UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
INSTITUTO DE CIÊNCIAS BÁSICAS DA SAÚDE
PROGRAMA DE PÓS-GRADUAÇÃO EM MICROBIOLOGIA
AGRÍCOLA E DO AMBIENTE

Rodolfo Krüger da Câmara Ribas

POSSIBILIDADES DE CONTROLE MICROBIANO NA ESTOCAGEM SIMULADA DE BIODIESEL E MISTURA B20

Porto Alegre
2023

Rodolfo Krüger da Câmara Ribas

POSSIBILIDADES DE CONTROLE MICROBIANO NA ESTOCAGEM SIMULADA DE BIODIESEL E MISTURA B20

Tese apresentada ao Programa de Pós-Graduação em Microbiologia Agrícola e do Ambiente do Instituto de Ciências Básicas da Saúde da Universidade Federal do Rio Grande do Sul como requisito parcial para a obtenção do título de doutor em Microbiologia Agrícola e do Ambiente.

Orientadora: Profª Drª Fátima Menezes Bento

Porto Alegre

2023

CIP - Catalogação na Publicação

Ribas, Rodolfo Krüger da Câmara
Possibilidades de controle microbiano na estocagem simulada de biodiesel e mistura B20 / Rodolfo Krüger da Câmara Ribas. -- 2023.
104 f.
Orientadora: Fátima Menezes Bento.

Tese (Doutorado) -- Universidade Federal do Rio Grande do Sul, Instituto de Ciências Básicas da Saúde, Programa de Pós-Graduação em Microbiologia Agrícola e do Ambiente, Porto Alegre, BR-RS, 2023.

1. Biodiesel. 2. Biodeterioração. 3. Biocida. 4. Clioquinol. 5. Estanho. I. Bento, Fátima Menezes, orient. II. Título.

Elaborada pelo Sistema de Geração Automática de Ficha Catalográfica da UFRGS com os dados fornecidos pelo(a) autor(a).

SUMÁRIO

ÍNDICE DE FIGURAS

ÍNDICE DE TABELAS

Siglas

°C – Grau Celsius
ABNT – Associação Brasileira de Normas Técnicas
ANP – Agência Nacional do Petróleo, Biocombustíveis e Gás Natural
ASTM – American Society for Testing and Materials
FTIR – Fourier transform infrared spectroscopy
g – grama
GC – Cromatografia Gasosa
GC MS – Cromatografia Gasosa com Espectrômetro de Massas
Kg – quilograma
M – molar
m^3 – Metro Cúbico
mg – miligrama
min – minuto
mL – mililitro
mm – milímetro
PCA – Plate Count Agar
pH – potencial hidrogeniônico
ppm – partes por milhão
TSA – Tryptone Soy Agar
UV – ultravioleta
µL – microlitro
µm – micrômetro

POSSIBILIDADES DE CONTROLE MICROBIANO NA ESTOCAGEM SIMULADA DE BIODIESEL E MISTURA B20

Autor: Rodolfo Krüger da Câmara Ribas

Orientadora: Profª Dra. Fátima Menezes Bento

RESUMO

A mistura de biodiesel ao óleo diesel no Brasil trouxe vantagens ambientais e estratégicas para a matriz energética mundial. No entanto, a presença de água e micro-organismos deteriogênicos no armazenamento causa formação de borra biológica e alterações do combustível, sendo recomendada a drenagem diária da água e adição de preservantes. O objetivo deste trabalho foi avaliar a atividade antimicrobiana residual de compostos organoestânicos, utilizados na síntese de biodiesel e dos antifúngicos clínicos 8-Hidroxiquinolina (8HQ) e Clioquinol (CLQ) - comparando-os com biocidas comerciais. Foram utilizados os microrganismos deteriogênicos *Aureobasidium pullulans*, *Pseudallescheria boydii, Penicillium citrinum, Pseudomonas aeruginosa*, *Bacillus pumilus* e um inóculo segundo norma E1256 (ASTM, 2018) (inóculo ASTM). Foram determinadas as concentrações mínima inibitória (CMI) e biocida (CMB) para estanho IV, CLQ, 8HQ e quatro biocidas comerciais (A, B, C e D). O efeito da depleção de micronutrientes (Ca^{++}, Fe^{++}, Mg^{++}) e da presença de EDTA foi comparado com o dos antifúngicos. O efeito sinérgico entre CLQ e o biocida C foi avaliado com o inóculo ASTM. Os testes foram feitos em placas de 96 poços ou frascos de 20 mL, a 30 ºC por até 28 dias, quando o pH foi medido e a produção de biomassa definida por contagem de Unidades Formadoras de Colônias ou por método gravimétrico. O combustível foi avaliado por análise de Infravermelho (FTIR) e cromatografia gasosa (GC). *B. pumilus* foi inibido pelo estanho, assim como *A. pullulans*. Os produtos A e B não foram eficazes a 2000 ppm. EDTA a 1000 ppm inibiu o inóculo ASTM e estimulou *P. boydii.* A análise de FTIR indicou degradação do combustível, e GC não indicou alteração do teor de ésteres. Em microcosmos, MIC x 8 não ocasionou efeito biocida ou biostático de CLQ ou 8HQ. Uma mistura de morfolinas, apresentou melhor inibição e efeito sinérgico junto a CLQ sobre o inóculo ASTM.

[1]Tese de Doutorado em Microbiologia Agrícola e do Ambiente – Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brasil. (104 p.), março, 2023.

POSSIBILITIES OF MICROBIAL CONTROL IN BIODIESEL AND B20 BLEND SIMULATED STORAGE

Author: Rodolfo Krüger da Câmara Ribas

Advisor: Profª Dra. Fátima Menezes Bento

ABSTRACT

The blending of biodiesel with diesel fuel in Brazil has brought environmental and strategic advantages to the global energy matrix. However, the presence of water and deteriorating microorganisms during storage leads to the formation of biological sludge and fuel alterations, making it advisable to drain water daily and add biocides. The aim of this study was to evaluate the residual antimicrobial activity of organotin compounds used in the synthesis of biodiesel, and of the clinical antifungals 8-Hydroxyquinoline (8HQ) and Clioquinol (CLQ), comparing them with commercial biocides. Deteriogenic microorganisms, including *Aureobasidium pullulans, Pseudallescheria boydii, Penicillium citrinum, Pseudomonas aeruginosa, Bacillus pumilus*, and an inoculum following ASTM E1259 standard (ASTM inoculum), were used. Minimum inhibitory concentrations (MIC) and biocide minimum bactericidal concentrations (MBC) were determined for tin IV, CLQ, 8HQ, and four commercial biocides (A, B, C, and D). The effects of micronutrient depletion (Ca^{++}, Fe^{++}, Mg^{++}) and the presence of EDTA were compared to those of the antifungals. The synergistic effect between CLQ and biocide C was evaluated using the ASTM inoculum. The tests were performed in 96-well plates or 20 mL vials at 30°C for up to 28 days, during which the pH was measured, and biomass production was determined by counting Colony-Forming Units or using gravimetric methods. The fuel was assessed by Infrared Analysis (FTIR) and gas chromatography (GC). B. pumilus was inhibited by tin, as was A. pullulans. Products A and B were not effective at 2000 ppm. EDTA at 1000 ppm inhibited the ASTM inoculum and stimulated P. boydii. FTIR analysis indicated fuel degradation, while GC did not show any alteration in ester content. In microcosms, MIC x 8 did not induce a biocidal or biostatic effect of CLQ or 8HQ. A mixture of morpholines showed better inhibition and synergistic effect when combined with CLQ on the ASTM inoculum.

[1]Doctoral Dissertation in Agricultural and Environmental Microbiology – Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil. (104 p.) march 2023.

1. INTRODUÇÃO

Combustíveis derivados do petróleo, como o óleo diesel, gasolina e o querosene de aviação, têm sido a principal fonte energética utilizada no mundo, porém, a busca por alternativas renováveis e de menor impacto ambiental é uma realidade. Neste sentido, desde 2008, o óleo diesel comercializado no Brasil é classificado como tipos A como o óleo diesel marítimo (sem biodiesel) ou tipo B, óleo diesel rodoviário (com adição de biodiesel). O percentual mínimo de biodiesel adicionado ao óleo diesel em 2023 é de 12% (constituindo a mistura B12) e a máxima de 15% (mistura B15). Com a implementação da Política Nacional de Biocombustíveis (RENOVABIO), existe uma previsão de aumento deste teor, tornando a mistura B15 obrigatória em 2023. No Brasil foram produzidos 6,3 bilhões de litros de biodiesel em 2022, colocando o Brasil como terceiro maior produtor e consumidor de biodiesel no mercado internacional.

Para a liberação e comercialização no mercado, 24 análises são mandatórias para o biodiesel, segundo a Resolução Nº 45 de 2014 (e devidas alterações) da Agência Nacional de Petróleo e Biocombustíveis. Caso o biodiesel não venha a ser comercializado no prazo de 30 dias após a emissão do Certificado de Qualidade pelo fabricante, o mesmo deverá ser submetido a novas análises. Devido à natureza do biocombustível, composto por ésteres de ácidos graxos, o biodiesel possui maior biodegradabilidade do que o diesel. Em caso de contaminação ambiental essa propriedade é considerada uma vantagem, mas é reconhecida como uma vulnerabilidade durante o armazenamento. Na medida que microrganismos estão presentes no combustível, e em situações de alta densidade microbiana e longos períodos de residência do combustível nos tanques pode ocorrer o comprometimento da qualidade final do produto.

Com a introdução de biodiesel na mistura com o diesel rodoviário verificou-se uma maior suscetibilidade à biodeterioração, devido principalmente a natureza da molécula e a higroscopicidade do biodiesel, resultando em maior incorporação de água durante o armazenamento. Como consequência, foi constatado, desde a introdução do biodiesel em misturas (diesel/biodiesel), um aumento na geração de sedimentos de natureza biológica e/ou química, com o comprometimento da qualidade final do produto armazenado. Estudos sobre o potencial de crescimento de isolados fúngicos filamentosos de lodos biológicos têm mostrado que este grupo de microrganismos apresenta capacidade de degradar hidrocarbonetos e ésteres de ácidos graxos, vantagem metabólica relacionada à presença de genes relacionados à degradação e captação e à grande superfície de contato formada pelas hifas. O micélio pode formar emaranhados de biomassa, ocasionando entupimento de filtros e bombas em tanques e caminhões. As consequências da contaminação microbiana durante o armazenamento estão associadas diretamente ao tempo de residência do

combustível e as rotinas de manutenção adotadas, como drenagens frequentes e limpeza dos tanques, como forma de minimizar a presença de água no fundo dos tanques.

Como estratégia de controle químico de microrganismos são utilizados biocidas, compostos químicos (orgânicos e/ou inorgânicos) com liberação de uso em outros países como Estados Unidos. No Brasil, vários estudos foram conduzidos avaliando a efetividade de moléculas naturais e/ou artificiais quanto ao potencial antimicrobiano. Os produtos devem atender a exigências importantes para o uso em combustível, tais como amplo espectro de ação (fungos e bactérias), coeficiente de partição que permita a solubilidade em ambas as fases óleo-água, baixo impacto ambiental e toxicológico. Dentre os compostos comerciais conhecidos com ação antimicrobiana estão as oxazolidinas, isotiazolonas e morfolinas. Compostos organoestânicos também são conhecidos como antimicrobianos, pelo que neste trabalho foi avaliado o efeito residual de um catalisador a base de estanho (síntese de biodiesel) sobre microrganismos deteriogênicos de diesel-biodiesel. Outro grupo de moléculas avaliado foram as quinolinas. A molécula mais conhecida desta classe é o clioquinol (5-cloro-7-iodo-8-hidroxiquinolina), um derivado de 8-Hidroxiquinolina (8HQ). Tanto o CLQ quanto a 8HQ possuem baixa irritabilidade e toxicidade tópica, bem como alta eficácia. O mecanismo de ação ocorre especialmente sobre a parede fúngica, atuando sobre a formação de hifas e pseudohifas**,** o que torna a molécula promissora como biocida contra fungos deteriogênicos.

2. HIPÓTESES:

✓ A presença residual de catalisadores organoestânicos utilizados na síntese de biodiesel pode ter atividade antimicrobiana.

✓ O uso de clioquinol ou hidroxiquinolina como aditivo no armazenamento de biodiesel e mistura B20 pode ter potencial como antimicrobiano, sem provocar alterações nas especificações dos combustíveis.

✓ Os antifúngicos clioquinol e/ou 8-hidroxiquinolina podem apresentar maior efetividade do que biocidas comerciais (Isotiazolonas e Morfolinas) na preservação de combustíveis armazenados.

3. OBJETIVOS

3.1 Objetivo Geral

Avaliar os efeitos antimicrobianos do resíduo de um catalisador a base de estanho utilizado na síntese de biodiesel, de moléculas derivadas do clioquinol sobre fungos deteriogênicos em biodiesel puro e na mistura diesel e biodiesel (B20) e de biocidas comerciais disponíveis no mercado para controle microbiano no armazenamento.

3.2 Objetivos Específicos

3.2.1 Avaliar a atividade biocida e/ou bioestática residual de um catalisador organoestânico utilizado na síntese de biodiesel de soja sobre um inóculo não caracterizado segundo norma ASTM 1259-18, sobre os fungos *Aureobasidium pullulans* e *Pseudallescheria boydii* e sobre as bactérias *Pseudomonas aeruginosa* e *Bacillus pumilus*, em microcosmos de 10 mL (estocagem simulada), observando a deterioração abiótica do combustível em comparação com a degradação observada no combustível inoculado.

3.2.2 Avaliar a atividade fungicida e/ou fungistática de 8-hidroxiquinolina e clioquinol (determinando Concentração Mínima Inibitória e Concentração Mínima Biocida), em condições ideais e em estocagem simulada (microcosmos) com um inóculo não caracterizado (Norma ASTM 1259-18) e com os fungos *Aureobasidium pullulans*, *Pseudallescheria boydii* e *Penicillium citrinum.*

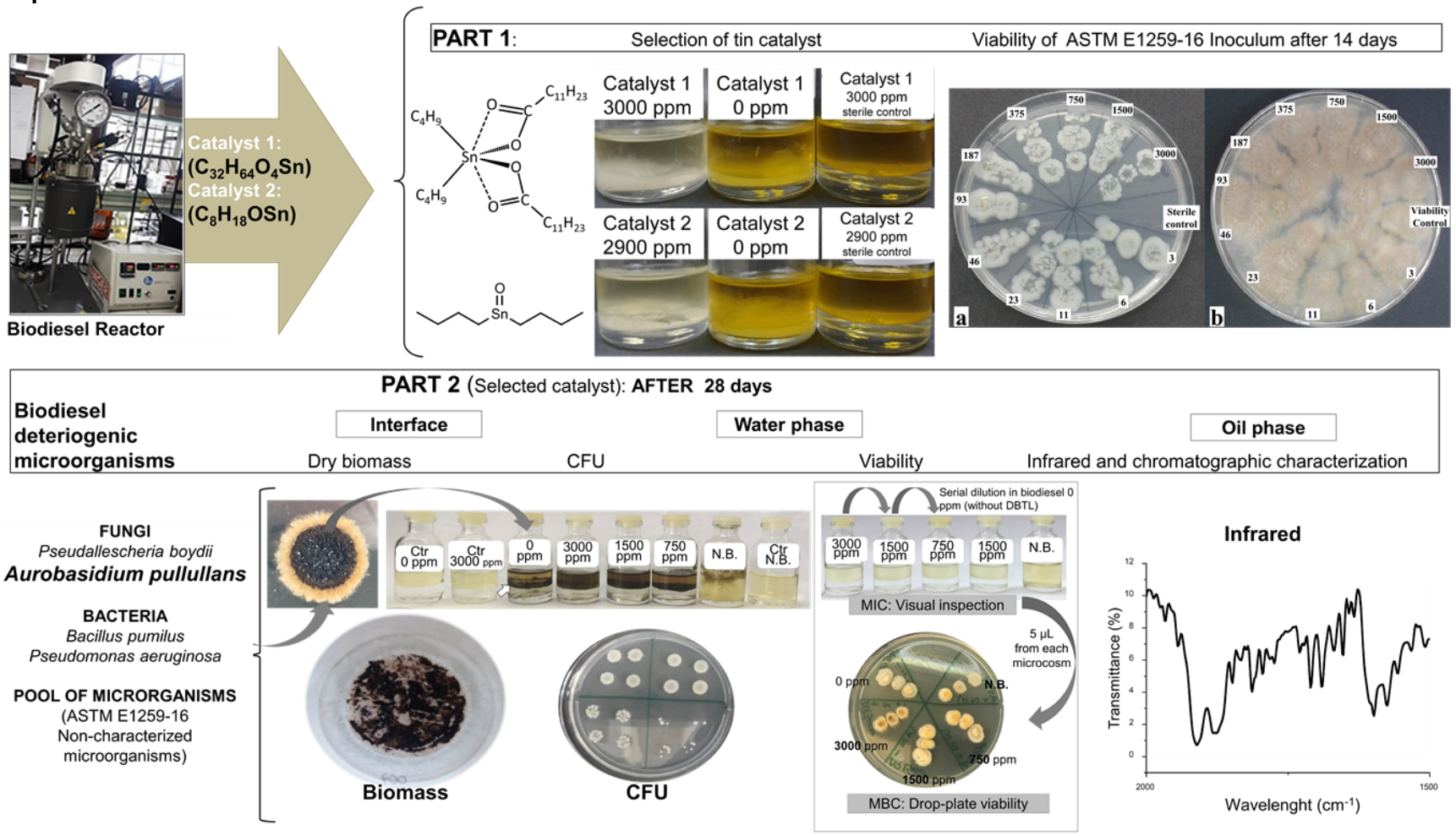
3.2.3 Avaliar atividade fungicida e/ou fungistática de compostos disponíveis comercialmente (determinando Concentração Mínima Inibitória e Concentração Mínima Biocida), em condições ideais e em estocagem simulada (microcosmos) com um inóculo não caracterizado (Norma ASTM 1259-18) e com os fungos *Aureobasidium pullulans*, e *Pseudallescheria boydii*.

3.2.4 Avaliar a presença de efeito sinérgico de biocidas comerciais com 8-hidroxiquinolina ou clioquinol.

5. CAPÍTULO I - Avaliação do efeito antimicrobiano de um catalisador organoestânico sobre microrganismos deteriogênicos e inóculo não-caracterizado em biodiesel (B100)

O artigo **Characterization of antimicrobial effect of organotin-based catalysts on diesel–biodiesel deteriogenic microorganisms** está redigido em inglês, foi formatado segundo as normas da revista **Environmental Monitoring and Assessment** e publicado em dezembro de 2020 sob o DOI https://doi.org/10.1007/s10661-020-08744-x

Graphical Abstract:



Environ Monit Assess (2020) 192: 802
https://doi.org/10.1007/s10661-020-08744-x

Characterization of antimicrobial effect of organotin-based catalysts on diesel–biodiesel deteriogenic microorganisms

Rodolfo Ribas & Juciana Clarice Cazarolli & Eid Cavalcante da Silva & Mario Roberto Meneghetti & Simoni Margareti Plentz Meneghetti & Fátima Menezes Bento

Received: 21 June 2020 / Accepted: 10 November 2020 / Published online: 1 December 2020

Abstract Organotin compounds are applied in several industrial reactions and can present antifungal and antibac- terial activities. Incorrect handling and storage practices of biodiesel and diesel–biodiesel blends can lead to microbial development, impacting its final quality. Concerning this problem, this work investigated the antimicrobial action of two organotin catalysts used in biodiesel production with four isolated microorganisms (*Bacillus pumilus*, *Pseu- domonas aeruginosa*, *Pseudallescheria boydii*, and *Aureobasidium pullulans*) and a pool of microorganisms (ASTM E1259 standard practice). Samples of soybean biodiesel with different concentrations of dibutyl tin dilaurate (catalyst 1) and di-n-butyl-oxo-stannane (catalyst 2) were prepared and added of mineral medium. The pool of microorganisms was inoculated and incubated at 30 °C and final biomass was weighed after 14 days. Thereafter, soybean biodiesel with catalyst 2 was used. Fungal bio- mass was weighted, and plate count was used to assess bacterial growth. Results show that catalysts 1 and 2 presented no inhibitory activity on the pool of microor- ganisms evaluated. A slight inhibitory activity was ob- served for *B. pumilus* and *A. pullulans* growth, but not for *P. boydii*, *P. aeruginosa*, or the pool of microorgan- isms. All experiments exhibited acidification higher than sterile control. Infrared analysis shows less microbiological degradation products in the tin-protected fuel with ASTM inoculum. These results suggest that these tin-based cata- lysts show no toxic effect on native microbial population and a slight effect on some isolated microbial population in laboratory scale and for the first time shows that these organotin compounds can be employed safely as biodiesel catalyst.

Keywords ASTM . Biocide . Biodeterioration . Microbial control . Preservative . Soybean

R. Ribas (*) : J. C. Cazarolli : F. M. Bento

Department of Microbiology, Immunology and Parasitology,
Fuels and Biofuels Biodeterioration Laboratory (LAB-BIO), Federal University of Rio Grande do Sul, Sarmento Leite Street 500, Porto Alegre, RS 90050-170, Brazil
e-mail: rodolfo.ribas@gmail.com

E. C. da Silva
Technology Center (CTEC), Federal University of Alagoas, Av. Lourival de Melo Mota, s/n°, Maceió, AL 57072-970, Brazil

M. R. Meneghetti : S. M. P. Meneghetti

Institute of Chemistry and Biotechnology, Group of Catalysis and Chemical Reactivity (GCAR), Federal University of Alagoas, Av. Lourival de Melo Mota, s/n°, Maceió, AL 57072-970, Brazil

Introduction

Brazil has increasingly added biodiesel to fossil diesel, and the National Biofuel policy—RenovaBio—promotes the use of a series of biofuels and at the same time encourages the obtention of Decarbonization Credits—i.e., the differ- ence in emissions between fossil fuels and biofuel (Salina et al., 2020). The use of biodiesel in Brazil started with a compulsory blend of 2% in fossil diesel in 2008 (B2), and today the mandatory blend has at least 12% biodiesel, and until 15% is allowed (RESOLUÇÃO Nº 16, DE 29 DE OUTUBRO DE 2018, 2018). The presence of biodiesel in diesel fuel is therefore a reality, which brings environmen- tal and strategic advantages. However, there are still chal- lenges to be overcome, such as the possibility of biodiesel deterioration in its transportation and storage (Passman, 2013;

Bücker et al., 2014, 2018; Bento et al., 2016; Cazarolli et al., 2020). One of the major problems ob- served in commercial use of biodiesel is its instability, which can occur during transport and storage under incor- rect conditions, compromising the final quality of the biofuel. The presence of free water in the fuels during its transport and storage systems can accelerate both abiotic (oxidation and hydrolysis) and biotic (microbial participa- tion) deterioration processes (Atadashi et al., 2013; Salina et al., 2020). Biodiesel has an amphiphilic nature, which increases the dissolution rate of diesel in water, facilitating its dissolution in water and microbial development (Gupta, 2020). Biodiesel is chemically defined as a mixture of monoalkyl fatty acid esters obtained by transesterification of vegetable or animal oils and fats with methanol or ethanol in the presence of a catalyst (acid or base) (Knothe & Gerpen, 2005; Nautiyal et al., 2020). The presence of water in tank bottoms is the most important factor influencing the extent of microbial growth in fuel tanks and systems. It is well documented that microorgan- isms (aerobic and anaerobic bacteria; filamentous fungi, yeasts) will find appropriate conditions for growth and they are most metabolically active at the oil–water inter- face (Fátima Menezes Bento et al., 2016; Cazarolli et al., 2020; Passman, 2013; Zimmer et al., 2017). Good house- keeping is recommended in Brazil by following Brazilian Standards such as ABNT NBR 15512 (ABNT, 2014) and 16732-19 (ABNT, 2019). The imperative for regular draining of water and the addition of chemical compounds exhibiting antimicrobial activity (biocides), to prevent or kill microorganisms are also cited. However, in Brazil, the use of biocides is still under evaluation, as there are still many clarifications to be made concerning their use and disposal in the environment (Luz et al., 2018). A chemical strategy in controlling microbial growth is the use of additives and/or antimicrobial compounds that can act as a fuel preservative at all stages of product handling (Passman, 2013).

Organotin compounds are used in many catalytic activities in industry (Hadi et al., 2019; Zubair et al., 2019) and have a broad spectrum of biological activity, especially as antifungal and antibacterial (Adeyemi et al., 2019; Butt et al., 2019; Hadi et al., 2019; Iqbal et al., 2015; Jamil et al., 2009; Javed et al., 2017). Biodiesel can be produced through several processes but transesterification, either by alkaline or acid cataly- sis, is typically used (Atadashi et al., 2013; Lam et al., 2010; Thangaraj et al., 2019). It is noteworthy that the class of organotins bearing four substituents groups, i.e., organotin (IV) compounds, are extensively consumed by timber, plastics, and paint industries in manufactur- ing processes as PVC polymerization and stabilization, as well as building of heat-insulating and waterproof materials., catalysis of rubber, polymide foams, and polyurethane (PU) (Erofeev et al., 2016; Finnegan et al., 2018; Matveeva et al., 2018; Yousif et al., 2016). It has been shown that organotin (IV) compounds also can serve as excellent catalysts for biodiesel transesterification and esterification, with many advan- tages over traditional ones such as no need for neutral- ization, no residual effluent production, little residual concentration in the final product, or even its immobili- zation in a heterogeneous phase (Hadi et al., 2019; Meneghetti et al., 2013; Zubair et al., 2019).

Organotin (IV) compounds can be broadly catego- rized in four types: R_4Sn (type I); R_3SnX (type II); R_2SnX_z (type III); $RSnX_3$ (type IV), R representing an organic substituent linked to the tin atom by a tin– carbon bond, and X standing for a substituent (organic or not). The antimicrobial activity of tin (IV) is reported in many organic associations (Adeyemi et al., 2019; Ashfaq et al., 2004; Butt et al., 2019; Hadi et al., 2019; Jamil et al., 2009; Javed et al., 2017). As it has been long shown, depending on its substituents, its antimicrobial activity will vary (Van der Kerk & Luijten, 1954). For instance, triorganotin compounds (bearing three C-Sn bonds) tend to be more toxic than its counterparts bear- ing two substituents (Appel, 2004; Barbosa et al., 2018; Hadi et al., 2019; Hoch, 2001; Song et al., 2006). Actually, antimicrobial activity seems to be directly proportional to R substituent, with little impact of X substituent (Baul, 2008; Doctor & Fox, 1982; Hadi et al., 2019; Pellerito et al., 2006).

Regarding its substituents, it has been observed that alkyl (saturated linear-derived) are more toxic than aryl (aromatic-derived) (Doctor & Fox, 1982; Hadi et al., 2019). It is also reported that the hydrophobicity of the entire molecule is directly related to toxicity (Adeyemi et al., 2018)—for a high antifungal activity the total number of carbon atoms in the alkyl groups of a trialkyl alkyltin compound should be about 9–12 for instance (Hadi et al., 2019). This hydrophobicity is related to the cell membrane permeability. Depending on the substituent present, it binds to CoA-binding pockets (active sites) causing different effects once inside the cell: it is possible that organotin molecules either bind to meta- bolic crucial proteins or to the double-stranded DNA in an intercalate form (Butt et al., 2019; Lu et al., 2017). In fact, energy metabolism impairment in abalones by tributyltin along with a series of secondary metabolites accumulation suggests impairment of several metabolic pathways (Javed et al., 2017; Lu et al.,

2017), while DNA binding by butyltin compounds is also related (Barbosa et al., 2018; Butt et al., 2019; Iftikhar et al., 2018), as well as histones (Osada et al., 2005). Dibutyltin compounds show excellent catalytic activity in biodiesel production (Mônica A. da Silva et al., 2019; Mônica Araújo da Silva et al., 2017; Meneghetti et al., 2013) and less hazardous effects than tributyltin (Boyer, 1989; Hoch, 2001; Iqbal et al., 2015). Considering the promising advantages of catalysis using diorganotin (IV) complexes are its efficiency, safety, many aspects related to antimicrobial properties of biodiesel produced in those conditions are still unknown. The aim of this work was to evaluate the antimicrobial activity of organometallic (IV) tin-based catalysts used in the pro- duction of biodiesel. Two catalysts were evaluated on an uncharacterized inoculum according to ASTM E1259- 18 standard (ASTM, 2018) and the best catalyst was also tested on a filamentous fungus (*Pseudallescheria boydii*), a yeast-like fungus (*Aureobasidium pullulans*), a Gram-negative bacterium (*Pseudomonas aeruginosa*), and a Gram-positive bacterium (*Bacillus pumilus*) as well as on uncharacterized inoculum.

Materials and methods

Biodiesel samples production using tin (IV) catalysts Biodiesel samples were obtained using an

methanol:oil:catalyst molar ratio of 400:100:1 at 150

°C, in a 100-mL stainless steel reactor with stirring and temperature control (Parr Instrument Company, 4590 series). After 2 h of reaction, the reaction mixture was washed three times with distilled water, dried in the presence of $MgSO_4$, and centrifuged. The catalysts used were dibutyl tin dilaurate (Bu_2Sn (Lau)$_2$, 1—CAS nr. 77-58-7), di-*n*-butyl-oxo-stannane (Bu_2SnO, 2—CAS nr. 818-08-6), and sodium methoxide (CAS nr 124-41-

4) to comparison. The yield of the transesterification reaction was determined by HPLC and expressed in terms of the percentage of fatty acid methyl esters (% FAMEs) produced. The amount of Sn present in the samples was determined by ICP-OS. All biodiesel sam- ples used for microbiological tests were sterilized using 0.22-μm membranes (Millipore, USA) in a kitassato system (sterilized as well) as soon as received and kept at 4 °C in glass bottles protected from light by aluminum foil for until one month for microbiological testing.

Microorganisms

Four deteriogenic biodiesel microorganisms were used: a filamentous fungus (*Pseudallescheria boydii*), a yeast- like fungus (*Aureobasidium pullulans*), a Gram- negative bacterium (*Pseudomonas aeruginosa*), and a Gram-positive bacterium (*Bacillus pumilus*). An uncharacterized inoculum was also tested according to ASTM E1259 standard practice (ASTM, 2018).

Fungi were identified by sequencing of the ITS re- gion, *B. pumilus* by sequencing of the 18S region of the rRNA, and *P. aeruginosa*, a clinical isolate identified by MALDI-TOF. The final inoculum concentration was 10^5 cells or spores mL^{-1}.

Preparation of inocula

The bacterial inocula (*B. pumilus* and *P. aeruginosa*) were prepared from culture in tryptic soy broth (TSB) after 24 h stirring (200 rpm) and incubation at 30 °C. The inocula of *P. boydii* and *A. pullulans* were prepared from 7 day cultivation cultures on malt agar in slanted tubes in incubator at 28 °C by adding 2 mL of sterile (0.85%) saline and 2 mL of Tween 80 liquid solution (0.01%). Uncharacterized inoculum was prepared ac- cording to ASTM E1259 (ASTM, 2018). Briefly, 2% of B100 mixture (previously filter-sterilized) was added to 100 mL Bushnell-Haas mineral medium in an Erlen- meyer flask, which was then inoculated with 5 mL of microbiological sludge from a B100 tank. The flask was incubated in an orbital shaker at 29 ± 1 °C, 200 rpm for 7 days. The final concentration of all inocula was 10^5 cells or spores mL^{-1}.

Microcosm preparation

Microcosm triplicates were prepared in 20-mL glass vials containing 4 mL of the oil phase (biodiesel with or without DBTDL) and 6 mL of Bushnell-Haas (BH) mineral broth as the aqueous phase. Three concentra- tions of DBTDL (3000, 1500, and 750 ppm) were tested

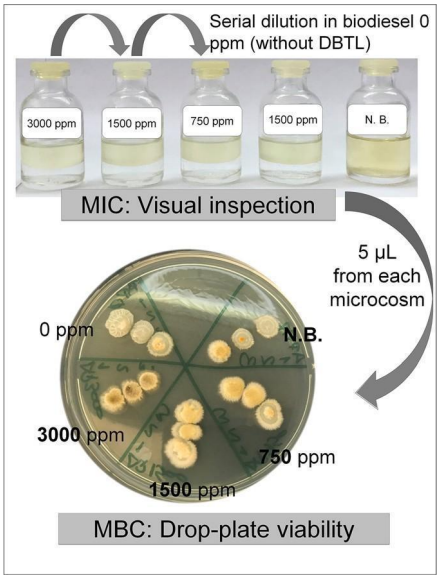


Fig. 1 Procedure used for the assembly and evaluation of the inhibitory and biocidal concentration of two tin-based catalysts used for biodiesel production with microorganisms during 14 days for bacteria and 28 days for fungi

and compared with biodiesel produced without the tin- based catalyst (0 ppm). Also, viability controls were made using TSB (bacteria) or Sabouraud broth (fungi and ASTM inoculum). All flasks were kept at 30 °C for 14 days (bacteria) or 28 days (fungi and ASTM inocu- lum) and analyzed by visual inspection and drop plate viability tests after 24, 48, and 72 h; 5, 7, and 14 days (bacteria); 21 and 28 days (fungi and ASTM inoculum). Drop plate tests were performed to evaluate MIC, inoc- ulating 5 µL of the microcosm aqueous phase in agar (Sabouraud for fungi, TSA for bacteria), and visual inspection of biomass production was made to evaluate MBC. Final biomass production was defined by cell- forming unit (CFU) count (bacteria) or gravimetric

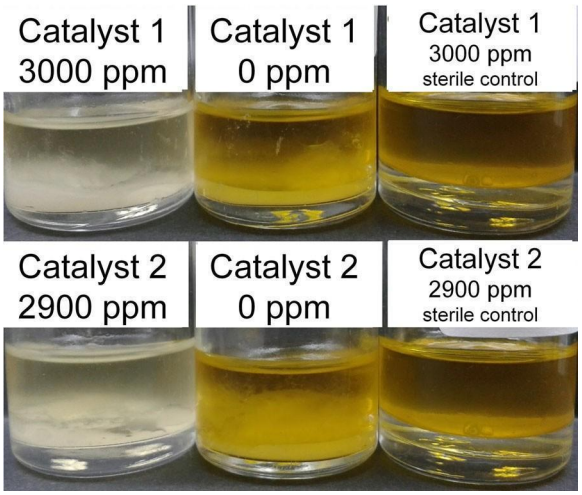


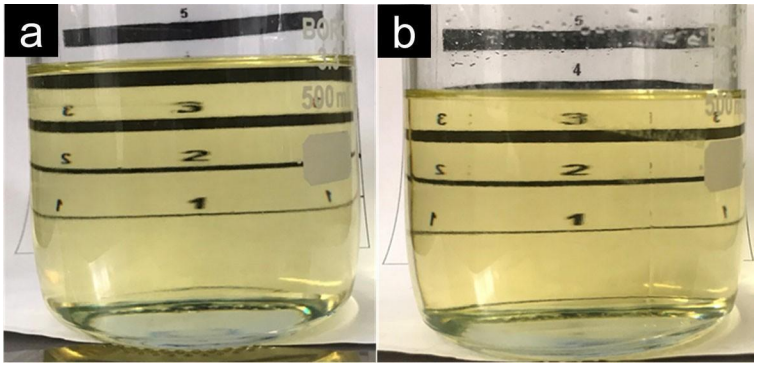
Fig. 3 Microcosm appearance of biodiesel produced with catalyst 1 (3000 ppm), catalyst 2 (2900 ppm), and without (0 ppm) tin- based catalyst in the presence of ASTM E1259 inoculum after 14 days. Sterile control contains no inocula

method after a 14-µm membrane filtration (fungi and uncharacterized inoculum).

CFU count—bacteria

After 14 days, an aliquot of the aqueous phase was serially diluted from 10^{-1} to 10^{-9} and 5 µL of each sample was deposited in triplicates of plate count agar plates. This drop plate method allows one to infer the final cell count of each bacterial microcosm similarly to a spread plate technique (Herigstad et al., 2001). Tripli- cate values were averaged and different dilutions were corrected accordingly. These procedures are summa- rized in Fig. 1.

Fig. 2 Visual aspect on HAZE scale according to ASTM D4176 (04-2019) (ASTM International. American Society for Testing and Materials., 2019): a Biodiesel without tin and b biodiesel pro- duced with $Bu_2Sn(Lau)_2$ con- taining 3000 ppm of tin



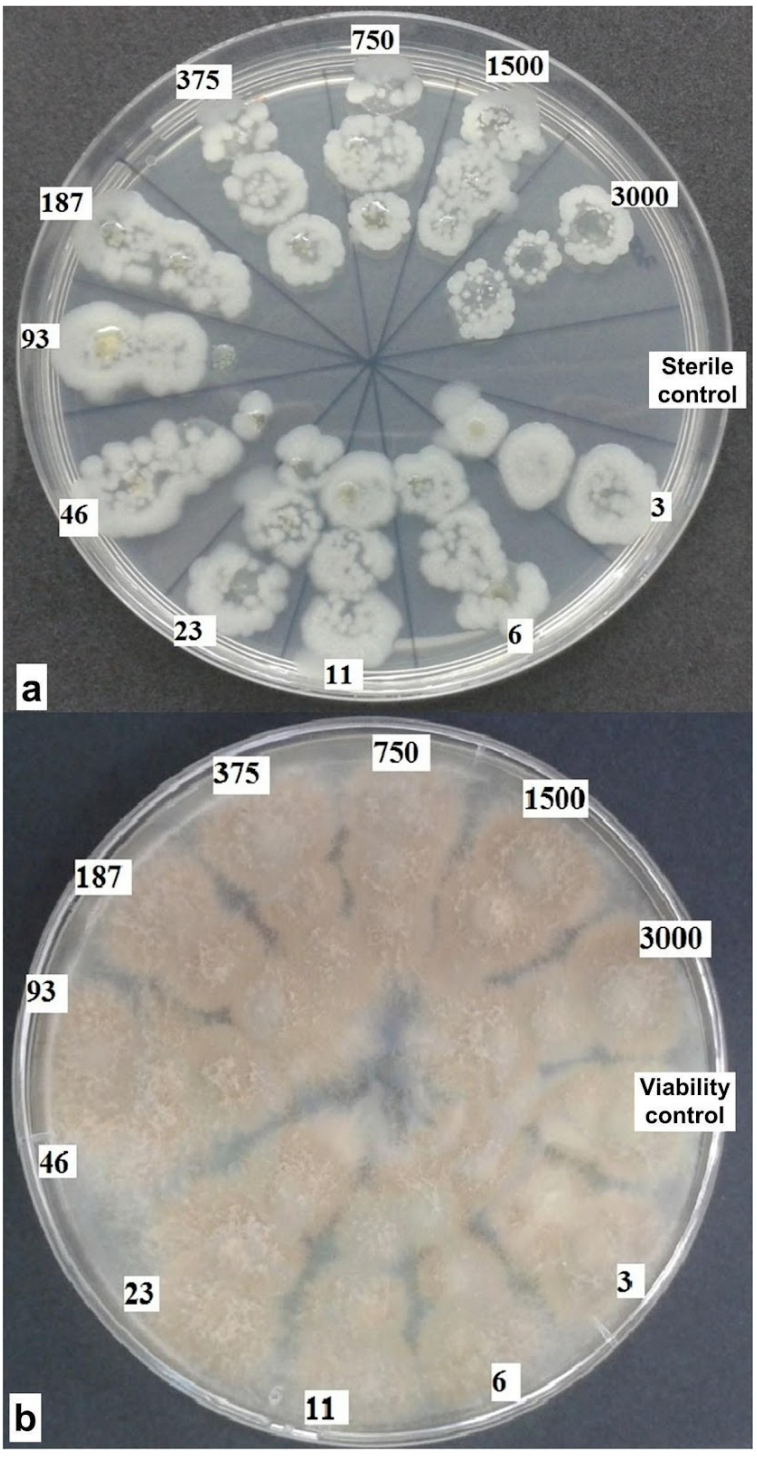


Fig. 4 Viability tests through drop plate technique in plate count agar (a) and potato dextrose agar (b). Numbers indicate concentration of organotin catalyst. Viability control contains no organotin compound

ppm →	3000	1500	750	375	187	93	46	23	11	6	0
Catalyst 1	31.3	37.1	29.7	26.4	35.4	26.8	26.6	22.3	30.9	23.9	21.9
Catalyst 2	38.1	31.2	25.1	31.2	29.6	27.5	27.8	22.8	26.3	29.4	27.7

Table 1 Biomass amount for each concentration tested of catalysts 1 and 2; (ppm), parts per million of each catalyst tested Biomass amount (mg)

Biomass weight—fungi and ASTM inocula

After 28 days, the biomass formed was filtered through previously weighed filter paper. To remove adhered fuel from the biomass, discs were filter- washed with 3 ml of hexane. They were then placed at 30 °C for 48 h and transferred to a dehydrating chamber for 24 h to remove water, and the dry weight was recorded. Biomass weight was calculated as final weight minus initial weight (in milligrams); triplicate values were averaged.

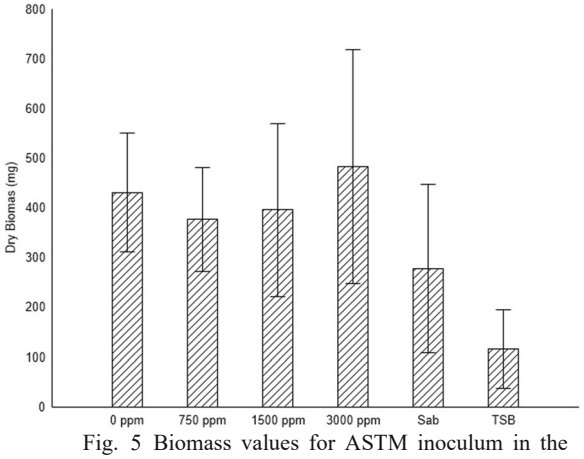


Fig. 5 Biomass values for ASTM inoculum in the presence (3000, 1500, and 750 ppm), and without tin (0 ppm) and viability control (SAB, Sabouraud nutrient broth; TSB, tryptic soy broth) after 28 days. Upper lines indicate standard error ($p = 0.05$)

Chromatographic analysis

The fatty acid compositions of the samples were determined by gas chromatography (GC) using the AOCS official methods Ce 1-62 and Ce 2-26 (AOCS, 1998) and, the instrumental precision of the method was evaluated through 5 injections of a sample, with the determination of the standard deviation.

Infrared analysis

Samples of the oil phase from initial and final times were analyzed by infrared spectroscopy with Fourier transform coupled to an attenuated total reflectance accessory (FTIR/ATR) in the Nicolet IR200 spec- trometer (Thermo Fisher, USA) with a zinc selenide crystal (ZnSe). The spectra were obtained in the range of 4000–900 cm^{-1}, with a resolution of 4 cm^{-1} and 32 scans at 25 °C.

ICP-OES characterization

The amount of tin was determined by inductively coupled plasma optical emission spectrometry (ICP

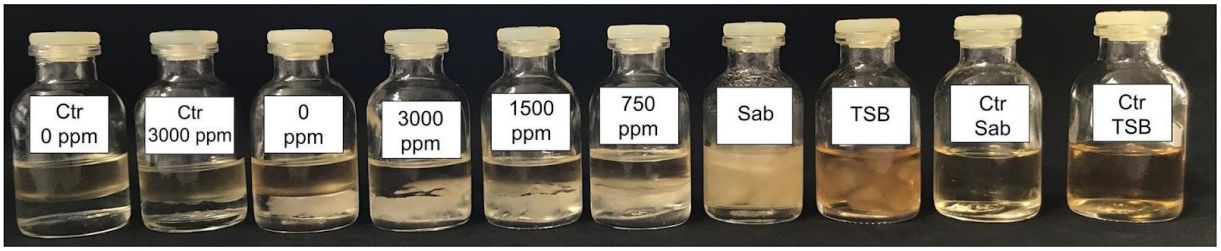


Fig. 6 Microcosm appearance of biodiesel produced with (3000, 1500, and 750 ppm) and without (0 ppm) tin-based catalyst in the presence of ASTM E1259 inoculum after 28 days. Sab, Sabouraud nutrient broth viability control. TSB, tryptic soy broth viability control. Ctr, Sterile controls

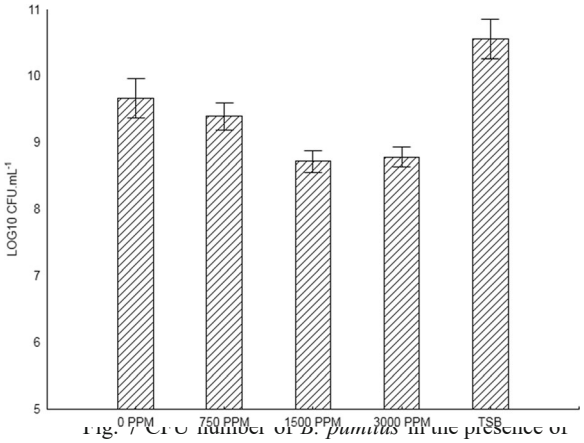


Fig. 7 CFU number of *B. pumilus* in the presence of tin (3000, 1500, and 750 ppm), without tin (0 ppm) and in the positive control (TSB) after 28 days. Upper lines indicate standard error (p = 0.05). *X*-axis initial cell inoculum of 10^5 cells mL^{-1}

OES) using Spectro Arcos equipment, after acid diges- tion of the samples.

Statistics

Statistical analyses of HRGC peaks percentage, pH, and biomass values (analysis of variance; Tukey test, p = 5%; biomass values used p corrected by Sidak), were performed with PAST (version 3.25). Microbial growth and pH graphics were built using Statistica 10 (Statsoft Inc., 2011).

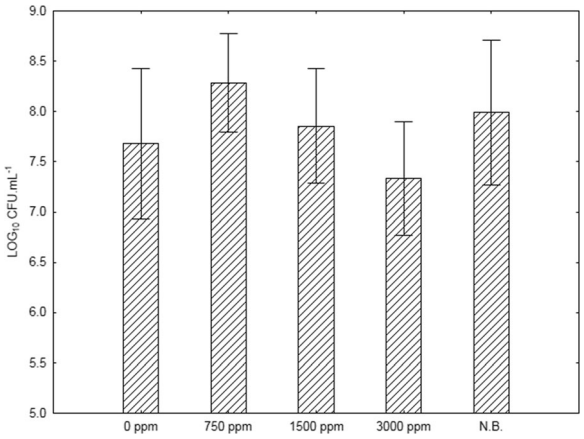


Fig. 8 CFU number of *P. aeruginosa* in the presence of tin (3000, 1500, and 750 ppm), without tin (0 ppm) and in the positive control (N.B) after 28 days. Upper lines indicate standard error (p = 0.05). *X*- axis corresponds to initial cell inoculum of 10^5 cells mL

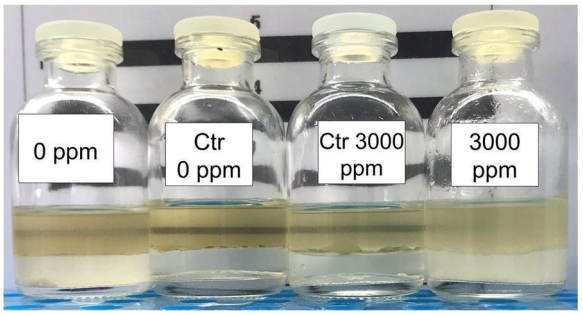


Fig. 9 Microcosm appearance of biodiesel produced with (3000 ppm) and without (0 ppm) tin-based catalyst, in the presence of *Pseudomonas aeruginosa* after 28 days. Ctr, sterile control. Lines behind flasks are from ASTM haze rating chart. Line number 2 is shown right behind the oil (upper) phase

Results and discussion

Biodiesel production and characterization

Biodiesel samples were obtained with > 96% ester con- tent (as determined by HPLC) using dibutyl tin dilaurate (Bu_2Sn $(Lau)_2$, 1) and di-*n*-butyl-oxo-stannane (Bu_2SnO, 2) as catalysts and, the tin content, determined by ICP-OS, was 3000 ppm in both samples. A sample of biodiesel produced using sodium methoxide as catalyst was used as comparison. Biodiesel characterization is in accordance to ANP specifications, and the ester content of 97.8%, 98.0%, and 97.5% were found for biodiesel produced using 1, 2, and sodium methoxide as catalyst, respectively. Concerning the viscosity and density values of 4.4, 4.7, and 4.5 mm2 s^{-1} and 887, 886, and

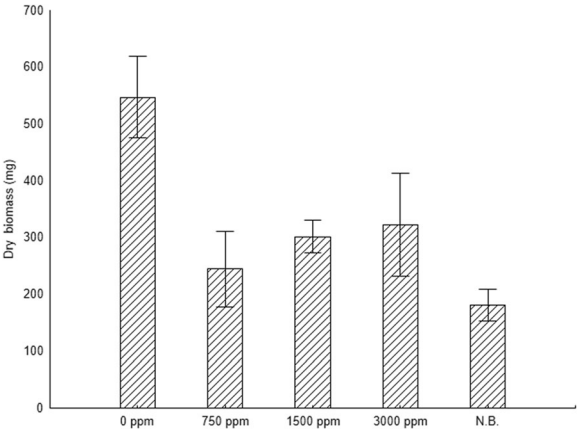


Fig. 10 Biomass values for *Aureobasidium pullulans* in the pres- ence (3000, 1500, and 750 ppm), and without tin (0 ppm) and viability control (N.B.—Sabouraud nutrient broth) after 28 days. Upper lines indicate standard error (p = 0.05)

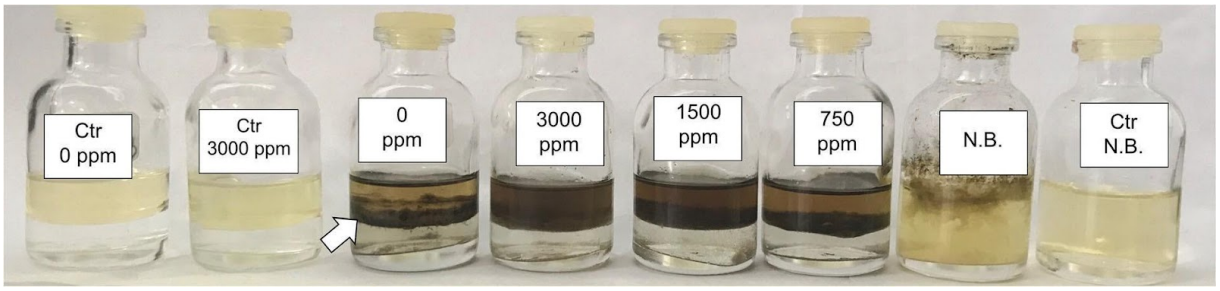


Fig. 11 Microcosm appearance of biodiesel produced with (3000, 1500 and 750 ppm) and without (0 ppm) tin-based catalyst in the presence of *A. pullulans* after 28 days. Arrow, melanin production. N.B., Sabouraud nutrient broth viability control. Ctr, sterile control vials

885 kg/m−^{3} were determined, respectively, for biodiesel produced using 1, 2, and sodium methoxide catalysts.

The visual aspect of control and the biofuels used in this experiment are in according to ASTM D4176 (04- 2019) HAZE scale (ASTM, 2019) for biodiesel produced using sodium methoxide and $Bu_2Sn\ (Lau)_2$ (Fig. 2).

Uncharacterized inoculum

Initially, the two catalysts (1 and 2) were tested only against the ASTM inoculum, exhibiting no inhibitory effect as evidenced by visual inspection (Figure 3) as well as viability tests (drop plate—Fig. 4) and biomass values (Table 1).

In the second part of the study, only biodiesel pro- duced by 1 ($Bu_2Sn\ (Lau)_2$) and sodium methoxide cat- alysts were used. Similar results to the previous exper- iment were observed: visible biomass formation was observed in all ASTM E1259 inocula (Fig. 5) as it can be confirmed by dry biomass results (Fig. 6).

Bacteria

After 14 days, the bacterium *B. pumilus* showed inhibi- tion proportional to the tin concentration (Fig. 7).

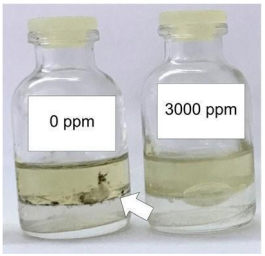


Fig. 12 Detail of microcosm appearance of biodiesel produced without (0 ppm) and with 3000 ppm tin-based catalyst in the presence of *A. pullulans* after 7 days. Arrow, biomass production

Conversely, *P. aeruginosa* was able to grow in all catalyst 1 concentrations tested (Fig. 8), exhibiting as well alterations in the texture of the aqueous phase (Fig. 9).

Fungi

The growth of the yeast-like fungus *A. pullulans* was also inhibited, with high residual tin concentrations, and melanin production by the fungus was observed in the microcosms (dark color—Fig. 10).

Melanin production began after 7 days of growth, which is indicated by a soon color change in biodiesel (Fig. 11).

Biomass production after 7 days also seemed to be lower in biodiesel with residual organotin compound (Fig. 12), in agreement with the final result after 28 days (Fig. 11).

The filamentous fungus *P. boydii* exhibits no differ- ence in final dry biomass results (Fig. 13) among

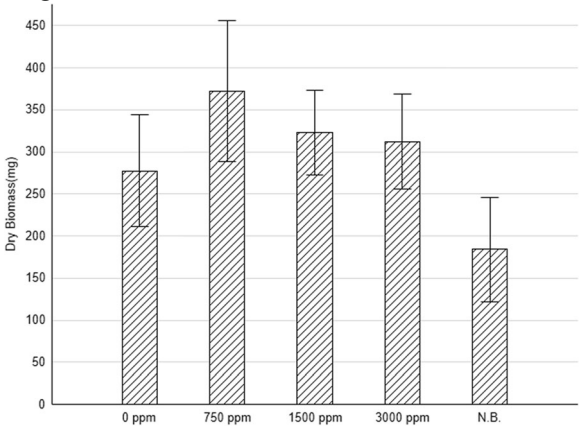


Fig. 13 Biomass values for *Pseudallescheria boydii* in the pres- ence (3000, 1500, and 750 ppm), and without tin (0 ppm) and viability control (N.B.—Sabouraud nutrient broth) after 28 days. Upper lines indicate standard error (p = 0.05)

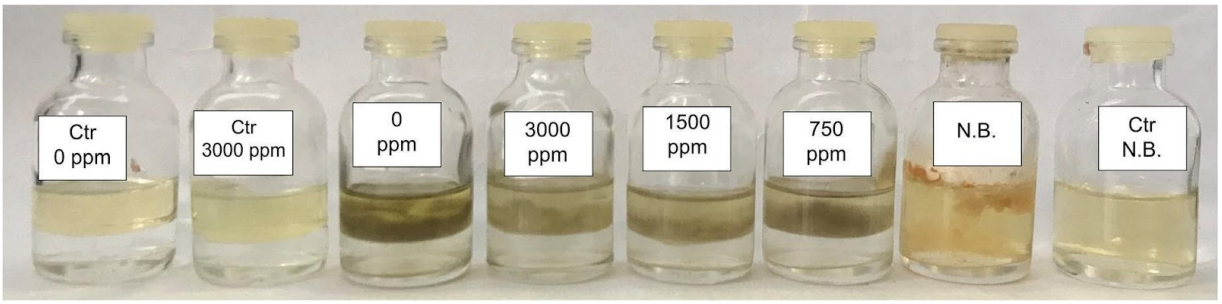


Fig. 14 Microcosm appearance of biodiesel produced without (0 ppm) and with (3000, 1500, and 750 ppm) tin-based catalyst in the presence of *Pseudallescheria boydii* inoculum after 28 days. N.B., Sabouraud nutrient broth viability control. Ctr, sterile controls

catalyst 1 concentrations, exhibiting visible biomass formation too (Fig. 14).

Despite this fact, visual inspection of microcosms show pronounced differences between *P. boydii* in 0 and 3000 ppm DBTD (Fig. 15).

As observed in Fig. 16, all experiments show acidi- fication in the aqueous phase.

Chromatographic and infrared analysis

After 28 days of inoculum, oil phase samples were char- acterized by infrared spectroscopy and by gas chroma- tography. Table 2 shows the fatty acids composition of the oil phase, determined by gas chromatography.

The same samples were characterized by medium infrared spectroscopy. Figure 17 presents the spectra for biodiesel as received, with 0 ppm (a) and 3000 ppm (b) of residual organotin.

In Fig. 18, it is shown infrared spectra for sterile control, which is, biodiesel in contact with BH medium after 28 days of storage. Both products (as received and sterile control) exhibit no difference among spectra.

On the other hand, the infrared spectrum of ASTM oil phase shows a different result, as shown in Fig. 19.

The ASTM E1259 inoculum was able to grow in all concentrations of the tin-based residual catalyst. It is reported that uncharacterized inocula such as these are capable of growth in biodiesel, as well as biodiesel degradation (Bücker et al., 2014, 2018)

B. pumilus showed inhibition values suggesting a biocidal and sporistatic effect, as reported in the litera- ture (Butt et al., 2019; Javed et al., 2017).

P. aeruginosa ability of growing in biodiesel is well reported (Bücker et al., 2014; De Azambuja et al., 2017; Jensen et al., 2017). Several strains of this species also produce a common emulsifier called Rhamnolipid (Cieśla et al., 2018; Fenibo et al., 2019; Kaskatepe & Yildiz, 2016). This substance has several applications, including environmental remediation of metal ions and organometallic compounds such as organotin com- plexes (Cieśla et al., 2018; Finnegan et al., 2018). As hydrophobicity and molecule partition in the aqueous phase are crucial to organotin (IV) toxicity (Adeyemi et al., 2018), this emulsifier may have led to a reduced antimicrobial effect in aqueous medium keeping it from entering the cell.

It is known that melanin production may have a variety of functions, from virulence factor to stress

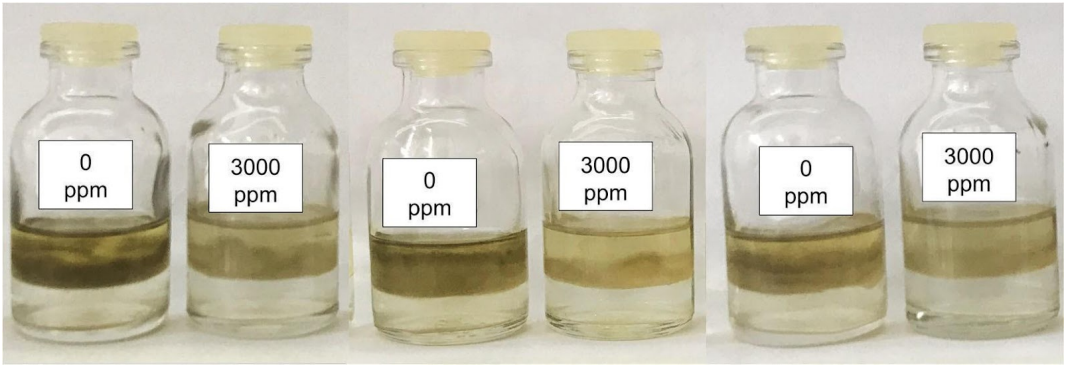


Fig. 15 Microcosms triplicate of *P. boydii* after 28 days of incubation

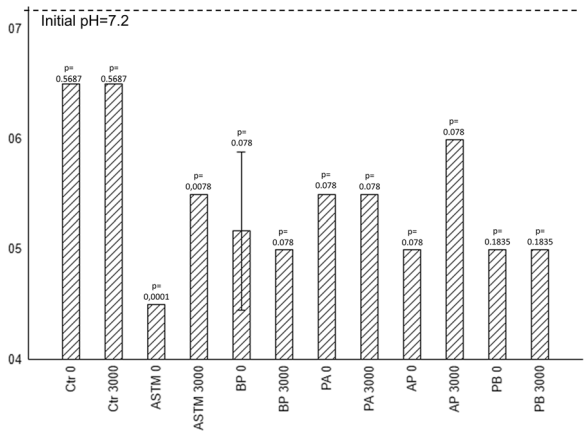


Fig. 16 Graphic showing final pH of each inoculum compared to sterile controls. Upper dotted lines indicate initial pH value (7.2). Spreadlines indicate standard error ($p = 0.05$). *P* values are relative to initial pH

response (Cordero & Casadevall, 2017; Wang et al., 2019). The fact that more melanin production by

A. pullulans is observed, in the absence of organotin, may indicate inhibition of its production, or inhibition of growth of the yeast itself. It is probable that both sce- narios would take place due to alterations in DNA expression, since it is reported that dibutyltin com- pounds can intercalate the DNA and bind histones (Javed et al., 2017; Osada et al., 2005).

It is known that *P. boydii* is an emergent pathogen, with antimicrobial resistance reported (Coates et al., 2020; Gilgado et al., 2006; Liu et al., 2019; Livermore, 2002; Yagüe et al., 2013). In spite of the incipient use of organotin substances for microbial con- trol, it is also reported that this substance already showed a medium effect over environmental isolates of *P. boydii* (Liu et al., 2019), which could explain its resistance.

pH medium alterations may indicate production of either secondary acid metabolites or hydrolysis products (Fátima M. Bento & Gaylarde, 2001; Bücker et al., 2011, 2014; Cazarolli et al., 2020)

The final pH observed for both sterile controls (0 and 3000 ppm) was 6.5, while for *B. pumilus* it was 6.0 and for *P. aeruginosa* was 5.5. Both fungi exhibited a final pH of 5.0 in both catalyst 1 concentrations. Since initial pH of aqueous phase was 7.2, the acidification observed in the sterile control was probably due the tendence of migration of FAMEs from the oil phase and hydrolysis products to the aqueous phase (Fátima

M. Bento & Gaylarde, 2001; Bücker et al., 2011, 2014; Cazarolli et al., 2020).

Previous studies show consistently that, despite the visible microbial growth evidenced by biomass for- mation and dry weight data, GC analysis of FAMEs after incubation seemed unaltered. It has been reported (Cazarolli et al., 2014) that several methyl esters migrate

Table 2 Fatty acid methyl esters (FAMEs) composition determined by gas chromatography. 0 ppm indicates biodiesel without residual organotin. 3000 ppm indicates biodiesel with 3000 ppm of residual organotin. AR indicates sample was analyzed as re- ceived. Ctr indicates control samples that were in contact with BH medium Ctr 1: sterile control for isolated microorganisms exper- iment; Ctr2: sterile for ASTM inoculum. ASTM, ASTM inocu- lum; BP, *Bacillus pumilus* inoculum experiment; AP, *Aureobasidium pullulans* inoculum experiment. Standard devia- tion is ± 1.0%

Samples	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	Linolenic acid
0 ppm AR	11.7	3.7	22.5	55.6	6.5
3000 ppm AR	12.5	4	23.3	55.3	4.9
0 ppm Ctr1	12.3	3.8	22.5	55.9	5.6
3000 ppm Ctr1	12.5	3.9	22.9	55.6	5.2
0 ppm Ctr2	12.8	4	23.4	54	5.9
3000 ppm Ctr2	12.9	3.9	23.3	54.1	5.8
0 ppm ASTM	13.4	3.7	22.3	54.5	6.2

Samples	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	Linolenic acid
0 ppm AR	11.7	3.7	22.5	55.6	6.5
3000 ppm AR	12.5	4	23.3	55.3	4.9
0 ppm Ctr1	12.3	3.8	22.5	55.9	5.6
3000 ppm Ctr1	12.5	3.9	22.9	55.6	5.2
0 ppm Ctr2	12.8	4	23.4	54	5.9
3000 ppm ASTM	13.4	3.8	22.6	54.2	6.1
0 ppm BP	13	3.6	22.6	54.7	6.1
3000 ppm BP	13.2	3.8	22.8	54.1	6.1
0 ppm AP	12.8	4.4	23.6	53.4	5.8
3000 ppm AP	12.8	3.6	22	55.1	6.4

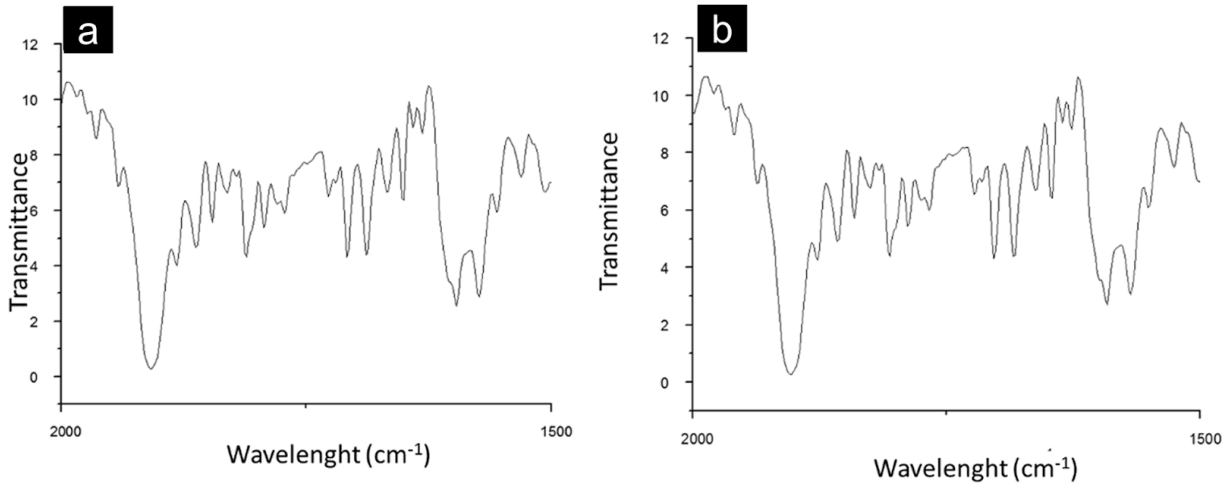


Fig. 17 Infrared spectra between 2000 and 1500 cm^{-1} of biodiesel as received, with 0 ppm (A) and 3000 ppm (B) of residual organotin

from the oil phase to the aqueous phase, thus providing a carbon source for microbial growth, and yet maintaining the FAMEs composition of the oil phase roughly the same. These findings also help to explain the spontane- ous pH reduction observed in the sterile controls.

Beker et al. (Beker et al., 2016) obtained similar results regarding FAMEs composition after microbial incubation, suggesting that this migration may have contributed to the microbial population nutrients for its growth, along with other biodiesel components such as glycerin, mono, di and triacylglycerides compounds (Beker et al., 2016; Bücker et al., 2011; Cazarolli et al., 2014).

The alteration around 1710 cm^{-1} observed in Fig. 19 (a and b) can be related to biodiesel degradation (Boelter et al., 2018; Pinho et al., 2014). This is an important result

as biomass observations (as shown in Fig. 6) also indicate inoculum growth. According to GC analysis, there is no modification in the fatty acid profile after 28 days and the changes in the carbonyl region observed in FTIR spectra may be related to acid formation, due to hydrolysis of TAGs and degradation reactions. These chemical species tend to migrate to the aqueous phase, causing the afore- mentioned pH reduction in the sterile controls (Beker et al., 2016; Bücker et al., 2011; Cazarolli et al., 2014).

These findings allow studies in larger scales to be outlined, i.e., field and industrial scales, conducted using safe and useful concentrations of the organotin residual concentrations after a scaling-up production with indus- trial purposes. Also, environmental models can be employed to ensure its safety such as plant and animal models in contact with residual bottom water.

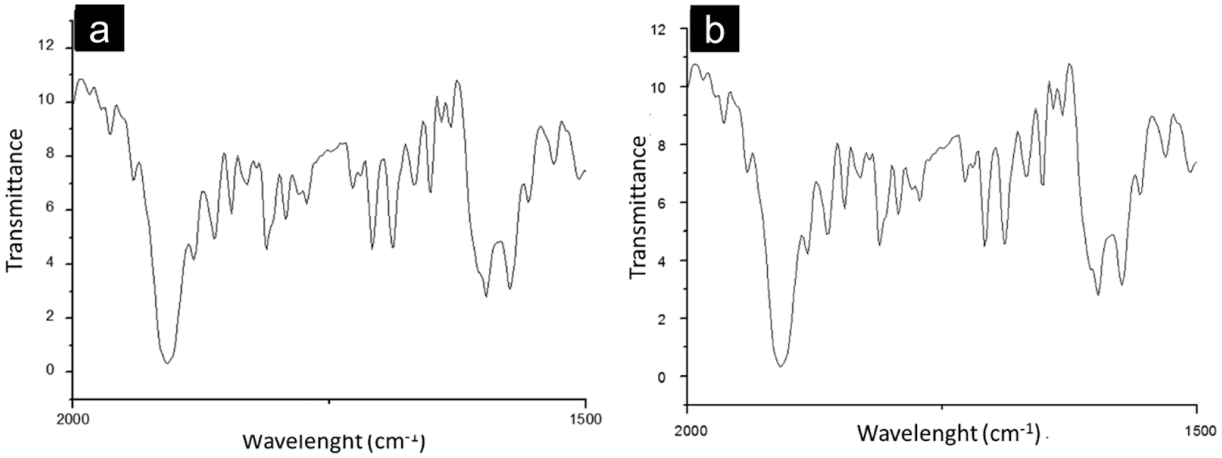


Fig. 18 Infrared spectra between 2000 and 1500 cm^{-1} of biodiesel with 0 ppm (A) and 3000 ppm (B) of residual organotin after 28 days in contact with BH medium

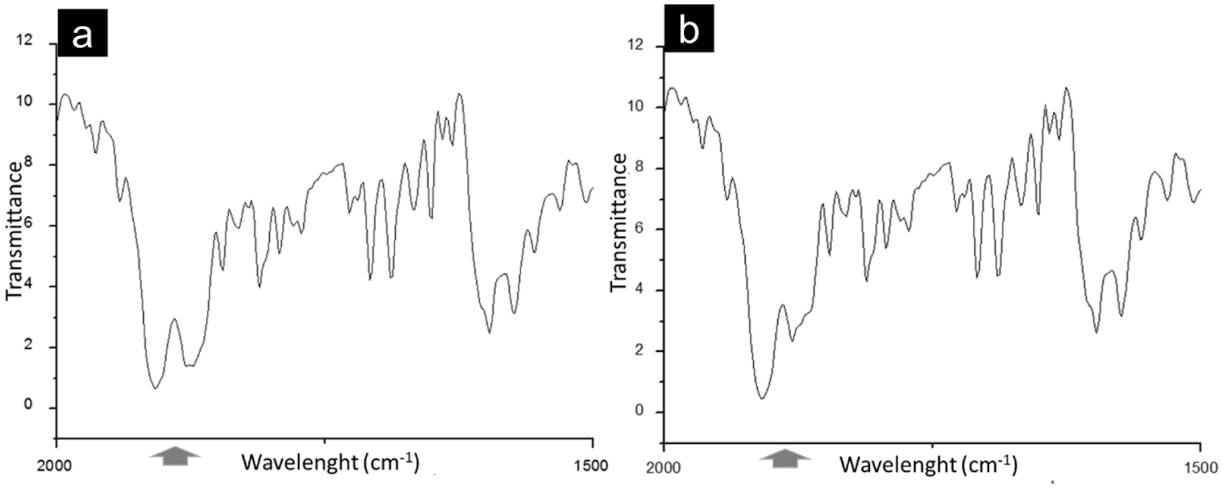


Fig. 19 Infrared spectra between 2000 and 1500 cm^{-1} of biodiesel with 0 ppm (A) and 3000 ppm (B) of residual organotin after 28 days in contact with ASTM inoculum. Arrow, region of wavelength related to carbonyl groups

Conclusions

The organotin compounds tested in the present work did not show any biocide effect over the microorganisms tested, an important novelty. Our findings in terms of microbial growth are in accordance to previous reports in the literature regarding the uncharacterized inoculum ASTM (Bücker et al., 2014, 2018) and the isolated organisms *P. boydii* (Boelter et al., 2018; Cazarolli et al., 2014; Martin-Sanchez et al., 2018), *A. pullulans* (Cazarolli et al., 2020), *B. pumilus* (Beker et al., 2016), and *P.aeruginosa* (Bücker et al., 2014; De Azambuja et al., 2017; Jensen et al., 2017). These results also suggest that the tin residual content did not present environmental concern regarding this catalyst, as it had low or no effect at all over the microorganisms tested.

Acknowledgments Laboratório de Biodeterioração de Combustíveis e Biocombustíveis—Universidade Federal do Rio Grande do Sul; Programa de Pós-Graduação em Microbiologia Agrícola e do Ambiente—Universidade Federal do Rio Grande do Sul; Grupo de Catálise e Reatividade Química, Universidade Federal do Alagoas; Programa de Pós-graduação em Engenharia Química—Centro de Tecnologia; Financiadora de Estudos e

Projetos; Coordenação de Aperfeiçoamento de Pessoal de Ensino Superior; Conselho Nacional de Pesquisa, Fundação de Amparo à Pesquisa do Estado de Alagoas.

Data availability The datasets generated during and/or analyzed during the current study are not publicly available due to ongoing research, but are available from the corresponding author on reasonable request.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interests.

References

ABNT, A. B. D. N. T. (2014). *NBR 15512: armazenamento,*

transporte, abastecimento e controle de qualidade de biodie- sel e/ou mistura óleo diesel/biodiesel. Rio de Janeiro, 2008. htt p :/ /w ww .dt . c o .kr/ cont ent s . h tm l? ar ti cl e_ no=20120713020105317490 01

ABNT, A. B. de. N. T.. (2019). *NBR 16732 - Combustíveis*

líquidos e sistemas associados — Requisitos para prevenção, monitoramento e controle de contaminação microbiana.

Adeyemi, J. O., Onwudiwe, D. C., Ekennia, A. C., Anokwuru, C. P., Nundkumar, N., Singh, M., & Hosten, E. C. (2019). Synthesis, characterization and biological activities of organotin (IV) diallyldithiocarbamate complexes. *Inorganica Chimica Acta, 485*(Iv), 64–72. https://doi.org/10.1016/j.ica.2018.09.085.

Adeyemi, J. O., Onwudiwe, D. C., Ekennia, A. C., Uwaoma, R. C., & Hosten, E. C. (2018). Synthesis, characterization and antimicrobial studies of organotin (IV) complexes of N- methyl-N-phenyldithiocarbamate. *Inorganica Chimica Acta, 477* , 148 – 159. https://doi.org/10 . 1016 /j.ica.2018.02.034.

AOCS - American Oil Chemists Society. (1998). Official methods and recommended practices of the AOCS. Champaign: A.O.C.S.

Appel, K. E. (2004). Organotin compounds: toxicokinetic aspects. *Drug Metabolism Reviews, 36*(3–4), 763–786. https://doi. org/10.1081/DMR-200033490.

Ashfaq, M., Khan, M. I., Kaleem Baloch, M., & Malik, A. (2004). Biologically potent organotin (IV) complexes of 2- maleimidoacetic acid. *Journal of Organometallic Chemistry, 689*(1), 238–245. https://doi.org/10.1016/j. jorganchem.2003.10.007.

ASTM, I. A. S. f. T. a. M. (2018). *Standard practice for evaluation of antimicrobials in liquid fuels boiling below 390°C* (pp. 1– 5). https://doi.org/10.1520/E1259-10.2.

ASTM, I. A. S. f. T. a. M. (2019). *ASTM D4176-04 (2019),*

Standard test method for free water and particulate contam- ination in distillate fuels (Visual Inspection Procedures). West Conshohocken: ASTM International. https://doi. org/10.1520/D4176-04R19.

Atadashi, I. M., Aroua, M. K., Abdul Aziz, A. R., & Sulaiman, N.

M. N. (2013). The effects of catalysts in biodiesel production: a review. *Journal of Industrial and Engineering Chemistry, 19*(1), 14–26. https://doi.org/10.1016/j.jiec.2012.07.009.

Barbosa, A. S. L., de Guedes, J. S., da Silva, D. R., Meneghetti, S.

M. P., Meneghetti, M. R., da Silva, A. E., de Araujo, M. V., Alexandre-Moreira, M. S., de Aquino, T. M., de Siqueira Junior, J. P., de Araújo, R. S. A., da Cruz, R. M. D., & Mendonça-Junior, F. J. B. (2018). Synthesis and evaluation of the antibiotic and adjuvant antibiotic potential of organotin

(IV) derivatives. *Journal of Inorganic Biochemistry, 180*(August 2017), 80–88. https://doi.org/10.1016/j.jinorgbio.2017.12.004.

Baul, T. S. B. (2008). Antimicrobial activity of organotin (IV) compounds: a review. *Applied Organometallic Chemistry, 22*(4), 195–204. https://doi.org/10.1002/aoc.1378.

Beker, S. A., da Silva, Y. P., Bücker, F., Cazarolli, J. C., de Quadros, P. D., do Peralba, M. C. R., Piatnicki, C. M. S., & Bento, F. M. (2016). Effect of different concentrations of tert- butylhydroquinone (TBHQ) on microbial growth and chem- ical stability of soybean biodiesel during simulated storage. *Fuel, 184* , 701 – 707. https://doi.org/10 . 1016 /j.fuel.2016.07.067.

Bento, F. M., & Gaylarde, C. C. (2001). Biodeterioration of stored diesel oil: studies in Brazil. *International Biodeterioration and Biodegradation, 47*(2), 107–112. https://doi.org/10.1016

/S0964-8305(00)00112-8.

Bento, F. M., do Peralba, M. C. R., Ferrão, M. F., Zimmer, A. R., De Azambuja, A. O., Barbosa, C. S., Bücker, F., Cazarolli, J. C., De Quadros, P. D., & Beker, S. A. (2016). Diagnóstico, monitoramento e controle da contaminação microbiana em biodiesel e misturas durante o armazenamento. In D. M. M. Pinho & P. A. Z. Suarez (Eds.), *Armazenagem e Uso de Biodiesel: problemas associados e formas de controle. (1a. ed.)* (1st ed., pp. 112–174). Brasília: CDT, UNB Centro de Apoio ao Desenvolvimento Tecnológico, Universidade Federal de Brasília - UNB.

Boelter, G., Cazarolli, J. C., Beker, S. A., de Quadros, P. D., Correa, C., Ferrão, M. F., Galeazzi, C. F., Pizzolato, T. M., & Bento, F. M. (2018). *Pseudallescheria boydii* and *Meyerozyma guilliermondii*: behavior of deteriogenic fungi during simulated storage of diesel, biodiesel, and B10 blend in Brazil. *Environmental Science and Pollution Research, 25*(30), 30410–30424. https://doi.org/10.1007/s11356-018-

3015-x.

Boyer, I. J. (1989). Toxicity of dibutyltin, tributyltin and other organotin compounds to humans and to experimental animals. *Toxicology, 55*(3), 253–298. https://doi.org/10.1016/0300-483X(89)90018-8.

Bücker, F., Aguiar, N., Fernando, L., Seminotti, R. J., Ruaro, C., Anastácio, F., Camargo, D. O., & Menezes, F. (2011). International biodeterioration & biodegradation impact of biodiesel on biodeterioration of stored Brazilian diesel oil. *International Biodeterioration & Biodegradation, 65*(1), 172–178. https://doi.org/10.1016/j.ibiod.2010.09.008.

Bücker, F., Barbosa, C. S., Quadros, P. D., Bueno, M. K., Fiori, P., te Huang, C., Frazzon, A. P. G., Ferrão, M. F., de Oliveira Camargo, F. A., & Bento, F. M. (2014). Fuel biodegradation and molecular characterization of microbial biofilms in stored diesel/biodiesel blend B10 and the effect of biocide. *International Biodeterioration and Biodegradation, 95*(PB), 346–355. https://doi.org/10.1016/j.ibiod.2014.05.030.

Bücker, F., de Moura, T. M., da Cunha, M. E., de Quadros, P. D., Beker, S. A., Cazarolli, J. C., Caramão, E. B., Frazzon, A. P. G., & Bento, F. M. (2018). Evaluation of the deteriogenic microbial community using qPCR, n-alkanes and FAMEs biodegradation in diesel, biodiesel and blends (B5, B10, and B50) during storage. *Fuel, 233*(November), 911–917. https://doi.org/10.1016/j.fuel.2017.11.076.

Butt, A. F., Ahmed, M. N., Bhatti, M. H., Choudhary, M. A., Ayub, K., Tahir, M. N., & Mahmood, T. (2019). Synthesis, structural properties, DFT studies, antimicrobial activities and DNA binding interactions of two newly synthesized organotin (IV) carboxylates. *Journal of Molecular Structure, 1191*(Iv), 291–300.

https://doi.org/10.1016/j. molstruc.2019.04.066.

Cazarolli, J. C., Guzatto, R., Samios, D., do Peralba, M. C. R., de Cavalcanti, E. H. S., & Bento, F. M. (2014). Susceptibility of linseed, soybean, and olive biodiesel to growth of the deteriogenic fungus. *Pseudallescheria boydii. International Biodeterioration and Biodegradation, 95*(PB), 364–372. https://doi.org/10.1016/j.ibiod.2013.09.025.

Cazarolli, J. C., Silva, T. L., da Camara Ribas, R. K., da Fontoura Xavier Costa, L., de Moura, T., Galeazzi, C. F., Dallé da Rosa, P., Kenne, D. C., Carvalho, Â. R., Valente, P., Pizzolato, T. M., Correa, C., Ferrão, M. F., Guedes Frazzon, A. P., & Bento, F. M. (2020). Deterioration poten- tial of *Aureobasidium pullulans* on biodiesel, diesel, and B20 blend. *International Biodeterioration & Biodegradation, 147* (500), 104839. https://doi.org/10.1016/j. ibiod.2019.104839.

Cieśla, J., Koczańska, M., & Bieganowski, A. (2018). An interac- tion of rhamnolipids with Cu2+ ions. *Molecules, 23*(2). https://doi.org/10.3390/molecules23020488.

RESOLUÇÃO No 16, DE 29 DE OUTUBRO DE 2018, 1 (2018).

Coates, A. R. M., Hu, Y., Holt, J., & Yey, P. (2020). Antibiotic combination therapy against resistant bacterial infections: synergy , rejuvenation and resistance reduction. *Expert Review of Anti-Infective Therapy, 18*, 1–15. https://doi.org/10.1080/14787210.2020.1705155.

Cordero, R. J. B., & Casadevall, A. (2017). Functions of fungal melanin beyond virulence. *Fungal Biology Reviews, 31*(2), 99–112. https://doi.org/10.1016/j.fbr.2016.12.003.

da Silva, M. A., dos Santos, A. S. S., Neto, A. J. S., Giertyas, C. J., Bortoluzzi, J. H., Meneghetti, M. R., & Plentz Meneghetti, S.

M. (2019). Evaluation of esterification of oleic acid and glycerol in the presence of organotin (IV) compounds. *European Journal of Lipid Science and Technology, 121*(6), 1–9. https://doi.org/10.1002/ejlt.201900103.

da Silva, M. A., dos Santos, A. S. S., dos Santos, T. V., Meneghetti, M. R., & Meneghetti, S. M. P. (2017). Organotin (IV) compounds with high catalytic activities and selectivities in the glycerolysis of triacylglycerides. *Catalysis Science & Technology, 7*(*23*), 5750–5757. https://doi.org/10.1039/C7CY01559C.

De Azambuja, A. O., Cazarolli, J. C., De Quadros, P. D., Bücker, F., Correa, C., Casagranda, J. C., Ferrão, M. F., Celikkol, S., Ogawa, A., Bonifay, V., Sunner, J. A., Beech, I. B., & Bento,

F. M. (2017). Effect of sulfur content on microbial composi- tion and biodegradation of a Brazilian diesel and biodiesel blend (B10). *Energy and Fuels, 31*(11), 12305–12316.
https://doi.org/10.1021/acs.energyfuels.7b02287.

Doctor, S. V., & Fox, D. A. (1982). Effects of organotin com- pounds on maximal electroshock seizure (Mes) responsive- ness in mice. i.tri(n-alkyl)tin compounds. *Journal of Toxicology and Environmental Health, 10*(1), 43–52. https://doi.org/10.1080/15287398209530229.

Erofeev, V., Bobryshev, A., Shafigullin, L., Zubarev, P., Lakhno, A., Darovskikh, I., & Tretiakov, I. (2016). Building heat- insulating materials based on the products of the transesterification of polyethylene terephthalate and dibutyltin dilaurate. *Procedia Engineering, 165*, 1455–

1459. https://doi.org/10.1016/j.proeng.2016.11.879.

Fenibo, E. O., Ijoma, G. N., Selvarajan, R., & Chikere, C. B. (2019). Microbial surfactants: the next generation multifunc- tional biomolecules for applications in the petroleum industry and i t s associated environmental remediation. *Microorganisms, 7*(11), 1–29. https://doi.org/10.3390
/microorganisms7110581.

Finnegan, C., Ryan, D., Enright, A. M., & Garcia-Cabellos, G. (2018). A review of strategies for the detection and remedi- ation of organotin pollution. *Critical Reviews in Environmental Science and Technology, 48*(1), 77–118. https://doi.org/10.1080/10643389.2018.1443669.

Gilgado, F., Serena, C., Cano, J., Gené, J., & Guarro, J. (2006). Antifungal susceptibilities of the species of the *Pseudallescheria boydii* complex. *Antimicrobial Agents and Chemotherapy, 50*(12), 4211–4213. https://doi.org/10.1128
/AAC.00981-06.

Gupta, P. K. (2020). Fate, transport , and bioremediation of bio- diesel and blended biodiesel in subsurface environment: a review. *Journal of Environmental Engineering, 146*(1), 1– 10. https://doi.org/10.1061/(ASCE)EE.1943-7870.0001619.

Hadi, A. G., Jawad, K., Ahmed, D. S., & Yousif, E. (2019). Synthesis and biological activities of organotin (IV) carbox- ylates: a review. *Systematic Reviews in Pharmacy, 10*(1), 26– 31. https://doi.org/10.5530/srp.2019.1.5.

Herigstad, B., Hamilton, M., & Heersink, J. (2001). How to optimize the drop plate method for enumerating bacteria. *Journal of Microbiological Methods, 44*(2), 121–129. https://doi.org/10.1016/S0167-7012(00)00241-4.

Hoch, M. (2001). Organotin compounds in the environment - an overview. *Applied Geochemistry, 16*(7–8), 719–743. https://doi.org/10.1016/S0883-2927(00)00067-6.

Iftikhar, S. H., Gilani, S. R., Taj, M. B., Raheel, A., Imtiaz-Ud- Din, Termizi, S. A., Al-Shakban, M., & Mohd Ali, H. (2018). Design, synthesis and biological evaluation of organotin (IV) complexes of flumequine and cetirizine. *Journal of the Serbian Chemical Society, 83*(4), 425–437. https://doi.org/10.2298/JSC161203070I.

Iqbal, H., Ali, S., & Shahzadi, S. (2015). Antituberculosis study of organotin (IV) complexes: a review. *Cogent Chemistry, 1*(1), 1–12. https://doi.org/10.1080/23312009.2015.1029039.

Jamil, K., Bakhtiar, M., Khan, A. K. R., Rubina, F., Rehana, R.,

Wajid, R., Qaisar, M., Khan, A. K. R., Danish, M., Awais,

M., Bhatti, Z. A., Rizwan, M., Naveed, A., Hussani, M., & Pervez, A. (2009). *Synthesis characterization and antimicrobial activities of novel organotin compounds* (Vol. 3, pp. 66– 71).

Javed, F., Ali, S., Shahzadi, S., Sharma, S. K., Qanungo, K., Munawar, K. S., & Khan, I. (2017). Synthesis, characteriza- tion, and biological activity of organotin (IV) complexes with 4-oxo-4-[3-(trifluoromethyl)phenylamino]butanoic acid. *Russian Journal of General Chemistry, 87*(10), 2409–2420. https://doi.org/10.1134/S1070363217100231.

Jensen, P. A., Zhu, Z., & van Opijnen, T. (2017). Antibiotics disrupt coordination between transcriptional and phenotypic stress responses in pathogenic bacteria. *Cell Reports, 20*(7), 1705–1716. https://doi.org/10.1016/j.celrep.2017.07.062.

Kaskatepe, B., & Yildiz, S. (2016). Rhamnolipid biosurfactants produced by pseudomonas Species. *Brazilian Archives of Biology and Technology, 59*(December), 1–16. https://doi. org/10.1590/1678-4324-2016160786.

Knothe, G., & Gerpen, J. V. (2005). In G. Knothe, J. Van Gerpen, & J. Krahl (Eds.), *The Biodiesel Handbook*. Urbana: AOCS Publishing. https://doi.org/10.1201/9781439822357.

Lam, M. K., Lee, K. T., & Mohamed, A. R. (2010).

Homogeneous, heterogeneous and enzymatic catalysis for transesterification of high free fatty acid oil (waste cooking oil) to biodiesel: a review. *Biotechnology Advances, 28*(4), 500–518. https://doi.org/10.1016/j.biotechadv.2010.03.002.

Liu, D. H., Sun, Y. Z., Kurtán, T., Mándi, A., Tang,

H., Li, J., Su,

L., Zhuang, C. L., Liu, Z. Y., & Zhang, W. (2019). Osteoclastogenesis regulation metabolites from the coral-associated fungus *Pseudallescheria boydii* TW-1024-3. *Journal of Natural Products, 82*(5), 1274–1282. https://doi.org/10.1021/acs.jnatprod.8b01053.

Livermore, D. M. (2002). Multiple mechanisms of antimicrobial resistance in *Pseudomonas aeruginosa*: our worst night- mare? *Clinical Infectious Diseases, 34*(5), 634–640. https://doi.org/10.1086/338782.

Lu, J., Feng, J., Cai, S., & Chen, Z. (2017). Metabolomic re- sponses of Haliotis diversicolor to organotin compounds. *Chemosphere, 168*, 860–869. https://doi.org/10.1016/j. chemosphere.2016.10.124.

Luz, G. V. S., Sousa, B. A. S. M., Guedes, A. V., Barreto, C. C., & Brasil, L. M. (2018). Biocides used as additives to biodiesels and their risks to the environment and public health: a review. *Molecules, 23* (2698). https://doi.org/10.3390
/molecules23102698.

Martin-Sanchez, P. M., Gorbushina, A. A., & Toepel, J. (2018). Quantification of microbial load in diesel storage tanks using culture- and qPCR-based approaches. *International Biodeterioration and Biodegradation, 126*, 216–223. https://doi.org/10.1016/j.ibiod.2016.04.009.

Matveeva, L., Efremova, M., & Baranets, I. (2018). Studies of the Morphology of waterproof coatings based on urethane iso- cyanate, alkyl-phenol-formaldehyde resin and dibutyltin dilaurate using the high-resolution optical microscopy technique. *Architecture and Engineering, 3*(2), 43–47. https://doi.org/10.23968/2500-0055-2018-3-2-43-47.

Meneghetti, S. M. P., Meneghetti, M. R., & Brito, Y. C. (2013). Transesterification, some applications and biodiesel produc- tion. *Revista Virtual de Quimica, 5*(1), 63–73. https://doi. org/10.5935/1984-6835.20130007.

Nautiyal, P., Subramanian, K. A., Dastidar, M. G., & Kumar, A. (2020). Experimental assessment of performance, combus- tion and emissions of a compression ignition engine fuelled with Spirulina platensis biodiesel. *Energy, 193*, 116861. https://doi.org/10.1016/j.energy.2019.116861.

Osada, S., Nishikawa, J. I., Nakanishi, T., Tanaka, K., & Nishihara, T. (2005). Some organotin compounds enhance histone acetyltransferase activity. *Toxicology Letters, 155*(2), 329–335. https://doi.org/10.1016/j.toxlet.2004.10.009.

Passman, F. J. (2013). Microbial contamination and its control in fuels and fuel systems since 1980 - a review. *International Biodeterioration and Biodegradation, 81*, 88–104. https://doi.org/10.1016/j.ibiod.2012.08.002.

Pellerito, C., Nagy, L., Pellerito, L., & Szorcsik, A. (2006). Biological activity studies on organotin (IV)n + complexes and parent compounds. *Journal of Organometallic Chemistry, 691*(8), 1733–1747. https://doi.org/10.1016/j. jorganchem.2005.12.025.

Pinho, D. M. M., Santos, V. O., Dos Santos, V. M. L., Oliveira, M.

C. S., Da Silva, M. T., Piza, P. G. T., Pinto, A. C., Rezende,999

M. J. C., & Suarez, P. A. Z. (2014). Evaluating the use of en 14078 for determination of biodiesel in diesel blends sold in the Brazilian market. *Fuel, 136*, 136–142. https://doi.org/10.1016/j.fuel.2014.07.043.

Salina, F. H., de Almeida, I. A., & Bittencourt, F. R. (2020). RenovaBio opportunities and biofuels outlook in Brazil. In

A. Sayigh (Ed.), *Renewable energy and sustainable build- ings* (pp. 391–399). Berlin: Springer International Publishing. https://doi.org/10.1007/978-3-030-18488-9.

Song, X., Zapata, A., & Eng, G. (2006). Organotins and quantitative-structure activity/property relationships. *Journal of Organometallic Chemistry, 691*(8), 1756–1760. https://doi.org/10.1016/j.jorganchem.2005.12.003.

Thangaraj, B., Solomon, P. R., Muniyandi, B., Ranganathan, S., & Lin, L. (2019). Catalysis in biodiesel production - a review. *Clean Energy, 3*(1), 2–23. https://doi.org/10.1093
/ce/zky020.

Van der Kerk, G. J. M. D., & Luijten, J. G. A. (1954). Investigations on organo-tin compounds. *III. The biocidal properties of organo-tin compounds. Journal of Applied Chemistry, 4*(6), 314–319. https://doi.org/10.1002
/jctb.5010040607.

Wang, M., Danesi, P., James, T. Y., Al-Hatmi, A. M. S., Najafzadeh, M. J., Dolatabadi, S., Ming, C., Liou, G. Y., Kang, Y., & de Hoog, S. (2019). Comparative pathogenicity of opportunistic black yeasts in Aureobasidium. *Mycoses, 62*(9), 803–811. https://doi.org/10.1111/myc.12931.

Yagüe, P., López-García, M. T., Rioseras, B., Sánchez, J., & Manteca, Á. (2013). Pre-sporulation stages of Streptomyces differentiation: state-of-the-art and future perspectives. *FEMS Microbiology Letters, 342*(2), 79–88. https://doi. org/10.1111/1574-6968.12128.

Yousif, E., Salimon, J., Salih, N., Jawad, A., & Win, Y. F. (2016). New stabilizers for PVC based on some diorganotin (IV) complexes with benzamidoleucine. *Arabian Journal of Chemistry, 9*, S1394–S1401. https://doi.org/10.1016/j. arabjc.2012.03.004.

Zimmer, A. R., Oliboni, A., Viscardi, S. L. C., Teixeira, R. M., Flores, M., & M, F. (2017). *Biodiesel blend (B10) treated with a multifunctional additive (biocide) under simulated stored conditions: a field and lab scale monitoring* (Vol. 14, pp. 627–636). https://doi.org/10.18331/BRJ2017.4.2.7.

Zubair, M., Sirajuddin, M., Haider, A., Hussain, I., Tahir, M. N., & Ali, S. (2019). Organotin (IV) complexes as catalyst for biodiesel formation: synthesis, structural elucidation and computational studies. In *Applied Organometallic Chemistry, August* (pp. 1–16). https://doi.org/10.1002
/aoc.5305.

7. DISCUSSÃO GERAL

Dois compostos organoestânicos (iv) presentes como resíduo da catálise de biodiesel, foram testados em inóculos ASTM e um deles foi também testado em espécies deteriogênicas isoladas, mostrando pouca ou nenhuma atividade sobre os microrganismos, em concentrações próximas às permitidas em lei para aditivos.

Segundo relatos da literatura, aditivos baseados em estanho com efeito antimicrobiano e incrustante, especialmente aqueles associados a radicais orgânicos, são poluentes ambientais importantes, ocasionando efeitos adversos na fauna aquática (Formalewicz, 2019; Chen, 2017; Monteiro, 2021). Assim, seu uso já foi proibido por exemplo na tinta usada em cascos de navios, como agente anti-incrustante (U.E., 2003; Sonak, 2009), uma vez que há desprendimento lento destes compostos da tinta para o oceano (Lagerstörm, 2017). No entanto, o efeito antimicrobiano apresentado pelo composto de organoestanho aqui testado foi baixo, sugerindo três hipóteses: que seu uso seja seguro em termos ambientais, que os microrganismos testados possuam, todos, resistência intrínseca ou adquirida; ou que os compostos de organoestanho presentes em combustível não afetem os microrganismos da mesma forma que em meio aquoso.

Segundo Van der Kerk & Luijten, 1954 a toxicidade dos compostos organometálicos de forma geral, incluindo os de organoestanho em seus diferentes estados de oxidação (ii e iv, os mais comuns em moléculas organoestânicas) relaciona-se ao radical orgânico por ele apresentado, o que aumenta sua penetração em biomembranas (Baul, 2008; Doctor & Fox, 1982; Hadi et al., 2019; Pellerito et al., 2006) Neste caso o dibutilestanho apresenta menos toxicidade do que tributilestanho, tanto pelo fato de possuir 2 substituintes orgânicos em vez de 3, quanto pelo fato destes substituintes possuírem caráter menos hidrofóbico do que outros substituintes orgânicos com maior número de carbonos (Doctor & Fox, 1982; Hadi et al., 2019). Desta forma, compostos como dibutil-estanho possuem excelente atividade catalítica na produção de biodiesel (Mônica A. da Silva et al., 2019; Mônica Araújo da Silva et al., 2017; Meneghetti et al., 2013) e apresentam menos perigo do que compostos de tributilestanho (Boyer, 1989; Hoch, 2001; Iqbal et al., 2015).

Testes toxicológicos e ecotoxicológicos, bem como de emissões gasosas, ainda necessitam ser realizados nesse sentido para garantir a segurança ambiental de seu uso. Também seria preciso verificar se a presença de resíduos de estanho no combustível poderiam causar algum efeito indesejado em diferentes partes do motor, principalmente deposições metálicas nos cilindros de combustão e nos bicos injetores.

Da mesma forma, a presença de 1000 ppm de 8-hidroxiquinolina em combustível B20 com meio BH apresentou precipitações metálicas no fundo da fase aquosa. Embora esta porção não esteja em contato direto com o combustível, indica a interação de metais

presentes na fase aquosa com o composto presente na fase combustível - ou seja, um nível de partição suficiente para que fossem alcançadas as duas fases, um dos requisitos para que um antimicrobiano possa ter seu uso considerado em sistemas de combustível. O Clioquinol apresentou efeitos antimicrobianos semelhantes aos observados para 8-Hidroxiquinolina, molécula da qual este é derivado. Estes testes em microcosmos indicam o efeito destas moléculas, em comparação com o observado por antimicrobianos comerciais, em um cenário mais próximo daquele encontrado em tanques de estocagem. O efeito sinérgico observado para o antimicrobiano C, na presença de Clioquinol, prevê ao menos 50% de redução da quantidade necessária de morfolinas aplicadas no combustível para sua efetividade, com apenas 16µg/L de clioquinol - em comparação com 250 µg/L de clioquinol sem morfolinas, e 1mg/L da mistura de morfolinas.

Segundo a especificação do diesel brasileiro, seguindo uma tendência mundial, a quantidade máxima de enxofre é de 10 µg/L. Por outro lado, diversos biocidas aprovados pela EPA, como isotiazolonas, possuem enxofre em sua constituição, o que já descredencia seu uso atualmente em misturas Bx. Uma das vantagens do resultado observado neste trabalho é justamente a ausência de enxofre em todas as moléculas testadas - tanto a mistura de morfolinas (Metileno-bis-morfolina / nitro metil-morfolina) quanto 8-hidroxiquinolina e seu derivado, clioquinol - são isentos de enxofre. Além disso, seu uso na dose de 16 ppm (Log25 vezes menor) foi suficiente para diminuir à metade a quantidade de morfolinas necessárias para o mesmo efeito inibitório, indicando um efeito sinérgico de uma molécula isenta de enxofre, precipitações ou outras alterações de especificação observáveis.

Este trabalho traz duas contribuições importantes no sentido de elucidar o efeito antimicrobiano de compostos em dois cenários: sua presença residual após a catálise (dibutil-organoestanho) e sua adição para controle microbiológico (clioquinol, 8-hidroxiquinolina e compostos comerciais). Observou-se que a associação de compostos de classes diferentes, com mecanismos de ação distintos sobre microrganismos, é uma importante alternativa a ser utilizada. A associação de antifúngicos em aplicações clínicas já é uma prática usual atualmente, mas esta mesma prática pode trazer benefícios em cenários como o industrial e de combustíveis, reduzindo impactos ambientais e econômicos.

Também é importante ressaltar que, assim como a adição de compostos ao combustível pode ocasionar interações e alterações em suas especificações, a associação de compostos biocidas também precisa ser feita criteriosamente, de modo a evitar alterações nas propriedades do combustível, dos próprios biocidas, e efeitos antagônicos sobre os microrganismos deteriogênicos. Em suma, a análise e escolha de compostos químicos para controle microbiológico em combustíveis deve ser feita de forma criteriosa, levando em conta as especificidades do cenário de armazenamento.

8. REFERÊNCIAS BIBLIOGRÁFICAS:

Abreu FR, Lima DG, Hamú EH, Einloft S, Rubim JC, Suarez PAZ. 2003. New Metal Catalysts for Soybean Oil Transesterification. 80: 601–604.

Adamkiewicz G, Liddie J, Gaffin JM. 2020. The Respiratory Risks of Ambient/Outdoor Air Pollution. *Clinics in Chest Medicine* 41: 809–824.

Adeyemi JO, Onwudiwe DC, Ekennia AC, Uwaoma RC, Hosten EC. 2018a. Synthesis, characterization and antimicrobial studies of organotin(IV) complexes of N-methyl-N-phenyldithiocarbamate. *Inorganica Chimica Acta* 477: 148–159.

Adeyemi JO, Onwudiwe DC, Ekennia AC, Uwaoma RC, Hosten EC. 2018b. Synthesis, characterization and antimicrobial studies of organotin(IV) complexes of N-methyl-N-phenyldithiocarbamate. *Inorganica Chimica Acta* 477: 148–159.

Al-Hawash AB, Dragh MA, Li S, Alhujaily A, Abbood HA, Zhang X, Ma F. 2018a. Principles of microbial degradation of petroleum hydrocarbons in the environment. *Egyptian Journal of Aquatic Research* 44: 71–76.

Al-Hawash AB, Dragh MA, Li S, Alhujaily A, Abbood HA, Zhang X, Ma F. 2018b. Principles of microbial degradation of petroleum hydrocarbons in the environment. *Egyptian Journal of Aquatic Research* 44: 71–76.

Allsopp D, Seal K, Gaylarde C, Bento FM, Shirakawa M, Saad DS. 2016. *Introdução à biodeterioração.*

Amann RI, Ludwig W, Schleifer KH. 1995. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. *Microbiological Reviews* 59: 143–169.

do Amaral BE (UFMG). 2018. ESTABILIDADE DE BLENDAS DE DIESEL E BIODIESEL EM CONDIÇÕES DISTINTAS DE TEMPERATURA E UMIDADE. Unpublished thesis, UFMG.

do Amaral BE, de Rezende DB, Pasa VMD. 2020. Aging and stability evaluation of diesel/ biodiesel blends stored in amber polyethylene bottles under different humidity conditions. *Fuel* 279: 118289.

Ambrozin ARP, Kuri SE, Monteiro MR. 2009. CORROSÃO METÁLICA ASSOCIADA AO USO DE COMBUSTÍVEIS MINERAIS E BIOCOMBUSTÍVEIS. *Quím. Nova* 32: 1910–1916.

ANP. Produção de Biodiesel - ANP.

ANP ANDPGNEB. 2013. *RESOLUÇÃO ANP Nº 50, DE 23.12.2013 - DOU 24.12.2013*.

ANP. 2014. Ranp 45 - 2014. *Agência Nacional do Petróleo, Gás Natural e*

Biocombustíveis.

ANP ANDOPGÁSNEB. 2019a. RESOLUÇÃO ANP Nº 791, DE 12.6.2019 - DOU 14.6.2019.

ANP. 2019b. Ranp 798 - 2019. : 2–3.

Appel KE. 2004. Organotin compounds: Toxicokinetic aspects. *Drug Metabolism Reviews* 36: 763–786.

Aranda DAG, Santos RTP, Tapanes NCO, Ramos ALD, Antunes OAC. 2008. Acid-Catalyzed Homogeneous Esterification Reaction for Biodiesel Production from Palm Fatty Acids. *Cata. Lett*: 20–25.

Araújo BQ, Nunes RCDR, de Moura CVR, de Moura EM, das Graças Lopes Citó AM, dos Santos Júnior JR. 2010. Synthesis and Characterization of Beef Tallow Biodiesel. *Energy & Fuels* 24: 4476–4480.

Ashfaq M, Khan MI, Kaleem Baloch M, Malik A. 2004. Biologically potent organotin(IV) complexes of 2-maleimidoacetic acid. *Journal of Organometallic Chemistry* 689: 238–245.

ASTM. 2018. ASTM E1259-18: Standard Practice for Evaluation of Antimicrobials in Liquid Fuels Boiling Below 390 °C.

ASTM. 2019. ASTM D4176-04 (2019), Standard Test Method for Free Water and Particulate Contamination in Distillate Fuels (Visual Inspection Procedures).

ASTM 2020. ASTM D6751-20: Standard Specification for Biodiesel Fuel Blend Stock (B100) for Middle Distillate Fuels. *ASTM International*.

Atabani AE, Silitonga AS, Badruddin IA, Mahlia TMI, Masjuki HH, Mekhilef S. 2012. A comprehensive review on biodiesel as an alternative energy resource and its characteristics. *Renewable and Sustainable Energy Reviews* 16: 2070–2093.

De Azambuja AO, Cazarolli JC, De Quadros PD, Bücker F, Correa C, Casagranda JC, Ferrão MF, Celikkol S, Ogawa A, Bonifay V, Sunner JA, Beech IB, Bento FM. 2017. Effect of Sulfur Content on Microbial Composition and Biodegradation of a Brazilian Diesel and Biodiesel Blend (B10). *Energy and Fuels* 31: 12305–12316.

Barbosa ASL, Guedes J de S, da Silva DR, Meneghetti SMP, Meneghetti MR, da Silva AE, de Araujo MV, Alexandre-Moreira MS, de Aquino TM, de Siqueira Junior JP, de Araújo RSA, da Cruz RMD, Mendonça-Junior FJB. 2018. Synthesis and evaluation of the antibiotic and adjuvant antibiotic potential of organotin(IV) derivatives. *Journal of Inorganic Biochemistry* 180: 80–88.

Bareggi SR, Cornelli U. 2012. Clioquinol: Review of its mechanisms of action and clinical uses in neurodegenerative Disorders. *CNS Neuroscience and Therapeutics* 18: 41–46.

Basu S, Bose C, Ojha N, Das N, Das J, Pal M, Khurana S. 2015. Evolution of

bacterial and fungal growth media. *Bioinformation* 11: 182.

Baul TSB. 2008. Antimicrobial activity of organotin(IV) compounds: A review. *Applied Organometallic Chemistry* 22: 195–204.

Bento M, Beech IB, Gaylarde CC, Englert GE. 2005. Degradation and corrosive activities of fungi in a diesel – mild steel – aqueous system. : 135–142.

Bento FM, Bücker F, Santestevan N, de Siqueira Cavalcanti EH, Zimmer AR, Gaylarde CC, Camargo FA. 2010. Impacto da adição de biodiesel ao óleo diesel durante a estocagem: um enfoque microbiológico e controle. *Revista Biodiesel* 14.

Bento FM, do Carmo Ruaro Peralba M, Ferrão MF, Zimmer AR, Azambuja AO De, Barbosa CS, Bücker F, Cazarolli JC, Quadros PD De, Beker SA. 2016a. Diagnóstico, monitoramento e controle da contaminação microbiana em biodiesel e misturas durante o armazenamento. (DMM Pinho and PAZ Suarez, Eds.). *Armazenagem e Uso de Biodiesel: problemas associados e formas de controle. (1a. ed.). Brasília: CDT, UNB.*: 112–174.

Bento FM, Gaylarde CC. 2001. Biodeterioration of stored diesel oil: Studies in Brazil. *International Biodeterioration and Biodegradation* 47: 107–112.

Bento FM, de Oliveira Camargo FA, Gaylarde CC. 2008. Biossurfactantes (IS de Melo and JL de Azevedo, Eds.). *Microbiologia Ambiental*: 647.

Bento FM, Peralba M do CR, Ferrão MF, Zimmer AR, De Azambuja AO, Barbosa CS, Bücker F, Cazarolli JC, Quadros PD De, Beker SA. 2016b. Diagnóstico, monitoramento e controle da contaminação microbiana em biodiesel e misturas durante o armazenamento. In: Pinho DMM,, In: Suarez PAZ, eds. **Armazenagem e Uso de Biodiesel: problemas associados e formas de controle. (1a. ed.). Brasília: CDT, UNB.** Brasília: Centro de Apoio ao Desenvolvimento Tecnológico, Universidade Federal de Brasília - UNB, 112–174.

Bento FM, Viscardi SLR, Daroda R, Menendez AG, Camargo FAO, Gaylarde CC. 2006. Suscetibilidade do óleo diesel com 2 e 5% de biodiesel á contaminação microbiana durante a estocagem. *Revista Biodiesel* 4: 24–26.

Berglund EC, Kiialainen A, Syvänen AC. 2011. Next-generation sequencing technologies and applications for human genetic history and forensics. *Investigative Genetics* 2: 1–15.

Bharathi D, Rajalakshmi G. 2019. Microbial lipases: An overview of screening, production and purification. *Biocatalysis and Agricultural Biotechnology* 22: 101368.

Bhardwaj KK, Gupta R. 2017. Synthesis of Chirally Pure Enantiomers by Lipase. *Journal of Oleo Science J. Oleo Sci* 66: 1073–1084.

Bharti R, Singh B. 2020. Green tea (Camellia assamica) extract as an antioxidant additive to enhance the oxidation stability of biodiesel synthesized from waste cooking oil. *Fuel* 262: 116658.

Bhuiya MMK, Rasul MG, Khan MMK, Ashwath N, Azad AK. 2015. Prospects of 2nd

generation biodiesel as a sustainable fuel — Part : 1 selection of feedstocks , oil extraction techniques and conversion technologies. *Renewable and Sustainable Energy Reviews*: 1–20.

Botkin DB, Saxe H, Araújo MB, Betts R, Bradshaw RHW, Cedhagen T, Chesson P, Dawson TP, Etterson JR, Faith DP, Ferrier S, Guisan A, Hansen AS, Hilbert DW, Loehle C, Margules C, New M, Sobel MJ, Stockwell DRB, Brasil M, Chel A, Kaushik G, Nataša A. Kablar, Kishore RH, Babu MT, Rao MPR, Sasidhar G, Elliott D, Callegari A, Bolognesi S, Cecconet D, Capodaglio AG, Salina FH, de Almeida IA, Bittencourt FR, Han F, Guo H, Hu J, Zhang J, Ying Q, Zhang H, Perera F, Jacobson MZ, BRASIL. 2019. Sources and health risks of ambient polycyclic aromatic hydrocarbons in China (A Sayigh, Ed.). *Alexandria Engineering Journal* 57: 134229.

Boury A, Zussa A, Campos C, Felix M, Felix M, Marin M, Ferreira L, Ferreira N, Toma S, Toyama M. 2019. Study of Biodiesel Production By Heterogeneous Catalysis Using Magnetic Nanoparticles Supported in Silica in a Packed Bed Reactor. *The Journal of Engineering and Exact Sciences* 5: 137–142.

Boyer IJ. 1989. Toxicity of dibutyltin, tributyltin and other organotin compounds to humans and to experimental animals. *Toxicology* 55: 253–298.

Brasil. 1996. *LEI COMPLEMENTAR Nº 87, DE 13 DE SETEMBRO DE 1996*. Brasil.

Brasil. 2004. Ministério da Agricultura, Pecuária e Abastecimento. : 9.

BRASIL. 1975. *Decreto nº 76.593, de 14 de Novembro de 1975 - Institui o Programa Nacional do Álcool e dá outras Providências*.

BRASIL. 2016. Lei Nº 13.263, de 23 de março de 2016. *Diario oficial da União*.

BRASIL. 2017a. Ministério de Minas e Energia. Programa RenovaBio. : 1–11.

BRASIL. 2017b. *Ministério de Minas e Energia. Programa RenovaBio*.

Brito YC, Ferreira DAC, Fragoso DMDA, Mendes PR, Oliveira CMJD, Meneghetti MR, Meneghetti SMP. 2012. Simultaneous conversion of triacylglycerides and fatty acids into fatty acid methyl esters using organometallic tin(IV) compounds as catalysts. *Applied Catalysis A: General* 443–444: 202–206.

Bruna C, Taís P, Andrzejewski F, Andrade SF, Fuentefria AM. 2020. In vitro antidermatophytic synergism of double and triple combination of clioquinol with ciclopirox and terbinafine. : 993–1001.

Bücker F, Aguiar N, Fernando L, Seminotti RJ, Ruaro C, Anastácio F, Camargo DO, Menezes F. 2011. International Biodeterioration & Biodegradation Impact of biodiesel on biodeterioration of stored Brazilian diesel oil. *International Biodeterioration & Biodegradation* 65: 172–178.

Bücker F, de Moura TM, da Cunha ME, de Quadros PD, Beker SA, Cazarolli JC, Caramão EB, Frazzon APG, Bento FM. 2018a. Evaluation of the deteriogenic microbial

community using qPCR, n-alkanes and FAMEs biodegradation in diesel, biodiesel and blends (B5, B10, and B50) during storage. *Fuel* 233: 911–917.

Bücker F, de Moura TM, da Cunha ME, de Quadros PD, Beker SA, Cazarolli JC, Caramão EB, Frazzon APG, Bento FM. 2018b. Evaluation of the deteriogenic microbial community using qPCR, n-alkanes and FAMEs biodegradation in diesel, biodiesel and blends (B5, B10, and B50) during storage. *Fuel* 233: 911–917.

Bushnell LD, Haas HF. 1941. THE UTILIZATION OF CERTAIN HYDROCARBONS BY MICROORGANISMS. *Journal of Bacteriology*: 653–673.

Butt AF, Ahmed MN, Bhatti MH, Choudhary MA, Ayub K, Tahir MN, Mahmood T. 2019. Synthesis, structural properties, DFT studies, antimicrobial activities and DNA binding interactions of two newly synthesized organotin(IV) carboxylates. *Journal of Molecular Structure* 1191: 291–300.

Callegari A, Bolognesi S, Cecconet D, Capodaglio AG. 2019. Production technologies, current role, and future prospects of biofuels feedstocks: A state-of-the-art review. *Critical Reviews in Environmental Science and Technology* 0: 1–53.

de Campos Júnior EO, Araújo DF, Souto HN, Campos CF, Pereira BB. 2020. Contamination and health risks assessment in a dam in the southeast region of Brazil using ecotoxicological methods. *Journal of toxicology and environmental health. Part A* 83: 404–411.

Canter N. 2012. The regulatory future of antimicrobial pesticides. *Tribology and Lubrication Technology* 68.

Carvalho AL De, Cardoso EA, Rocha GO Da, Teixeira LSG, Pepe IM, Grosjean DM. 2016. Carboxylic acid emissions from soybean biodiesel oxidation in the EN14112 (Rancimat) stability test. *Fuel* 173: 29–36.

Cazarolli JC, Boelter G, Lima AMDL De, Hengles T, Correa C, Peralba MCR, Ferrão MF, Cavalcanti EHS, Bisol M V, Bezerra KS, Filho NRA, Bento FM. 2018a. Nature of insoluble material found in the bottom of soybean biodiesel storage tank: Chemical and microbiological approach. *Journal of the Brazilian Chemical Society* 29: 2034–2045.

Cazarolli JC, Boelter G, Lima AMDL De, Hengles T, Correa C, Peralba MCR, Ferrão MF, Cavalcanti EHS, Bisol M V, Bezerra KS, Filho NRA, Bento FM. 2018b. Nature of insoluble material found in the bottom of soybean biodiesel storage tank: Chemical and microbiological approach. *Journal of the Brazilian Chemical Society* 29: 2034–2045.

Cazarolli JC, Guzatto R, Samios D, do Carmo Ruaro Peralba M, de Siqueira Cavalcanti EH, Bento FM. 2014a. Susceptibility of linseed, soybean, and olive biodiesel to growth of the deteriogenic fungus Pseudallescheria boydii. *International Biodeterioration and Biodegradation* 95: 364–372.

Cazarolli JC, Guzatto R, Samios D, Peralba M do CR, Cavalcanti EH de S, Bento FM.

2014b. Susceptibility of linseed, soybean, and olive biodiesel to growth of the deteriogenic fungus Pseudallescheria boydii. *International Biodeterioration and Biodegradation* 95: 364–372.

Cazarolli JC, Silva TL, da Camara Ribas RK, da Fontoura Xavier Costa L, de Moura T, Galeazzi CF, da Rosa PD, Kenne DC, Carvalho ÂR, Valente P, Pizzolato TM, Correa C, Ferrão MF, Frazzon APG, Bento FM. 2020. Deterioration potential of Aureobasidium pullulans on biodiesel, diesel, and B20 blend. *International Biodeterioration & Biodegradation* 147: 104839.

Chanakaewsomboon I, Tongurai C, Photaworn S. 2020. Journal of Environmental Chemical Engineering Investigation of saponification mechanisms in biodiesel production : Microscopic visualization of the effects of FFA , water and the amount of alkaline catalyst. *Journal of Environmental Chemical Engineering* 8: 103538.

Chel A, Kaushik G. 2018. Renewable energy technologies for sustainable development of energy efficient building. *Alexandria Engineering Journal* 57: 655–669.

Chellappa T, Rosa JGDSS, Santos DLF, Boufleur RN, Paiva IVDL, Lèger T, Chellappa NT, Nascimento RM. 2019. Oxidative stability of blended sunflower and castor biodiesel by Rancimat and static mode P-DSC. *Energy Sources, Part A: Recovery, Utilization and Environmental Effects* 00: 1–10.

Chen YA, Liu PWG, Whang LM, Wu YJ, Cheng SS. 2019. Biodegradability and microbial community investigation for soil contaminated with diesel blending with biodiesel. *Process Safety and Environmental Protection* 130: 115–125.

Chesnau HL. 2003. Remediation Techniques. In: Passman FJ, ed. FUEL AND FUEL SYSTEM MICROBIOLOGY. ASTM International, .

Christensen E, McCormick RL. 2014. Long-term storage stability of biodiesel and biodiesel blends. *Fuel Processing Technology* 128: 339–348.

Conama. 1997. RESOLUÇÃO CONAMA nº 230, de 22 de agosto de 1997 (Revoga a Resolução no 20/96). : 462–463.

CONAMA. 2002. Resolução nº 313, de 29 de outubro de 2002. *Ministério do Meio Ambiente*: 85–91.

Contini E, Gazzoni D, Aragão A, Mota M, Marra R. 2018. Série, desafios do agronegócio brasileiro: Parte 1, complexo soja - Caracterização e desafios tecnológicos. *Nota Técnica - EMBRAPA*: 35.

Corzo G, Revah S. 1999. Production and characteristics of the lipase from Yarrowia lipolytica 681. *Bioresource Technology* 70: 173–180.

Cyplik P, Schmidt M, Szulc A, Marecik R, Lisiecki P, Heipieper HJ, Owsianiak M, Vainshtein M, Chrzanowski Ł. 2011. Relative quantitative PCR to assess bacterial community dynamics during biodegradation of diesel and biodiesel fuels under various aeration

conditions. *Bioresource Technology* 102: 4347–4352.

Ding WQ, Liu B, Vaught JL, Palmiter RD, Lind SE. 2006. Clioquinol and docosahexaenoic acid act synergistically to kill tumor cells. *Molecular Cancer Therapeutics* 5: 1864–1872.

Doctor S V., Fox DA. 1982. Effects of organotin compounds on maximal electroshock seizure (Mes) responsiveness in mice. i.tri(n-alkyl)tin compounds. *Journal of Toxicology and Environmental Health* 10: 43–52.

Dodos GS, Tsesmeli CE, Zannikos F. 2017. Evaluation of the antimicrobial activity of synthetic and natural phenolic type antioxidants in biodiesel fuel. *Fuel* 209: 150–161.

Dunn RO. 2010. Other Alternative Diesel Fuels from Vegetable Oils and Animal Fats (G Knothe, J Van Gerpen, and J Krahl, Eds.). *The Biodiesel Handbook: Second Edition*: 241–263.

El-Aziz AMA, Shaker MA, Shaaban MI. 2019. Enhanced Biocatalytic Activity of Recombinant Lipase Immobilized on Gold Nanoparticles. *Current Pharmaceutical Biotechnology* 20: 497–505.

Erofeev V, Bobryshev A, Shafigullin L, Zubarev P, Lakhno A, Darovskikh I, Tretiakov I. 2016. Building Heat-insulating Materials Based on the Products of the Transesterification of Polyethylene Terephthalate and Dibutyltin Dilaurate. *Procedia Engineering* 165: 1455–1459.

Escher F, Wilkinson J. 2019. A economia política do complexo Soja-Carne Brasil-China. *Revista de Economia e Sociologia Rural* 57: 656–678.

Fernández-Álvarez P, Vila J, Garrido-Fernández JM, Grifoll M, Lema JM. 2006. Trials of bioremediation on a beach affected by the heavy oil spill of the Prestige. *Journal of Hazardous Materials* 137: 1523–1531.

Fernandez-Calvino D, Baath E. 2010. Growth response of the bacterial community to pH in soils di¡ering in pH ˚.

Ferreira AB, Cardoso AL, da Silva MJ. 2012. Tin-Catalyzed Esterification and Transesterification Reactions: A Review. *ISRN Renewable Energy* 2012: 1–13.

Fickers P, Marty A, Nicaud JM. 2011. The lipases from Yarrowia lipolytica: Genetics, production, regulation, biochemical characterization and biotechnological applications. *Biotechnology Advances* 29: 632–644.

Fickers P, Nicaud JM, Destain J, Thonart P. 2003. Overproduction of lipase by Yarrowia lipolytica mutants. *Applied Microbiology and Biotechnology* 63: 136–142.

Fickers P, Nicaud JM, Gaillardin C, Destain J, Thonart P. 2004. Carbon and nitrogen sources modulate lipase production in the yeast Yarrowia lipolytica. *Journal of Applied Microbiology* 96: 742–749.

Finnegan C, Ryan D, Enright AM, Garcia-Cabellos G. 2018. A review of strategies for the detection and remediation of organotin pollution. *Critical Reviews in Environmental*

Science and Technology 48: 77–118.

da Fonseca MMB, Minnicelli CF, Silva-Portela R de CB, de Farias MF, dos Santos PRS, Fernandes GJT, Agnez-Lima LF. 2019. Unlocking and functional profiling of the bacterial communities in diesel tanks upon additive treatment. *Fuel* 236: 1311–1320.

Franklin RB. 2016. Zinc Ionophore (Clioquinol) Inhibition of Human ZIP1-Deficient Prostate Tumor Growth in the Mouse Ectopic Xenograft Model: A Zinc Approach for the Efficacious Treatment of Prostate Cancer. *International Journal of Cancer and Clinical Research* 3: 1–11.

Fregolente PBL, Fregolente LV, Wolf MacIel MR. 2012. Water content in biodiesel, diesel, and biodiesel-diesel blends. *Journal of Chemical and Engineering Data* 57: 1817–1821.

Fuentefria AM, Pippi B, Lana DFD, Donato KK, de Andrade SF. 2018. Antifungals discovery: an insight into new strategies to combat antifungal resistance. *Letters in Applied Microbiology* 66: 2–13.

Gassen J, Bento F, Frazzon A, Ferrão M, Marroni I, Simonetti A. 2015. Growth of Paecilomyces variotii in B0 (diesel), B100 (biodiesel) and B7 (blend), degradation and molecular detection. *Brazilian Journal of Biology* 75: 541–547.

Gaylarde CC, Bento FM, Kelley J. 1999. Microbial contamination of stored hydrocarbon fuels and its control. *Revista de Microbiologia* 30: 1–10.

Ghosh P, Jaffe SB, Group CM, Process E. 2006. Detailed Composition-Based Model for Predicting the Cetane Number of Diesel Fuels. : 346–351.

Giakoumis EG, Sarakatsanis CK. 2019. A comparative assessment of biodiesel cetane number predictive correlations based on fatty acid composition. *Energies* 12: 1–30.

Gupta PK. 2020. Fate , Transport , and Bioremediation of Biodiesel and Blended Biodiesel in Subsurface Environment : A Review. *J. Environ. Eng.* 146: 1–10.

Hadi AG, Jawad K, Ahmed DS, Yousif E. 2019a. Synthesis and biological activities of organotin (IV) carboxylates: A review. *Systematic Reviews in Pharmacy* 10: 26–31.

Hadi AG, Jawad K, Ahmed DS, Yousif E. 2019b. Synthesis and biological activities of organotin (IV) carboxylates: A review. *Systematic Reviews in Pharmacy* 10: 26–31.

Van Hamme JD, Singh A, Ward OP. 2003. Recent Advances in Petroleum Microbiology Recent Advances in Petroleum Microbiology. *Microbiol. Mol. Biol. R.* 67: 503–549.

Han F, Guo H, Hu J, Zhang J, Ying Q, Zhang H. 2020. Sources and health risks of ambient polycyclic aromatic hydrocarbons in China. *Science of the Total Environment* 698: 134229.

Hasan MM, Rahman MM. 2017. Performance and emission characteristics of biodiesel–diesel blend and environmental and economic impacts of biodiesel production: A

review. *Renewable and Sustainable Energy Reviews* 74: 938–948.

He BB, Thompson JC, Routt DW, Gerpen JH Van. 2007. Moisture Absorption in Biodiesel and its Petro-Diesel Blends. *Applied Engineering in Agriculture* 23: 71–76.

Hill G. 2003. Sampling Methods for Detecting Microbial Contamination in Fuel Tanks and Systems. *Fuel and Fuel System Microbiology: Fundamentals, Diagnosis, and Contamination Control*: 14–23.

Hill EC, Hill GC. 2008a. Microbial Contamination and Associated Corrosion in Fuels, during Storage, Distribution and Use. *Advanced Materials Research* 38: 257–268.

Hill EC, Hill GC. 2008b. Microbial Contamination and Associated Corrosion in Fuels, during Storage, Distribution and Use. *Advanced Materials Research* 38: 257–268.

HIRAKURI M, CONTE O., PRANDO A, de; CASTRO C, JUNIOR AB. 2019. Diagnóstico da produção de soja na macrorregião sojícola 4. : 119.

Hoch M. 2001. Organotin compounds in the environment - An overview. *Applied Geochemistry* 16: 719–743.

Howarth RW. 1988. ECOSYSTEMS. : 89–110.

IBGE. 2022. Levantamento Sistemático da Produção Agrícola - Sistema IBGE de Recuperação Automática - SIDRA.

Iftikhar SH, Gilani SR, Taj MB, Raheel A, Imtiaz-Ud-Din, Termizi SA, Al-Shakban M, Ali HM. 2018. Design, synthesis and biological evaluation of organotin(IV) complexes of flumequine and cetirizine. *Journal of the Serbian Chemical Society* 83: 425–437.

Iqbal H, Ali S, Shahzadi S. 2015. Antituberculosis study of organotin(IV) complexes: A review. *Cogent Chemistry* 1: 1–12.

Jacobson MZ. 2010. Short-term effects of controlling fossil-fuel soot, biofuel soot and gases, and methane on climate, Arctic ice, and air pollution health. *Journal of Geophysical Research Atmospheres* 115.

Jaeger KE, Reetz MT. 1998. Microbial lipases form versatile tools for biotechnology. *Trends in Biotechnology* 16: 396–403.

Jakeria MR, Fazal MA, Haseeb ASMA. 2014. Influence of different factors on the stability of biodiesel: A review. *Renewable and Sustainable Energy Reviews* 30: 154–163.

Jamil K, Bakhtiar M, Khan AKR, Rubina F, Rehana R, Wajid R, Qaisar M, Khan AKR, Danish M, Awais M, Bhatti ZA, Rizwan M, Naveed A, Hussani M, Pervez A. 2009. Synthesis characterization and antimicrobial activities of noval organotin compounds. 3: 66–71.

Javed F, Ali S, Shahzadi S, Sharma SK, Qanungo K, Munawar KS, Khan I. 2017. Synthesis, characterization, and biological activity of organotin(IV) complexes with 4-oxo-4-[3-(trifluoromethyl)phenylamino]butanoic acid. *Russian Journal of General Chemistry* 87: 2409–2420.

Jesus RA De, Conceição P Da, Silva JPV Da, Brainer NS, Meneghetti SMP,

Meneghetti MR. 2019. Different strategies to anchor organotin methoxides on silica and their (Re)use as heterogeneous catalysts for transesterification reactions. *Journal of the Brazilian Chemical Society* 30: 1976–1987.

KASERER H. 1905. Ueber die oxydation des wasserstofes und des methane durch mikroorganismen (Sur l'oxydation de l'hydrogène et du méthane par les microorganismes). *Z landw Versuchsw in Osterreich* 8: 789–792.

der Kerk GJMD Van, Luijten JGA. 1954. Investigations on organo-tin compounds. III. The biocidal properties of organo-tin compounds. *Journal of Applied Chemistry* 4: 314–319.

Kleinberg MN, Rios MAS, Buarque HLB, Parente MM V, Cavalcante CL, Luna FMT. 2019. Influence of Synthetic and Natural Antioxidants on the Oxidation Stability of Beef Tallow Before Biodiesel Production. *Waste and Biomass Valorization* 10: 797–803.

Knothe G. 2009. Improving biodiesel fuel properties by modifying fatty ester composition. *Energy and Environmental Science* 2: 759–766.

Knothe G, Gerpen J Van. 2005a. *The Biodiesel Handbook* (G Knothe, J Van Gerpen, and J Krahl, Eds.). AOCS Publishing.

Knothe G, Gerpen J Van. 2005b. *The Biodiesel Handbook* (G Knothe, J Van Gerpen, and J Krahl, Eds.). AOCS Publishing.

Knothe G, Sharp CA, Ryan TW. 2006. Exhaust emissions of biodiesel, petrodiesel, neat methyl esters, and alkanes in a new technology engine. *Energy and Fuels* 20: 403–408.

Komariah LN, Dewi TK, Ramayanti C. 2019. Study on corrosion behavior of storage tanks filled with biodiesel and the blends. *IOP Conference Series: Materials Science and Engineering* 543.

Kostenidou E, Martinez-valiente A, Mili BR, Marques B, Temime-roussel B, Durand A, André M, Liu Y, Louis C, Vansevenant B, Ferry D, Laffon C, Parent P, Anna BD. 2021. Technical note : Emission factors , chemical composition , and morphology of particles emitted from Euro 5 diesel and gasoline light-duty vehicles during transient cycles. : 4779–4796.

Kumar N. 2017. Oxidative stability of biodiesel : Causes , effects and prevention. *Fuel* 190: 328–350.

Kumar H, Sarma AK, Kumar P. 2020. A comprehensive review on preparation, characterization, and combustion characteristics of microemulsion based hybrid biofuels. *Renewable and Sustainable Energy Reviews* 117: 109498.

Lawan I, Zhou W, Idris AL, Jiang Y, Zhang M, Wang L, Yuan Z. 2020. Synthesis, properties and effects of a multi-functional biodiesel fuel additive. *Fuel Processing Technology* 198: 106228.

Ławniczak Ł, Woźniak-Karczewska M, Loibner AP, Heipieper HJ, Chrzanowski Ł. 2020. Microbial degradation of hydrocarbons—basic principles for bioremediation: A review.

Molecules 25: 1–19.

Leng L, Wei L, Li W, Xu X, Leng S, Li J, Zhou W. 2019. Determination and comparison of the activation energies of biodiesel microemulsion and biodiesel blends. *Energy Sources, Part A: Recovery, Utilization and Environmental Effects* 0: 1–10.

Leonardo RS, Valle MLM, Dweck J. 2017. Evaluation of different aging procedures on biodiesel thermal degradation process. *Journal of Thermal Analysis and Calorimetry* 130: 541–547.

Leung DYC, Wu X, Leung MKH. 2010. A review on biodiesel production using catalyzed transesterification. *Applied Energy* 87: 1083–1095.

Li Y, jie Liu T, jie Zhao M, Zhang H, qin Feng F. 2019. Screening, purification, and characterization of an extracellular lipase from Aureobasidium pullulans isolated from stuffed buns steamers. *Journal of Zhejiang University: Science B* 20: 332–342.

Lisiecki P, Chrzanowski Ł, Szulc A, Ławniczak Ł, Białas W, Dziadas M, Owsianiak M, Staniewski J, Cyplik P, Marecik R, Heipieper HJ. 2014. Biodegradation of diesel / biodiesel blends in saturated sand microcosms. 116: 321–327.

Livramento Silva T, Clarice Cazarolli J, Mara Pizzolato T, Paula Guedes Frazzon A, Bertoni Mann M, Frazzon J, Kochenborger John D, Flôres Ferrão M, Henrique Rampelotto P, Bezerra D'Alessandro E, Roberto Antoniosi Filho N, Menezes Bento F. 2022. Microbial sludge formation in Brazilian marine diesel oil (B0) and soybean methylic biodiesel blends (B10 and B20) during simulated storage. *Fuel* 308.

Luz GVS, Sousa BASM, Guedes A V, Barreto CC, Brasil LM. 2018. Biocides Used as Additives to Biodiesels and Their Risks to the Environment and Public Health: A Review. *Molecules* 23.

Magarette OB, Onyekachi ON, Verla AW, Enyoh CE, Johnpaul M. 2022. Optimization and effect of varying catalyst concentration and trans-esterification temperature on the yield of biodiesel production from palm kernel oil and groundnut oil. 5: 55–69.

Mairizal AQ, Awad S, Priadi CR, Hartono DM, Moersidik SS, Tazerout M, Andres Y. 2020. Experimental study on the effects of feedstock on the properties of biodiesel using multiple linear regressions. *Renewable Energy* 145: 375–381.

Manaf ISA, Embong NH, Khazaai SNM, Rahim MHA, Yusoff MM, Lee KT, Maniam GP. 2019. A review for key challenges of the development of biodiesel industry. *Energy Conversion and Management* 185: 508–517.

Mariano AP, de Arruda Geraldes Kataoka AP, de Franceschi de Angelis D, Bonotto DM. 2007. Laboratory Study on the Bioremediation of Diesel Oil Contaminated Soil From a Petrol Station. *Brazilian Journal of Microbiology* 38: 346–353.

Martin-Sanchez PM, Gorbushina AA, Toepel J. 2018a. Quantification of microbial load in diesel storage tanks using culture- and qPCR-based approaches. *International*

Biodeterioration and Biodegradation 126: 216–223.

Martin-Sanchez PM, Gorbushina AA, Toepel J. 2018b. Quantification of microbial load in diesel storage tanks using culture- and qPCR-based approaches. *International Biodeterioration and Biodegradation*.

Mataqi K, Jose S, Mathew B, Al-saffar S. 2019. International Journal of Pharma Sciences and Scientific Research Assessment and Control of Biomass Growth in Fuel Storage Tanks at the KNPC Phase II : Evaluation of the Effectiveness of Biocides in Controlling the Microorganisms Causing Problems in Oil Pr. 5: 5–12.

Matveeva L, Efremova M, Baranets I. 2018. Studies of the Morphology of Waterproof Coatings Based on Urethane Isocyanate, Alkyl-Phenol-Formaldehyde Resin and Dibutyltin Dilaurate Using the High-Resolution Optical Microscopy Technique. *Architecture and Engineering* 3: 43–47.

Meade TW. 1975. Subacute myelo-optic neuropathy and cioquinol. : 157–169.

Mendow G, Veizaga NS, Querini CA. 2011. Bioresource Technology Ethyl ester production by homogeneous alkaline transesterification : Influence of the catalyst. *Bioresource Technology* 102: 6385–6391.

Meneghetti MR, Meneghetti SMP. 2015. Sn(iv)-based organometallics as catalysts for the production of fatty acid alkyl esters. *Catalysis Science and Technology* 5: 765–771.

Meneghetti SMP, Meneghetti MR, Brito YC. 2013. Transesterification, some applications and biodiesel production. *Revista Virtual de Quimica* 5: 63–73.

Menezes RS (org. . 2016. *BIODIESEL NO BRASIL: IMPULSO TECNOLÓGICO*. UFLA.

Menezes LNB, Suarez PAZ, Ghesti GF. 2022. Diesel-Like Biofuels Production Using Fatty Waste. *J. Braz. Chem. Soc.* 33: 550–555.

Miller NJ, Mudge SM. 1997. The Effect of Biodiesel on the Rate of Removal and Weathering Characteristics of Crude Oil Within Artificial Sand Columns. *Spill Science and Technology Bulletin* 4: 17–33.

Ministério de Minas e Energia;, Agência Nacional do Petróleo Gás Natural e Biocombustíveis. 2018. *RESOLUÇÃO Nº 758, DE 23 DE NOVEMBRO DE 2018 Regulamenta*. Brazil.

MIYOSHI M. 1895. Die Durchbohrung von Membramen durch Pilzfaden. *Jahrbuch für Wissenschaftliche Botanik* 28: 269–289.

MME M de M e E, EPE E de PE. 2022. *Balanço energético nacional*.

Moser BR. 2014. Impact of fatty ester composition on low temperature properties of biodiesel-petroleum diesel blends. *Fuel* 115: 500–506.

Mustapha AO, Sarumi AD, Adewuyi SA, Oluwatobi E, Adebayo BR, Adams RO, Abdulsalam ZJ. 2022. Using Response Surface Methodology to Optimize Biodiesel

Production from Sweet Almond (Prunusamygdalus Dulcis) and Jatropha (Jatropha Curcas) Seed Oils. 2: 1–15.

Napp AP, Pereira JES, Oliveira JS, Silva-Portela RCB, Agnez-Lima LF, Peralba MCR, Bento FM, Passaglia LMP, Thompson CE, Vainstein MH. 2018. Comparative metagenomics reveals different hydrocarbon degradative abilities from enriched oil-drilling waste. *Chemosphere* 209: 7–16.

Nataša A. Kablar. 2019. Renewable Energy: Wind Turbines, Solar Cells, Small Hydroelectric Plants, Biomass, and Geothermal Sources of Energy. *Journal of Energy and Power Engineering* 13: 162–172.

Nautiyal P, Subramanian KA, Dastidar MG, Kumar A. 2020. Experimental assessment of performance, combustion and emissions of a compression ignition engine fuelled with Spirulina platensis biodiesel. *Energy* 193: 116861.

Nomanbhay S, Ong MY. 2017. A review of microwave-assisted reactions for biodiesel production. *Bioengineering* 4.

Novato M, Lacerda MI. 2017. Innovative Energy & Research RenovaBio-Towards a New National Biofuel Policy and a Truly Sustainable World. 6.

Ogunkunle O, Ahmed NA. 2019. A review of global current scenario of biodiesel adoption and combustion in vehicular diesel engines. *Energy Reports* 5: 1560–1580.

Oliveri V, Vecchio G. 2016. 8-Hydroxyquinolines in medicinal chemistry: A structural perspective. *European Journal of Medicinal Chemistry*.

Osada S, Nishikawa JI, Nakanishi T, Tanaka K, Nishihara T. 2005. Some organotin compounds enhance histone acetyltransferase activity. *Toxicology Letters* 155: 329–335.

Passman FJ. 2011. D 6469-99 Standard Guide for Microbial Contamination in Fuels and Fuel Systems (FJ Passman, Ed.). *Fuel and Fuel System Microbiology: Fundamentals, Diagnosis, and Contamination Control*: 11–81.

Passman FJ. 2013. Microbial contamination and its control in fuels and fuel systems since 1980 - a review. *International Biodeterioration and Biodegradation* 81: 88–104.

Passman FJ, Dobranic JK. 2005. Relative biodegradability of B-100 biodiesel and conventional low sulfur diesel fuels. *9th International Conference on Stability, Handling and Use of Liquid Fuels 2005* 2: 1001–1070.

Passman FJ, Kelley J, Whalen P. 2019. Interlaboratory study comparing two fuel microbiology standard test methods. *International Biodeterioration and Biodegradation* 141: 17–23.

De Paulo AA, Da Costa RS, Rahde SB, Vecchia FD, Seferin M, Dos Santos CA. 2016. Performance and emission evaluations in a power generator fuelled with Brazilian diesel and additions of waste frying oil biodiesel. *Applied Thermal Engineering* 98: 288–297.

Pellerito C, Nagy L, Pellerito L, Szorcsik A. 2006. Biological activity studies on

organotin(IV)n+ complexes and parent compounds. *Journal of Organometallic Chemistry* 691: 1733–1747.

Pereira-Meireles FV, Rocha-Leão MHM, Sant'Anna GLJ. 1997. A Stable Lipase from Candida lipolytica: Cultivation Conditions and Crude Enzyme Characteristics. *Applied biochemistry and biotechnology* 63: 73–85.

Pereira JES. 2018. PROSPECÇÃO E CARACTERIZAÇÃO DE ISOLADOS MICROBIANOS PARA A BIORREMEDIAÇÃO DE AMBIENTES MARINHOS CONTAMINADOS POR PETRÓLEO E MISTURAS DE ÓLEO DIESEL/BIODIESELJosé. *Tese de Doutorado. PPBCM/UFRGS.*: 30–55.

Pereira GG, Garcia RKA, Ferreira LL, Barrera-Arellano D. 2017. Soybean and Soybean/Beef-Tallow Biodiesel: A Comparative Study on Oxidative Degradation During Long-Term Storage. *JAOCS, Journal of the American Oil Chemists' Society* 94: 587–593.

Perera F. 2018. Pollution from fossil-fuel combustion is the leading environmental threat to global pediatric health and equity: Solutions exist. *International Journal of Environmental Research and Public Health* 15.

Peters GP, Andrew RM, Canadell JG, Friedlingstein P, Jackson RB, Korsbakken JI, Le Quéré C, Peregon A. 2019. Carbon dioxide emissions continue to grow amidst slowly emerging climate policies. *Nature Climate Change*: 2–5.

Pimentel F. 2011. O fim da Era do Petróleo e a Mudança do Paradigma Energético Mundial: Perspectivas e Desafios para a Atuação Diplomática Brasileira. *rEV. bRA. eST. dEF.* 2: 185–187.

Pinho DMM, Santos VO, Santos VML Dos, Oliveira MCS, Silva MT Da, Piza PGT, Pinto AC, Rezende MJC, Suarez PAZ. 2014. Evaluating the use of en 14078 for determination of biodiesel in diesel blends sold in the Brazilian market. *Fuel* 136: 136–142.

Pinho DMM, Suarez PAZ. 2017. Do Óleo de Amendoim ao Biodiesel- Histórico e Política Brasileira para o Uso Energético de Óleos e Gorduras From Peanut Oil to Biodiesel-History and Brazilian Policyfor the Energetic Use of Fats and Oils Do Óleo de Amendoim ao Biodiesel- Histórico e Polí. 9: 39–51.

Pippi B, Lopes W, Reginatto P, Émili F, Silva K, Rocha A, José R, Pozza G, Henning M, Fernandes S, Meneghello A. 2019. New insights into the mechanism of antifungal action of 8-hydroxyquinolines. *Saudi Pharmaceutical Journal* 27: 41–48.

Pollera C, Lucchini B, Formentin E, Bareggi S, Poli G, Ponti W. 2005. Evaluation of anti-prionic activity of clioquinol in an in vivo model (Mesocricetus auratus). *Veterinary Research Communications* 29: 253–255.

Polshettiwar V, Luque R, Fihri A, Zhu H, Bouhrara M, Basset JM. 2011. Magnetically recoverable nanocatalysts. *Chemical Reviews* 111: 3036–3075.

Priel T, Aricha-Tamir B, Sekler I. 2007. Clioquinol attenuates zinc-dependent β-cell

death and the onset of insulitis and hyperglycemia associated with experimental type I diabetes in mice. *European Journal of Pharmacology* 565: 232–239.

Rabiei K, Sarrafzadegan N, Ghanbari A, Shamsipour M, Hassanvand MS, Amini H, Yunesian M, Farzadfar F. 2020. The burden of cardiovascular and respiratory diseases attributed to ambient sulfur dioxide over 26 years. *Journal of Environmental Health Science and Engineering* 18: 267–278.

Rajaeifar MA, Tabatabaei M, Aghbashlo M, Hemayati SS, Heijungs R. 2019. Biodiesel Production and Consumption: Life Cycle Assessment (LCA) Approach. In: Tabatabaei M,, In: Aghbashlo M, eds. **Biodiesel: From Production to Combustion**. Cham: Springer International Publishing, 161–192.

Ramadhani AN, Harimawan A, Devianto H. 2018. Water content effect on biofilm formation and bio-corrosion process in biodiesel-diesel storage tank. *International Journal of Engineering and Technology(UAE)* 7: 2009–2012.

Ramos MJ, Fernández CM, Casas A, Rodríguez L, Pérez Á. 2009. Influence of fatty acid composition of raw materials on biodiesel properties. *Bioresource Technology* 100: 261–268.

Rauch ME, Graef HW, Rozenzhak SM, Jones SE, Bleckmann CA, Kruger RL, Naik RR, Stone MO. 2006. Characterization of microbial contamination in United States Air Force aviation fuel tanks. *Journal of Industrial Microbiology and Biotechnology* 33: 29–36.

dos Reis Carraro A, da Silva César A, Conejero MA. 2018. Sebo Bovino: Potencial para produção de biodiesel no brasil. *AgroAnalysis*: 21–22.

Ribas RK da C, dos Santos Carboni D, Cazarolli JC, Flôres SH, Ramirez-Castrillon M, Valente P. 2019. Nitrogen source and pH interact and modulate lipase secretion in a non-clinical strain of Candida parapsilosis. *Acta Scientiarum - Biological Sciences* 41.

Rocha A, Taufer R, Keli B, Staudt J, Carine T, Joaquim AR, Pippi B, Antonio M, Aline R, Zimmer R, Verlindo B, Gustavo DA, Alexandre S, Fuentefria M, Andrade SF De, Andreza S, Martins F, Lettieri M, Francisco T. 2018. hydroxyquinolines with few fungal lines. : 1–11.

Roszak DB, Colwell RR. 1987. Complexity in natural and cultural systems. *MICROBIOLOGICAL REVIEWS* 51: 365–379.

Saifuddin N, Samiuddin A, Kumaran P. 2015. A Review on Processing Technology for Biodiesel Production. *Trends in Applied Sciences Research* 10: 1–37.

Salina FH, de Almeida IA, Bittencourt FR. 2020. RenovaBio Opportunities and Biofuels Outlook in Brazil (A Sayigh, Ed.). *Renewable Energy and Sustainable Buildings*: 391–399.

Sari CN, Hertadi R, Gozan M, Roslan AM. 2019. Factors Affecting the Production of Biosurfactants and their Applications in Enhanced Oil Recovery (EOR). A Review. *IOP Conference Series: Earth and Environmental Science* 353.

Sazzad BS, Fazal MA, Haseeb ASMA, Masjuki HH. 2016. Retardation of oxidation and material degradation in biodiesel: A review. *RSC Advances* 6: 60244–60263.

Schleicher T, Werkmeister R, Russ W, Meyer-Pittroff R. 2009. Microbiological stability of biodiesel-diesel-mixtures. *Bioresource Technology* 100: 724–730.

Serra TM, Mendona DR De, Silva JPV Da, Meneghetti MR, Meneghetti SMP. 2011. Comparison of soybean oil and castor oil methanolysis in the presence of tin(IV) complexes. *Fuel* 90: 2203–2206.

Sharma V, Duraisamy G, Arumugum K. 2020. Impact of bio-mix fuel on performance, emission and combustion characteristics in a single cylinder DICI VCR engine. *Renewable Energy* 146: 111–124.

Shimada Y, Sugihara A, Nagao T, Tominaga Y. 1992. Induction of Geotrichum candidum lipase by long-chain fatty acids. *Journal of Fermentation and Bioengineering* 74: 77–80.

Shorot S, Gaurav K, Rajaeifar MA, Tabatabaei M, Aghbashlo M, Hemayati SS, Heijungs R. 2019. Biodiesel Production and Consumption: Life Cycle Assessment (LCA) Approach (M Tabatabaei and M Aghbashlo, Eds.). *Biodiesel: From Production to Combustion* 5: 161–192.

Sia CB, Kansedo J, Tan YH, Lee KT. 2020. Evaluation on biodiesel cold flow properties, oxidative stability and enhancement strategies: A review. *Biocatalysis and Agricultural Biotechnology* 24: 101514.

Silitonga AS, Shamsuddin AH, Mahlia TMI, Milano J, Kusumo F, Siswantoro J, Dharma S, Sebayang AH, Masjuki HH, Ong HC. 2020. Biodiesel synthesis from Ceiba pentandra oil by microwave irradiation-assisted transesterification: ELM modeling and optimization. *Renewable Energy* 146: 1278–1291.

Silva TL, Cazarolli JC, Pizzolato TM, Frazzon APG, Mann MB, Frazzon J, John DK, Ferrão MF, Rampelotto PH, D'Alessandro EB, Filho NRA, Bento FM. 2022. Microbial sludge formation in Brazilian marine diesel oil (B0) and soybean methylic biodiesel blends (B10 and B20) during simulated storage. *Fuel* 308.

da Silva MA, dos Santos ASS, Neto AJS, Giertyas CJ, Bortoluzzi JH, Meneghetti MR, Plentz Meneghetti SM. 2019. Evaluation of Esterification of Oleic Acid and Glycerol in the Presence of Organotin(IV) Compounds. *European Journal of Lipid Science and Technology* 121: 1–9.

da Silva MA, dos Santos ASS, dos Santos TV, Meneghetti MR, Meneghetti SMP. 2017a. Organotin(IV) compounds with high catalytic activities and selectivities in the glycerolysis of triacylglycerides. *Catalysis Science & Technology* 7: 5750–5757.

da Silva MA, dos Santos ASS, dos Santos TV, Meneghetti MR, Meneghetti SMP. 2017b. Organotin (IV) compounds with high catalytic activities and selectivities in the

glycerolysis of triacylglycerides. *Catalysis Science & Technology* 7: 5750–5757.

de Siqueira Cavalcanti EH, de Souza AG, dos Santos IMG, Bento FM. 2016. Armazenamento, Estabildiade e Problemas Associados (RS Menezes, Ed.). *BIODIESEL NO BRASIL: IMPULSO TECNOLÓGICO*: 167–187.

de Siqueira Cavalcanti EH, Zimmer AR, Bento FM, Ferrão MF. 2019. Chemical and microbial storage stability studies and shelf life determinations of commercial Brazilian biodiesels stored in carbon steel containers in subtropical conditions. *Fuel* 236: 993–1007.

Soares IP, Fávaro LC de L, Quirino BF, Costa PPKG, Souto B de M, Franco PF, Brunale PP de M, Arantes AFB, Araújo ACB de, Moraes BR de L, Oliveira NG de. 2019. Monitoramento Químico e Microbiano da Mistura Diesel/Biodiesel Durante Armazenamento Simulado. *BOLETIM DE PESQUISA E DESENVOLVIMENTO - EMBRAPA ENERGIA* 23.

Solomon S, Plattner GK, Knutti R, Friedlingstein P. 2009. Irreversible climate change due to carbon dioxide emissions. *Proceedings of the National Academy of Sciences of the United States of America* 106: 1704–1709.

Song C. 2020. *Chemistry of Diesel Fuels*. CRC Press.

Song X, Zapata A, Eng G. 2006. Organotins and quantitative-structure activity/property relationships. *Journal of Organometallic Chemistry* 691: 1756–1760.

Soriano AU, Martins LF, de Assumpção Ventura ES, de Landa FHTG, de Araújo Valoni É, Faria FRD, Ferreira RF, Faller MCK, Valério RR, de Assis Leite DC, do Carmo FL, Peixoto RS. 2015. Microbiological aspects of biodiesel and biodiesel/diesel blends biodeterioration. *International Biodeterioration and Biodegradation* 99: 102–114.

Stamps BW, Bojanowski CL, Drake CA, Nunn HS, Lloyd PF, Floyd JG, Emmerich KA, Neal AR, Crookes-Goodson WJ, Stevenson BS. 2020a. In situ Linkage of Fungal and Bacterial Proliferation to Microbiologically Influenced Corrosion in B20 Biodiesel Storage Tanks. *Frontiers in Microbiology* 11: 1–13.

Stamps BW, Bojanowski CL, Drake CA, Nunn HS, Lloyd PF, Floyd JG, Emmerich KA, Neal AR, Crookes-Goodson WJ, Stevenson BS. 2020b. In situ Linkage of Fungal and Bacterial Proliferation to Microbiologically Influenced Corrosion in B20 Biodiesel Storage Tanks. *Frontiers in Microbiology* 11: 1–13.

Sulistyarini DH. 2019. Optimization of Blending Octane Number Premium Composition and Blending Sulfur Content Diesel Composition In Refinery I Ru IV Cilacap. *IOP Conf. Series: Materials Science and Engineering* 494.

Sundus F, Fazal MA, Masjuki HH. 2017. Tribology with biodiesel: A study on enhancing biodiesel stability and its fuel properties. *Renewable and Sustainable Energy Reviews* 70: 399–412.

Tan SX, Lim S, Ong HC, Pang YL. 2019. State of the art review on development of ultrasound-assisted catalytic transesterification process for biodiesel production. *Fuel* 235:

886–907.

TAUSSON WO, ALESHINA WA. 1932. Uber die bakterielle sulatreduktion bei Anwesenheit der Kohlenwasserstoffe. *Microbiology* 1.

Taylor LT, Jones DM. 2001. Bioremediation of coal tar PAH in soils using biodiesel. *Chemosphere* 44: 1131–1136.

Teixeira LSG, Couto MB, Souza GS, Filho MA, Assis JCR, Guimarães PRB, Pontes LAM, Almeida SQ, Teixeira JSR. 2010. Characterization of beef tallow biodiesel and their mixtures with soybean biodiesel and mineral diesel fuel. *Biomass and Bioenergy* 34: 438–441.

Thangaraj B, Solomon PR, Muniyandi B, Ranganathan S, Lin L. 2019. Catalysis in biodiesel production - A review. *Clean Energy* 3: 2–23.

Torsvik V, Øvreås L. 2002. Microbial diversity and function in soil: from genes to ecosystems. *Current opinion in microbiology* 5: 240–5.

Varjani SJ. 2017. Microbial degradation of petroleum hydrocarbons. *Bioresource Technology* 223: 277–286.

Vormittag EMPA, Rodrigues CG, de André PA, Saldiva PHN. 2018a. Assessment and valuation of public health impacts from gradual biodiesel implementation in the transport energy matrix in Brazil. *Aerosol and Air Quality Research* 18: 2375–2382.

Vormittag EMPA, Rodrigues CG, de André PA, Saldiva PHN. 2018b. Assessment and valuation of public health impacts from gradual biodiesel implementation in the transport energy matrix in Brazil. *Aerosol and Air Quality Research* 18: 2375–2382.

Waikhom D, Ngasotter S, Devi LS, Devi S, Singh AS. 2020. Role of Microbes in Petroleum Hydrocarbon Degradation in the Aquatic Environment: A Review. *International Journal of Current Microbiology and Applied Sciences* 9: 2903–2990.

Wehbe M, Malhotra AK, Anantha M, Lo C, Dragowska WH, Santos N Dos, Bally MB. 2017. Development of a copper-clioquinol formulation suitable for intravenous use.

Woźniak-Karczewska M, Lisiecki P, Białas W, Owsianiak M, Piotrowska-Cyplik A, Wolko Ł, Ławniczak Ł, Heipieper HJ, Gutierrez T, Chrzanowski Ł. 2019a. Effect of bioaugmentation on long-term biodegradation of diesel/biodiesel blends in soil microcosms. *Science of the Total Environment* 671: 948–958.

Woźniak-Karczewska M, Lisiecki P, Białas W, Owsianiak M, Piotrowska-Cyplik A, Wolko Ł, Ławniczak Ł, Heipieper HJ, Gutierrez T, Chrzanowski Ł. 2019b. Effect of bioaugmentation on long-term biodegradation of diesel/biodiesel blends in soil microcosms. *Science of the Total Environment* 671: 948–958.

Yemashova NA, Murygina VP, Zhukov D V., Zakharyantz AA, Gladchenko MA, Appanna V, Kalyuzhnyi S V. 2007. Biodeterioration of crude oil and oil derived products: A review. *Reviews in Environmental Science and Biotechnology* 6: 315–337.

Yousif E, Salimon J, Salih N, Jawad A, Win YF. 2016. New stabilizers for PVC based on some diorganotin(IV) complexes with benzamidoleucine. *Arabian Journal of Chemistry* 9: S1394–S1401.

Zhai S, Yang L, Cindy Q. 2010. Tumor cellular proteasome inhibition and growth suppression by 8-hydroxyquinoline and clioquinol requires their capabilities to bind copper and transport copper into cells. : 259–269.

Zhang X, Petersons C, Reece D, Haws R, Möller G. 1998. BIODEGRADABILITY OF BIODIESEL IN THE AQUATIC ENVIRONMENT. *Transactions of the American Society of Agricultural Engineers* 41: 1423–1430.

Zhao B. 2017. Why will dominant alternative transportation fuels be liquid fuels, not electricity or hydrogen? *Energy Policy* 108: 712–714.

Zimmer AR. 2014. MONITORAMENTO E CONTROLE DA CONTAMINAÇÃO MICROBIANA DURANTE O ARMAZENAMENTO SIMULADO DE MISTURAS DIESE/BIODIESEL COM USO DE BIOCIDA. : 180.

Zimmer AR, Oliboni A, Viscardi SLC, Teixeira RM, Flores M, M F. 2017. Biodiesel blend (B10) treated with a multifunctional additive (biocide) under simulated stored conditions : a field and lab scale monitoring. 14: 627–636.

Zubair M, Sirajuddin M, Haider A, Hussain I, Tahir MN, Ali S. 2019. Organotin (IV) Complexes as Catalyst for Biodiesel Formation: Synthesis, Structural Elucidation and Computational Studies. *Applied Organometallic Chemistry*: 1–16.