG}).

	Experimental Biogas Yield	Potential electricity generation (Eq. 2)	*In natura* orange production for juice industry	Orange peel from juice production	Potential annual electricity generation (EG)
Two-stage reactor	18.21 m^3/ton OP	0.01697 MWh/ton OP	11,487,649 ton	5,743,824 ton OP	97.51 $x10^3$ MWh/year
Control reactor	11.62 m^3/ton OP	0.01409 MWh/ton OP			80.94 $x10^3$ MWh/year

	Emission factor*	EG	Avoided GHG emissions (A_{GHG})
Two-stage reactor	0.0927 tCO_{2eq}/MWh	97.51 $x10^3$ MWh/year	9.05 $x10^3$ tCO_{2eq}/year
Control reactor		80.94 $x10^3$ MWh/year	7.5 $x10^3$ tCO_{2eq}/year

* Ministry of Science, Technology, Innovation, and Communication (MCTIC, 2019)

3.4. Conclusion

Anaerobic digestion of OP waste was evaluated in one and two-stage reactors. The two-stage system efficiently reduced the organic load (50%) and produced biogas with higher methane content than the one-stage reactor, resulting in a corresponding 20% increase in methane production. Interestingly, the acidogenic reactor appears to play a role in reducing recalcitrant or inhibitory compounds, permitting robust methane production in the methanogenic reactor without the need for other pretreatment resulting in an accumulated methane yield of 0.79 L/gSVT. Besides, the anaerobic reactor was able to start up with a waste composed of high solids, a potential strategy for implementation in UASB reactors. The analysis suggested that orange peels biogas using the two-stage reactor approach could provide up to 0.2% of the electrical power used in São Paulo state while offsetting between 7.5×10^3 and 9.05×10^3 tCO_2eq/year. Moreover, the electric energy generation is equivalent to 0.0085 kWh/kg of *in nature* orange destined for juice processing, which could account for 11% of the demand of the industrial orange processing. Further consideration of this robust, reliable technology for producing renewable energy from agro-industrial solid residues may be an attractive option in Brazil and other agriculture-producing nations. This strategy would improve both energy production and energy efficiency and effectively contribute to the reduction of the citrus industry's carbon footprint. Ultimately, anaerobic digestion of OP waste could represent an essential contribution to local and regional development.

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Capítulo 4–Discussão geral

Primeiramente, a *análise bibliométrica* que foi realizada, nos anos compreendidos entre 1900 e 2019, usando o programa Web of Science mostrou que as publicações se concentram ao estudo do processo de fermentação para produção de etanol, atividades anti-inflamatórias de flavonoides, uso da casca de laranja para remoção de corantes, produção de metano por digestão anaeróbica, análise econômica de uma biorrefinaria e, diferentes técnicas de pré-tratamento. Assim, as áreas mais significativas foram associadas às seguintes áreas de pesquisa: engenharia (19,2%), biotecnologia/microbiologia aplicada (13,91%), ecologia em ciências ambientais (13,59%), química (12,30%) e biocombustíveis (11,65).

Quanto aos principais artigos e autores os resultados mostram que aqueles mais citados são os mais antigos, devido ao seu tempo de indexação. O artigo mais citado (748 citações) é intitulado "Chemistry and uses of pectin - A review" o qual trata das diferentes aplicações para a indústria farmacêutica e de alimentos. Outros estudos publicados com maior número de citações estavam relacionados com os temas de remoção de corantes de soluções aquosas usando a casca de laranja como adsorvente principalmente nas águas residuárias da indústria têxtil, e depois, alguns fazendo referência à produção de biocombustíveis, entre eles o artigo mais citado é "Production of biofuels, limonene and pectin from citrus wastes". Redes de colaboração dos autores também foram reportadas no trabalho entre as quais estão: Universidade de Bharathiar, Índia, desenvolvendo pesquisa em colaboração com os co-autores da Arábia Saudita e dos Estados Unidos, Universidade de Boras (Suécia), Universidade de Toronto (Canadá), Universidade de Córdoba (Espanha), Universita Mediterranea di Reggio Calabria (Itália), entre outros.

As revistas que apresentam maior número de publicações são a Bioresource Technology, Waste of Management e Journal of Cleaner Production, revistas com alto fator de impacto que têm foco nas áreas de pesquisa relacionadas à biomassa, tratamento de resíduos biológicos, bioenergia, bio-transformações, análise de sistemas de biofontes, tecnologias associadas à conversão ou produção, geração de resíduos sólidos, e avaliações ambientais. Os países e instituições envolvidos na análise bibliométrica da casca de laranja foram classificados pelo número de publicações e os resultados mostram que a Espanha é o país mais produtivo, seguido pela China e Estados Unidos.

Assim, os resultados sugerem que a indústria de alimentos demanda uma grande quantidade de energia para o processamento de matérias-primas assim como na questão relacionada ao descarte e destino adequados de resíduos agroindustriais como a casca de laranja. Considerando que, na indústria cítrica, a maior parte da energia é empregada para processar apenas 50% de toda a fruta para produzir suco e que os resíduos sólidos agroindustriais da laranja representam altos custos, torna-se obrigatório o desenvolvimento de tecnologias viáveis para agregar valor a esse resíduo. Um exemplo é o conceito de biorrefinaria que mostra a possibilidade de obter biocombustíveis como biogás e etanol e outros produtos de alto valor agregado, como pectina e limoneno. Além disso, na queima de biogás, a energia elétrica poderia ser utilizada pela própria indústria, reduzindo seu custo global, enquanto o excedente de energia eventual poderia ser vendido e/ou distribuído para outras indústrias contribuindo para a redução de energia não renovável na matriz energética.

Por outro lado, *os resultados dos ensaios experimentais* que utilizaram os reatores de uma fase (controle) e fases separadas (reator acidogênico e reator metanogênico) mostram que a composição físico-química inicial da casca de laranja consiste em 14,77% de lignina total sendo 7,34% solúvel e 7,44% de lignina insolúvel, estes resultados são similares aos descritos na literatura. O teor de lignina relativamente baixo sugere que a casca de laranja pode ter potencial como substrato para o processo de digestão anaeróbia. Em relação ao conteúdo de matéria orgânica, a casca de laranja é composta principalmente de pectina e celulose, compostos conhecidos por serem facilmente degradados pelo processo. O teor de nitrogênio da casca foi de 1,10% e sua relação C / N foi de 24:1 considerada uma boa relação para a digestão.

Os resultados relacionados aos sólidos totais (ST) do reator controle mostram que o valor inicial foi aproximadamente 10% e depois atingiu uma aparente estabilidade de 5% após 20 dias de processo. Pelo contrário, o conteúdo de ST do reator acidogênico permaneceu relativamente estável a um valor de aproximadamente 6-7% nos primeiros 15 dias de operação, e depois caiu precipitadamente, um comportamento similar foi apresentado pelo reator metanogênico. Assim, os Sólidos Voláteis Totais (SVT) no reator acidogênico mostraram uma eficiência de redução de 48% até o dia 43, e o reator metanogênico de 50%. Os resultados de eficiência de redução de carga orgânica em termos de demanda química de oxigênio indicam umaumento durante os primeiros dias de operação para os reatores acidogênicos e controle, consistentes com a hidrólise feita à carga orgânica colocada nos reatores. Após esse período inicial, observa-se uma fase de estabilização.

Em relação à alcalinidade, ela aumentou constantemente, sendo maior no reator metanogênico do que no reator acidogênico. O pH foi corrigido com NaOH 6 N para garantir condições ideais, as flutuações de pH são atribuídas à produção de ácidos graxos voláteis sendo os mais produzidos no caso do reator acidogênico os ácidos acético e propiônico. No reator metanogênico e controle, os ácidos orgânicos foram completamente em maior quantidade do que no reator acidogênico e com produtos principais como ácido acético e butílico.

Os resultados de desempenho da digestão anaeróbia em função da composição do biogás mostram que o conteúdo de hidrogênio do biogás do reator acidogênico apresentou valores médios de aproximadamente 5%, com picos de 35%. Enquanto o reator metanogênico imediatamente começou a produzir metano, com concentrações máximas próximas de 70%. Em comparação, o reator controle precisou de oito dias para começar a produzir metano, e a concentração máxima atingida foi de apenas 60%, o que não foi mantido ao longo do experimento.

O rendimento de metano do experimento no reator metanogênico foi de 0,79 L/gSVT, comparado com 0,49 L/gSVT no reator controle. Além disso, o tempo de retenção hidráulico (HRT) foi de 25,7 dias para os três reatores. Resultados similares foram encontrados por Martín et al. (2010) na digestão anaeróbia de casca de laranja com pré-tratamento de destilação a vapor para extrair o D-limoneno, no qual obtiveram um HRT de 26 dias e um rendimento de metano de 0,60 L/gSVT. No entanto, a composição de metano no reator deles foi de 56-59%.

Estes resultados são consistentes com o reator acidogênico que serve como um tipo de pré-tratamento para a digestão anaeróbia e a possível remoção do D-limoneno, uma vez que esse composto é conhecido por inibir a digestão anaeróbia. Adicionalmente, estes resultados mostram que o uso da digestão anaeróbia e o controle dos parâmetros operacionais podem permitir a digestão da casca de laranja sem uma etapa explícita de pré-tratamento. Isso confere vantagens em termos de custo e simplicidade do processo. Assim mesmo, os resultados de volume acumulado de biogás mostram que o reator controle produz mais gás, 20.000 ml, seguido pelo acidogênico (13.000 ml) e depois o reator metanogênico produzindo (10.000 ml). Entretanto a soma dos volumes de gás do reator acidogênico e metanogênico excede a do reator de controle. Adicionalmente, a composição do metano e do volume de biogás no reator de duas fases é significativo devido que produz 20% mais metano do que o reator controle, representando uma melhoria significativa da abordagem de digestão anaeróbia em fases separadas ou duas fases.

Por outro lado, os resultados de microscopia eletrônica de varredura (MEV) mostram que a superfície da alimentação de cascas de laranja possui uma estrutura lignocelulósica complexa com estruturas porosas irregulares e heterogêneas além de canais e cavidades, podendo corresponder a estruturas de celulose e lignina sem uma direção bem definida. As imagens de MEV obtidas do sólido digerido nos reatores acidogênico, metanogênico e controle, ajudam a visualizar como a comunidade microbiana decompôs a matéria orgânica e a converte em materiais mais simples e dissolvidos, ideais para a digestão, especialmente, comparando imagens do sistema de fases separadas, em comparação com o observado no reator controle, mostrando uma digestão mais robusta conforme maiores concentrações de metano observadas.

Nos cálculos de potencial de geração de eletricidade (GE) e mitigação de emissões de gases de efeito estufa foram calculados, estimou-se que a produção de suco de cítricos no Estado de São Paulo (Brasil) produz aproximadamente $5,7 \times 10^6$ toneladas de cascas de laranja anualmente. Por sua vez, os resultados mostram que o potencial de geração de energia elétrica pode ser de até $97,5 \times 10^3$ MWh / ano de acordo com os dados obtidos na parte experimental. Esta energia gerada a partir do biogás de cascas de laranja poderiaser usada de forma local ou vendida de volta à rede em caso de ter em excesso. A geração de energia elétrica referida calculada foi de 0,0085 kWh / kg de laranja in natura, representando 11% da demanda do processamento industrial de laranja, estimado em 0,077 kWh / kg de laranja in natura processada na indústria de sucos. Por outro lado, a produção de energia elétrica das cascas de laranja reduziria entre $7,5 \times 10^3$ e $9,05 \times 10^3$ tCO2eq/ano, compensando as emissões da indústria cítrica do Estado de São Paulo. Como estratégia para reduzir as pegadas de carbono, os efeitos colaterais ambientais associados às emissões de GEE, bem como economizar custos com a energia elétrica obtida da rede nacional, a digestão anaeróbia em fases separadas é apresentada como uma tecnologia robusta e confiável.

Capítulo 5–Conclusão geral

Conclui-se que este trabalho é original por abordar tecnologias de valorização de resíduos procedente da indústria de processamento de suco de laranja, cascas de laranja, através da digestão anaeróbia mesofílica em reatores do tipo fases separadas visando à obtenção de biogás. Foram avaliados valores de geração de eletricidade (GE) e emissões evitadas de GEE e sua aplicação na indústria cítrica, assim como análise bibliométrica da produção científica global de resíduos agroindustriais de laranja para produzir bioenergia.

A análise bibliométrica indicou que a casca de laranja é um substrato promissor para vários processos biotecnológicos, podendo contribuir para uma indústria mais ecológica, e as principais conclusões obtidas foram:

- Os trabalhos publicados estabeleceram soluções teóricas para o descarte de resíduos de laranja no meio ambiente, e a rede colaborativa internacional foi eficiente no desenvolvimento de muitos produtos industriais com resíduos agroindustriais de laranja;
- As publicações se concentram ao estudo do processo de fermentação para produção de etanol, atividades anti-inflamatórias de flavonoides presentes na casca de laranja, produção de metano por digestão anaeróbia, biorrefinaria, e diferentes técnicas de pré-tratamento;
- As áreas de pesquisa mais trabalhadas foram associadas às seguintes: engenharia (19,2%), biotecnologia/microbiologia aplicada (13,91%), ecologia em ciências ambientais (13,59%), química (12,30%) e combustíveis energéticos (11,65);
- A análise dos principais artigos e autores mostra que aqueles mais citados são os mais antigos, e que o artigo mais citado contém 748 citações com aplicações para a indústria farmacêutica e de alimentos;
- Os trabalhos com maior números de citações estão relacionados com os temas de remoção de corantes de soluções aquosas por casca de laranja, casca de laranja como adsorvente na remoção do ácido violeta 17, efeitos biológicos da hesperidina com atividade anti-inflamatória e analgésica, produção de biocombustíveis (produção de etanol e biogás);
- As principais redes de colaboração entre os autores estão na Índia, Arábia Saudita, Estados Unidos, Suécia, Canadá, Espanha e Itália.
- Os periódicos mais importantes mostram maiores publicações nas áreas de pesquisa relacionadas à biomassa, tratamento de resíduos biológicos, bioenergia,

biotransformações, tecnologias associadas à conversão ou produção, geração de resíduos sólidos, tratamento e descarte e avaliações ambientais;

- Dos países e instituições envolvidos na análise bibliométrica da casca de laranja Espanha é o país mais produtivo em termos de publicações, seguida pela China e Estados Unidos;
- Pré-tratamentos de casca específicos em um conceito de biorrefinaria mostram que é possível obter biocombustíveis como biogás e etanol e outros produtos de alto valor agregado, como pectina e limoneno;
- A queima de biogás poderia ser utilizada pela própria indústria, através da energia elétrica, reduzindo seu custo global, enquanto o excedente de energia eventual poderia ser vendido para a rede, contribuindo para a redução de energia não renovável na matriz energética;
- O trabalho apresenta um diagnóstico útil das tendências de pesquisa em resíduos agroindustriais de laranja que podem subsidiar futuras tomadas de decisão nesse campo da ciência.

A digestão anaeróbica das cascas de laranja como resíduo agroindustrial foi avaliada em reatores de uma e duas fases e as principais conclusões obtidas foram:

- O sistema de duas fases ou fases separadas reduz eficientemente a carga orgânica e produz uma porcentagem maior de metano que o reator de uma fase, com um aumento de 20% na produção de metano;
- Os conteúdos de sólidos voláteis totais do reator acidogênico mostram maior eficiência de redução de 46% até o dia 43, e do reator metanogênico de 50% resultando em uma boa redução de carga orgânica;
- As flutuações de pH são atribuídas à produção de ácidos graxos voláteis sendo os mais produzidos o ácido acético e propiônico no reator acidogênico e no reator metanogênico e controle, os ácidos orgânicos produzidos foram o ácido acético e o butílico;
- A composição do biogás mostra que o conteúdo de hidrogênio do biogás do reator acidogênico com picos de 35% e no reator metanogênico com concentrações máximas próximas de 70%, maiores que o reator controle;

- O volume acumulado de biogás mostra que o reator de controle produz mais gás, 20.000 ml, seguido pelo acidogênico (13.000 ml) e depois o reator metanogênico produzido (10.000 ml), e a soma dos volumes de gás do reator acidogênico e metanogênico excede a do reator de controle;
- A microscopia eletrônica de varredura (MEV) do digerido dos reatores acidogênico, metanogênico e controle mostra que a comunidade microbiana decompôs a matéria orgânica e converteu em materiais mais simples e dissolvidos, ideais para a digestão;
- O reator acidogênico desempenha um papel na redução de compostos recalcitrantes ou inibidores, permitindo produção robusta de metano no reator metanogênico, sem a necessidade de outro pré-tratamento;
- O biogás de casca de laranja, usando a abordagem de reator de duas fases, pode fornecer até 0,4% da energia elétrica utilizada na indústria cítrica do estado de São Paulo, enquanto compensa entre $7{,}5 \times 10^3$ e $9{,}05 \times 10^3$ tCO_2eq / ano, mostrando ser uma tecnologia robusta e confiável para a produção de energia renovável a partir de cascas de laranja;
- A queima de biogás pode subsidiar um mecanismo de desenvolvimento limpo, empregando um suprimento de energia mais renovável para a indústria cítrica, e esta estratégia melhoraria a produção de energia para a redução da pegada de carbono da indústria cítrica.

Além disso, como sugestões para trabalhos futuros se recomenda realizar a quantificação do D-Limoneno para saber exatamente quanto desse composto foi retirado depois de realizar a digestão anaeróbia em fases separadas, testar outro tipo de metodologias para substituir o pré-tratamento na casca de laranja como substrato da digestão anaeróbia, fazer uma análise técnico-econômica do processo de digestão anaeróbia das cascas de laranja com o objetivo de avaliar sua viabilidade, estudar e analisar o biodigerido que fica como resíduo depois do processo de processo para conhecer melhor sua aplicabilidade e características físico-químicas e finalmente,aplicar modelos matemáticos para fazer predições do sistema e para conseguir aumentar a escala do experimento atingindo volumes mais próximos à indústria.

Capítulo 6–Memorial do mestrado

Artigos submetidos a periódicos internacionais

Paper 1: Two-stage anaerobic digestion of orange peel without pre-treatment: experimental evaluation and application to São Paulo state, submetido à revista Journal of Environmental Chemical Engineering.

Paper 2: Bioenergy Production from orange industrial waste: A bibliometric case study, submetido na revista Biofuels, Bioproducts & Biorefining.

Participação em Eventos Internacional:

13º SLACA - Simpósio Latino Americano de Ciência de Alimentos, Campinas, SP, BRASIL, 2019.

Apresentação de pôster em Eventos Internacional:

13º SLACA - Simpósio Latino Americano de Ciência de Alimentos, Vol. 10, pp.1-3, Campinas, SP, BRASIL, 2019.

Capítulo 7– Referências Bibliográficas

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