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Consumo e biodegradação de plástico por larvas de inseto: um estudo sobre *Galleria mellonella*

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Dissertação submetida ao Programa de Pós-Graduação em Biociências da Universidade Federal de Ciências da Saúde de Porto Alegre como requisito para a obtenção do grau de Mestre.

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SUMÁRIO

LISTA DE ABREVIATURAS

PE, Polyethylene
PS, Polystyrene
PVC, Polyvinyl chloride
PP, Polypropylene
iPP, Isotactic PP
sPP, Syndiotactic PP
aPP, Atactic PP
LDPE, Low-density polyethylene
HDPE, High density polyethylene
EPS, Expanded polystyrene
HIPS, High impact polystyrene
ISO, International Organization for Standardization
ASTM, American Society for Testing and Materials
FTIR, Fourier Transformed Infrared Spectroscopy
GPC, Gel Permeation Chromatography
TGA, Thermogravimetric Analysis
SEM, Scanning Electronic Microscopy
AFM, Atomic Force Microscopy
XPS, X-ray Photoelectron Spectroscopy
GC-MS, Gas Chromatography coupled to Mass Spectroscopy
HPLC, High Performance Chromatography
NMR, Nuclear Magnetic Resonance spectroscopy (NMR)
^{13}C, Radiolabelled Carbon
PUR, Polyurethane
PET, Polyethylene Terephthalate
UV, Ultra Violet
PTLDPE, Pretreated LDPE
UTLDPE, Untreated LDPE
SCS, Sole carbon source
PBS, Poly(butylene succinate)

PBSA, Poly(butylene succinate-co-butylene adipate)
PCL, Polycaprolactone
PLA, Polylactic Acid
CEL, Cellulose
PE-1, LDPE foam, which contains pink color additives
PE-2, LDFE film, which is colorless without additives
XRD, X-ray Diffraction
DDGS, Dried distillers' grains with soluble
CEL-ca, Cellulose Cardboard
PE-oxo, Oxo-degradable polyethylene
OAT, Oatmeal
LLDPE, Linear Low-density Polyethylene
EVA, Ethylene-Vinyl Acetate
TGA-SDTA, Thermo Gravimetry Differential Thermal Synchronous Analyzer
PPS, Polyphenylene Sulfide
PE-WC, PE Waxcomb
OTUs, Operational Taxonomic Units.
LC-DAD-MS, Liquid Chromatography coupled to Diode Array Detection and Mass Spectrometry
FEG-SEM-EDS, Field Emission Gun Scanning Electron Microscopy coupled Energy Dispersive Spectroscopy
PBS, Phosphate Buffered Saline
DNA, Desoxyribonuceic Acid
dsDNA, double-stranded DNA
HS, High-Sensitivity
dNTP, Deoxyribonucleotide Triphosphate
OTUs, Operational Taxonomic Units
PcoA, Principal Coordinate Analysis
ANOVA, Analysis of Variance
BPC, Base Peak Chromatograms
MF, Molecular Formula
RT: Retention Time

Hts, High-Throughput Sequencing

Mt, Milhões de toneladas

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RESUMO

O polietileno (PE) representa aproximadamente 30% da demanda total de plásticos, e é formado a partir da condensação do monômero etileno (C_2H_4). O alto peso molecular, o caráter hidrofóbico e a ausência de grupos hidrolisáveis e oxidáveis naestrutura química, tornam o PE inerte e resistente à biodegradação. Assim, o aumento no uso desses materiais tem provocado acúmulo de resíduos plásticos sólidos, os quais são prejudiciais a vida terrestre e marinha. Estudos visando novas alternativas para o tratamento desses resíduos têm ganhado destaque. Em 2017 houve o primeiro relato na literatura sobre a capacidade de larvas do inseto *Galleria mellonella* biodegradar filmes de PE, a uma taxa superior às de biodegradação microbiana já descritas na literatura. Interessantemente, essa capacidade pode ser relacionada com a ecologia natural do inseto, uma vez que seu habitat natural são as colmeias de abelha, material constituído por substâncias químicas ricas em ligações C-C e C-H, assemelhando-se à estrutura do PE. No entanto, o trabalho pioneiro sofreu críticas da comunidade científica, uma vez que os dados obtidos não eram suficientes para afirmar a biodegradação do PE, e criou a perspectiva de avaliar esse processo por larvas de *G. mellonella*. Desse modo, os objetivos do presente trabalho são: (i) revisar a literatura sobre tratamentos de resíduos plásticos e sobre biodegradação por larvas de insetos; (ii) verificar experimentalmente se as larvas de *G. mellonella* são capazes de biodegradar o PE ou apenas o convertem em microplástico e o excretam; (ii) entender a rota de ingestão do PE pelas larvas de *G. mellonella*, e (iii) avaliar o envolvimento da microbiota dessas larvas no processo de biodegradação do PE. Inicialmente determinamos o período larval de maior consumo do PE, verificamos que uma única larva, pesando 130 mg, é capaz de ingerir 17,22 ± 1,29 mg de PE por dia. Análises físico-químicas, incluindo FTIR, LC-MS, FEG-SEM-EDS e XPS, realizadas em diferentes sítios corporais (hemolinfa, casulo e fezes) de larvas alimentadas com PE, por 7 dias, ou com dieta laboratorial indicaram a presença de substâncias de metabolização distintas exclusivamente em larvas alimentadas PE. A excreção de compostos com características muito semelhantes à estrutura do PE foi observada nas fezes das larvas alimentas com PE. Baseado nestas análises, é possível concluir que parte do PE fragmentado mecanicamente pelas larvas é metabolizado e absorvido e outra parte é eliminada nas fezes das larvas como PE. Para verificar o processo de biomineralização do plástico após sua ingestão por larvas de *G. mellonella* foi realizado um ensaio de respirometria a fim de quantificar o CO_2 de dois grupos de larvas: (i) alimentadas com PE e (ii) sem alimentação. No entanto, a produção de do metabólito final de biodegradação aeróbia do PE, não foi alterada. Assim ainda é necessário esclarecer se o PE não é biomineralizado no metabólito final ou se esse gás é utilizado por enzimas do animal para manutenção do pH intestinal. Dados preliminares da análise do sequenciamento do gene *rRNA 16S* indicam que uma alimentação com PE altera o microbioma intestinal das larvas, sugerindo a participação da microbiota bacteriana no processo de digestão do PE. Desse modo, esse estudo contribui no entendimento do processo de biodegradação de PE utilizando larvas de *G. mellonella*, o que pode futuramente auxiliar em novas soluções biotecnológicas inovadoras para gerenciar o desafiador problema dos resíduos plásticos no meio ambiente.

Palavras-chaves: polietileno, biodegradação, larvas de insetos, microbiota, *Galleria mellonella*.

ABSTRACT

Polyethylene (PE) represents approximately 30% of the total demand for plastics and is formed from the ethylene monomer (C_2H_4) condensation. Its high molecular weight, the hydrophobic character, and the absence of hydrolyzable and oxidizable groups in the chemical structure, make PE inert and resistant to biodegradation. Thus, the use increase of these materials has caused a solid plastic waste accumulation, which is harmful to terrestrial and marine life. Studies aiming new alternatives to treatment of these residues have gained prominence. In 2017, there was the first report in the literature on the ability of *Galleria mellonella* larvae to biodegrade PE films, at a rate higher than that biodegradation microbial described in the literature. Interestingly, this ability can be related to the insect's natural ecology, since its natural habitat is beehives, a material formed by chemical substances rich in C-C and C-H bonds, similar PE structure. However, the scientific community criticized the pioneering work, since the data obtained were not sufficient to affirm the PE biodegradation, and created the perspective of evaluating this process by *G. mellonella* larvae. Thus, the objectives of the present work are: (i) to review the literature on plastic waste treatments and on biodegradation by insect larvae; (ii) experimentally verify whether the *G. mellonella* larvae are capable biodegrade PE, or just convert it into microplastic and excrete it; (ii) understand the route of PE ingestion by *G. mellonella* larvae, and (iii) evaluate the microbiota involvement of these larvae in the PE biodegradation process. Initially, we determined the larval greatest period PE consumption, and we saw a single larva, weighing 130 mg, is capable to ingeste 17.22 ± 1.29 mg of PE per day. Physico-chemical analyzes, including FTIR, LC-MS, SEM-FEG-EDS and XPS, performed at different larvae body sites (hemolymph, cocoon and feces) fed with PE, for 7 days, or with laboratory diet indicated were presen only in PE-fed larvae. The compounds with characteristics very similar to the PE structure excretion was observed in larvae fed with PE the feces. Based on these analyzes, it is possible to conclude PE mechanically fragmented by the larvae part is metabolized and absorbed and another part is eliminated in the feces. To verify PE biomineralization process after ingestion by *G. mellonella* larvae, a respirometry test was performed, to quantify the CO_2 the group larvae: (i) fed with PE and (ii) starved. However, the production of the PE final aerobic biodegradation metabolite was not altered. Thus, it is still necessary to clarify whether PE is not biomineralized in the final metabolite or whether this gas is used by animal enzymes to maintain intestinal pH. Preliminary data from sequencing of the *16S rRNA* gene indicate that feeding with PE alters the intestinal larvae microbiome, suggesting the bacterial microbiota participation in the PE digestion process. In this way, this study contributes to the understanding PE biodegradation by *G. mellonella* larvae, which may in the future assist in new innovative biotechnological solutions to manage the challenging plastic waste.

Keywords: plastic, polyethylene, biodegradation, larvae insect, microbiota, *Galleria mellonella*.

1 INTRODUÇÃO

Plásticos são formados a partir de uma longa cadeia polimérica (SCOTT, 1999) e podem ser facilmente moldados em diferentes formas para dar origem a um produto de interesse (ANDRADY, 2015). Aliado a isto, algumas características físico-químicas, como a sua durabilidade, leveza, resistência à ataques microbianos e influências ambientais, tornam esse material amplamente utilizado em diversas áreas industriais (ANDRADY; NEAL, 2009). Como resultado dessa ampla utilização, observa-se uma elevada produção de plástico, estimando-se uma produção de 368 milhões de toneladas no ano de 2019 (PLASTICS EUROPE, 2020). E, desde 1950, quando o plástico foi criado, até 2019 foram produzidos 8,5 bilhões de toneladas no mundo (GEYER et al., 2017; PLASTICS EUROPE, 2020).

Dentre os diversos plásticos existentes, o polietileno (PE) ganha destaque uma vez que esse polímero representa 29,8% da demanda total de produtos de termoplásticos (PLASTIC EUROPE, 2020). O PE é amplamente utilizado em embalagens de alimentos, produtos farmacêuticos e hospitalares, brinquedos, utilidades cosméticas, entre outros (MEDEIROS; FERREIRA; CARCIOFI, 2017). No entanto, esse polímero apresenta algumas características físico-químicas que o tornam inerte e resistente à degradação, como o alto peso molecular, o caráter hidrofóbico e a ausência de grupos hidrolisáveis e oxidáveis, sendo que os últimos são conhecidos por facilitar processos de adesão microbianae de reação química (GAUTAM; BASSI; YANFUL, 2007). Desse modo, dado o grande contraste entre a longa duração e o rápido e elevado consumo de materiais plásticos, observa-se um elevado acúmulo desse material no ambiente, ocasionando danos à vida terrestre e marinha (SHAH, et al., 2008; ROY, et al., 2011).

Atualmente o tratamento de resíduos plásticos se dá por basicamente três processos: a reciclagem, a incineração e o despejo desses materiais em aterros sanitários (PLASTIC EUROPE, 2020). Apesar da reciclagem ser o processo de tratamento de resíduos sólidos mais recomendado, dados da literatura indicam que esse processo não é amplamente aplicado e, consequentemente, uma grande quantidade de resíduos é depositada no meio ambiente (UNEP, 2016; RAGAERT et al., 2017). Em 2018, a União Europeia, Noruega e Suíçareciclaram cerca de 12,39 Mt (32,5%) de resíduos de plástico (PLASTICS EUROPE, 2020). Neste mesmo ano, o Brasil reciclou apenas 1,28% de resíduo plástico (CUFFARI, 2019), no entanto, esse valor é controverso para a Associação Brasileira da Indústria de

Plástico, que mostra que o país atingiu 22,1% de reciclagem de resíduos de origem plástica (ABIPLAST, 2019). Ainda sobre os métodos de gerenciamento de resíduos sólidos plásticos, sabe-se que no cenário mundial, cerca de 15% do resíduo plástico é tratado pelo processo de incineração, o que acarreta uma emissão de 2,7 toneladas de CO_2 na atmosfera para cada 1 tonelada de plástico queimado (WIT et al., 2019). Em 2018, a União Europeia, Noruega e Suíça utilizaram 42,6% desses resíduos para recuperação de energia, pelo processo de incineração. Além disso, no mesmo ano, esses países despejaram 29,4% de resíduos plásticos em aterros sanitários (PLASTICS EUROPE, 2019), esse dado para o Brasil é de 67,54% (CUFFARI, 2019).

No entanto, ainda não há um tratamento ideal para resíduos plásticos uma vez que os três tratamentos tradicionais apresentam aspectos negativos: (i) o processo da reciclagem de plásticos é considerado uma técnica economicamente desfavorável, devido a necessidade de tratamentos prévios, como por exemplo etapas de limpeza, o que acarreta em custos superiores quando comparado a utilização de uma matéria-prima virgem (SCOTT, 2000; OJEDA et al., 2009); (ii) o processo de incineração acarreta na liberação de uma elevada quantidade de gases na atmosfera e; (iii) a deposição em aterro sanitário pode afetar drasticamente o ecossistema presente no local, levando a uma diminuição na qualidade do solo (SHAH et al., 2008; ROY et al., 2011; MACHADO et al., 2018).

O aprimoramento de outras técnicas para o tratamento desses resíduos plásticos pode contribuir com a solução para o acúmulo desses materiais presentes no ambiente (MARTÍNEZ et al., 2015). Uma dessas técnicas é a degradação, que pode ocorrer por meios abióticos e bióticos e se inicia por mudanças físico-químicas na estrutura do plástico que levam à deterioração da funcionalidade do material. Então, posteriormente, fenômenos físicos, químicos e/ou biológicos induzem cisões de ligações e transformações químicas, que dão origem a novos grupos químicos funcionais, alterando as propriedades mecânicas, elétricas e ópticas do material (SHAH et al., 2008; ESMAEILI et al., 2013; MORRO et al., 2019). Estudos reportados na literatura apontam a biodegradação de PE por micro-organismos (Tabela 1). Eles utilizam análises gravimétricas e/ou análises de superfície de polímero, sendo relatada a presença de grupos químicos como carbonilas, cetonas, vinil e metila, indicativas de modificação do material.

Tabela 1 - Biodegradação de PE por micro-organismos.

Classificação	**Micro-organismo**	**Local de isolamento**	**Perda de peso do plástico (%)**	**Tempo de incubação (dias)**	**Referência**
Bactéria Gram positiva	*Rhodococcus ruber*	Solo de despejo de PE	7,5%	56	SILVAN, SZANTO, PAVLOV (2006)
	Bacillus sphericus	Bactéria marinha (isolado da zona pelágica)	10%	180	SUDHAKAR, et al. (2008)
	Bacillus cereus		2,5%		
	Kocuria palustres		1%	30	HARSHVARDHAN, JHA (2013)
	Bacillus pumilus		1,5%		
	Bacillus subitilis		1,75%		
	Arthrobacter sp.	Depósito de PE no Golfo de Mannar	12%	30	BALASUBRAMANIAN et al. (2010)
Bactéria Gram negativa	*Pseudomonas syringae*	Solo	11,3%	120	YOON, JEON, KIM (2012)
	Pseudomonas aeruginosa		11%		KYAW, et al. (2012
	Pseudomonas		9%		

	putida				
	Chelatococcus	Compostos bacterianos	N.D*	80	HARSHVARDHAN, JHA (2013)
Fungo	*Aspergillus niger*	Solo	8,2%	126	ESMAEILI, et al. (2013)
	Trametes versicolor	Madeira	N.D*	12	IIYOSHI, TSUTSUMI, NISHIDA (1998)
	Phanerochaete spp.				
	Penicillium simplicissimum	N.D*		90	YAMADA-ONODERA, et al. (2001)
	Fusarium redolens	Solo	0,39%	730	ALBERTSSON, BÁNHIDI, (1980)

N.D*: Não Determinado

No entanto esse processo é extremamente lento e segundo Chamas e colaboradores (2020), o PE consegue realizar o processo de degradação a uma taxa de 11 µm ano^{-1}, sendo assim, os autores estimam que uma garrafa desse material permaneça cerca de 116 a 500 anos, quando em ambiente marinho e terrestre, respectivamente. Desse modo, a necessidade da busca de alternativas biotecnológicas que possam futuramente auxiliar no tratamento de resíduos plásticos no ambiente, vem ganhando destaque e mostram uma abordem recente na comunidade científica mundial: a utilização de larvas de inseto para biodegradação plástica.

O primeiro relato de uma larva capaz de biodegradar poliestireno (PS) ocorreu em 2010, quando Miao e colaboradores descreveram a capacidade da larva de *Zophodas morio* em ingerir e biodegradar diferentes tipos de plástico. Além disso, outras 8 diferentes espécies de larvas de insetos também já foram relatadas como capazes de biodegradar plástico: *Plodia interpunctella, Achroia grisella, Galleria mellonella, Tenebrio molitor, Tenebrio obscurus, Alphitobius diaperinus, Plesiophthalmus davidi* e *Tribolium castaneum.* O Capítulo II desse documento traz um compilado desses estudos, mostrando resultados importantes e discussões sobre o tema.

Nesse sentido, Bombelli, Howe e Bertocchini (2017) foi o primeiro estudo que relatou a capacidade de larvas de *G. mellonella* em biodegradar PE, fazendo isso a uma taxa de 0,23 mgcm^{-2} h^{-1}, o que é considerada muito superior quando comparada àquelas relatadas na literatura para micro-organismos (bactérias e fungos, citados anteriormente). No entanto, esse trabalho pioneiro para biodegradação plástica envolvendo larvas de *G. mellonella* sofreu críticas da comunidade científica, isso pois os dados apresentados se mostraram inconclusivos para a confirmação da biodegradação de PE por essas larvas. Esse fato criou a perspectiva de avaliar se a atividade digestória do plástico (hidrocarbonetos de alto peso molecular) por *G. mellonella* deriva da ação enzimática do próprio animal ou de ações enzimáticas de sua microbiota. Desde 2017 até o presente momento foram publicados 13 estudos na literatura sobre que visam entender o processo de biodegradação de plástico por larvas de *G. mellonella*, por isso é importante o conhecimento sobre algumas características biológicas desse inseto.

G. mellonella faz parte da família Pyralidae, da ordem dos lepidópteros e pode ser encontrada em todo o mundo (KWADHA et al., 2017). Esse inseto possui quatro fases em seu ciclo de vida, sendo eles: ovo, larva, pupa e mariposa e o tempo para completar o ciclo de vida pode levar semanas a meses, dependendo de fatores como a temperatura, exposição a luz e disponibilidade de comida (NIELSEN; BRISTER, 1979; GULATI; KAUSHIK, 2004). Desse modo, sabe-se que a alimentação mais intensa desse inseto ocorre nos primeiros estágios de vida (NIELSEN; BRISTER, 1979) de 28 dias a 6 meses ocorre sua fase larval e o estágio pupal leva entre 1 e 9 semanas (WILLIAMS, 1997; ELLIS; GRAHAM; MORTENSEN, 2013). Posteriormente ocorre a metamorfose em mariposa macho e fêmea, onde essas acasalam para que recomece o ciclo de vida do inseto de *G. mellonella*, como mostra a Figura 1.

Figura 1 - Estágios de desenvolvimento do inseto de *G. mellonella*. (a) larva de *G. mellonella* no último estágio larval, atingindo 3 cm (b) larva envolta por um casulo de seda produzido, (c) pupa (d) fase adulta de mariposa. Fonte: RAMARÃO, 2012.

Essas larvas são conhecidas popularmente como traça da cera, pois possuem como nicho natural as colmeias, local onde se alimentam de cera de abelha. Este material é constituído majoritariamente por cerina (formada por ácido cerótico) e miricina (éster de áciso palmítico e álcool miricílico), substâncias químicas ricas em ligações C-C e C-H (DICKMAN, 1933), assemelhando-se com a estrutura química do PE. Portanto, esse fato pode sugerir que a biodegradação inata do PE por estas larvas, relacionada com a ecologia do animal.

Apesar do aumento no número de trabalhos na área com experimentos de biodegradação bem-sucedidos e promissores utilizando larvas de insetos e espécies da microbiota intestinal, a compreensão de como ocorre a clivagem biológica dos polímeros plásticos é extremamente limitada. Ainda, as enzimas envolvidas e a importância dos fatores limitantes impostos pela fisiologia animal e pelas características físicas da matriz polimérica, permanecem amplamente desconhecidos. Desse modo, o presente trabalho visa contribuir na compreensão do processo de biodegradação de PE por larvas de *G. mellonella*, o qual poderá auxiliar, futuramente, a gerenciar o problema do acúmulo de resíduo plástico no ambiente.

Sendo assim, esse trabalho está dividido em (i) Capítulo I, onde é mostrado características físico-químicas dos termoplásticos mais utilizados no mundo, bem como, dados importantes sobre o gerenciamento de resíduos desses materiais, além de apresentar técnicas utilizadas para detecção de degradação do plástico, (ii) Capítulo II, onde foi abordado um artigo de revisão crítica sobre o tema, abrangendo um compilado de todos os 46 estudos que envolvem a biodegradação de plástico por 9 diferentes espécies de larvas de insetos, (iii) Capítulo III, no qual possui um artigo original abordando o tema da biodegradação de PE utilizando larvas de *G. mellonella* e (iv) Capítulo IV, onde consta experimentos realizados na padronização de ensaios para estudo da biodegradação de PE por de larvas do inseto *G. mellonella* em nosso laboratório.

3 OBJETIVOS

3.1 OBJETIVO GERAL

Este trabalho visa determinar se as larvas do inseto *Galleria mellonella* são capazes de biodegradar o plástico polietileno e contribuir na compreensão de como ocorre este processo.

3.2 OBJETIVOS ESPECÍFICOS

- Realizar uma revisão sobre as características físico-quimicas dos termoplásticos mais utilizados no mundo, abrangendo dados sobre o descarte desses materiais e as técnicas utilizadas para avaliação do processo de degradação dos plásticos;
- Realizar um levantamento na literatura sobre o consumo e a biodegradação de plásticos por larvas de insetos;
- Padronizar ensaios de biodegradação de PE por larvas de *G. mellonella*;
- Determinar o estágio larval de *G. mellonella* capaz de consumir a maior quantidade de PE;
- Determinar se as larvas de *G. mellonella* são capazes de metabolizar o PE;
- Avaliar se as larvas de *G. mellonella* são capazes de biomineralizar o PE até o produto de biodegradação aeróbia (CO_2);
- Propor uma rota de digestão do PE após sua ingestão por larvas de *G. mellonella*;
- Realizar um estudo piloto metagenômico para verificar a diversidade bacteriana no intestino de larvas de *G. mellonella* após alimentação com PE.

CAPÍTULO I

Nesse capítulo será apresentado parte do referencial teórico do trabalho, que aborda as características físico-quimicas dos termoplásticos mais utilizados no mundo, dados sobre o gerenciamento de resíduos sólidos desses materiais e técnicas para avaliar a degradação plástica. Desse modo, esse capítulo está redigido na forma de artigo científico em língua inglesa, conforme as normas da revista "Journal of Environmental Sciences". O manuscrito será submetido para publicação em breve.

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Plastic waste: characterization and management techniques

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Abstract

Plastics are synthetic polymers and over the years, their stability has been continuously improved. This fact made this material replace paper and other cellulose-based packaging, since these polymers have superior physic-chemical properties and resistance to many environmental factors, such as water and most water-borne microorganisms, giving a remarkable quality of low degradability. Thus, an increase in world production is observed, since its invention, to the present day. In 2019, is estimated that 368 million tons of plastics were produced globally, and this dramatic increase in the use of plastic materials was not accompanied by a corresponding development of procedures for their safe disposal or degradation. Currently, there are three methods to manage plastic waste: (i) recycling, (ii) incineration with or without energy recovery and (iii) landfill disposal. However, these techniques may be economically and/or sustainably unfeasible. Thus, alternatives methodologies, such as, biodegradation need be more explored. Herein we present an overview about the physicochemical features, production and, the current methods of waste management applied for the most used plastics in the world. In addition, we bring a section that shows the possible forms of plastic degradation (abiotic and biotic), also showing techniques for the validation of these methods.

Keywords: plastic waste; plastic management; recycling; incineration; landfill; degradation plastic.

1. Introduction

Plastics are a synthetic polymer, and this material represents one of the most essential and widely used materials in the world. In mid of century XX, plastics have begun to substitute natural products. This is due to its strength, lightness, stable physicochemical properties, resistance to changes caused by degradation and environmental factors (Andrady, 2015; Andrady and Neal, 2009; Jambeck et al., 2015; Scott, 1999a). For the plastic polymerization, generally, common monomers used for the synthesis of the plastics, such as ethylene and propylene, are commonly derived from fossil hydrocarbons (Mueller, 2006; Amobonye et al., 2020).

This polymeric structure makes them materials have low maintenance requirements, weather resistance, low toxicity, transparency, and low price, increasing their application in many industrial, commercial, and agricultural activities (Jenkins et al., 2020; Amobonye et al., 2020). Thus, in 2019, about 368 million tons of plastic were produced globally and it is estimated that the European Union was responsible for the production of 57.9 million tons (Plastics Europe, 2020). However, several physicochemical characteristics make the plastic a material inert in the nature, such as: (i) its highly stable C-C and C-H covalent bonds, (ii) its high molecular weight, (iii) its lack of readily oxidizable and/or hydrolyzable groups, and (iv) its hydrophobic. This fact became the biodegradation process extremmaly slow and hard in this material (Gautam et al., 2007). As a result of this the plastic pollution/accumulation in the environment constitutes an environment increasingly threat to natural ecosystems and public health (Shah et al., 2008).

Currently, there are three ways to manage plastic waste: (i) recycling, (ii) incineration with or without energy recovery and (iii) landfill disposal. However, these processes have some obstacles. The first, recycling, can be an economically unfeasible technique, this because to obtain the final product can be more costly than the value of the raw material to obtain that same product (Ojeda et al., 2011a; Scott, 1999b). Moreover, the incineration process can cause hazardous effects on living organisms and to the releasing of CO_2 into the atmosphere. Lastly, when plastic wastes are buried in soil, it can affect the drainage patterns, disturb the ecosystem, and decrease the soil quality. In this sense, the inappropriate management of these residues has led to the deposition of at least 100 million tons of plastics per year in the environment, causing terrestrial and marine pollution and damage to life (Machado et al., 2018; Roy et al., 2011; Shah et al., 2008). An alternative to treatment waste disposal problems is the enhancement of technologies related to plastic degradation process (Kundungal et al., 2019). In this context, this review brings information about the physicochemical features, production and the current methods of waste management applied for the most used plastics in the world.

2. Plastics

The word plastic originates from the Greek "*plastikos*", which can be defined as "capable of being molded in different shapes" (Fried, 1995). Since 1950 until 2016, the plastic production increased 200 times, considering an annual production projectionin increase of 4% per year since 2000 (Wit et al., 2019). Chemically, plastics are formed by a long polymeric chain with repeated units of a given structure (monomer) composed by organic or inorganic elements, such as carbon, silicon, hydrogen, nitrogen, oxygen and chlorine (Fried, 1995; Scott, 1999a; Seymour, 1986). They are classified according to its resistance to temperature in thermoplastics and thermosets (Borrelly, 2002; Pitt et al., 2011).

Thermoplastics chains are linked by weak connections of the Van der Waals type. Thus, when they are exposed to high temperatures, chemical bonds get weaker, giving to the material flexible features that can even change its physical state to a viscous liquid. However, when this material is submitted to milder temperatures, it can return to solid state. This feature provides an advantage for the processing of these polymers, while confers to the material lower resistance to extreme temperatures (Borrelly, 2002; Pitt et al., 2011). Some thermoplastics examples are polyethylene (PE), polystyrene (PS), polyvinyl chloride (PVC) and polypropylene (PP), presented in Figure 1.

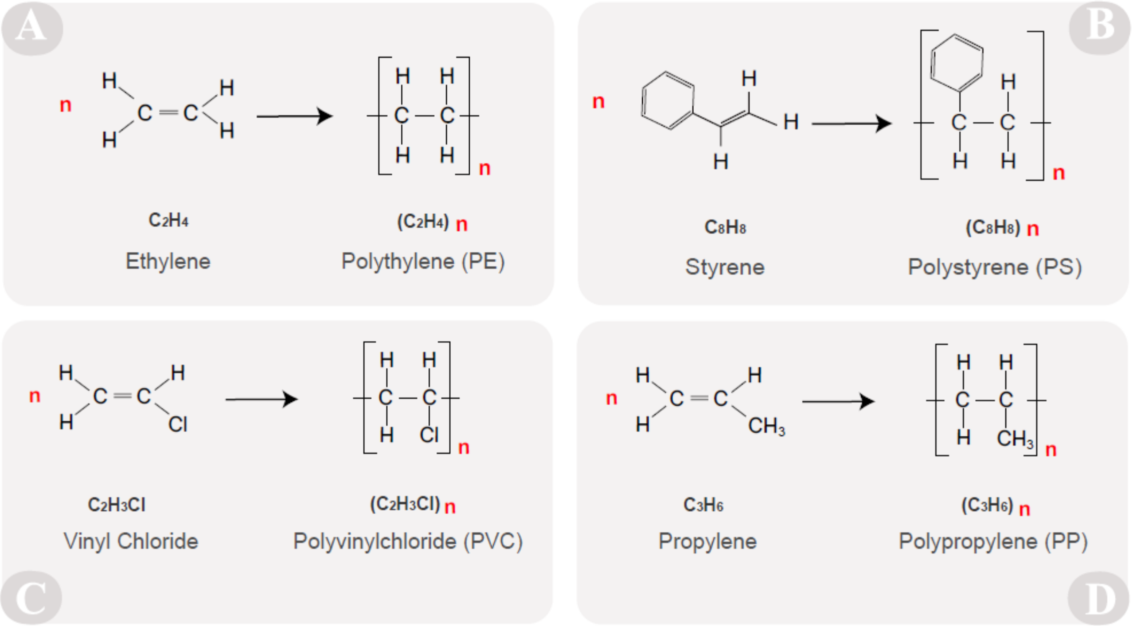


Figure 1: Examples of thermoplastics and its generic polymerization reactions: Polymerization reaction of a) the ethylene monomer into PE; b) the styrene monomer into PS; c) the vinyl chloride monomer into PVC; d) the propylene monomer into PP. The index “n” indicates the degree of polymerization.

On the other hand, thermosets processing leads in the formation of crosslinking bonds between the polymeric chains caused by high temperature and pressure. Thus, when a polymer undergoes the heating process and it is subsequently exposed to milder temperatures, the material gets more rigid and the polymer will show greater resistance to high temperatures. Some thermosets examples are phenol-formaldehyde, epoxy resins, and urea-formaldehyde (Borrelly, 2002; Pitt et al., 2011).

The packaging segment is one of the main plastic world demanders. Currently, most thermoplastics are used in packaging industry are non-biodegradable polymers obtained from petroleum as polyolefins. These materials present very interesting features, as the low density when compared to other solid materials, force-weight ratio significant high, good stability to mechanical shocks, chemical and corrosion resistance. While the desirable advantages of these materials had result in a great improvement of people life quality, the excessive consumption and the inappropriate discard of plastic became a great environmental concern (Farzi et al., 2019; Harshvardhan and Jha, 2013; Ragaert et al., 2017). Plastics have been accumulated in the oceans, soils, seabed, and isolated islands since the 50’s decade, time in which the massive production of plastic products began. About 13 million tons of plastics are dumped inside the oceans from all over the world each year, which can be ingested by fish, sea birds and other organisms (Skariyachan et al., 2018). The single-use plastic materials are the primarily responsible for environmental pollution and they usually are manufactured from thermoplastics PE, PP, PVC, and PS (Harshvardhan and Jha, 2013; Montazer et al., 2020; Ragaert et al., 2017).

2.1 Polyethylene (PE)

PE is the termoplastic more produced globally and is defined as semicrystalline polymer. Its flexibility and physicochemical properties vary according to the relative amount of amorphous and crystalline phases used (Doak, 1986; Harshvardhan and Jha, 2013; Montazer et al., 2020; Ragaert et al., 2017). PE is formed by polymerization of the ethylene monomer, during the reaction, the C=C bond in each ethylene monomer breaks, and two electrons form a C-C bond together with other two ethylene monomers (Figure 1a). The obtained polymerization through the monomers coupling using its multiples bonds is called polymerization by addition (Peacock, 2000).

The PE polymer molecular weight can vary according to the type of synthesis performed and the reaction conditions of the system in which the ethylene polymerization occurs. PE can present different densities and tridimensional structures according to different manufacture processes: Ultra Low-Density Polyethylene (ULDPE); Linear Low-Density Polyethylene (LLDPE); Low Density Polyethylene (LDPE); High Density Polyethylene (HDPE); and Ultra High Molecular Weight Polyethylene (UHMWPE) (Harshvardhan and Jha, 2013; Montazer et al., 2020; Peacock, 2000; Ragaert et al., 2017). LDPE and LLDPE have an average molecular weight of 50,000 $g.mol^{-1}$, while the HDPE and UHMWPE present an average molecular weight of 200,000 and 6,000.000 $g.mol^{-1}$, respectively (Kurtz, 2016). The chains present in HDPE and LLDPE are more organized compared to LDPE. The HDPE chains do not show branches, while in LLDPE structure it is noted the presence of short branches. LDPE does not have an organized structure and presents a significant amount of short and long branches in its polymeric chain (Peacock, 2000).

The distinct types of PE are used for distinct applications (Peacock, 2000). UHMWPE is used in chemical, textile, and mining industries due to its bigger strength and hardness (Kurtz, 2016; Miranda et al., 2020). HDPE is applied in products as toys, milk bottles, shampoo bottles, pipes, and houseware, which are manufactured with a more resistant and hard resin. LLDPE is widely used in coatings, general tarps, diapers and pads production, housewares, and toys (Abiplast, 2018; Coutinho et al., 2003; Peacock, 2000; PlasticsEurope, 2019). LDPE has many applications, mainly in products made of a flexible and transparent resin. It is major used as films for food packaging and plastic bags due to its resistance, flexibility, and low toxicity properties, and for hospital and pharmaceutical products, toys, cosmetics, and coating of materials destined for construction (Coutinho et al., 2003). PE represents about 29.8% of the demanded plastics in the European Union and in this sense, HDPE and LDPE/LLDPE account for 12.4% and 17.4%, respectively (PlasticsEurope, 2020).

2.2 Polystyrene (PS)

The German chemists Fritz Stastny and Karl Bulchholz were primarily responsible for the PS discovery at Basf laboratory in 1949 (Rubin, 1990). It is a synthetic plastic characterized as homopolymer and is obtained from the styrene (Figure 1b). Although PS presents a thermoplastic behavior, its use is limited due to the smaller impact resistance. In order to overcome it, PS blends and styrene copolymers are widely used in industry (Amianti and Botaro, 2008). Expanded Polystyrene (EPS) and High Impact Polystyrene (HIPS) are the most used types of PS. The styrene polymerization in water has as final product pearls with 3 mm diameter, approximately. For EPS production, the polymer undergoes by a 50-fold-expansion process using steam and then the polymer can be molded according to the final product. At end, the expanded pearls consist of just 2% (m/v) of PS; therefore, for recycling process, it is recommended the polymer compaction (Amianti and Botaro, 2008; Bledzki and Gassan, 1999; Selke and Wichman, 2004).

PS represents 7.1% of all worldwide thermoplastics production, which can be explained by its diverse applications as plastic cups, egg trays, food packaging (dairy and fishery), building insulation, electrical and electronic equipment, inner liner for fridges, eyeglasses frames. PS and EPS represents approximately 6.2% of the demanded plastics in the European Union (Abiplast, 2018; PlasticsEurope, 2020).

2.3 Polyvinyl chloride (PVC)

PVC contains three basic bonds: C-C, C-H and C-Cl (Figure 1c). Although vinyl chloride (PVC monomer) has low reactivity, its radical is highly reactive and therefore PVC is manufactured by radical polymerization techniques, including suspension, mass, or emulsion polymerization (Saeki and Emura, 2002; Wypych, 2020).

Approximately 80% of the world production of PVC is obtained by suspension polymerization, as shown in Figure 1c. The production of paste-type resin by emulsion is 12% in the world and micro-suspension polymerization is 3% and 5% in the USA and Japan, respectively (Wypych, 2020). The suspension technique uses water to keep the system temperature-controlled, a low-cost process. The initiator is dissolved in the monomer (vinyl chloride) and to this mixture is added a specific suspending agent, mostly poly (vinyl alcohol). The agitation process begins, and polymerization occurs according with an increase in temperature. The final products are pearls with a size of 50 to 200 μm, which are subsequently filtered, washed and dried, and can then be used in several applications (Nass, 1976; Saeki and Emura, 2002; Wypych, 2020).

PVC production has grown about 4% per year since 1990 and the global demand for this polymer is expected to increase about 3.2% per year until 2021. In 2013, China was the largest producer of PVC resins, which represented about of 29.96% of global PVC production (Yu et al., 2016). PVC can be used for window frames, profiles, floor and wall coverings, pipes, cable insulation, garden hoses, inflatable pools, etc. It represents about 10% of the demanded plastics in the European Union (Abiplast, 2018; PlasticsEurope, 2020; Yu et al., 2016).

2.4 Polypropylene (PP)

PP is a linear hydrocarbon polymer that can contain a little unsaturation or not. The presence of a methyl group in chain backbone can change the PP properties in many ways, such as the introduction of a steric center and the possibility of different stereostructures (Arutchelvi et al., 2008; Gahleitner and Paulik, 2017). There are at least three different stereoisomers of PP: isotactic PP (iPP), syndiotactic PP (sPP), atactic PP (aPP), among others. In iPP structure, all methyl groups are on the same side of the chain backbone, and this type presents the highest crystallinity degree (Gahleitner et al., 1999; Hisham A. Maddah, 2016; Housmans et al., 2009). In sPP structure, the methyl groups are on alternating sides, which confers a medium level of crystallinity, while in aPP structure, the methyl groups are arranged randomly along the chain, which turns aPP an amorphous polymer (Gahleitner and Paulik, 2017; Hisham A. Maddah, 2016).

The catalyst systems used during the polymerization process of propylene into PP (Figure 1d) are very sensible to impurities, which can decrease of the catalyst activity and affect the isotacticity of PP (Arutchelvi et al., 2008; Gahleitner et al., 2011; Gahleitner and Paulik, 2017; Housmans et al., 2009). To improve PP properties, co-monomers can be inserted in the polymerization process (Housmans et al., 2009). Currently, the most used co-monomer is ethylene, although 1-butene and higher olefins were also reported (Gahleitner and Paulik, 2017).

First commercial use of PP resins occurred in 1957 in form of iPP as fibers, films, pipes, bottles and injection moldings (Hisham A. Maddah, 2016). The annual production of PP in 1960, 1980 and 2012 was 100 thousand tons, 6.1 million tons and 53 million tons, respectively (Gahleitner and Paulik, 2017) and the fast grown of PP production can be associated to the availability of the cheap monomer. PP resins present characteristics and properties such as chemical resistance, effective water and gas barrier properties, high stability, among others (Hisham A. Maddah, 2016; Pires et al., 2019). PP and PE resins have many similarities in their properties, such as chemical resistance toward solvents and electrical properties (Arutchelvi et al., 2008; Gahleitner and Paulik, 2017). It represents about 19.4% of the demanded plastics in the European Union (Abiplast, 2018; Pires et al., 2019; PlasticsEurope, 2020).

3. Management of plastic wastes

Plastic accumulation in the environment brings conservation problems and causes damage to the society and the world economy. It has been estimated that 4% of all oil and gas demand is turned into plastic production (Iea, 2018), giving an idea of pollution magnitude caused by plastics. Plastic wastes can be found at different scales and locations, i.e. at macro, micro and/or nano dimensions in soils, freshwaters and oceans (Murphy et al., 2016). Moreover, it has been showed that humans and animals are continuously ingesting plastics residues from food or drinking water, and the consequences of these intakes are still unknown (Kosuth et al., 2018).

The main conventional methods used for plastic waste management are recycling, incineration, and landfill disposal and allternatively, chemical treatment and thermal degradation are also used (Kundungal et al., 2019). The plastic waste amount generated by 28 countries of Europe Union plus Norway and Switzerland (28EU+NO/CH) was 29.1 Mt (Mt =10^6 tons) in 2018. From this amount, 9.5 Mt was recovered by recycling (32.5%) and 12.4 Mt (42.6%) was used in energy recovery processes; however, 24.9% of the waste amount went to landfills (PlasticsEurope, 2020).

None of these are methods that effectively solves the problem of plastic waste management; in addition, some of them can also lead to other negative impacts to environment. Therefore, new alternatives for the management of these residues are extremely necessary. Biodegradation processes, which present low cost and eco-friendly features, represent an option with great potential, but need that deserve to be optimized for this purpose.

3.1 Recycling

Recycling is the transformation of a given product aiming to the material reuse. According to the final product obtained for reuse, the recycling process can be classified as primary, secondary, and tertiary (Al-Salem et al., 2009; Singh et al., 2017). In primary recycling, the transformation of the plastic products occurs by standard methodologies and gives origin to new products, which possess economic value similar to the originals. Secondary recycling combines processes that generate a final product with lower added value when compared to the original. Lastly, the tertiary recycling is characterized using technological processes to transform polymeric products in chemical inputs and fuels (Al-Salem et al., 2009; Ragaert et al., 2017; Singh et al., 2017; Yu et al., 2016).

Recycling is the most recommended solids treatment process, however, it may be economically unfeasible, as the process to obtain the raw material for a given product, through recycling, can be more costly in relation to the value of the material, press virgin to obtain this same product (Ojeda et al., 2011a; Scott, 1999b). Moreover, literature data indicate that it is not widely applied and consequently a high amount of waste is deposited in the environment (Ragaert et al., 2017; UNEP, 2016). In 2016, the European countries (28EU+NO/CH) (PlasticsEurope, 2018), United State of America (USEPA, 2019) and Japan recycled about 8.4 Mt, 3.2 Mt and 2.4 Mt of plastic waste, respectively (Institute, 2020).

3.2 Incineration

Incineration of plastic waste generally is performed as a closed and controlled industrial process, and the gas emission amount is regulated by the environmental agencies (Wit et al., 2019). This process can include gasification, pyrolysis or/and arc plasma (temporary gas state) technology, which can turn it very expensive and hard (Jamil et al., 2019). The advantage of incineration is the fast decreasing of the discarded plastic waste volume (about 80%) (Franchetti and Marconato, 2006).

In the last decades, the incineration of plastic wastes has been used for energy recovery, once the energy content of plastic is comparable with heating oil. However, it requires advanced pollution control measures since toxic and noxious dioxins, derived from chemical additives incorporated during the plastic synthesis, can be released in the process, affecting the drainage patterns, disturbing the ecosystem and decreasing the soil quality process (Jamil et al., 2019).

The waste incineration increases CO_2 emission levels, and it is estimated that these levels can triple by the year 2030 (Wit et al., 2019), which can lead to the natural ecosystem damage by accelerating climate changes (Jamil et al., 2019; Ren et al., 2019; UNEP, 2016). Currently, about 15% of plastic production is treated by this process (Economics, 2016), emitting about 2.7 tons of CO_2 in the atmosphere for every 1 ton of burnt plastic (Wit et al., 2019). In 2016, the European countries (28EU+NO/CH) (PlasticsEurope, 2018), USA (USEPA, 2019) and Japan (Institute, 2020) incinerated with energy recovery about 11.3 Mt, 5.3 Mt and 1.0 Mt of plastic waste, respectively.

3.3 Landfill disposal

Landfills are used to dispose tons of waste in remote locations, generally away from the cities. They are prepared to stow the great amount of plastics materials, which will be a long time exposed under natural environment conditions or it will be used to incineration and energy generation (incineration with energy recovery) (Franchetti and Marconato, 2006). Plastics can be anaerobically biodegraded by microorganisms in sediments and in landfills, producing methane as biodegradation final product (biomineralization). However, the breakdown of plastics, including petroleum-based plastics, is extremely difficult and slow, which makes the landfill disposal not efficient (Shah et al., 2008), and leads to waste accumulation and serious pollution problems (Harshvardhan and Jha, 2013) (Jamil et al., 2019).

In addition, landfills require large area and can act as vectors of infectious diseases if inappropriately monitored (Skariyachan et al., 2018), mainly when hospital waste are disposal without the proper treatment (Jamil et al., 2019). It has been estimated that from 1950 until 2015, approximately 4,900 million tons of plastic waste were discarded annually in landfills and natural environments of all world. This amount represents about 60% of all the plastic materials ever produced (Montazer et al., 2019). In 2016, the European countries (28EU+NO/CH) (PlasticsEurope, 2018), USA (USEPA, 2019) and Japan (Institute, 2020) disposed in landfills about 7.4 Mt, 26.3 Mt and 0.5 Mt of plastic waste, respectively.

4. Environmental plastic degradation: abiotic and biotic roles factors

Environmental plastic degradation can occur by abiotic and biotic ways. The process initiates through physicochemical changes in plastic structure that lead to the functionality deterioration of the material, causing changes to occur on the plastic surface. After, physical, chemical, and/or biological phenomena induce bonds break and chemical transformations, which originate new functional chemical groups in the polymer matrix. These modifications change mechanical, electrical and/or optical properties of the material, and it can cause physical damage, such as cracks, cleavages, phase separation or delamination (Esmaeili et al., 2013; Morro et al., 2019; Ojeda et al., 2011b; Shah et al., 2008; Yang et al., 2014).

Chemical and structural factors can affect the plastic degradation in the environment such as: surface conditions (surface area, hydrophilic and/or hydrophobic properties, surface porosity); first order structures (chemical structure, water diffusivity, molecular weight and its distribution); and high order structures (glass transition temperature, melting temperature, modulus of elasticity, crystallinity and crystal structure, pore size, distribution and geometry) (Kjeldsen et al., 2019). According to the biodegradability technical standards defined in ISO (International Organization for Standardization, 2020) and ASTM (International Organization for Standardization, 2020) and ASTM (American Society of Testing and Materials, 2020), plastic materials are considered:

- Biodegradable, when it breaks down to basic elemental components (water, biomass, and gas) with the aid of microorganisms (bacteria, fungi and algae), i.e., the material should be fully assimilated, leaving no residues or fragments of plastic (microplastic) in the natural environment.
- Degradable, when it breaks down to smaller subunits and loses its original properties, i.e., the decomposition is caused by specific environmental conditions, excluding the naturally occurring microorganisms.
- Compostable, when it degrades to carbon dioxide, water and inorganic compounds due to biological processes (customized mixtures of microorganisms in controlled conditions), without leaving traces of visually distinguishable residues or toxic residues.

4.1 Abiotic degradation

Abiotic degradation initiates by thermal or hydrolytic stimuli or by exposition to ultraviolet radiation (UV), leads to change of physicochemical properties of plastics. In this process, there is the combination of some degradation types (Kjeldsen et al., 2019), as:

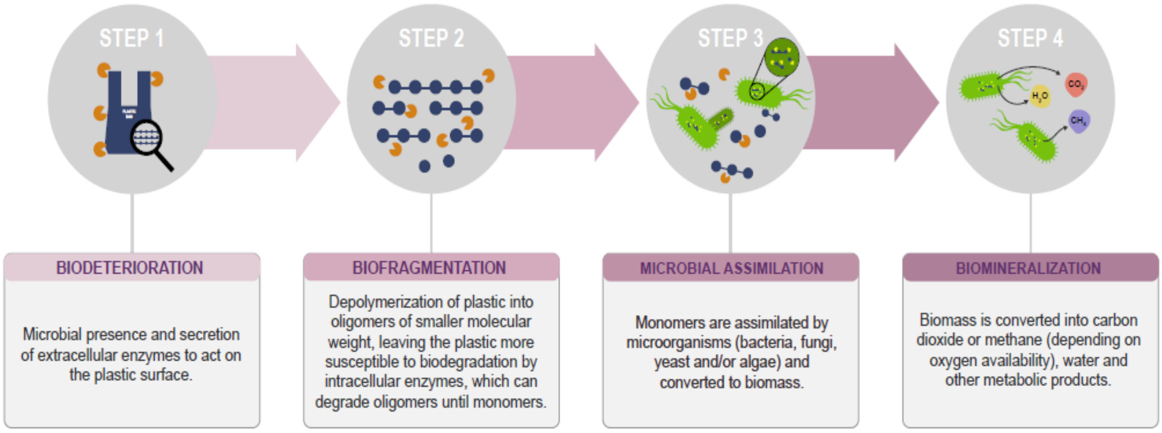
- Mechanical degradation: involves physical forces (compression, tension and shear forces) in the environment that can damage the plastic, such as air and water turbulence, snow pressure and animal tearing;
- Photo-degradation: solar UV-radiation initiates chemical reactions, leading to destabilization of plastic structural characteristics;
- Thermal degradation: exposition to heat influences the plastic organized framework through of thermo-oxidative reactions;
- Chemical degradation: exposition to atmospheric pollutants or agrochemicals can lead to a chemical breakdown; oxygen is the most important factor to plastic oxidative degradation.

In this way, the material surface is firstly degraded, and then it remains exposed and available to chemical and/or enzymatic attack (Gewert et al., 2015). In addition to the surface damage promoted by abiotic factors such as solar light (Mehmood et al., 2016; Vimala and Mathew, 2016), heat (Bonhomme et al., 2003; El-Shafei et al., 1998; Sudhakar et al., 2008) or both (Fontanella et al., 2010; Koutny et al., 2009), some microorganisms can initiate an oxidation process through the hydroperoxidation (Montazer et al., 2020a). Some studies have shown that during microbial degradation of plastics, the abiotic deterioration or the addition of oxidant chemical agents, such as nitric acid (Hadad et al., 2005; Hasan et al., 2007), can act as a pre-treatment to turn plastic more fragile and sensible to additional oxidation catalyzed by microbial enzymes. In general, this phase is characterized by the increasing in the access points for further enzymes action, as well as by the decreasing of physical properties of the plastic (Montazer et al., 2020a).

4.2 Biotic degradation

Biotic degradation is the result of the material degradation caused by a biological activity through enzymatic action. Initially, biodegradation can be described as the breakdown of plastic monomers or polymers due to biological process, where carbon dioxide and water (aerobic conditions) or methane (anaerobic conditions) are generated (Kjeldsen et al., 2019). The biotic degradation process can be dependent of many factors, as environment (soil, water, pH, temperature, moisture, O_2 availability, nutritional supply and presence of microorganisms), starting material composition (plastic chain length and strength of interactions, formulation and additives presence) and the plastic material shape (surface area) (Artham and Doble, 2008; Franchetti and Marconato, 2006; Gu, 2003; Kjeldsen et al., 2019). It is important to mention that the plastic physicochemical properties, such as the stable C-C and C-H bonds, the absence of hydrolysable and oxidant groups; the high molecular weight and the limited solubility in polar solvents (Gautam et al., 2007) confer to the biodegradation process extreme slowness in environmental conditions (Sangeetha Devi et al., 2016).

The general mechanism of plastic biodegradation processes can be divided in three subsequent steps as shown in Figure 2: (1) biodeterioration, (2) biofragmentation, (3) microbial assimilation, and (4) biomineralization (Kjeldsen et al., 2019; Montazer et al., 2020a; Tosin et al., 2019).



BIODETERIORATION

Microbial presence and secretion of extracellular enzymes to act on the plastic surface.

BIOFRAGMENTATION

Depolymerization of plastic into oligomers of smaller molecular weight, leaving the plastic more susceptible to biodegradation by intracellular enzymes, which can degrade oligomers until monomers.

MICROBIAL ASSIMILATION

Monomers are assimilated by microorganisms (bacteria, fungi, yeast and/or algae) and converted to biomass.

BIOMINERALIZATION

Biomass is converted into carbon dioxide or methane (depending on oxygen availability), water and other metabolic products.

Figure 2: Schematic illustration about the general mechanism of the plastic biodegradation process in the environment. In steps 1 to 3, the orange structures represent extracellular enzymes acting on the polymer surface, the blue structure refers about the plastic surface (in Step 1) and the carbonic chain (in Steps 2 and 3) forming oligomers. In steps 3 and 4, the yellow structures represent intracellular enzymes acting on oligomers (up to 500 g/mol), converting them into monomers, which are then biomineralized into H_2O, CO_2 and/or CH_4, and green structures represents the microorganism (bacteria) doing the biodegradation process.

Extracellular enzymes produced by several organisms, and excreted in the surrounding environment, can act directly on plastic surface causing its biodeterioration (Step 1 – Figure 2). Subsequently, during plastic biofragmentation (Step 2 – Figure 2), it will be released small cleavage fragments (oligomers with molar weight of about 500 g/mol) through of plastic depolymerization. Some microorganisms living around the material can assimilate plastic oligomers (up to 500 g/mol), which can, at this point, permeate through microbial cell wall and cell membrane to be hydrolyzed into monomers by the intracellular enzymes. In microbial assimilation step (Step 3 – Figure 2), microorganisms metabolize the oligomer plastics for their own growth and energy uptake, i.e., microbial monomers are assimilated by these microorganisms to generate cellular biomass. Environmental factors, such as pH, temperature and moisture content can affect both fragmentation and microbial degradation rate. In the biomineralization step (Step 4 – Figure 2), biomass is completely metabolized by microorganisms as elementary constituent for macromolecule synthesis and mineralized into inorganic substances, such as carbon dioxide or methane (depending on oxygen availability), and water. Conditions with absence of oxygen are described as aerobic, limited oxygen as anoxic and without oxygen as anaerobic (Bonhomme et al., 2003; Kjeldsen et al., 2019; Montazer et al., 2020a, 2019; Tosin et al., 2019). The formation of distinct final products is related to the different microbial metabolism, which depends of oxygen availability, and the biodegradation route that the plastic material undergoes in biomineralization (Gu et al., 2000):

- Aerobic conditions: usually observed in natural ways by living organisms. The total plastic biomineralization generates carbon dioxide (CO_2) and water as end products;
- Anoxic conditions: usually observed in composting processes and in the soil, resulting in CO_2 and water as end products;
- Anaerobic conditions: usually observed in sediments and landfills. The total plastic biomineralization generates methane (CH_4) and water as end products, although some microorganisms as fungi can not metabolize plastics under this condition.

4.3 Techniques used to evaluate the plastic degradation

Several techniques can be used to evaluate the plastic degradation, caused by natural or simulated abiotic and biotic degradations (Table I). Some of them provide more robust data than others and this must be taken into account to predict whether there is indeed a plastic degradation. The importance was highlighted of sensitive techniques applied for the determination of plastic biodegradation, because only the gravimetric analysis is not enough to verify the changes in the physicochemical structure of the plastic, as well as it can not be used to identify degradation product (Ren et al., 2019). Moreover, the lack of standardization of some test methodologies is a huge problem and contribute for discrepancies in results obtained by distinct studies. It is necessary to control the assay conditions directly involved in biodegradation process, such as temperature, humidity, microbial inoculum, number of animals and other factors, as previously described.

Biodegradation process is facilitated by the decreasing in polymer molecular weight, and this property can be evaluated by GPC analyses. It occurs since material needs to be transported across the cell membrane to be metabolized. Thus, smaller units such as monomers, dimers and oligomers are more easily degraded and mineralized (Tokiwa et al., 2009). In addition, the initial microbial colonization of plastic polymers increases according to higher hydrophilicity of the material. Thus, hydrophilic surfaces have higher surface energies, which provides lower contact angles with water and promotes microbial attachment to the polymer surface, leading to acceleration in degradation rate (Chamas et al., 2020). The presence of this hidrophilic groups can be evaluate using FTIR, NMR, GC-MS and HPLC analyses. Moreover, others techniques are performed for the analysis the process, such as the mineralization of plastic polymers, that has been demonstrated by isotopic tracking and the quantification of CO_2 release using the respirometric method (Yang, et al., 2015).

Table I: Techniques used to evaluate plastic biodegradation.

Aim	Main analytical techniques used	Parameters	References
Characterization and evaluation of plastic properties before and after degradation	Fourier Transformed Infrared Spectroscopy (FTIR)	Plastic functional chemical groups	(Arutchelvi et al., 2008; Hakkarainen and Albertsson, 2004; Jamil et al., 2019; Miranda et al., 2020; Montazer et al., 2020; Raddadi and Fava, 2019; Restrepo-Flórez et al., 2014; Sangeetha Devi et al., 2016; Shah et al., 2008)
	Gel Permeation Chromatography (GPC)	Molar weights and molar weight distribution	(Arutchelvi et al., 2008; Hakkarainen and Albertsson, 2004; Jamil et al., 2019; Miranda et al., 2020; Montazer et al., 2020; Raddadi and Fava, 2019; Restrepo-Flórez et al., 2014; Sangeetha Devi et al., 2016)
	Thermogravimetric Analysis (TGA)	Plastic thermal stability	(Arutchelvi et al., 2008; Jamil et al., 2019; Miranda et al., 2020; Raddadi and Fava, 2019)
	Gravimetry	Mass loss	(Jamil et al., 2019; Montazer et al., 2020; Raddadi and Fava, 2019; Restrepo-Flórez et al., 2014; Shah et al., 2008)
	Scanning Electronic Microscopy (SEM)	Plastic surface erosion	(Arutchelvi et al., 2008; Hakkarainen and Albertsson, 2004; Jamil et al., 2019; Miranda et al., 2020; Montazer et al., 2020; Raddadi and Fava, 2019; Restrepo-Flórez et al., 2014; Sangeetha Devi et al., 2016; Shah et al., 2008)
	Atomic Force Microscopy (AFM)	Roughness of plastic surface	(Jamil et al., 2019; Restrepo-Flórez et al., 2014; Shah et al., 2008)
	X-ray Photoelectron Spectroscopy (XPS)	Elemental composition of plastic surface	(Jamil et al., 2019; Shah et al., 2008)

Evaluation (identification and quantification) of intermediate and end degradation products from plastics	Gas Chromatography coupled to Mass Spectroscopy (GC-MS)	Intermediate and end degradation products: gases	(Arutchelvi et al., 2008; Jamil et al., 2019; Sangeetha Devi et al., 2016)
	High Performance Chromatography (HPLC)	Intermediate and end degradation products: soluble compounds	(Bombelli et al., 2017; Ghatge et al., 2020; Jamil et al., 2019)
	Nuclear Magnetic Resonance spectroscopy (NMR)	Intermediate and end degradation products: soluble compounds	(Arutchelvi et al., 2008; Hakkarainen and Albertsson, 2004; Jamil et al., 2019; Montazer et al., 2020; Shah et al., 2008)
Estimative of plastic biotic degradation through microbial growth or metabolism	Radiolabeled Carbon (^{13}C) (Respiration)	Plastic degradation and $^{13}CO_2$ or $^{13}CH_4$ production	(Jamil et al., 2019; Raddadi and Fava, 2019; Shah et al., 2008)
	Aerobic and Anaerobic Biodegradation (Respiration)	CO_2 or CH_4 production by carbon mineralization: Amount of produced CO_2/CH_4 or polymer carbon that has disappeared over time.	(Jamil et al., 2019; Miranda et al., 2020; Montazer et al., 2020a; Restrepo-Flórez et al., 2014)
	Microorganism cultivation	Microbial growing and development in artificial medium containing plastic as solely carbon source	(Jamil et al., 2019; Montazer et al., 2020, 2019)

In addition, it is necessary that when studies address issues plastic biodegradation, show techniques that assess the material characterization before and after the process, to answer: how the polymer was treated? What changes this material underwent to affirm that the process occurred? What step the process are this material? Therefore, the importance of exploring and applying techniques is noted (Table I) to assess the plastic degradation process, providing important answers regarding the material and microorganism evolution responsible for the biodegrading action.

5. Conclusion and future prospects

This article has highlighted significant research on the increasing demand for plastics in various industrial sectors. Thus, we note that the physicochemical characteristics of these plastic materials lead to products that are difficult to degrade in the environment. Therefore, we also point out current data on the management of plastic waste across the planet and we saw that, due to the high consumption of these materials with short duration (disposable products), this polymeric material accumulates in nature, which damages terrestrial and marine life. The understanding of the plastic biodegradation process, as well as the characterization of this process through analytical techniques, are necessary and are shown throughout this article.

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CAPÍTULO II

Nesse capítulo será apresentado parte do referencial teórico do trabalho, que traz uma revisão crítica, compilando todos os estudos que tratam sobre biodegradação de plásticos por larvas de insetos. Ainda nesse capítulo, são abordados os aspectos biológicos desses animais e o contexto histórico sobre essa ação biodegradadora. Desse modo, ele está redigido na forma de artigo científico em língua inglesa, conforme as normas da revista “Science of the Total Environmental”. O manuscrito será submetido para publicação em breve.

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Plastics: progress and perspectives on consumption and biodegradation by insect larvae

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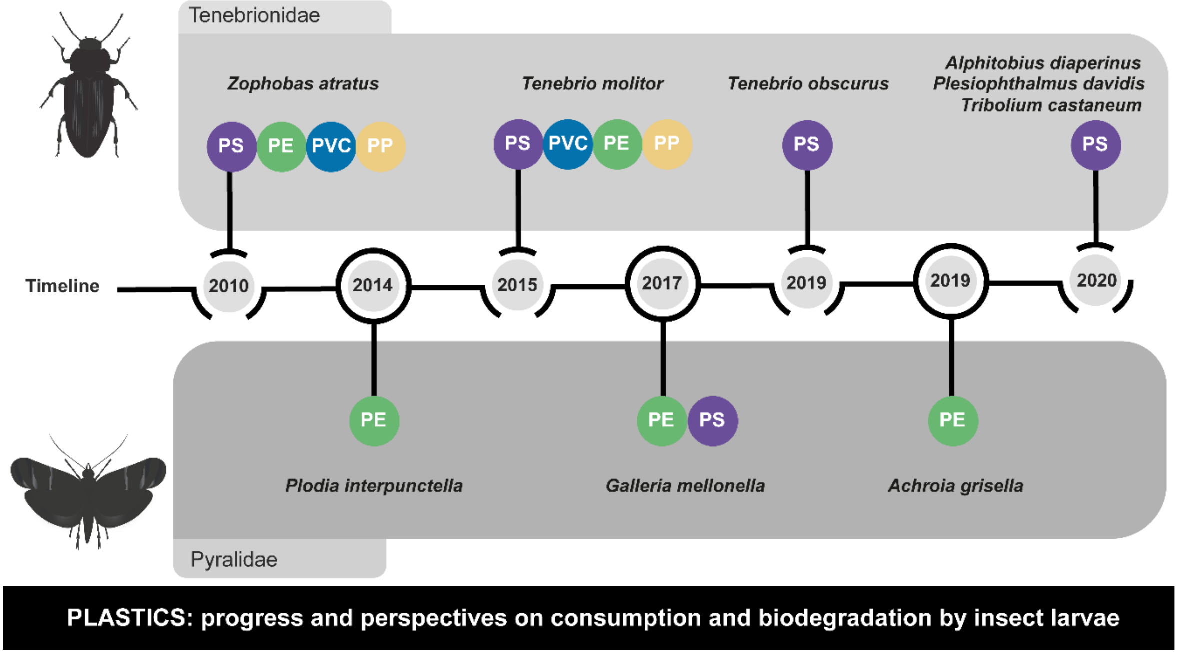
Graphical Abstract



Highlights

- Larvae of nine insect species have been shown to consume and even biodegrade plastics;
- Coleopteran larvae are mostly associated with PS biodegradation;
- Lepidopteran larvae are mostly associated with PE biodegradation;
- PS biodegradation may be ubiquitous within the *Tenebrio* genus, where it is microbial-dependent;
- Microbial action might not be fully essential for PE biodegradation by *G. mellonella*.

Abstract

Plastics are synthetic polymers designed to meet the very different needs of thousands of end products. Physicochemical properties of these materials provide resistance to many environmental factors, such as water and most water-borne microorganisms, conferring a remarkable quality of low degradability. It is estimated that about 368 million tons of plastics were produced globally in 2019. The obvious contrast between the remarkable durability and the short service time of most plastic products leads to the deposition of at least 100 million tons of plastics per year in nature, resulting in terrestrial and marine pollution, and damage to life. Recycling, incineration with or without energy recovery, and landfill disposal are the main methodologies applied in plastic waste management, however they are insufficient to handle the current amount of waste generated worldwide. Interestingly, a number of studies since 2010 have shown the potential of some insect larvae to consume and even to biodegrade different types of plastics, at rates higher than those reported for microbial degradation processes. Therefore, this research topic bears critical implications for future management of plastic residue. This review discusses a compilation of studies about plastic consumption and biodegradation by larvae of different insects (the lepidopterans *Achroia grisella*, *Galleria mellonella* and *Plodia interpunctella*, and the coleopterans *Alphitobius diaperinus*, *Plesiophthalmus davidis*, *Tenebrio molitor*, *Tenebrio obscurus*, *Tribolium castaneum* and *Zophodas atratus*). Moreover, the biological aspects, including insect ecology, and the role of gut microbiota in this process were also addressed. Uncovering and understanding the chemical processes behind the innate plastic biodegradation by insect larvae will open the perspective to new eco-friendly innovative biotechnological solutions for the challenge of plastic waste in the environment.

Keywords: plastics; biodegradation; insect larvae; larval microbiota, waste management.

1. Introduction

Plastics are synthetic polymers formed by a long polymer chain, which can be easily molded into different shapes. In the mid-twentieth century, plastics have begun to substitute natural products (horn, waxes, natural rubber, and resins) in many industry areas due to their strength, lightness, stable physicochemical properties, and resistance to changes due to environmental factors (Andrady, 2015; Andrady and Neal, 2009; Jambeck et al., 2015; Scott, 1999). Chemically, these materials consist of a long polymeric chain with repeated units of a given structure (monomer) composed by organic or inorganic elements (Fried, 1995; Scott, 1999a; Seymour, 1986). The most used plastics in 2019 were PE (29.8%), PP (19.4%), PVC (10%), PUR (7.9%), PET (7.9%) and PS (6.2%) (PlasticsEurope, 2020).

The chemical structure of plastics makes these materials inert in nature and confers a remarkably low degradability (Gautam et al., 2007). They are applied to food packaging, pharmaceutical products, cosmetics, detergents, and chemical products (PlasticsEurope, 2016). In 2019, about 368 million tons of plastic were produced globally, and it is estimated that the European Union was responsible for the production of 57.9 million tons (PlasticsEurope, 2020). Plastics used in packaging industry are non-biodegradable polymers obtained from petroleum as polyolefins, which present very appealing features, such as low density, high force-weight ratio, good stability to mechanical shocks, and chemical and corrosion resistance. Such materials can improve human activities in many ways, however, excessive consumption and the inappropriate disposal of plastics turned them into a great environmental concern (Farzi et al., 2019; Harshvardhan and Jha, 2013; Ragaert et al., 2017). In particular, single-use plastic materials are recognized as primarily responsible for environmental pollution (Harshvardhan and Jha, 2013; Montazer et al., 2020a; Ragaert et al., 2017).

Currently, there are three ways to manage plastic waste: (i) recycling, (ii) incineration with or without energy recovery, and (iii) landfill disposal. However, each of these processes have important caveats. Recycling is generally considered an economically unfeasible technique, since the process to obtain the final product can be more costly than the value of the raw material to obtain that same product (Ojeda et al., 2011; Scott, 1999). Incineration, in turn, can cause hazardous effects on living organisms due to the release of CO_2 into the atmosphere. Lastly, when plastic waste is buried in soil, it can affect the drainage patterns, disturb the ecosystem, and decrease the soil quality (Machado et al., 2018; Roy et al., 2011). Hence, the inappropriate management of plastic residues has led to the deposition of at least 100 million tons of plastics per year in the environment, which results in terrestrial and marine pollution and damage to life (Shah et al., 2008).

An alternative to cope with plastic disposal problems is the development of plastic biodegradation technologies (Kundungal et al., 2019a). Plastic biodegradation has been reported in a series of studies published over the past 30 years. However, there is a consensus that the process of biodegradation by microorganisms is extremely slow under environmental conditions. Microbial activity may be physically limited by the plastic substrate, given the material insolubility in aqueous medium, the high molar weight of the polymer, and the eventual release of toxic substances during the process (Arutchelvi et al., 2008; Eubeler et al., 2010; Gu, 2003; Hakkarainen and Albertsson, 2004; Koutny et al., 2009; Peixoto et al., 2017; Restrepo-Flórez et al., 2014). Microbial biodegradation processes can be monitored through characterization and evaluation of plastic properties before and after exposure to microorganisms using several techniques, such as, FTIR, GPC, TGA, SEM, AFM, XPS and surface gravimetry (Arutchelvi et al., 2008; Hakkarainen and Albertsson, 2004; Jamil, Kumar, and Batool, 2019; Miranda et al., 2020; Montazer, et al., 2020a;

Raddadi and Fava, 2019; Restrepo-Flórez, et al., 2014; Sangeetha Devi et al., 2016; Shah, et al., 2008).

Literature from the mid'40s report a great problem related to food deterioration caused by larvae belonging to some coleopterans and lepidopterans insect species, which were able to eat food packaging composed of polymeric materials (Essig et al., 1943; Gerhardt and Lindgren, 1954). At that time, there was an emphasis on finding repellent products or resistant materials to avoid insect penetration in packaging films. Since 2010, a series of studies have explored this uncommon ability of some insect larvae to consume and even to biodegrade different types of plastics at a rate sometimes higher than microorganisms. The general goal of these studies is to understand the biochemical pathways involved in this process to propose new biotechnological alternatives for plastic waste treatment processes.

In this context, the present review discusses a compilation of the published studies about insect larvae capability of chewing, eating and, eventually, biodegrading plastics. The literature search was performed in Science Direct; Web of Science and PubMed databases using the following keywords: Biodegradation and Plastic and Larvae; Biodegradation and Plastic and Insect; Plastic-eating and Larvae; Plastic-eating and Insect; Plastic-eating and Waxworms; Plastic-eating and Mealworms; Biodegradation and Polystyrene and Insect; Biodegradation and Polyvinyl chloride and Insect; Mineralization and Polyethylene and Larvae; Mineralization and Polystyrene and Larvae; Greater wax moth and Plastic; Mealworms and Plastic; Insect and Plastic; Larvae and Plastic. All 46 articles about these subjects published online up to December 31st 2020 were included

2. Biological aspects of insect species whose larvae were reported to consume and/or biodegrade plastic

Three species of Lepidoptera and six species of Coleoptera were reported in literature about plastic consumption or biodegradation.

The complete life cycle of insects presents 4 stages: eggs, larvae, pupae, and adults, with sexual dimorphism in the adult stage. Noteworthy, the length of each insect life cycle stages can vary according to several factors, such as temperature, humidity, nutrition, and age of the parents (Mariod et al., 2017; Silhacek and Murphy, 2006; Singkum et al., 2019).

Adult lepidopterans emerge as moths or butterflies. They possess a suction-type oral apparatus, formed by a spiro thrombus. The masticatory system present in lepidopteran larvae, composed of a pair of jaws, is atrophied in the adult. Thus, differently from larvae, adults do not cause damage to vegetation (Nomura et al., 2006; Moura et al., 2019). Adult coleopterans emerge as beetles. Some of them have robust chewing jaws that are used to break seeds or gnaw wood; others present thin and sharp jaws, with a groove or channel through which the insect feeds. These chewing mouthpiece is also found in the larvae of these animals (Borror and Delong, 1969; Moura et al., 2019). Both larvae and adults of Coleopteran order have great agricultural relevance since they feed on all plant parts, from the root to the nectar.

2.1 Lepidoptera: Pyralidae

2.1.1 *Achroia grisella* - Lesser wax moth

The lesser wax moths are cosmopolitan and their natural habitats are honeybee hives, where they damage combs and cause serious economic losses for beekeepers (Chalup et al., 2018; Nomura et al., 2006). The larvae of this insect feed on the remains of stored food and organic debris, whereas adults, because they have a stunted trunk, do not eat or drink, but remain in the vicinity of the bee colony during its brief longevity (7 to 14 days) (Greenfield and Coffelt, 1983). The whole life cycle can last from 41 to 64 days, while adulthood usually lasts one week (Nomura et al., 2006; Singh, 1962).

2.1.2 *Galleria mellonella* - Greater wax moth

The greater wax moth, also known as honeycomb moth, is a ubiquitous pest of the honeybee and, similar to *A. grisella*, they cause damage to honeybees and hives. The lesser wax moth, *A. grisella*, is a closely related species, but less destructive and less common than *G. mellonella* (Kwadha et al., 2017). Beeswax is the natural food of these larvae, a material mostly composed by cerin (formed by kerotic acid) and by myrinic (an ester of palmitic acid and myricyl alcohol), both presenting a chemical structure rich in by C-C and C-H bonds (Chalup et al., 2018; Dickman, 1933). The greater wax moth has a wide distribution around the world. The life cycle varies from weeks to months. Eggs take 3 to 30 days to hatch into larvae, which start to feed on the natural beeswax diet. The most intense feeding is observed in the larval stage, which can last about 28 days to 5 months (Williams, 1997). The pupal stage takes from 1 to 9 weeks, after which they develop into adults, a stage that can last about 3 weeks (Ellis et al., 2013; Nomura et al., 2006; Shimanuki, 1981). Adult males are smaller than females (Cardoso et al., 2007). Oviposition begins 4 to 10 days after the female emerges from the cocoon and matches with a male. As opposed to most lepidopterans, *G. mellonella* displays unique mating behaviour (Williams, 1997). A representation of *G. mellonella* morphology can be observed in Figure 1.

2.1.3 *Plodia interpunctella* - Indian meal moth

The indian meal moths have been often reported to infest grades of whole wheat or graham flour, maize, cornmeal, dried fruits, nuts, seeds, powdered milk, chocolate, and dry pet food. Moreover, *P. interpunctella* also invade, attack, and damage honeybee colonies and hive products. Because it is able to adapt to different climates, this insect can be found in stored products and/or in warehouses worldwide, with a cosmopolitan distribution in commercial and residential environments (Robinson, 2005). However, this animal is very common in Florida, where it successfully lives outdoors (Robinson, 2005; Shepard, 1943). The female can lay 40 to 400 eggs, depending on environmental conditions, such as temperature. The oviposition period is approximately 18 days, and the eggs hatch after 4-8 days at room temperature. The adult animals vary in length from 2 to 25 days, while the larval period is around 29 to 282 days, and the pupal stage lasts from 4 to 33 days (Tzanakakis, 1959). The literature also reports that the shortest egg-to-adult cycle is 27 days (animals fed with figs), and the longest 305 days (animals fed with plums) (Hamlin et al., 1931). A representation of *P. interpunctella* morphology can be observed in Figure 1.

2.2 Coleoptera: Tenebrionidae

2.2.1 *Alphitobius diaperinus* – Lesser mealworm

The lesser mealworm is considered a minor pest of stored products and is a problem in certain poultry operations (Vaughan et al., 1984). According to Pfeiffer and Axtell (1980), with the advent of large-scale poultry production in the USA, this insect has become the most abundant beetle inhabiting litter and manure in both the broiler and egg industry. Such infestations become a health problem because the lesser mealworm has been shown to harbor several types of poultry pathogens including helminths (Elowni and Elbihari, 1979), and *Escherichia coli* and *Salmonella typhimurium* (Casas et al., 1968). Interestingly, in the mid-1980s, it has become a structural pest in houses in which panels of PS or PP are used for insulation. This insect is a small, shiny black beetle and it has been associated with the cosmopolitan stored products pests; its most common synonyms are *A. laevigatus, A. piceus* and *A. ovatus*. Reports have shown the distribution of this animal in various regions of the world, such as East Africa and America (Vaughan et al., 1984). At 32.2 ºC, the optimal development temperature, the average egg-to-adult lifespan is 45.6 days; it drops to 42.3 days at 37 ºC, while no development is observed at 10 ºC. The time from egg hatching to the last larval stage can vary from 105 days at 15 ºC to 33.7 days, when animals are at 37 ºC, while pupal development can vary from 9 to 6 days (Wilson and Miner, 2013).

2.2.2 *Plesiophthalmus davidis* - Darkling beetle

The dark beetle occurs mainly in subtropical and tropical areas of the eastern hemisphere and they are also found in the eastern Palearctic region (Bremer, 2014) in mixed forests. The larvae and adults are known to feed on rotten wood, and the larvae can often serve as a source of animal nutrition. Information about its life cycle is scarce in the literature.

2.2.3 *Tenebrio molitor* - Yellow mealworm

The yellow mealworm larvae and beetles feed on flour and other grain products, although they are not associated with damage to crop plantations (Howard, 1955). Interestingly, the initial larval and pupal stages are rich in protein and considered a popular dish in some countries, where their potential as a farmable product has been compared to conventional livestock (Li et al., 2013; Mariod et al., 2017; Zaelor and Kitthawee, 2018).

Mealworm beetles are indigenous to Europe and are distributed worldwide (Mariod et al., 2017; Peng et al., 2019). Females can lay 300–400 eggs, from which the larvae are hatched in 5 to 12 days. The larval period varies from 22 to 100 days, while the pupal period is about 8 days (Simon et al., 2013). A representation of *T. molitor* morphology can be observed in Figure 1.

2.2.4 *Tenebrio obscurus* - Dark mealworm

The dark mealworm is commonly recognized as a pest of stored grain, although it has been used in recent years as an animal feed additive due to its high protein content (Bai et al., 2018; Mariod et al., 2017; Robinson, 2005). This species is cosmopolitan, although they are generally distributed in temperate regions (Mariod et al., 2017; Peng et al., 2019). The larval stage can vary from 12 up to 169 days, depending on the temperature and humidity, as well as the pupa stage and adult life (12 up to 3days) (Fiore, 1960). *T. obscurus* is very closely related to *T. molitor* (Bai et al., 2019, 2018).

2.2.5 *Tribolium castaneum* - Red flour beetle

The red flour beetle is considered a worldwide pest of stored products, and this insect can be found anywhere where grains or other dry food are stored (Bergerson and Wool, 1986). A female can lay up to 450 eggs. The time to complete its life cycle is variable, but it can last 40 days under certain conditions (Farias, 2015).

2.2.6 *Zophobas atratus* (synonym as *Zophobas morio* or *Zophobas rugipes*)- Giant mealworm beetle

The giant mealworm beetle is also known as super worm or king worm (Kim et al., 2015). As most pest species of tenebrionids, the giant mealworm is associated with damaged stored food, and it is found in guano deposits, *i.e.* accumulated bat excrement. *Z. atratus* is one of the most common insects that have been grown with the purpose to produce food (Cortes Ortiz et al., 2016; Zaelor and Kitthawee, 2018). This species is native from South and Central America (Kim et al., 2015) and it is capable of exploring organic waste. *Z. atratus* adult beetles are very similar in appearance to *T. molitor* and *T. obscurus*, but they are bigger, measuring about 25–30 mm long (Park et al., 2013). A representation of *Z. atratus* morphology can be observed in Figure 1.

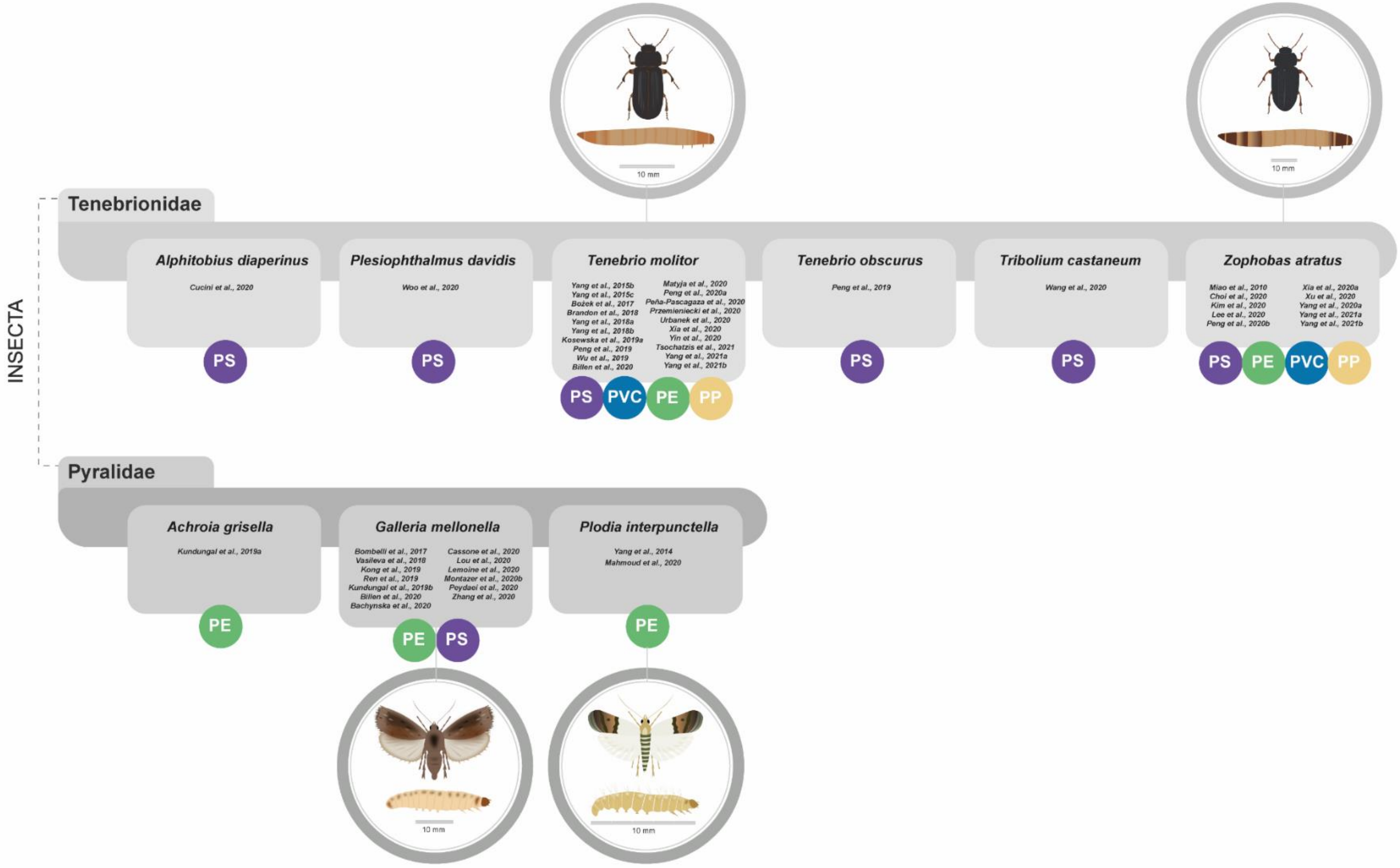


Figure 1: Insects belonging to the families of beetles (*Coleoptera: Tenebrionidae*) or moths (*Lepidoptera: Pyralidae*) reported in the literature as capable of consuming and/or biodegrading PE, PS, PVC, and PP plastics. Grey boxes present the references and circles depict adult insects (top) and the respective larvae (bottom). Scale bars: 10 mm.

3. Plastic biodegradation by insects

As early as 1943, studies already focused on the description of insects capable of penetrating food plastic packaging, in order to evaluate food security. Only in 2010, however, this phenomenon began to be studied by research groups interested in the process of biodegradation of plastic materials. This new subject has attracted the attention of the scientific community, as well as the general public, due to the implications of plastic accumulation in the environment. Surely, the identification and understanding of the biological and chemical mechanisms involved in plastic digestion by insects and/or by their microbiota, could bring forward new innovative biotechnological solutions to manage the plastic waste problem.

The definition of (bio)degradation concepts and guidelines to study biodegradation processes are available from organizations such as ISO and ASTM. Considering that, until recently, plastic biodegradation processes were only described for microorganisms, there is currently a lack of standardized tests and concepts related to plastic biodegradation by insects. In this context, using as reference the studies published in this field in recent years, we adopted herein the term “plastic biodegradation” by insect larvae to refer to processes where plastic biofragmentation products are generated by the animal.

3.1 First descriptions of plastics-penetrating insects

Early reports of insects penetrating plastic-packed food, especially dried fruits, date from 1943. Essig and co-workers highlighted the need to understand insect biology to control the species capable of penetrating stored products (Essig et al., 1943). At that time, as World War II unfolded, it was essential to maintain food supplies for long periods and protected from insects. A number of studies were developed during the 40’s which reported the penetration of plastics by larvae and adult insects, with the aim of obtaining answers about packaging material resistance (Table I).

Rhyzopertha dominica, a species of polyphagous Coleopteran insect belonging to the *Bostrichidae* family, stood out for its great capability of penetrating plastic packaging materials, both in larval and adult stages (Gerhardt and Lindgren, 1954; Riudavets et al., 2007; Sreenathan et al., 1960). Their capacity to penetrate PE, polyurethane, PVC, fluorinated carbon, cellophane and polyester packaging was further investigated. As result, the authors found that PE and cellophane were more easily penetrated by *R. dominica* adults, in accordance with reports by Sreenathan and collaborators (1960) (Highland and Wilsons, 1981). Importantly, when larvae and adults were offered plastics together with another type of food, both were able to penetrate and consume the plastic packaging more efficiently than the alternative energy source (Cline, 1978; Gerhardt and Lindgren, 1954; M. Davey and Amos, 1961).

These findings represent the first evidence that some insect species are able to chew and/or penetrate various packaging materials, serving as a foundation for more recent studies that report the ability of different coleopteran and lepidopteran insects to consume and biodegrade plastics.

Table I: Studies reporting larva and/or adult insects capable of penetrating package materials.

Subject species	**Common name**	**Insects able to chew the packages**	**Package materials**	**Reference**
Oryzaephilus surinamensis	Sawtoothed grain beetle	All insects, except *Oryzaephilus surinamensis*	Many types of plastic and paper packaging	(Essig et al., 1943)
Tribolium confusum	Confused flour beetle			
Gnatocerus cornutus	Broad-horned flour beetle			
Tribolium destructor	Dark flour beetle			
Stegobium paniceum	Drugstore beetle			
Rhyz opertha dominica	Lesser grain borer			
Plodia interpunctella	Indian meal moth			
Tenebroides mauritanicus	Cadelle beetle			
Tenebroides mauritanicus	Cadelle beetle	*Tenebroides mauritanicus*	Cellophane PE, pliofilm, saran resin, aluminum foil	Gerhardt and Lindgren, 1954)
Tribolium confusum	Confused flour beetle	*Tribolium confusum*		
Dermestidae	Dermestid beetle	*Stegobium paniceum*		
Stegobium paniceum	Drugstore beetle	*Plodia interpuctella*		
Blattella germanica	German cockroach	*Rhyzopertha dominica*		
Sitophila granarius	Wheat weevil	*Ephestia kuehniella*		
Plodia interpunctella	Indian meal moth			
Rhyzopertha dominica	Lesser grain borer			
Ephestia kuehniella	Mediterranean flour moth			
Sitophilus oryzae	Rice weevil			
Oryzaephilus surinamensis	Sawtoothed grain beetle			

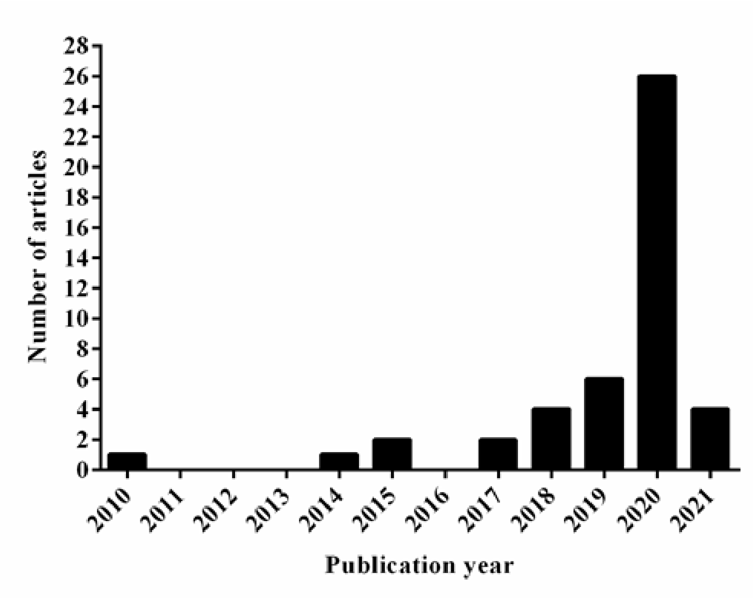
Sitophilus oryzae *Rhyzopertha dominica* *Tribolium castaneum* *Oryzaephilus surinamensis*	Rice weevil Lesser grain borer Red flour beetle Sawtoothed grain beetle	*Rhyzopertha dominica* showed the best results while *Oryzaaephilus surinamensis* the worst. All insects penetrated the packings	PE and cellophane	(Sreenathan et al., 1960)
Rhyzopertha dominica *Dermestes maculatus* *Lasioderma serricorne* *Sitophilus oryzae* *Tribolium castaneum* *Oryzaephilus mercator* *Trogoderma granarium* *Ephesia cautela*	Lesser grain borer Hide beetle Cigarette beetle Rice weevil Red flour beetle Merchant grain beetle Khapra beetle Tropical warehouse moth	*Rhyzopertha dominica*	PE and Kraft paper	(M. Davey and Amos, 1961)
Tribolium castaneum *Cryptolestes pusillus* *Oryzaephilus mercator* *Cathartus quadricollis* *Tenebroides mauritanicus* *Dermestes maculatus* *Trogoderma variabile*	Red flour beetle Flat grain beetle Merchant grain beetle Square-necked grain beetle Cadelle beetle Hide beetle Warehouse beetle	*Tenebroides mauritanicus* *Trogoderma variabile*, *Lasioderma serricorne* *Corcyra cephalonica* *Ephestia cautella* *Plodia interpunctella*	Aluminum foil, Kraft paper, PE, PP, PVC and polyester	(Cline, 1978)

Lasioderma serricorne	Cigarette beetle			
Corcyra cephalonica	Rice moth			
Ephestia cautella	Tropical warehouse moth			
Plodia interpunctella	Indian meal moth			
Rhizopertha dominica	Lesser grain borer	*Rhyzopertha dominica*	PE, PVC, PET, and polyester	(Highland and Wilsons, 1981)
Rhyzopertha dominica	Lesser grain borer	All insects	PP, PE, aluminum and cigarette paper	(Riudavets et al., 2007)
Sitophilus oryzae	Rice weevil			
Lasioderma serricorne	Cigarette beetle			
Lasioderma serricorne	Cigarette beetle	*Lasioderma serricorne*	PE, PVC, PP and cellophane	(Allahvaisi et al., 2009)

3.2 Plastic consumption and biodegradation by insect larvae

The first study investigating plastic consumption and biodegradation by insect larvae was published in 2010 (Miao et al., 2010). Since then, and until December 31, 2020, 46 articles were published in the literature about this topic, indicating an increasing interest in this subject (Figure 2) and the most relevante informations about this teme are present inTables II, III, IV and V (organized according plastic used).

(a)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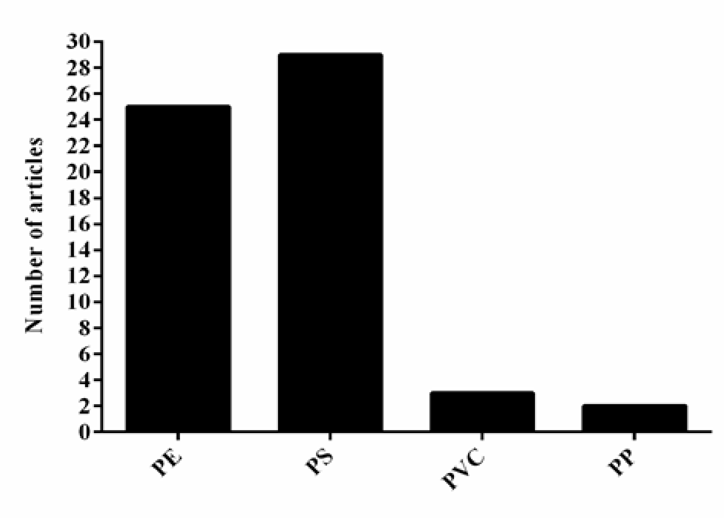
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PP

(c)

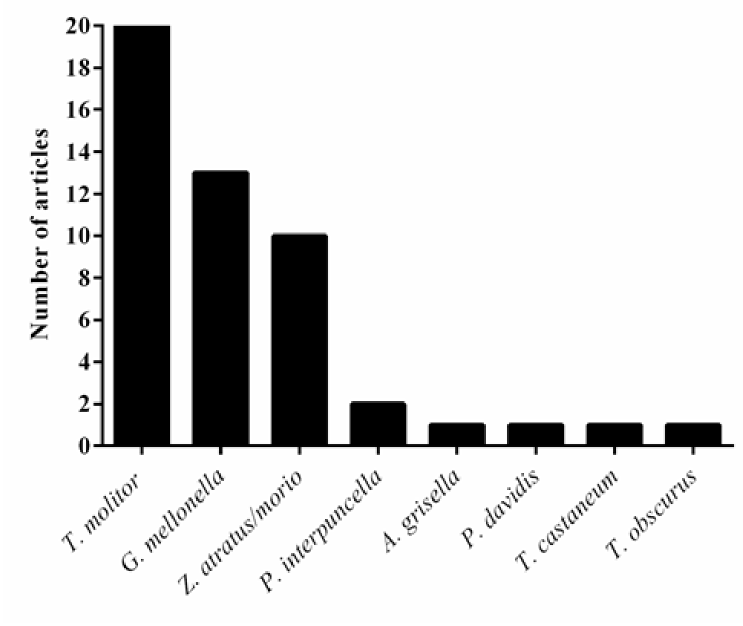
Number of articles
0
2
4
6
8
10
12
14
16
18
20
T. molitor
G. mellonella
Z. atratus/morio
P. interpuncella
A. grisella
P. davidis
T. castaneum
T. obscurus

Figure 2: Number of articles about plastic biodegradation by insect larvae. (a) Number of articles published per year since 2010 (when the first report about plastic biodegradation by insect larvae was published), up to December 31, 2020 (2021 citations refer to articles published online before December 31, 2020). (b) Number of articles about the consumption and/or biodegradation of specific types of plastic by insect larvae. (c) Number of articles about the consumption and/or biodegradation of plastics by larvae of different insect species.

The articles focused on the understanding of the biological process associated to the consumption and digestion of four types plastics by larvae from 9 different insects (the lepidopterans *Achroia grisella*, *Galleria mellonella* and *Plodia interpunctella*, and the coleopterans *Alphitobius diaperinus*, *Plesiophthalmus davidis*, *Tenebrio molitor*, *Tenebrio obscurus*, *Tribolium castaneum* and *Zophobas atratus*). Moreover, one study also evaluated the ability of *T. molitor* adults to consume plastics (Peña-Pascagaza et al. 2020).

Table II: Consumption and biodegradation of PE by insect larvae and by their gut microbiota.

Type of PE	**Insect**	**Biological sample, experiment duration**	**Larval instar, weight or size**	**Microorganism**	**Plastic consumption** (mass units or percent weight loss – WL)	**Biofragmentation products**	**Reference**
HDPE films	*A. grisella*	8 days, live larvae	3rd instar	N.D*	1.83 mg of PE/day/larva	N.D* in this step	(Kundungal et al., 2019a)
		16 days, live larvae	3rd instar	N.D*	N.D* in this step	Alcohol, bonds C=O and C-O	
PE bags	*G. mellonella*	12 h, live larvae	N.D*	N.D*	92 mg per 100 larvae	N.D* in this step	(Bombelli et al., 2017)
		14 h, larvae homogenate	N.D*	N.D*	13% WL or 0.23 mg/cm²/h	Ethylene glycol	
Various types of PE		90 days, live larvae	N.D*	N.D*	Up to 51.0 ± 6.5 µg of PE/day per 200 larvae	N.D*	(Vasileva et al., 2018)
Commercial grade LDPE		7 days, live larvae	4th instar	N.D*	WL by 15 larvae: UPTLDPE$^{\#}$: 18.57 ± 1.8 % PTLDPE$^{\#\#}$: 55.8 ±1.2 %	Alcohol, bonds C=O and C-O	(Kundungal et al., 2019b)
LDPE powder type, 500 micron		14 days, live larvae	2nd instar	N.D*	N.D*	Fatty acids	(Kong et al., 2019)
LDPE bag		24 h and 72 h, live larvae	4th and 5th instar	N.D*	250 µg/larva on day 1 125 ug/larva on day 3	Products of enzymatic assays	(Lemoine et al., 2020)
LDPE films		12 h, live larvae	Last instar	N.D*	Holes with 6.3 mm and pittings with 14 µm	N.D* in this step	(Peydaei et al., 2020)
		20 days, salivary glands homogenate			N.D* in this step	C-O-C, C=O and C-O	
PE films		31 days, culture period	N.D*	*Enterobacter* spp. D1	N.D*	Alcohols, esters, and acids were increased	(Ren et al., 2019)

						in culture supernatant as a result of the D1 treatment	
LDPE and HDPE microplastic particules		28 days, culture period	N.D*	*Aspergillus flavus*	WL: 3.90 ± 1.18%	C-O-C and -OH	(Zhang et al., 2020)
LDPE		No access	No access	*Lysinibacillus fusiformis*, *Bacillus aryabhattai*, and *Microbacterium oxydans*	No access	Linear alkanes and unknow putative LDPE hydrolysis products	(Montazer et al., 2020b)
LDPE films		60 weeks, culture period	4rth and 5th instar	*Acinetobacter* spp.	N.D*	Glycol	(Cassone et al., 2020)
PE foam		28 days, live larvae	15-25 mm long	*Bacillus* ssp. and *Serratia* spp.	1.95 g per 150 larvae	C=O and C-O	(Lou et al., 2020)
Commercial LDPE fruit bags		89 h, live larvae	N.D*	N.D*	0.54 mg/day/larva	-	(Billen et al., 2020)
LDPE film	*P. interpunctella*	60 days, culture period	N.D*	*Enterobacter asburiae* YT1 *Bacillus* spp. YP1	6.1% WL (YT1) 10.7% WL (YP1)	Carbonyl groups	(Yang et al., 2014)
PE ordinary daily and domestic used		60 days, culture period	5th and last instar	*Enterobacter tabaci* strain YIM Hb-3	N.D*	Alcohols, ethers, phenols, alkanes, aromatics, carboxylic	(Mahmoud et al., 2020)

				Bacillus subtilis subsp. Spizizenii strain NBRC 101239		acids, aldehydes, and ketones	
LDPE particles and PE mulching film	*T. molitor*	10 days, culture period	N.D*	*Acinetobacter* sp. Strain NyZ450 *Bacillus* sp. strain NyZ451	18% WL, by the consortium	O-H and C=O (bacteria alone and consortium)	(Yin et al., 2020)
Commercial LDPE fruit bags		38 days, live larvae	N.D*	N.D*	0.023 mg/day/larva	-	(Billen et al., 2020)
LDPE foam PE-1^{+} and PE-2^{++}		60 days, live larvae	Weight: 70-80 mg/larvae	*S. marcescens*	PE1^{+}: 3.33 ± 0.02 mg/day PE2^{++}: 3.45 ± 0.04 mg/day per 100 larvae	C-O, C=O and R-OH	(Yang et al., 2021a)
LDPE foam		32 days, live larvae	Weight: 75-85 mg/larvae	*Kosakonia* sp.	PE: 0.87 ± 0.0 g PE + co-fedding: 1.1 ± 0.12 g per 120 larvae	Carbonyl and carboxylic acid groups	(Brandon et al., 2018)
Microplastic LDPE		1 month, live larvae	3rd and 4th instar	N.D*	WL per 500 larvae (different locations) Guangzhou: 36.9% Tai'an: 22.0% Shenzhen: 29.7%	C-O and C=O	(Wu et al., 2019)
Oxo-degradable-PE and PE-regranulate		2 months, culture period	N.D*	*Pantoea*	N.D*	N.D*	(Przemieniecki et al., 2020)

PE foam	*Z. atratus*	33 days (live larvae)	About 5 cm long	N.D*	Strain G China) 58.7 ± 1.8 mg LDPE/day per 100 larvae Strain M (U.S.) 57.1 ± 2.5 mg LDPE/day per 100 larvae (with co-feeding)	C=O, C=C, C-O and R-OH	(Peng et al., 2020b)
N.D*		60 days, culture period	4th instar	*P. aeruginosa*	WL 0.6 ± 0.0057% /day	C=O and O-H	(Lee et al., 2020)

*ND: not determined in the published article.

#PTLDPE: pretreated LDPE.

##UTLDPE: unpretreated LDPE.

+PE1: LDPE foam, which contains pink color additives.

++PE2: LDPE film, which is colorless without additives.

No access: only abstract available.

Table III: Consumption and biodegradation of PS by insect larvae and by their gut microbiota

Type of PS	**Insect**	**Biological sample, experiment duration**	**Larval instar, weight or size**	**Microorganism**	**Plastic consumption** (mass units or percent weight loss – WL)	**Biofragmentation products**	**Reference**
PS foam	*G. mellonella*	28 days, live larvae	15-25 mm long	*Bacillus* ssp. And *Serratia* spp.	0.88 g per 150 larvae	C=O and C-O	(Lou et al., 2020)
α ^{13}C-labeled and β ^{13}C-labeled PS	*T. molitor*	30 days, live larvae	3rd and 4th instar	N.D*	31.0 ± 1.7% WL per 500 larvae	Alkyl and methyl-C, phenyl derivatives groups, CO and CO_2	(Yang et al., 2015b)
α ^{13}C-labeled and β ^{13}C-labeled PS		60 days, culture period	3rd and 4th instar	*Exiguobacterium* sp. YT2	WL: 7.4 ±0.4%	Carbonyl groups	(Yang et al., 2015c)
Styrofoam		21 days, live larvae	3rd and 4th instar	N.D*	0.22g per 50 larvae (4.8 mg PS/g insect/day)	N.D*	(Kim et al., 2020)
Expanded PS		120 days, live larvae	N.D*	N.D* in this steep	100 mg per 45 larvae	N.D*	(Peña-Pascagaza et al., 2020)
		3 weeks, culture period		Larvae: *Bacillus* sp. *Bacillus anthracis* *Stenotrophomonas* sp.	N.D* in this step	N.D*	

			Adults: *Bacillus* sp. *Bacillus anthracis* *Pantoea agglomerans* *Erwinia persicina*			
Styrofoam	32 days, live larvae	5 th and 7th instar	*Erwinia* was the most prevalent genus	up to 45.59 ± 0.58 mg/day per 100 larvae	Carbonyl groups and double carbon bonds	(Yang et al., 2018a)
Styrofoam	2 weeks, culture period	N.D*	*Mixta tenebrionis* spp. Nov.	N.D*	N.D*	(Xia et al., 2020b)
EPS	7 days, live larvae	Weight: 72–80 mg/larvae	N.D*	13% WL per 100 larvae	2,4,6-Triphenyl-1-hexene, 1,3,5-triphenylcyclohexane, the volatiles acetophenone, cumyl alcohol and 2,4-di-tert butylphenol	(Tsochatzis et al., 2021)
PS foam	32 days, live larvae	Weight:75-85 mg/ larvae	N.D*	Up to 23.5 ± 0.1 mg/day per 100 larvae (with co-feeding)	Carbonyl and hydroxyl groups	(Yang et al., 2018b)

	50 days, live larvae	About of 20-50 mg	N.D*	51.4% WL per 46 larvae (with co-feeding)	N.D*	(Matyja et al., 2020)
PS, PSr, PSp and EPS§	21 days, culture period	About of 20-40 mg	*Serratia marcescens, Klebsiella oxytoca* and *Pseudomonas aeruginosa*	N.D*	N.D*	(Urbanek et al., 2020)
PS foam	60 days, live larvae	Weight: 70-80 mg/larvae	*Dyella* spp., *Lysobacter* spp., and *Leptothrix* spp. were significantly associated with with PS feeding	4.27 ± 0.09 mg/day per 100 larvae	C-O, C=O and R-OH	(Yang et al., 2021a)
PS thick plates	21 days, live larvae	About 2.5 cm long	N.D*	9% WL per 20 larvae	N.D*	(Bożek et al., 2017)
PS foam	32 days, live larvae	Weight: 75-85 mg/larvae	*Citrobacter* sp.	0.57 ± 0.2 g per 120 larvae 0.98 ± 0.11 g per 120 larvae (with co-feeding)	Carbonyl and carboxylic acid groups	(Brandon et al., 2018)
PS microplastic	1 month, live larvae	3rd and 4th instar	N.D*	WL by 500 larvae (different locations) Guangzhou: 57.5%	C-O and C=O	(Wu et al., 2019)

					Tai’an: 34.4% Shenzhen: 51.4%		
Styrofoam		10 weeks, live larvae	N.D*	N.D*	WL: 12.4 % per 100 larvae 13.4% per 100 larvae (with co-feeding)	N.D*	(Kosewska et al., 2019a)
PS		2 months, culture period	N.D*	*Agrobacterium*, *Nitrosomonas* and *Nitrospira*	WL: 12.4 % per 100 larvae	N.D*	(Przemieniecki et al., 2020 and Kosewska et al., 2019)
Styrofoam		31 days, live larvae	2 cm long	*Enterobacteriaceae*, *Spiroplasmataceae*, and *Enterococcaceae*	24.30 ± 1.34 mg/day/larva 33.23 ± 0.8 mg/day /larva (with co-feeding)	Carbonyl group and double carbon bonds	(Peng et al., 2019)
Styrofoam	*T. obscurus*	31 days, live larvae	2 cm long	*Enterobacteriaceae*, *Spiroplasmataceae*, and *Enterococcaceae*	32.44 ± 0.51 mg/day /larva 39.24 ± 1.73 mg/day /larva (with co-feeding)	Carbonyl group and double carbon bonds	(Peng et al., 2019)
Styrofoam	*T. obscurus*	31 days, live larvae	2 cm long	*Enterobacteriaceae*, *Spiroplasmataceae*, and *Enterococcaceae*	32.44 ± 0.51 mg/day /larva 39.24 ± 1.73 mg/day /larva (with co-feeding)	Carbonyl group and double carbon bonds	(Peng et al., 2019)

PS foam	*A. diaperinus*	30 days, live larvae	7–10 mm long	*Pseudomonas, Kocuria, Cronobacter, Pseudogracilibacillus Virgibacillus, Aspergillus, Trichoderma, Penicillium*	20% WL per 17,500 larvae	N.D*	(Cucini et al., 2020)
PS film and PS pellets	*T. castaneum*	60 days, culture period	4 mm long and 1 mg	*Acinetobacter* sp. AnTc-1	WL:12.14 ± 1.4%	N.D*	(Wang et al., 2020)
Styrofoam	*P. davidis*	14 days, live larvae	N.D*	N.D* in this step	34.27 ± 4.04 mg/larva	O-H, C=C and C-O	(Woo et al., 2020)
		20 days, culture period		*Serratia* sp. Strain WSW	N.D* in this step	C=O and C-O	
No access	*Z. atratus*	No access	No access	2.4 g PS per kilo larvae per day	No access	No access	(Miao and Zhang, 2010)
PS foam		27 days, live larvae	No access	N.D*	WL: 6.5%	N.I**	(Choi et al., 2020)

PS foam	28 days, live larvae	3rd and 4th instar	N.D* in this step	0.58 mg/day/larva	Alkyl and methyl carbon, CO_2	(Yang et al., 2020)
PS film	21 days, live larvae	3rd and 4th instar	N.D* in this step	1.4 g per 50 larvae (2.9 mg PS/ g insect/day)	C=O in larval frass	(Kim et al., 2020)
	60 days, culture period of extracted gut		*Pseudomonas* spp. DSM 50071	N.D* in this step	N.D* in this step	
EPS	33 days, live larvae	5 cm long	N.D*	Strain G (China) 61.5 ± 1.6 mg/day per 100 larvae Strain M (U.S). 30.3 ± 7.7 mg/day per 100 larvae (with co-feeding)	C=O, C=C, C-O and R-OH	(Peng et al., 2020b)
Styrofoam	60 days, culture period	4th instar	*P. aeruginosa*	Daily WL: 0.098 ± 0.026 %	C=O and O-H	(Lee et al., 2020)

*ND: not determined in the published article

**NI: not identified by experimental tests performed in the published article.

§PS: larvae fed with raw polystyrene, 1 mm thick plates were prepared; - PSr: larvae fed with processed polystyrene by extrusion, injection molding and grinding. - PSp: larvae fed with commercially available material for parcels; - EPS: larvae fed with commercially available insulation material expanded polystyrene.

No access: only abstract available.

Table IV: Consumption and biodegradation of PP by insect larvae and by their gut microbiota.

Type of PP	Insect	Biological sample, experiment duration	Larval instar, weight or size	Microorganism	Plastic consumption (mass units or percent weight loss – WL)	Biofragmentation products	Reference
PP foam	*T. molitor*	35 days, live larvae	59.6 ± 2.2 mg/larva and 2.0-2.5 cm long	*Kluyvera* sp.	1.0 ± 0.4 mg/day per 100 larvae 1.6 ± 0.3 mg/day per 100 larvae (with co-feeding)	Carbonyl, hydroxyl, and aldehyde groups	(Yang et al., 2021b)
PP foam	*Z. atratus*	35 days, live larvae	3.0-4.0 cm long and 611.0 ± 12.7 mg/larvae	*Citrobacter* sp. and *Enterobacter* sp.	3.1 ± 0.4 mg/ day per 100 larvae 3.6 ± 0.4 mg/day per 100 larvae (with co-feeding)	Carbonyl, hydroxyl, and alkene groups	
PP and PPS beads		60 days, culture period	4th instar	*P. aeruginosa*	WL (daily): PPS: 0.53 ± 0.016% PP: 0.025 ± 0.012%	C=O and O-H	(Lee et al., 2020)

Table V: Consumption and biodegradation of PVC by insect larvae and by their gut microbiota

Type of PVC	Insect	Biological sample, experiment duration	Larval instar, weight or size	Microorganism	Plastic consumption (mass units or percent weight loss – WL)	Biofragmentation products	Reference

	Z. atratus	No access	No access	No access	No access	No access	(Miao and Zhang, 2010)
PVC commercially available	*T. molitor*	21 days, live larvae	About 2.5 cm long	N.D*	WL: 3% by 20 larvae	N.D*	(Bożek et al., 2017)
PVC microplastic		1 month, live larvae	3rd and 4th instar	N.D*	WL 500 larvae (different locations) Guangzhou: 48.4% Tai'an: 34.0% Shenzhen: 57.0%	C-O and C=O	(Wu et al., 2019)
PVC microplastic powders		16 days, live larvae	4th-6th instar and 2 cm long	*Streptococcaceae, Spiroplasmataceae, Enterobacteriaceae,* and *Clostridiaceae*	36.6 ± 6.8 mg/day per 100 larvae	R-OH, C=O, CHCl and CCl	(Peng et al., 2020a)

*ND: not determined in the published article.

No access: only abstract available.

3.2.1 *Galleria mellonella* (Lepidoptera: Pyralidae)

In 2017, a study by Bombelli and co-workers reported for the first time the consumption of plastic by *G. mellonella* larvae. The authors observed a consumption of 92 mg of plastic after PE bags were in contact with 100 larvae during 12 h. To investigate if the action of the larvae masticatory system alone accounted for PE breakdown, they evaluated the effect of larval homogenates on PE films. They reported a significant difference in PE films covered with larvae paste for 14 h, corresponding to 13% weight loss compared with untreated samples, or 0.23 mg/cm^2/h - a degradation rate higher than those demonstrated by microorganisms. Their results also suggested the presence of a degradation intermediate product, ethylene glycol, in films analyzed by FTIR. The authors proposed that PE degradation by *G. mellonella* larvae could be related to the animal's natural habitat, and the fact that they feed on beeswax (Bombelli et al., 2017). Subsequently, the consumption of different PE types by *G. mellonella* larvae was also investigated using ~200 larvae for 90 days. Consumption rates ranging from 1.5 ± 0.5 to 51.0 ± 6.5 μg/day were observed, depending on the PE type analyzed (Vasileva et al., 2018).

Importantly, the FTIR data presented by Bombelli et al. (2017), which indicated bands related to ethylene glycol as possible products of PE degradation, was questioned by the scientific community. Critics pointed out that the bands may also be related to residual lipids and proteins from the larvae homogenate that were not fully removed before film analysis (Weber et al., 2017). Moreover, in 2020, another research group tried to reproduce experiments with larval homogenates (*G. mellonella* and *T. molitor*) using PE and PS films to analyze their plastic-degrading activity. For both species, the authors found no difference in film weight, nor changes on the plastic surface, after exposure to the homogenates (Billen et al., 2020), although they also reported PE consumption by live larvae. Importantly, instead of replacing the homogenate every 2 h as described by Bombelli, Billen's experimental protocol refrained from changes during the analysis (24 h and 48 h), to avoid mechanical disruption of the plastic films, as suggested by Weber et al. Thus, the authors suggested that the PE mass loss reported by Bombelli et al., could have occurred due to mechanical disruptions during the washes, and these results highlighted that the use of a larval homogenate is not a feasible technique to evaluate plastic degradation. Moreover, it is reasonable to consider that the larval chewing process must play a fundamental role to facilitate plastic ingestion and subsequent degradation, since it favors the deterioration and fragmentation of the polymer.

In 2019, Kundungal et al. investigated whether pre-treating PE with UV radiation affected degradation by *G. mellonella*. LDPE films were exposed to direct sunlight during 15 days from 8 am to 4 pm. PE consumption rate increased up to 37% in UV-treated vs. non-treated plastic. This because, the authors reported a weight loss of 18.57±1.8% for UTLDPE, and 55.8±1.2% for PTLDPE (Kundungal et al., 2019b). Although there are no other studies about the influence of pretreatment on plastic degradation by insect larvae, this result indicates that UV exposure may facilitate the next stages of plastic degradation by these animals, just like in environmental biodegradation.

Bachynska and colleagues studied how the addition of PE to an artificial nutrient feeding affected biological parameters in *G. mellonella*. The addition of PE to the artificial diet in proportions of 1/4 up to 1/3 did not influence the biological parameters and viability, however when PE amounted to 1/2 or 3/4 of the diet, pupae viability decreased by 23% and 61%, respectively. They showed that the development phase when the artificial diet contained ½ of PE was 30 days longer than in the control group, and that increasing PE quantity in the artificial medium led to the reduced viability, indicating that PE can not be digested by these larvae. They pointed out that larvae and/or microorganisms have enzymes that can disintegrate the plastic, but that the larvae are not capable of completely digesting PE (Bachynska et al., 2020).

Using more robust methodologies, two recent transcriptomic studies have indicated that *G. mellonella* larvae are equipped with an expanded set of enzymes when compared to closely related lepidopterans (Kong et al., 2019; LeMoine, et al., 2020), possibly related to its exclusive natural food being honeycomb containing beeswax. Briefly, Kong and co-workers (2019) showed the first evidence that *G. mellonella* supports microbiota-independent metabolism of long-chain hydrocarbons, such as PE, but they also indicated that PE is metabolized by partial interaction between the host and its gut microorganisms. Using larvae fed with beeswax, they showed that the gene families related to the formation of long-chain fatty acids from long-chain hydrocarbon (e.g. lipases and cytochromes enzymes) were not overexpressed in the *G. mellonella* gut microbiota, while those related to short-chain fatty acids metabolism were upregulated. Therefore, they proposed that hydrocarbon wax is first depolymerized or hydrolyzed into long-chain fatty acids by the host, and not by the intestinal microbiota, and that both may act on decomposing short-chain fatty acids, suggesting an advantage for the microbiota. On the other hand, LeMoine and collaborators (2020) proposed that after the initial processing of PE by *G. mellonella* gut microbial community, the larvae utilize several key enzymes (such as CYP, ADH, ALDH) to decompose the reduced alkane chains into short-chain fatty acids, which can undergo β-oxidation and enter the Krebs cycle. These authors highlighted that long alkanes, such as PE, are very resistant to degradation, and some enzymes capable of breaking them down (including laccases, lignin peroxidases and manganese peroxidases) have been identified in microorganisms, but not yet in animals, not even in *G. mellonella* gut transcriptomes.

LeMoine et al also observed that *G. mellonella* ability to consume PE decreases with time. The amount of PE exceeded 250 μg/larva on the first day, but was reduced by approximately half on the subsequent 2 days. Both studies importantly contributed to the understating of PE digestion by *G. mellonella* larvae; however, it is still unclear how PE is degraded in the insect body. The specific enzymes involved in this process are unknown, especially those related to the first step of hydrocarbon decomposition into low molecular weight alkanes. Moreover, there is no consensus between the two transcriptomic studies regarding what drives the first step of PE-degradation: insect or microbial enzymes.

Peydaei et al. (2020) analyzed the if salivary glands of *G. mellonella* could be involved in PE biodegradation. By exposing PE films to salivary glands homogenates during 20 days, the authors verified the formation of pitting and degradation intermediates, such as carbonyl groups, indicating that salivary glands could assist in biodegradation. Furthermore they performed a proteomic study of the salivary glands obtained from PE-fed larvae during 10 days. Proteins that were significantly induced during consumption of PE revealed that the *G. mellonella* underwent general changes in energy levels, and that the enzymatic pathways related to fatty acid beta oxidation were stimulated (Peydaei et al., 2020).

A handful of studies report on the role of gut larvae microbiota in PE-biodegrading activity. Some of them use traditional *in vitro* culture techniques for microbial isolation while others apply metagenomic approaches to investigate changes in bacterial community. Ren et al. (2019), performed a screening with gut larvae homogenates, using a liquid medium in which PE (1%) was the sole carbon source, at 37 °C and 220 rpm during 31 days. The microorganism that showed best growth in this medium was identified as *Enterobacter* sp. D1. After 14 days of treatment with D1 suspension, roughness, depressions, and cracks appeared on PE film surface. In these samples, FTIR showed the presence of carbonyl functional groups and ether groups in films, while LC-MS revealed the presence of certain alcohols, esters, and acids in the supernatant, which represent bacterial metabolism during PE biodegradation. These observations evidenced the bacterial ability to degrade PE and the possible role of *G. mellonella* microbiota in this process (Ren et al., 2019). Following a similar methodology, Zhang et al. (2020) screened microorganism with potential to biodegrade LDPE and HDPE using as sole carbon source SCS-LDPE or SCS-HDPE media, according to the ASTM standard (ASTM G22 -76), at 28ºC and 150 rpm during 30 days. A fungal strain PEDX3 was isolated and identified as *Aspergillus flavus*. After 28 days of exposure to the fungal culture, damage was observed on the HDPE surface, the weight of the material decreased by 3.9025 ± 1.18%, the molecular weight was also decreased and peaks corresponding to the -OH, C = O and C-O-C groups were found in FTIR analyzes. Importantly, the authors verified that expression of two laccase-like multicopper oxidases genes (LMCOs), AFLA_006190 and AFLA_053930, was upregulated during PE biodegradation process, making them candidate PE-degrading enzymes (Zhang et al., 2020). In addition, Montazer et al., (2020b) isolated three bacterial species (*Lysinibacillus fusiformis*, *Bacillus aryabhattai*, and *Microbacterium oxydans*) from *G. mellonella* larvae gut. Exposed to each of these bacteria, PE films presented weight loss and PE hydrolysis products were observed in the growth medium. The authors also evaluated PE degradation by a bacterial consortium. To their three isolates, they added three other strains previously reported to degrade LDPE (*Cupriavidus necator* H16, *Pseudomonas putida* LS46 and *P. putida* IRN22). The consortium was shown to be more effective in degrading LDPE than individual species, as confirmed by the identification of linear alkanes and other unknown putative LDPE hydrolysis products through GC analysis. (Montazer et al., 2020b).

The contribution of *G. mellonella* intestinal microbiota to PE biodegradation was also approached by Cassone et al. (2020). Using biochemical assays, they showed *G. mellonella* larvae excrete the metabolic by-product glycol in their feces, confirming that they consume and metabolize LDPE. However, excretion of glycol was reduced when larvae were treated with antibiotics, suggesting a role for the microbiota. A metagenomic analysis of the bacterial community present in *G. mellonella* gut showed that larvae fed with PE during 24 h, in comparison to those starved, presented a relative increase in the genera *Escherichia-Shigella, Pantoea, Pseudocitrobacter, Salmonella, Serratia* and *Citrobacter* while the genera *Acetobacter, Asaia, Gluconacetobacter* and *Swaminathania* showed reduced abundance. After 72 h, the only significant differences were an increase in the genera *Aeromonas, Ottowia* and uncultured *Burkholderiaceae* in the PE-fed group. No significant differences were observed between animals fed on PE or honeycomb at 24 h. However, at 72 h, *Acetobacter* and *Ottowia* showed higher abundance in larvae fed with plastic. Sixty-weeks *in vitro* culture of the gut from larvae previously fed with PE (for 4 days) led to the isolation of *Acinetobacter* spp., a genus that has been repeatedly associated with PE biodegradation (Cassone et al., 2020).

Studies about plastic degradation by *G. mellonella* larvae are focused mostly on PE polymer. A single study, conducted by Lou and co-workers (2020), had also evaluated PS besides PE. They showed that these larvae are able to consume PS and PE plastics at similar rates, with a weight loss of 0.88 g of PS and 1.95 g of PE, during 28 days with 150 larvae. Polymer biodegradation occured, as indicated by the formation of C=O and C-O containing functional groups and long-chain fatty acids in the residual polymers. The study also showed that gut bacterial communities comprise simple core microbiomes, which are altered when a mixed diet (plastic with beeswax or wheat bran) is provided. Overall, *Bacillus cereus* and

Serratia marcescens were significantly associated with the PS and PE diets, with *S. marcescens* being more prominent in the PE-fed gut microbiome. *Pseudomonas, Geobacillus,* and *Propionibacterium* were also significantly associated with the PS-fed group when bran was the control diet (Lou et al., 2020).

Based on this set of studies, it is likely that the gut microbiota plays an important role in PE digestion by *G. mellonella*, but it might not be strictly required for this activity. Until December 2020 there was no study in the literature that has investigated whether biomineralization products are generated during PE degradation by *G. mellonella* larvae. Considering these few studies specifically addressing microbiota involvement, it is clear that there is no consensus about species and genera of microorganisms that can participate in PE biodegradation, nor about enzymes and their action on degradation cascade. At this point, a possible role of *G. mellonella* salivary gland microbiota remains to be evaluated.

3.2.2 *Plodia interpunctella* (Lepidoptera: Pyralidae)

Yang et al. (2014) showed the ability of *P. interpunctella* larvae to chew and eat PE films, and went on to focus on the isolation of microorganisms from the larvae gut. They identified the bacteria *Enterobacter asburiae* YT1 and *Bacillus* sp. YP1. After 28 days of incubation with bacterial cultures, PE films presented pits and cavities, as demonstrated by SEM and AFM images, and carbonyl groups were detected with XPS and FTIR analyses. In the presence of bacteria, the mass of PE films decreased at a rate of 6.1 ± 0.3% (strain YT1) and 10.7 ± 0.2% (strain YP1), PE molecular weight decreased about 6-13%, and lower molecular weight fragments were formed in the culture suspension after 60 days. These findings indicate a depolymerization/cleavage of PE, and a PE-biodegrading activity by these strains (Yang et al., 2014). In 2015, the authors reported the complete genome of the strain *Bacillus* sp. YP1 (Yang et al., 2015a).

In 2020, another study evaluated the effect of bacterial isolates from *P. interpunctella* larvae gut on PE biodegradation. It showed that larvae fed on a wheat bran diet yielded a significantly higher number of colonies than other diets. Using the larval microbiota as inoculum on a carbon-free basal agar medium with pieces of plastic for 14 days at 30 ºC the authors isolated two bacteria, which were identified as *Enterobacter tabaci* YIM Hb-3 (B1) and *Bacillus subtilis* subsp. *Spizizenii* NBRC 101239 (B2), the same genera isolated in previous work (Yang et al., 2014). PE samples were characterized by SEM and FTIR, after 60 days of exposure to bacterial cultures. Cavities and depressions on the PE surface were observed, which gradually increased with increasing incubation time, for both bacterial isolates. FTIR analyses of bacteria-exposed films showed the formation of alcohols, ethers, phenols, alkanes, aromatics, carboxylic acids, aldehydes, and ketones as degradation products (Mahmoud et al., 2020). Biomineralization products were not evaluated on either works.

3.2.3 *Tenebrio molitor* (Coleoptera: Tenebrionidae)

Together with *G. mellonella*, *T. molitor* is another insect that stands out in the number of publications reporting on its ability to degrade plastics, and its larvae were the first among insects to be shown degrading a persistent petroleum-based plastic, like PS, up to mineralization end-products. In 2015, Yang and collaborators reported that larvae fed with Styrofoam, a PS product, as the sole diet survived and behaved like those fed with bran over a period of 1 month. Using several techniques, including solid-state ^{13}C cross-polarization/magic angle spinning nuclear magnetic resonance (CP/MAS NMR) spectroscopy, they showed the cleavage of long-chain PS molecules and the formation of depolymerized metabolites in the larval gut. Importantly, they confirmed that ^{13}C-labeled PS was partially biomineralized to $^{13}CO_2$, and the remaining labeled carbon was incorporated into lipids (Yang et al., 2015b). Subsequently, they verified that the suppression of gut microbiota by gentamicin impaired the ability of the larvae to depolymerize PS, and that in this case $^{13}CO_2$ was not produced as a biomineralization end-product. By setting up cultures using PS films as the only carbon source and *T. molitor* larvae gut as inoculum, they isolated 13 bacteria among which the strain *Exiguobacterium* sp.YT2 was identified and studied. This microorganism developed as biofilms on PS surface, changed physico-chemical properties of the surface, degraded about 7.5% of PS, lowered the molecular weight of the residual PS pieces, and promoted the release of water-soluble products over a 60-day incubation period. The essential role of gut bacteria in PS biodegradation and biomineralization by *T. molitor* was evidenced by this study, and the presence of PS-degrading bacteria in larval gut was confirmed (Yang et al., 2015c). Therefore, these authors proposed a symbiotic multi-step PS biodegradation process for *T. molitor* larvae as follows: (i) firstly the polymer is fragmented into smaller particles by chewing, which increases the contact surface of the plastic for the action of microorganisms and extracellular enzymes, (ii) the ingested particles are depolymerized into small-molecule products by extracellular enzymes secreted by gut microbiota, (iii) products are mainly degraded or mineralized into CO_2 by various microorganisms and/or the larvae, and limited carbons from the products are further assimilated into the biomass; and (iv) finally the residual fragments and other intermediates are excreted in the larvae feces (Yang et al., 2015b, Yang et al., 2015c). The ability of *T. molitor* larvae to consume and biodegrade PS is not retained by adults, as recently shown by Peña-Pascagaza et al. 2020. *T. molitor* adults were allowed to feed on PS plates only, but after 15 days of incubation, surfaces did not exhibit significant changes. The apparent inability of adults to feed on PS could be attributed to larval-to-adult jaw structure changes that prevent adults from breaking and ingesting PS.

Further, another research group showed that *T. molitor* larvae from 22 different countries share the ability to eat and metabolize PS, supporting the hypothesis that this feature is independent of the geographic origin of the mealworms, and likely to be genetically-programmed and ubiquitous in members of this species (Yang et al., 2018a). By analyzing larval microbiomes, the data corroborate that gut microbiome plays a role in PS depolymerization and biodegradation. The microbiome of animals from different sources differs, however when mealworms were fed PS, the microbial community changed and converged on similar patterns independent of the larvae source (*Erwinia* was the most prevalent genus). This was possibly a direct result of PS consumption (*i.e.* a changing community better equipped to biodegrade PS), or an indirect response (*i.e.* a response to nutrient deprivation due to the limited diet). The authors hypothesized that PS depolymerization may be induced by gut microorganisms that are directly in contact with PS, or by synergistic effects involving gut microbiota and larvae digestive enzymes within the gut. In this context, during a screening for plastic-degrading gut bacteria associated with mealworms, the bacterial isolate BIT-26^T was identified as a new species of *Mixta* genus (*Erwiniaceae* family), and proposed as *Mixta tenebrionis* sp. nov. However, further analyses are still to be performed to confirm PS degradation activity by this bacterium (Xia et al., 2020a). Peña-Pascagaza et al. (2020) evaluated the growth of bacteria from the digestive tract of *T. molitor* (larval and adult stages) in PS as the sole source of carbon. Nine bacterial strains were isolated from *T. molitor* gut: five strains from larvae (*Bacillus anthracis*, *Bacillus* sp. and 3 *Stenotrophomonas* sp. isolates), and four strains from adult beetles

(*Bacillus anthracis*, *Bacillus* sp., *Pantoea agglomerans* and *Erwinia persicina*) (Peña-Pascagaza et al., 2020). As highlighted by the authors, the differences in the microbial composition within the digestive tracts of larvae versus adult *T. molitor* could be related to the loss of the ability of the adult to use PS as a source of food.

PS metabolization by *T. molitor* larvae was also demonstrated by the identification of chemical compounds produced during PS consumption, using GC-MS untargeted screening. PS monomers (styrene, α-methyl styrene), PS oligomers (mostly trimers), and two groups of fatty acids (saturated acids such as undecylic, myristic and palmitic acid, and unsaturated acids such as oleic acid) were identified from larval homogenates that received PS feeding for up to 7 days. All of these fatty acids were found in large quantities, except undecylic acid, which was present only at residual levels. Some esters (methyl linoleate, ethyl linoleate and ethyl palmitate) and amides (tetradecanamide, hexadecanamide and oleamide) related to fatty acids were also formed after 3 and 7 days, most probably due to enzymatic transformation. Total biodegradation rate at the end of 7 days was close to 13% (Tsochatzis et al., 2021).

The factors affecting consumption and biodegradation rates and the ability of PS-fed *T. molitor* larvae to complete their life cycle were also investigated. Yang et al. (2018b) showed that PS consumption and PS-biodegradation by *T. molitor* larvae increased more than 50% when they were co-fed with bran in comparison to the larvae that consumed PS only. Mealworms fed PS alone developed into pupae and subsequently into beetles. Temperature also influenced larval development; survival rate was lower at higher temperatures (30°C), and higher at mild temperatures (20-25 °C). However, PS consumption by these larvae is higher at 30 °C, which can be explained by a general lower metabolism at lower temperatures. This work indicated that the capacity to consume and degrade PS can be maintained, and perhaps enhanced, through selective breeding. In the same context, Matyja et al. (2020) explored the Dynamic Energy Budget, a mathematical model used to assess changes in physiological processes of different organisms, to study the effects of PS diet on *T. molitor* larval growth, development and survival. They found that *T. molitor* larvae when co-fed with oats presented the highest PS consumption rate (51.4% per 46 larvae during 50 days). The findings indicated that changes in larval development when fed with PS are mainly caused by the reduction in reserves density and the reaction of the organism to an insufficient food supply. Impaired ability to complete the life cycle was observed in larvae fed on PS only (Matyja et al., 2020). Mealworms were reported to lose a substantial fraction (up to 20%) of their body mass when fed only with PS, during 21 days, for all 4 different PS-type materials evaluated (Urbaneck et al., 2020). Moreover, short-term survival of *T. molitor* larvae with PVC or PS feed only was also reported (Peng et al., 2020a, Peña-Pascagaza et al., 2020). Pupation was prevented, and cannibalism was also observed after 20 days of *T. molitor* larvae eating PS or PE (Yang et al. 2021a). These results add to the evidence that these insects are not able to complete their life cycle when plastics are provided as the only food source, and that co-feeding is important.

A breeding investigation with different types of PS was performed to investigate changes in gut microbiome diversity (Urbanek et al., 2020). *T. molitor* gut microbiota is strongly related to the PS-type supplied: the highest number of cultured bacteria was observed for larvae fed with PS from material commercially available for parcels, which was also the material fastest consumed by mealworms possibly due to its expanded properties and lightness. On the other hand, larvae fed with commercially available EPS showed a lower number of bacteria in their guts. The predominant bacterial species was *Enterobacter hormaechei*. In accordance with Peña-Pascagaza et al., (2020), the larval bacterial community was shown to be differently diverse from the beetle bacterial community. *E. hormaechei* was not present in adults, which harbored *Lactococcus lactis* (41.42%) and *Lactococcus garvieae* (40.88%), the latter also found in larvae. The authors isolated three bacterial species from *T. molitor* gut *(Serratia marcescens, Klebsiella oxytoca* and *P. aeruginosa*) that showed degrading ability on the plates with bioplastics (PBS, PBSA and PCL), but not PS, as a sole carbon source (Urbanek et al., 2020).

Populations of *T. molitor* ingest and use PS as a food and energy source for a survival period, and this behavior is not limited to PS, including PE, PVC, PP, PLA and CEL. The study developed by Bozek and co-workers addressed PVC and PLA besides PS biodegradation by *T. molitor*. They showed that a group of 20 larvae was able to reduce in 3, 9 and 12% the weight of PVC, PS and PLA, respectively, in 21 days. They indicated that the energy stored by the animals as lipids and carbohydrates reservoirs, can maintain the basic metabolic rates with all plastic feed. For this reason, larvae can survive on a plastic-only diet, but lose weight constantly (Bożek et al., 2017). Importantly, Brandon et al (2018) showed that PE can be consumed even more rapidly than PS by *T. molitor* larvae, and that about 49.0% of ingested PE is converted into a putative gas fraction (CO_2), suggesting PE biomineralization. Microbiome analyses of larvae gut indicated the involvement of two bacterial species, *Citrobacter* spp. and *Kosakonia* spp., in PE and PS biodegradation, respectively, indicating the adaptability of the gut microbiota of larvae for biodegradation of plastics with different chemical structures (Brandon et al., 2018). *Acinetobacter* sp. strain NyZ450 and *Bacillus* sp. strain NyZ451 were isolated from *T. molitor,* and co-culture assays exhibited better results for PE films degradation (rate of 18% in 10 days), including molecular weight reduction, compared to pure cultures (Yin et al., 2020). Recently, Yang et al. (2021a) performed experiments on plastic biodegradation by *T. molitor* larvae using: (i)

LDPE foam (PE-1), which contains pink color additives and presents lower molecular weight; (ii) LDFE film (PE-2), which is colorless, without additives, and presents higher molecular weight; and (iii) PS. In this work, in contrast to Brandon et al. (2018), the highest rate of consumption was observed for PS (4.27 ± 0.09 mg/ day per 100 larvae), followed by PE-1 and PE-2 (3.33 ± 0.02 and 3.45 ± 0.04 mg/day per 100 larvae, respectively). Moreover, EPS and PE-1 (with lower molecular weight) were broadly depolymerized while depolymerization of PE-2 (with higher molecular weight) was limited. The authors comment that this fact can be related to the selective depolymerization of lower molecular weight polymers at a higher rate than the longer chain polymers, and the possible crosslinking reactions during depolymerization and biodegradation. Treatment with antibiotic gentamicin resulted in inhibition of EPS depolymerization, however, residual PE depolymerization was still observed during gentamicin treatment, indicating that PS biodegradation by *T. molitor* is dependent on intestinal microorganisms, but degradation PE seems to be less dependent on, or independent of microbiota. Great differences in the gut microbiota were observed for the second generation of larvae fed with PS or PE-1, including significant higher abundance of *Coprococcus* genus in larvae fed with PS. Differential abundance analysis indicated *Dyella* spp., *Lysobacter* spp., and *Leptothrix* spp. as significantly associated with PS feeding (Yang et al., 2021a). Similarly, Yang et al., 2021b showed that PP can be biodegraded by *T. molitor* larvae via gut microbe-dependent depolymerization. They showed that *T. molitor* larvae fed with PP foam as the sole diet consumed PP at 1.0 ± 0.4 mg/100 larvae/day but when PP foam was supplemented with wheat bran, the consumption rate was enhanced by 68.11%. GPC analyses of the larvae feces indicated limited extent PP depolymerization, but oxidation and biodegradation were confirmed. The inhibition of gut microbiota with gentamicin inhibited PP depolymerization, and high throughput *16S rRNA* sequencing revealed that *Kluyvera* was predominant in PP-fed larvae.

A comparative study on distinct plastics (PE, PS and PVC) consumption by *T. molitor* larvae from three different Chinese cities (Guangzhou, Tai'an and Shenzhen) was performed. The larvae were able to consume all plastic types. After 30 days, the highest consumption was observed for PS in the Guangzhou region, reaching a rate of 57.5% weight loss. In Tai'an, the best result was also for PS, although this rate was 34.4%, and similar to the PVC consumption. Larvae from Shenzhen consumed PVC in greater quantities (57.0%) than other materials. Using XRD, FTIR, TGA and GPC assays, the authors showed that PS and LDPE are metabolized by mealworms from all three regions. Larvae from Guangzhou were unable to degrade PVC, unlike Tai'an and Shenzhen, which also suggests that biological routes used for plastic digestion can differ according to the chemical composition of the material (Wu et al., 2019). Additionally, the ability of *T. molitor* larvae to consume rigid PVC microplastic powders as the sole diet during 16 days was also reported (about 37 mg/day by 100 larvae). A broad depolymerization of PVC was observed, while released chloride was counted as about 3% of the ingested PVC, indicating limited biomineralization of the plastic. Feces contained about 35% of residual PVC polymer, and chlorinated organic carbons. As shown for PS, suppression of gut microbiota with antibiotics severely inhibited PVC depolymerization, indicating that the PVC biodegradation by *T. molitor* depends on its gut microbes. The consumption of PVC was significantly associated with four bacterial families: *Streptococcaceae, Spiroplasmataceae, Enterobacteriaceae,* and *Clostridiaceae* (Peng et al., 2020a).

T. molitor larvae were assessed for their ability to consume PS, in a study by Kosewska and collaborators (2019) addressing *T. molitor* larvae ability to consume PS, the authors report that with DDGS as a nitrogen source, mealworms (n=100) can ingest more PS (13.2%) in comparison to those exclusively feeding of plastic (12.4%) for 10 weeks. Also, the enzymatic activity in the animal's digestive tract was evaluated with different diets consisting of PS only, PS with DDGS, and the control diets (oats and oats with DDGS). Enzymatic activity was greater in the PS-fed larvae than in those that received oats. The authors highlight a high adaptability of this animal and the rapid multiplication of intestinal microorganisms, which assist in the digestion of mealworm under diets that are not typical. The highest activity was observed for the ß-glucosidase activity tests and amygdalin oxidation fermentation. High activity was also demonstrated in the fermentation-oxidation tests of arabinose and reduction of nitrogen compounds, which can be linked to the enrichment of the substrate with a nitrogen source. On the other hand, it is important to note that the addition of DDGS activated proteolytic processes in both diets. In addition, PS-feed has intensified the fermentation-oxidation reaction of most sugars, including glucose, mannitol, and melibiose (Kosewska et al., 2019).

T. molitor larvae fed with (i) CEL-ca, (ii) PS, (iii) PE-oxo, (iv) PE-reg and (v) OAT as control substrate also had their gut microbiome and enzymatic activities analyzed. Microbial communities from animals under oat and cellulose diet grouped together, the two types of PE formed another group, while PS diet showed the highest dissimilarity. The highest relative abundance of bacteria colonizing the gut was found with PE-oxo feeding. Bacterial genus *Pantoea* was present in a higher proportion in larvae fed both PE diets. *Lactococcus* and *Elizabethkingia* bacteria were found in larvae fed with all plastic diets, and potential diazotrophic bacteria, such as *Agrobacterium* spp., *Nitrosomonas* spp., *Nitrospira* spp., as well as the less abundant phylum *Verrucomicrobia*, were characteristics of microbiota from PS-fed larvae. The presence of *Verrucomicrobia* suggested the possibility of protein synthesis from atmospheric nitrogen by the intestinal microbiota. Additionally, the study performed analyses of enzymes produced by the animal digestive tract cells as well as by symbiotic bacteria, showing that *T. molitor* digestive tract lysates (with symbionts) were far more active than the microorganisms alone, and also the concentrations of enzymes secreted by digestive tract homogenates were higher than those secreted by bacteria alone. The authors pointed out that enzymatic activity was generally stable across different diets, but esterase activity increased in larvae fed both PE types (Przemieniecki et al., 2020).

3.2.4 *Zophobas atratus* (synonym of *Zophobas morio* or *Zophobas rugipes* – Coleoptera: Tenebrionidae)

The first description of the consumption of plastics by insect larvae reported in the literature was in 2010 by Miao and Zhang. The authors evaluated the consumption of LDPE, LLDPE, EVA, PS and PVC microplastics by *Z. morio* larvae, and found that the larvae consumed 2.4 g of PS/kg of larvae/day. However, the study did not provide solid data on biodegradation for all plastics. Based on TGA-SDTA analysis of egested frass, changes in the physical properties of residual PVC and PS were observed, but not for LDPE and EVA, indicating the need for future studies.

When PS degradation was further evaluated in subsequent studies, it was observed that, for a group of 10 larvae and after 27 days of exposure to the plastic, there was a 6.5% - reduction in PS foam weight while animals had 16.3% weight loss (Choi et al., 2020). Yang et al., 2020 showed that *Z. atratus* larvae are capable to use Styrofoam (PS) as their sole diet (0.58 mg PS/day/larva) as well as bran, over a 28-day period, and that the consumption rate was 4 times higher than those reported for *T. molitor* larvae (Yang et al. 2015b). Analyses of feces demonstrated that the depolymerization of long-chain PS molecules and the formation of low molecular weight products occurred in the larval gut, and that PS is biomineralized to CO_2 during a 16-day test period. As reported for *Tenebrio* species, PS-degrading capability of *Z. atratus* was inhibited by the antibiotic suppression of gut microbiota, indicating that gut microbiota contributed to PS degradation (Yang et al., 2020). Because the amount of plastic ingested by each animal is related to its weight, PS consumption rates per weight of larvae were determined by Kim and co-workers (2020). During the 21-day period, fifty *Z. atratus* larvae consumed 1.42 g of PS whereas fifty *T. molitor* ingested 0.22 g from the same amount of PS supply, but the average consuming rate per 1 g *T. molitor* (4.8 mg/day) was around 1.7 times higher than that measured in *Z. atratus* (2.9 mg/day). Thus, *T. molitor* showed better efficiency than *Z. atratus* for PS ingestion. For both species, around 90% of larvae survived after the 21-day period. *Pseudomonas aeruginosa* DSM 50071 was isolated from the gut microbiota of *Z. atratus* larvae; the expression of S-formylglutathione hydrolase and serine hydrolase increased 2- and 7-fold, respectively, in bacteria grown in a PS-supplemented nutrient-limited medium. The essential role of serine hydrolase in PS biodegradation was further confirmed by blocking its activity with a specific inhibitor. For this, the authours performed an assay using 1 g of tert-butyl 4-[{2-fluorophenyl}carbamoyl]piperazine-1-carboxylate), with differents concentrations in liquid LCFBM, including 1 g of PS beads and *Pseudomonas* sp. DSM 50071. The authors suggest that this enzyme plays an important role in mediating the hydrolysis step to depolymerize PS and/or intermediates formed from depolymerized PS to small molecules in the biodegradation process (Kim et al., 2020).

Besides PS (EPS), Peng et al. (2020b) also evaluated PE (LDPE) biodegradation by *Z. atratus* larvae from strains from different regions: Guangzhou, China (strain G) and Marion, Illinois, USA (strain M). These larvae received different diets (with or without bran supplementation) for 33 days. The highest rate of degradation was achieved by strain G, with PS + bran or cabbage feeding, showing specific plastic consumption rates of 77.0 ± 2.5 mg/100 larvae/day. Moreover, two different depolymerization patterns were observed for EPS during biodegradation by *Z. atratus*: a broad depolymerization by strain G, and limited extent depolymerization by strain M. Meanwhile, a limited depolymerization of LDPE was observed for both strains. Interestingly, when larvae were treated with gentamicin, no depolymerization occurred for either plastics, indicating that both PS and PE biodegradation by Z. *atratus* is microbial-dependent, similarly to what has been shown for PS and *Tenebrio* genus (Peng et al., 2019). The authors pointed out that *Z. atratus* larvae can biodegrade PS and PE at higher rates compared with *T. molitor* and *T. obscurus* larvae, and it may be due to the larger size and the intrinsic aggressive habit of foraging by *Z. atratus*.

PP biodegradation by *Z. atratus* larvae has also been reported (Yang et al., 2021b). PP consumption rate by *Z. atratus* was 3.1 ± 0.4 mg/100 larvae/day, and co-feeding with wheat bran enhanced PP consumption rate by 39.70%. GPC analyses of residual PP in larvae feces demonstrated a limited-extent depolymerization, but TGA analysis indicated that feces contained not only residual PP but also degradation products. Suppression of gut microorganisms by antibiotic treatment with gentamicin indicated that PP depolymerization by *Z. atratus* larvae depends on the action of its microbiota, similarly to *T. molitor*. *Citrobacter* sp. and *Enterobacter* sp. were strongly associated with PP diet (Yang et al., 2021b).

Lee et al. (2020) isolated plastic-degrading gut microbes from superworms using LCFBM medium supplemented with PS at 25 ºC, 180 rpm, for 60 days. The *16S rRNA* sequencing analysis identified the isolates as *P. aeruginosa*. Elemental composition analysis showed strong oxygen signals on the PS beads surfaces incubated with *P. aeruginosa*, which were not found in the control group, indicating that oxidation had occurred during PS degradation. The authors also performed assays with three other types of plastics (PPS, PP and PE), with plastic beads being incubated with *P. aeruginosa* in the liquid LCFBM media. A quantitative analysis showed a daily average weight loss of 0.64% ± 0.057 for PE, PPS had a weight loss of 0.53% ± 0.016 for PPS, 0.098% ± 0.026 for PS, and 0.025% ± 0.012 for PP. Moreover, elemental composition analyses showed strong oxygen signals on the PE and PPS beads surfaces, in contrast, much weaker oxygen signals were detected on the PP beads. Therefore, FTIR analyses were conducted and identified C=O and O-H on PP surface. *P. aeruginosa*-mediated PP biodegradation had occurred, with lower efficiency than that for PE, PS, or PPS. Together, the results demonstrate that *P. aeruginosa* efficiently degrades PE, at a rate much faster than for any of the other plastics tested. This group also identified several enzymes that participate in plastic degradation, including serine hydrolase secreted from *P. aeruginosa*. Thus, the superior results found for PE might be because depolymerases secreted from *P. aeruginosa* can mediate PE depolymerization better than the other plastics kinds, or because certain specific PE-degrading enzymes are more highly expressed than others (Lee et al., 2020).

Recent studies focused on *in vitro* isolation of microorganisms from the gut of *Z. atratus* larvae identified two novel bacterial species. The strain BIT- B35^T showed highest 16S rRNA sequence similarity (98.1%) to *Escherichia fergusonii* ATCC 35469T and *Citrobacter koseri* LMG 5519T, and was proposed as a novel species of a novel genus within the family *Enterobacteriaceae,* named *Intestinirhabdus alba* gen. nov., sp. nov. (Xu et al., 2020). The strain BIT- d1^T showed highest *16S rRNA* sequence similarity (98.0%) to *Myroides pelagicus* SM1^T, and after integrative analyses was considered to represent a novel species within the genus *Myroides* and family *Flavobacteriaceae*, for which the name *Myroides albus* sp. nov was proposed (Xia et al., 2020b).

3.2.5 Single reports of insect larvae consumption and degradation of plastics

Other preliminary studies have described the ability of distinct insects, such as *A. grisella, T. obscurus, P. davidis, T. castenum* and *A. diaperinus,* to consume and even degrade PE and PS polymers. Along with assays investigating larvae activity, the role of microbiota in this process has been also addressed. These studies show the great potential for exploration of this field and its progress and fast expansion in a short time since the first reports were published.

The Lepidopteran *A. grisella* is featured in only one publication about its ability to digest plastics. Kundungal and co-workers (2019a) investigated a second generation of larvae, produced after a cycle developed with a PE or PE plus waxcomb (PE-WC) diet. In the first generation, PE consumption was 42.5 ± 2.1%, when this plastic was available alone, and 69.6 ± 3.2% for PE-WC co-fed worms. The second-generation larvae reached a PE consumption rate of 18.6 ± 2.1% (PE only), and 44.9 ± 3.2% (PE-WC co-fed). The results showed that larvae fed with PE-WC in the second generation degrade PE at par with the first generation. However, an decrease in this consumption is observed in the absence of co-feeding. Moreover, this group of larvae had a difficulty to complete their cycle life, while worms that received PE-WC co-feeding eventually completed their life cycle, evolved to be mature larvae, and then developed into pupae and moth. It indicates that co-feeding provides larvae more energy to perform plastic degradation (Kundungal et al., 2019a).

The first, and so far only, study involving plastic biodegradation by *T. obscurus* was conducted by Peng et al. (2019). They showed that *T. obscurus* larvae from two distinct origins were able to chew and ingest PS, and that the consumption rates and extent of depolymerization were higher than the equally sized *T. molitor* larvae. With expanded PS foam as the sole diet, the specific PS consumption rates for *T. obscurus* and *T. molitor* at similar sizes (2.0 cm, 62−64 mg per larva) were 32.44 ± 0.51 and 24.30 ± 1.34 mg by 100 larvae per day, respectively. After 31 days, the molecular weight of residual PS in feces of *T. obscurus* decreased by 26.03%, a remarkably higher reduction than that found for *T. molitor* (11.67%). Co-feeding corn flour to *T. obscurus* and wheat bran to *T. molitor* increased total PS consumption by 11.6% and 15.2%, respectively. As shown for *T. molitor, T. obscurus* PS degradation was also dependent on gut microbial community composition. After PS feeding, a community shift was mainly associated with the distribution of *Spiroplasmataceae*, *Enterococcaceae*, and *Enterobacteriaceae* families. For instance, the ingestion of PS resulted in an higher relative abundance of *Enterobacteriaceae* in *T. obscurus,* changing from 2.09% to 29.21% and 20.52% on day 6 and day 11, respectively, and reaching a higher abundance more quickly in *T. obscurus* than in *T. molitor* (Peng et al., 2019). The results indicate that microbial-dependent PS biodegradability may be ubiquitous within the *Tenebrio* genus.

A new PS-degrading insect species, the darkling beetle *P. davidis* was reported by Woo et al. (2020). They showed a consumption of 34.27 ± 4.04 mg PS per larva during 14 days. FTIR analyses of feces from PS-fed larvae presented peaks corresponding to O-H and C=C, and XPS confirmed the chemical modifications on the PS film by the presence of a C-O peak. Moreover, GPC revealed a decrease of Mw and Mn, confirming PS biodegradation. The authors discussed a possible PS conversion into CO_2, but further studies are needed to confirm the formation of biomineralization products. *Serratia* sp. strain WSW was isolated from the gut of PS-fed larvae, presenting 99.65% *16S rRNA* sequence similarity to the

Serratia plymuthica strain 31upmr by, and was able to grow on and modify PS films. Gut microbiota of the control group [freshly caught specimens from the wild and was put into hibernation at 4°C until the extraction, (<2 weeks)] had 5 OTUs of the genus *Lactococcus, Aquabacterium, Buttiauxella, Raoultella,* and *Serratia*, while the PS-fed larvae had one more OTU of the genus *Enterococcus*. The genus *Serratia* and *Lactococcus* increased in 6-and 10-fold, respectively, when the larvae were PS-fed. According to Yin et al., 2020, the complex gut flora is more efficient at degrading PS than a single bacterium. Further studies are needed to define the cooperative role of multiple microorganisms in PS biodegradation and the full biochemical pathway of PS biodegradation.

Cucini et al. (2020) showed the PS consumption by *A. diaperinus* larvae. Using 17500 larvae, the weight of PS decreased from 20 g (time zero) to 16 g (day 30) (s = 2.83), totalizing 4 g (20%) of PS consumption during 30 days of incubation. Gut microbiota of PS-fed larvae was characterized using a NGS metagenomic approach, targeting both bacteria and fungi. The bacterial genera *Pseudomonas, Kocuria, Cronobacter, Pseudogracilibacillus* and *Virgibacillus* and the fungi genera *Aspergillus, Trichoderma* and *Penicillium* were generally found to be significantly more abundant in microbiota of larvae fed on PS.

Larvae of *T. castaneum* were observed chewing and eating extruded polystyrene foam. The authors observed that the PS intake had influence on larvae gut microbial constitution, in which the bacteria related to PS degradation (*Acinetobacter* sp. and *Enterococcus* sp.) were enriched by the progress of PS-feeding. *Acinetobacter* sp. AnTc-1 was isolated from the larval gut and presented 99.52% similarity by 16S rDNA, to *Acinetobacter vivianii* NIPH 2168(T). The isolate showed high adherence to hexadecane, and a series of assays (GPC, ^{1}H NMR, TGA and SEM) were performed in residual PS to verify bacterial PS-degrading activity. After 60 days of incubation with bacterial culture, PS showed a 12.14 ± 1.4% weight loss and a reduced thermal stability. The PS molecular weight distribution was shifted to shorter chains due the decrease of 13–25% on its molecular weights. The aliphatic portion of PS chain increased after degradation, and the appearance of new peaks could indicate changes in PS molecular structure, as well as a slow depolymerization process. After 30 days of incubation, SEM images showed many cavities and pits, the presence of biofilm and bacterial cells adhered on PS surface. The authors concluded that one or more bacterial species with PS-degrading ability may exist in the gut of *T. castaneum* larvae and they highlighted that further studies are needed to demonstrate the PS consumption rate by the larvae and to understand the PS biodegradation process in the larvae digestive tract (Wang et al., 2020).

4. Conclusions

In this review we presented the current proof-of-concept stage related to consumption of plastics by insect larvae, based on papers published online up to December 2020. In particular, we highlighted (i) the plastic feeding preference for larvae of different insect species, (ii) the current understanding about the ability of these animals to consume, biodegrade and even biomineralize plastic materials, (iii) the *state of the art* regarding hypothetical biodegradation routes, and (iv) the role/influence of the insect microbiota in plastic degradation processes. Additionally, we compiled information about the biology of the different insects: *A. grisella, G. mellonella, P.interpunctella, A. diaperinus, P. davidis, T. molitor, T. obscurus, T. castaneum* and *Z. atratus*.

G. mellonella and *T. molitor* are the most studied insects in the context iof plastic degradation (Figure 1). Interestingly, the larvae of coleopteran species are mostly associated with the consumption and biodegradation of PS, while the lepidopteran larvae are more related to consumption and biodegradation of PE. We still do not know which products are formed at the end of this process, but studies have indicated that *T. molitor* larvae are capable of generating HCl from PVC and CO_2 from PS, reaching a complete degradation up to biomineralization-end products. However, this ability was not yet studied for other insects and other plastic materials. Moreover, biodegradation of PS, PP and PVC by *T. molitor* has been shown to be dependent on intestinal microorganisms, while PE biodegradation by *T. molitor* seems to be less dependent on or independent of microbiota. PS biodegradation by *T. obscurus* has been shown also dependent on gut microbial community composition, indicating that microbial-dependent PS biodegradation may be ubiquitous within the *Tenebrio* genus. Additionally, PS, PP and PE biodegradation by *Z. atratus* was also reported to be microbial-dependent. For *G. mellonella,* it is likely that gut microbiota plays an important role in PE-digesting process, but it seems that microbial action might not be essential for this activity in some cases. More studies are necessary to answer this question.

Despite these successful and promising biodegradation experiments using insect larvae and their gut microbiome species, the understanding of the biological cleavage of non-hydrolyzable plastics is extremely limited. The mechanisms, the enzymes involved, and the relative importance of limiting factors imposed by animal physiology and physical characteristics of the polymer matrix independently of chemical structure remain largely unknown. Specially for *Tenebrio* and *Zophobas* larvae, many studies have also shown that larval microbiota is intrinsically linked to the plastic metabolism; however, it is difficult to prove which microbial genera or families are responsible for enhanced plastic degradation, because only a few plastic-degrading bacteria have been isolated. Moreover, digestion in whole organisms appears to be more effective than by isolated gut bacteria. The chewing action by larvae can lead to polymer fragmentation, which can highly contribute to the increase in plastic surface area for biodegradation. Then, physicochemical treatments related to the ingestion, reaction with larval gut contents, and the degradation by a gut microbial consortium, together with the activity of enzymes secreted by the larvae, are also possibly critical steps for the success of rapid PS degradation by these insects.

At present, it is still difficult to imagine that larvae could be utilized directly as a solution toward the global crisis of plastic pollution. Considering the gut microbiome of the larvae seems to be the key in biodegradation, the intestinal bacterial community of these larvae can be a promising source of plastic degrading microorganisms. More research needs to be done on replicating the gut process and conditions through bacterial cultures, to fully understand the synergic actions between larvae digestion and microbial metabolism, and to better understand the enzymatic systems involved in the biodegradation of plastics. Surely these findings could inspire a technological approach to solve the problems of plastic waste and microplastic pollution.

CRediT authorship contribution statement

Andressa F. Pivato and Gabriela M. Miranda: Conceptualization, Methodology, Investigation, Writing – Original Draft and Visualization.

Janira Prichula: Methodology, Investigation, Writing – Original Draft and Visualization. Jeane E. A. Lima and Rosane A. Ligabue: Writing – Review & Editing.

Adriana Seixas: Supervision, Project Administration, Writing – Review & Editing. Danielle S. Trentin: Supervision, Project Administration, Funding acquisition, Writing – Review & Editing.

Declaration of competing interest

The authors declare no conflict of interest.

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CAPÍTULO III

Nesse capítulo serão apresentadosos resultados experimentais obtidos durante a realização do presente estudo. Ele está redigido na forma de artigo científico em língua inglesa conforme as normas da revista “Environmental Science and Technology”.

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Polyethylene consumption by *Galleria mellonella* larvae: from ingestion to excretion

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Abstract

Polyethylene (PE) represents approximately 30% of the global demand for plastic products and physicochemical features make it an inert material; consequently, biodegradation is an extremely slow process. Fast PE biodegradation was reported by *Galleria mellonella* larvae, however, there is no consensus how this process occurs, and who is responsible for the first step in the PE-degrading pathway: animal or microbial enzymes. Thus, this study aims evaluate biological and chemical modifications into animal during PE ingestion. As results, one larva (130 mg) ingested 17.22 ± 1.29 mg of PE day^{-1} and evolve into the adult moth stage. PE-fed larval body sites were analyzed: LC-DAD-MS indicated metabolization products (hemolymph, cocoon, and feces), SEM-FEG-EDS and FTIR brings difference in morphology and chemical structure (cocoon and feces), however, also indicated PE excretion (feces). CO_2 quantification during respirometry assays do not change; therefore, it remains to be clarified if PE is not biomineralized. Bacterial gut community was analyzed by HTS of partial amplification of *16S rRNA* gene and revealed that PE-fed selects for a distinct microbiota when compared to laboratorial diet-fed group. This study contributes the understanding PE biodegradation by *G. mellonella* larvae, which may be biotechnologically explored for new solutions regarding plastic waste treatment.

Keywords: *Galleria mellonella*, polyethylene, biodegradation, enzymes, microbiota.

1. Introduction

Polyolefins are a long polymeric chain and can be easily molded into different shapes[1,2]. Allied to this, their durability and lightness make them widely used in several industrial areas[3]. As a result, in 2019, about 368 million tons of plastic were produced globally. Among the existing plastics, polyethylene (PE) stands out since this polymer represents 30% of the total demand for plastic products of petrochemical origin[4]. PE is a polyolefin widely used in food packaging, pharmaceutical and hospital products, toys, and cosmetic utilities, given some physical-chemical characteristics that make it inert and resistant to degradation[5]. However, the contrast between the remarkable durability and the short lifecycle time of PE products has led to the deposition of at least 100 million tons of plastics per year in the nature, resulting in terrestrial and marine pollution and damage to life[6].

Recycling and biodegradation processes are approaches that have been studied in order to reverse the high accumulation of this polymer in the environment and in landfills[7,4]. However, the plastic recycling process is considered economically unfeasible due to the need for previous cleaning steps, resulting in higher costs when compared to the final product and excessive consumption of water. Moreover, polyolefins biodegradation in the environment is still an extremely slow process[7,8]. Chamas et al. (2020) estimated that an HDPE bottle material after discarded remains around 116 to 500 years in a marine and terrestrial environment, respectively[9].

Environmental polymer biodegradation process is dependent on microbial activity and comprises 4 steps: (i) firstly, the extracellular enzymes produced by environmental microorganisms are secreted and led to the biodeterioration of the polymer surface; (ii) then the polymer is depolymerized into small cleavage fragments (oligomers with molecular weight around 500 g/mol); after, (iii) the microorganisms assimilate these fragments and metabolize them into monomers by intracellular enzymes, using them as an energy source; and finally, (iv) the biomass is biomineralized into inorganic substances, such as carbon dioxide or methane (depending on the availability of oxygen) and water[10,11].

It is important to mention that usually the polymer is not directly colonized and attacked by microorganisms. Oxidation processes caused by exposition to UV light and/or oxidizing agents can be performed in the polymer surface, as well as occur naturally in the environment, in order to create functional groups, which are required to facilitate the microbial adhesion to the substrate, consequently improving the polymer hydrophilicity[12].

Plastic consumption by larvae insects was described in a pioneer study in 2010, which was conducted with *Zophobas morio* larvae and polystyrene (PS)[13]. In 2017, the fast PE biodegradation was reported by the larvae of the insect *Galleria mellonella*, at a rate of 0.23 mg cm^{-2} h^{-1}, which is a higher rate than to those rates demonstrated by microorganisms. These results attracted attention for improvement and optimization of plastic biodegradation research. *G. mellonella* is a moth of the family *Pyralidae* family and *Lepidopteran* order and it is found throughout the world[14]. It is a holometabolic insect and presents four life cycle stages: egg, larva, pupa, and adult moth[15].

During all larval stages the animal spin silk, a proteinaceous polymer secreted by the specialized exocrine silk glands, and at the last instar, the larvae spin a cocoon of silk for itself and enter the pupal stage[16]. It is commonly known as the greater wax moth, since it has hives as a natural niche, where larvae feed on beeswax which is composed mainly by cerin and miricin, substances rich in C-C and C-H bonds[17], also a feature of PE chemical structure.

The wax worm ecology may indicate an innate ability of *G. mellonella* larvae to break down a chemical bound not generally susceptible to biodegradation (C-C), such as those found in PE. In this sense, thirteen studies have been reported about the plastic biodegradation by *G. mellonella*. Based in this relates, it is likely that microbiota gut plays an important role in PE-digesting process by *G. mellonella*, however, there are no consensus about this role. Transcriptomic studies regarding who is responsible for this first step in the PE-degrading process: animal or microbiota enzymes. Works in which the larvae microbiota was studied, it is verified that there is no consensus about species and genera of microorganisms that can help in PE biodegradation, neither their enzymes nor their steps of action on degradation cascade[18,19].

Importantly, the action of a possible microbiota from *G. mellonella* salivary glands remains to be evaluated, once Peydaei et al., (2020) showed changes in protein expression in this larvae body sites/organ when the animal was PE-fed[20]. Until now, there is study in the literature that has investigated whether biomineralization products are generated during PE-degrading process by *G. mellonella* larvae. In this sense, it is necessary a study that compiles information about the influences of the larva's body sites/organ on the PE biodegradation, as well as on the verification of the biomineralization of plastic to its final metabolite (CO_2) and the role of the larva's intestinal microbiota in this process. Therefore, this study aims to contribute to the understanding of PE biodegradation by *G. mellonella* larvae, evaluating the biological, physical and chemical modifications in the animal after PE ingestion.

2. Materials and Methods

2.1 Materials

2.1.1 *Galleria mellonella*

G. mellonella larvae were grown in the laboratory and the larvae kept at 28ºC throughout its life cycle. From the egg stage until reaching the ideal size for the experiment (weight avarage130 mg, length 2 cm), the larvae were fed a laboratory diet for about 2 months. This diet consists in wheat flour (14.7%), coarse wheat bran (14.7%), wheat germ (14.7%), powdered milk (29.4%), liquid honey (8.83%), glycerol (8.83%) and brown sugar (8.83%), until be used in experiments.

2.1.2 Polyethylene (PE)

Low-density polyethylene (LDPE, code PB 608 from Braskem Company) films with 0.06 mm thickness were obtained in a hydraulic press (Marconi Company, load capacity = 15 ton) at 140 °C and 4 tons for 2 minutes, with avarege molar mass 90.000 g/mol. Films dimensions varied according to the assays.

2.2 Methods

2.2.1 Determination of PE consumption rate according to larval stage period.

To determine the *G. mellonella* larval stage, in which the highest PE consumption occurs, groups of five larvae were divided based on body weight (60 mg, 90 mg, 130 mg, 180 mg, 215 mg and 250 mg). In this assay, a single larva was in contact with a 2.5 cm² PE film. The PE and larvae masses were measured at the beginning (0 h) and after 6, 10 and 24 h of exposure.

2.2.2 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR analyses were performed to evaluate the structural characteristics changes on the surface (up to 1-2 µm deep) of larvae body sites (cocoon and feces) after PE consumption by *G. mellonella* larvae. For this was used samples from two larvae groups were used: (i) PE-fed for 7 days and (ii) that had been fed with laboratorial diet, as control. For this assay, it was used larvae with an average 130 mg weight, which showed best PE consumption results. Perkin Elmer Instruments Spectrum One FT-IR Spectrometer (USA) equipment was used and the spectrum acquisition was performed with the UATR sample accessory (universal total attenuated reflectance accessory) in the wavenumber range from 4000 to 500 cm^{-1} available at the Laboratory of Spectroscopy at PUCRS.

2.2.3 Scanning Electron Microscopy with Field Emission coupled with Dispersive energy spectroscopy (SEM-FEG-EDS)

SEM-FEG-EDS analyses were performed to examine samples micro and nanoscale characteristics and detect elemental chemical composition on the surface (up to 1-2 µm deep) of samples larvae sites body (feces and cocoon). For this, it was used two larvae groups: (i) PE-fed for 7 days and (ii) that had been fed with laboratorial diet, as control. For this test, it was used larvae with an average 130 mg weight, which showed best PE consumption results. The samples were coated with gold and the SEM-FEG images were obtained in FEI Inspect F50 equipment with a voltage of 20 kV in the mode of secondary electrons (SE). This assay was performed at the Central Laboratory of Microscopy and Microanalysis (LabCEMM) of PUCRS.

2.2.4 X-ray photoelectron Spectroscopy (XPS)

XPS analysis is a sensitive surface analysis method that analyzes the top 10 nm of the sample with an X-ray of Mgα of 1253 eV. For this assay, it was evaluating the cocoon from two larvae groups: (i) PE-fed for 7 days and (ii) that had been fed with laboratorial diet, as control. For this test, it was used larvae with an average 130 mg weight, which showed best PE consumption results.

2.2.5 Liquid Chromatography coupled to Diode Array Detection and Mass Spectrometry (LC-DAD-MS)

The LC-DAD-MS analyses were performed in an UFLC Shimadzu LC-20AD, which was coupled to diode array detector and a mass spectrometer MicroTOF-Q III (Bruker Daltonics). For the chromatographic analyses, a Kinetex C18 column (2.6 µm, 150 mm x 2.1 mm, Phenomenex) was applied. The mobile phase was composed by ultrapure water (A) and acetonitrile (B) added formic acid 0.1% and the following gradient elution profile was applied: 0 to 2 min - 3% of B, 2 to 25 min – 3-25% of B, 25 to 40 min – 80% of B. The flow rate was 0.3 µL/min and the chromatographic column was maintained at 50 ºC during the analyses. The samples larvae sites body (hemolymph, feces and cocoon) from two groups [(i) PE-fed for 7 days and (ii) that had been fed with laboratorial diet - blank control] were analyzed by LC-DAD-MS. For hemolymphs collection 10 animals were needed for each sample. For this, with the scalpel, a cut was made close to the last proleg, thus it was possible to bleed the larvae. Thus, with a 20 µl pipette, the contents of each animal were collected. This procedure occurred until reaching the volume of 200 µl hemolymph for each sample and after this was lyophilized. For the cocoon, collection was used five animals for each sample and for 100 mg of feces, collection was necessary next 100 animals for each sample. These animals were kept in previously sterile, by autoclave (121 ºC and 1,1 atm during 20 min), glass jars. This analysis occurs in triplicate. The samples were extracted with methanol and ultrapure water (9:1, v/v), cleaned by hexane liquid-liquid extraction, filtered by PTFE syringe filters (Millex 0.22 mm x 13 mm, Millipore), and injected on chromatographic system.

2.2.6 Aerobic biodegradation determination assay

To evaluate the possibility of PE biomineralization into CO_2 by *G. mellonella* larvae, it was performed the aerobic biodegradation assay using desiccators as bioreactors. Larvae, previously fed with laboratorial diet until they achieved an average weight of 130 mg, were separated in two groups: (i) 40 larvae in presence of 2500 mg of PE for feeding and (ii) 40 larvae without food (used as control). The groups were kept in the bioreactor during 8 days, at 28 °C. The CO_2 produced from each biometer flask was collected in 0.5 M KOH solution and titrated with a 0.25 M HCl solution at 0, 48, 144 and 192 h. This method and the calculation of trapped CO_2 were based on the standards ASTM D5988-18 and ISO 17556:2012[21]. The assay was performed in duplicate at distinct moments: two bioreactors with the larvae group with PE presence, two bioreactors with the larvae group starving (control 1) and one bioreactor control 2, where there are only PE film.

2.2.7 Bacterial community diversity determined by high-throughput *16S rRNA* gene sequencing

Groups of 10 larvae (average of 130 mg) were fed with laboratorial diet or PE for 7 days and these animals were kept in previously sterile, by autoclave (121 ºC and 1,1 atm during 20 min), glass jars. For gut collection, the larvae were submerged in 70% alcohol for 1 min and with the aid of sterile scalpels, the intestinal contents were removed. A pool of 10 intestines was transferred to an eppendorf tube containing 200 µL of sterile PBS solution pH 7.0, and DNA extraction was performed using the E.Z.N.A. Stool DNA Kit according to the manufacturer's standard protocol. The DNA quality was verified using the NanoDrop ND-1000 (Thermo Fisher Scientific,Waltham, MA, USA) and DNA concentration was determined using the Qubit double-stranded DNA (dsDNA) high-sensitivity (HS) assay kit (Life Technologies). The partial amplification of *16S rRNA* gene (V4 region) was performed using primers 515F and 806R, previously identified as suitable for bacteria (17). Amplification was performed in a 25 µL mixture, consisting of 12.5 ng of genomic DNA, 1.5mM $MgCl_2$, 0.2 µM of each primer, 200 µM of each dNTP, 2 U Platinum Taq DNA Polymerase Platinum (Invitrogen™), and 1X reaction buffer. Amplification was carried out in a BioRad MyCycler Thermocycler (BioRad, USA) according to the following program: initial denaturation at 94°C for 3 min, followed by 30 cycles of 94ºC for 30 s, 55ºC for 30 s, 72ºC for 30 s a final cycle at 72°C for 5 min. The amplicons were purified using Agencourt AMPure XP beads following manufacturer instructions. The library was constructed using the Nextera XT Index Kit (Illumina) sample preparation kit and sequencing was conducted on an Illumina MiSeq platform (Illumina Inc., San Diego, California, USA) using the MiSeq Reagent Nano Kit, v2 - 500 cycles.

Bioinformatics analysis of *16S rRNA* amplicons were performed as described[22] in Kozich et al., 2013 using Mothur v1.43[23]. Briefly, raw sequence data were combined into contigs, removing sequences with ambiguous bases and contigs longer than 293 bp. Duplicated sequences were unified to reduce computational resources, and the remaining sequences were aligned to the SILVA database version 132. Chimeric sequences were searched using VSEARCH[24] and removed. Sequences were clustered using the Bayesian classifier, using an 80% confidence bootstrap score[25]. Undesirable sequences from *Archaea*, chloroplasts and mitochondria were removed. Later, sequences were split into groups and assigned to Operational Taxonomic Units (OTUs) at a 3% dissimilarity level. Subsequent analyses were performed in RStudio using the phyloseq package[26].

Stacked abundance barplots were done filtering taxa with less than 1% abundance. Alpha diversity metrics (Observed, Shannon, Simpson, invSimpson, Chao1, ACE and Fisher), were estimated using the *plot_richness* function in phyloseq. Beta diversity metrics were estimated using the weighted UniFrac distances and performing a Principal Coordinate Analysis (PcoA) implemented in the phyloseq package.

2.2.8 Statistical analysis

One-way analysis of variance (One-way ANOVA) was used to analyze PE consumption and body weight of larvae in accordance to different larval stages. p-value <0.5 were considered significant (Graphpad Prism 6.0).

3. Results and Discussion

We evaluated PE consumption by *G. mellonella* larvae grouped according to body weight as indicative of distinct larval stage. It was observed a high variation in PE consumption, ranging from 7.14 ± 1.89 up to 17.22 ± 1.29 mg/day/larva. Thus, highest PE consumption by a single *G. mellonella* larva (17.22 ± 1.29 mg per day) was obtained for animals with an average weight of 133.32 ± 3.45 mg (Figure 1a).

Previous studies have reported distinct rates of PE consumption by *G. mellonella* larvae; however, they do not mention the highet consumption according to larval stage evaluated. In this sense, Kundungal et al., (2019) showed the 15 specimes *G. mellonella* (fourth instar larvae) capacity to consumption until 2.5 g of PE with a pre-treatmente with UV, and 0.83 g of PE without this pre-treatment, in 7 days[27]. LeMoine et al., (2020) performed a PE comsumption assay by *G. mellonella* fifth instar and showed a 250 μg/larva in 24 h[19].

The first report described that a group of 100 larvae consume 92 mg of PE during 12 h, indicanting that a single larva ingests 0.92 mg of PE during 12 h and provides the estimate rate of about 1.84 mg of PE per larva per day[14]. Another work reported a rate of consumption of 0.54 mg of PE per day for a single *G. mellonella* larva[28]. In our study, we observed similar values for larvae weighing 250 mg (7.14 ± 1.89 mg of PE/per larva/per day), but higher amount of PE was consumed by larvae weighting 130 mg, indicating that the larval stage strongly influences PE consumption rate. In addition, our results indicate that LDPE-feed only for 7 days bring an 11.96 ± 4.5 mg weight loss per larva, when this animal had a 130 mg initial weight (as mentioned, in this stage occurs the highest PE consumption). This weight loss increased to 26.68 ± 2.90 mg when the initial weight larvae was 250 mg. This fact shows us that PE is poor nutritive source and the larvae do not increase its weight with this alimentation, indicating that a feed supplementary is necessary at firsts larvae stage.

Another point that may impact in the PE consumption rate by the larvae are the animal rearing conditions, which can lead to changes in the animal physiology and microbiota. In this sense, a study conducted with polystyrene (PS) and larvae of the insect *Tenebrio molitor* showed that larvae from 22 different geographic regions are capable of consuming the plastic. However, the consumption rate varied according to region and larval weight (from 20.71 ± 0.51 mg of PS per day to 8.46 ± 0.14 mg of PS per day by 100 larvae, with initial weight of 73.98 ± 0.81 mg and 46.34 ± 0.89 mg, respectively)[29].

The capability of *G. mellonella* larvae to consume a high amount of PE is highlighted when compared with other larvae insects belonging to the families of beetles (Coleoptera: Tenebrionidae) and moths (Lepidoptera: Pyralidae). For the coleopterans, a consumption of 0.59 ± 0.02 mg of PE per day using a single *Zophobas atratus* larva[30] and of about 3 mg per day by 100 *T. molitor* larvae (which gives 0.03 mg per day per larva) were reported[31]. While for the lepidopteran *Achroria grisella*, known as the lesser wax moth, a consumption of 1.8 mg per day by single larva was described[32]. These observations are in line with the natural insect niche, indicating an innate ability of these larvae that are fed with beeswax to deal with PE substrates.

3.2 The influence of PE feeding on *G. mellonella* life cycle

Although *G. mellonella* has shown to ingest PE in all larval stages, and 130 mg weighting animals presented the highest rate of PE consumption, it was observed a growing reduction in the larvae body weight according to higher initial weight (Figure 1a). However, this animal is capable be turn into pupae when compared to the control group (laboratorial diet-fed larvae). Importantly, even under a nutritionally poor diet, 130 mg-larvae fed with PE was able to perform the entire metamorphosis process to evolve into the adult moth stage (Figure 1b) and to reproduce. Adults did not differ physically (in size and color) when compared to control group (laboratorial diet-fed larvae) (Figure 1b). It indicates that *G. mellonella* larvae metabolized PE and used it as an energy source. However, it will be important to analyze the fertility control of these adult animals, which will be performed by counting the eggs of adult moths that, as larvae, were fed with: (i) a laboratory diet and (ii) a diet with PE, for 7 days.

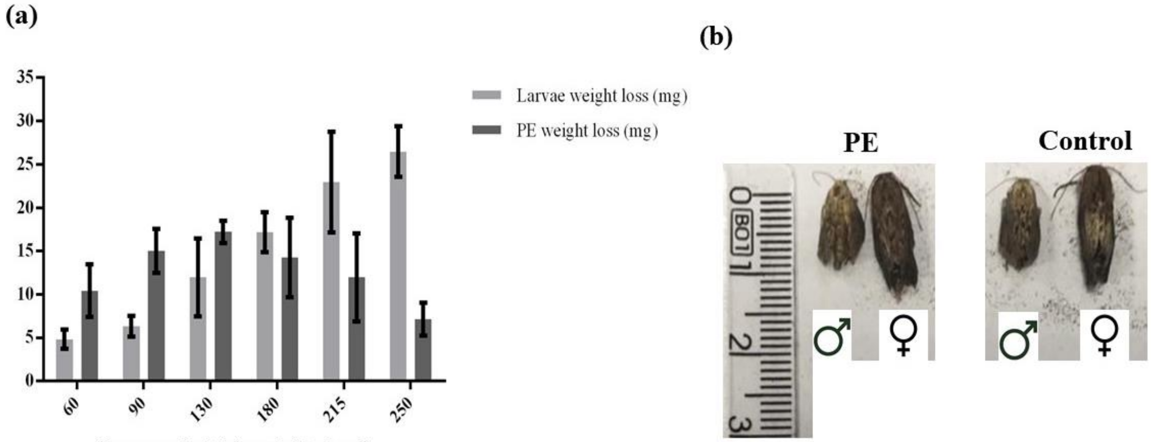


Figure 1. PE consumption by *G. mellonella* larvae. (a) PE consumption (mg) according to larval weight (mg). Light gray bars indicate the weight loss of a single *G. mellonella* larva during 24 h PE feeding. Dark gray bars indicate PE film weight loss after 24 h exposed for consumption by a single *G. mellonella* larva. The x-axis indicates the weight average of larvae in the beginning of assays (n=5). The averages of PE weight loss are significant (p=0.0012) by ANOVA test. (b) Adult moths of *G. mellonella* insect. At the left-side, moths evolved from PE-fed larvae during 14 days after reaching an average weight of 130 mg. At the right-side, moths evolved from laboratorial diet-fed larvae (control). In each side, the smallest and the largest indicate the male and the female, respectively.

3.3 PE metabolization by *G. mellonella* larvae: physics-chemical analyses in different body sites

The base peak chromatograms (BPC) from hemolymph obtained from PE-fed larvae and laboratorial diet-fed larvae presented little difference. Three compounds were found exclusively in the hemolymph of PE-fed larvae, which was not present in control (Figure 2, Table 1).

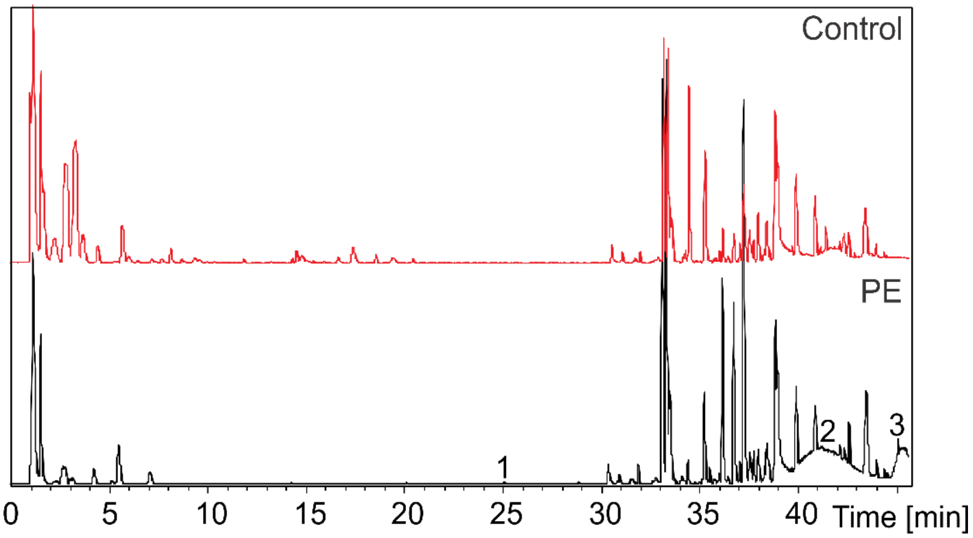


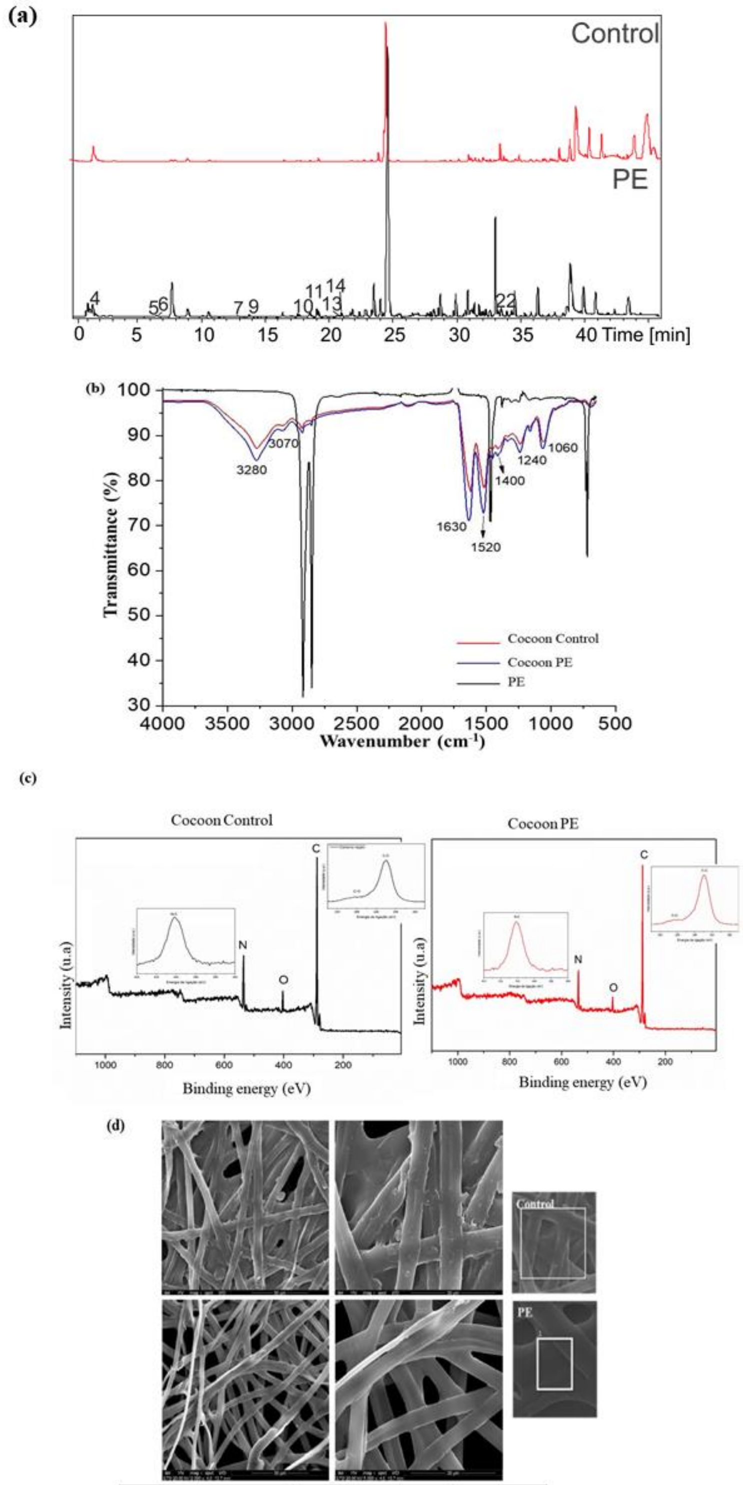
Figure 2. Base peak chromatograms (BPC) obtained in positive ion mode and the exclusive peaks annotated from hemolymph. On the top the chromatogram for laboratorial diet-fed larvae (control); below, the chromatogram for PE-fed larvae during 7 days after reaching an average weight of 130 mg. The spectral data of peaks 1-3 are described on Table 1.

This finding indicates that after ingestion, the polymer is biofragmented and its metabolites are absorbed, appearing on insect circulation. Similarly, as occur in blood circulation, it is expected that the residence time of substances on hemolymph will be influenced by kinetic of biodistribution, organ-specific accumulation and clearance process.

LC-DAD-MS chromatograms of larvae fed with PE and laboratorial diet (control) are very distinct, accounting for the presence of exclusive nineteen peaks (up to *m/z* 474,

Table 1) of cocoon produced by PE-fed larvae (Figure 3a). These results demonstrated the chemical changes (including nitrogen compounds) in cocoons when larvae are fed with PE. In this sense, it is possible to investigate the presence bacteria nitrogen-assimilating PE biodegrade. This is because it is known that nitric oxide (NO) is synthesized by microbes and diffuses through the outer membrane to its interface with PE and in the extracellular environment, NO is oxidized by O_2 to reactive nitrogen dioxide, which then interacts with the free radical from the PE chain, resulting in the formation of nitro (C-O-N-O)[33]. Interestingly Peixoto et al., (2016) reported the presence of a nitro group (NO) formation in PE film samples treated with bacterial degrading strains of this polymer (*Comamonas, Delftia* and *Stenotrophomonas*)[34].

FTIR spectrum of control group cocoon (Figure 3b) presented bands consistent with the composition of the cocoon, such as seroin and fibroin proteins, in both samples, and that PE polymer is not excreted in the cocoon, which were in accordance with elementary composition provided by XPS (Figure 3c). SEM-FEG images of control group cocoon showed more compact structure than those from PE-fed larvae (Figure 3d). Moreover, it was also observed visually when larvae cocoons of both groups were collected to perform all these analyses. This difference in cocoon morphology is expected, once the PE feeding is nutritionally poorer related to laboratorial diet and PE-fed larvae present an extended life cycle. EDS analysis also indicated that chemical elemental composition (%C, %N, and %O) on both samples' surfaces did not change significantly (Figure 3d). These results highlighted the importance to determine the chemical composition by LC-DAD-MS analyses, since the exclusive metabolites from cocoon of PE-fed larvae were compounds with low ion intensities. The constituents with higher ion intensities (including the nitrogen compounds) were similar between cocoons of PE- and laboratorial diet-fed larvae. Therefore, these findings allow us to propose that PE metabolites appearing on insect blood circulation are transported to silk glands and used in silk cocoon production.



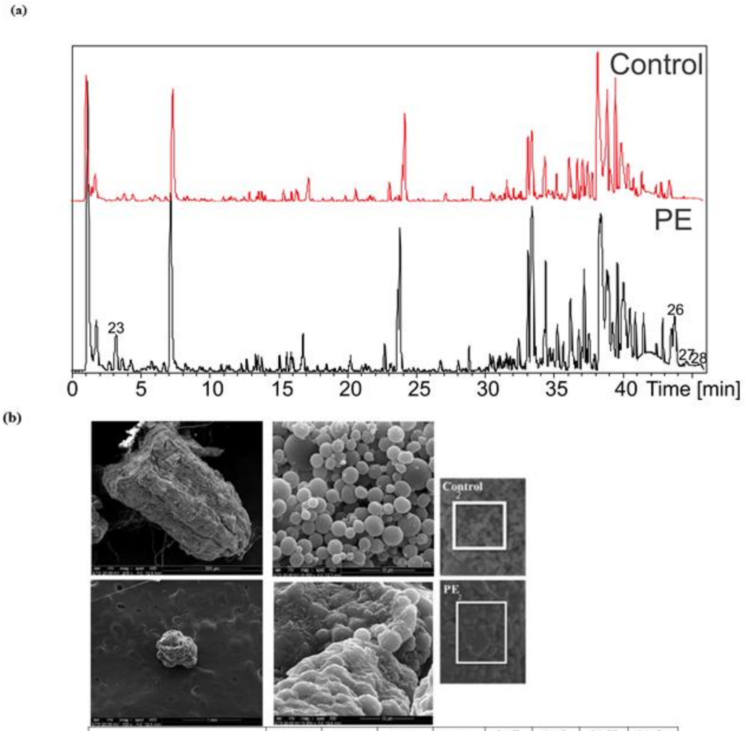
	% C	% N	% O	% K	% Ca
Cocoon Control	53,77	20,32	25,4	0,42	0,21
Cocoon PE	53,69	19,705	26,295	0,345	-

Figure 3. Physico-chemical analyses of cocoon produced by *G. mellonella*. Cocoon samples were collected from (i) laboratorial diet-fed larvae (control) (ii) PE-fed larvae during 7 days after reaching an average weight of 130 mg. (a) Base peak chromatograms

(BPC) obtained in positive ion mode and their annotated peaks (description of spectral data in Table 1). (b) FTIR spectra: red line corresponds to control group, blue line to PE-fed larvae and black line to PE polymer (material). (c) XPS spectra: black line corresponds to control group, and red line corresponds to PE-fed larvae. (d) SEM-FEG-EDS images and elemental composition.

When analyzing the feces from PE and laboratorial diet fed larvae (control) by LC-DAD-MS, it was observed differences in some peaks, indicating six exclusive compounds for PE-fed larvae. These components presented nitrogen or oxidized constituents and with up 42 carbons (Figure 4a). SEM-FEG images of control group feces presented structures with spherical morphology, suggesting the presence of coccus and/or yeast-rich microbiota. However, feces from PE-fed larvae displayed a layer covering these structures (Figure 4b), showing some diference when compared with the group control (laboratorial diet), and suggesting the presence of PE in these excretion feces. This change may be due to the total release of PE (without any type of physical-chemical modification) or due to the release of biofragmentation products of this polymer. EDS analysis showed an increasing in carbon (4.3%) and oxygen (2.6%) contents and decreasing in nitrogen (11.2%) content in feces of PE-fed larvae, indicating an effect of PE consumption in chemical composition (Figure 4b).

FTIR spectra of feces obtained from PE-fed larvae presented several peaks related to PE polymer (Figure 4c), corroborating with EDS results. Furthermore, when colored plastic bag fragments replaced colorless PE films, colored plastic wrapped in the feces and gut was observed under the optical microscope (Figure 4d). Based on this set of findings, we can conclude that in addition to the presence of PE biofragmentation products in larvae body sites, it also occurs the elimination of PE polymer in the larvae feces.



	% C	% N	% O	% Mg	% P	% S	% K	% Ca
Feces Control	32,87	28,72	31,67	0,39	1,78	-	3,78	0,79
Feces PE	37,15	17,53	34,31	1,90	5,03	-	4,08	-

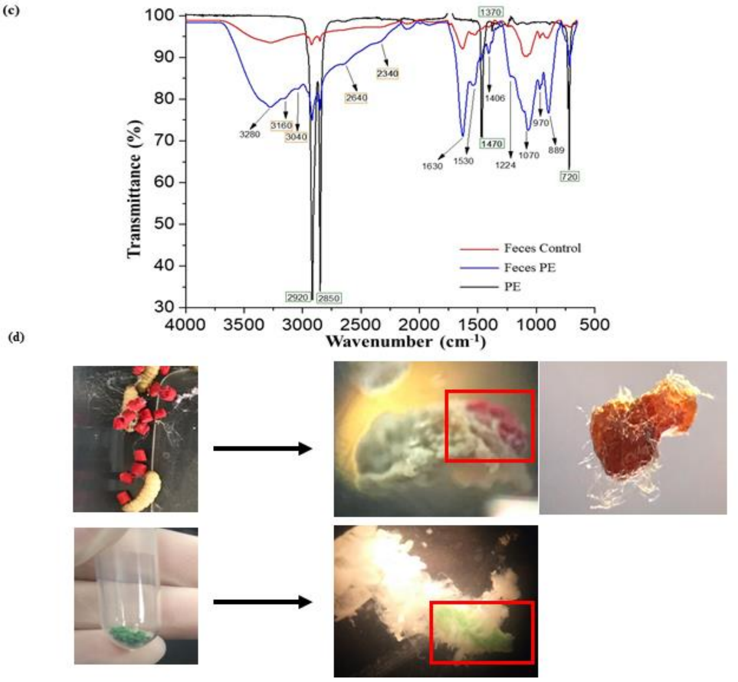


Figure 4. Physico-chemical analyses of feces produced by *G. mellonella* larvae. Feces samples were collected from (i) laboratorial diet-fed larvae (control) (ii) PE-fed larvae during 7 days after reaching an average weight of 130 mg. (a) Base peak chromatograms (BPC) obtained in positive ion mode and their annotated peaks (description of spectral data in Table 1). (b) SEM-FEG-EDS images and elemental composition. (c) FTIR spectra: red line corresponds to control group, blue line to PE-fed larvae and black line to PE polymer

(material). (d) Optical microscopy of the feces and the gut of larvae fed with colored PE plastic.

Table 1. Metabolites observed from samples (hemolymph, cocoon, and feces) of larvae feed with PE by LC-DAD-MS analyses.

Peak	**RT (min)**	**UV (nm)**	**MF**	**Positive (*m/z*)**		**Negative (*m/z*)**
				MS [M+H]$^+$	**MS/MS**	**MS [M-H]$^-$**
Hemolymph						
1	25.1	-	$C_{12}H_8O_7$	265.0335	-	-
2	41.5	-	$C_{40}H_{71}N_9O_5$	758.5649	-	-
3	45.1	-	$C_{42}H_{73}N_9O_5$	784.5839	184	-
Cocoon						
4	1.8	268, 360	$C_{10}H_9NO_4$	208.0592	-	-
5	6.5	-	$C_{17}H_{13}N_5O_5$	368.0965	-	-
6	6.6	246, 344	$C_{10}H_7NO_4$	206.0438	-	204.0313
7	12.8	-	$C_{21}H_{32}N_6O_6$	465.2442	-	-
8	17.4	-	$C_{16}H_{24}O_9$	-	-	359.1355
9	13.7	-	$C_{17}H_{24}N_6O_4$	377.1915	-	-
10	17.6	-	$C_{14}H_{24}N_8O_6$	401.1890	-	-
11	19.1	-	$C_{21}H_{32}N_6O_6$	435.2676	-	-
12	19.8	-	$C_{23}H_{32}O_6$	-	-	403.2096
13	20.6	-	$C_{17}H_{36}N_2O_6$	449.2837	-	-
14	21.0	-	$C_{17}H_{33}NO_6$	348.2351	-	-
15	23.0	-	$C_{10}H_{18}O_4$	-	-	201.1143
16	23.3	-	$C_{16}H_{32}O_6$	-	-	319.2132
17	27.8	-	$C_{26}H_{38}O_6$	-	-	445.2562
18	28.0	-	$C_{27}H_{36}O_6$	-	-	455.2414
19	30.2	-	$C_{21}H_{40}N_2O_7$	-	-	431.2774
20	33.5	-	$C_{25}H_{37}N_3O_6$	-	-	474.2624
21	33.6	-	$C_{19}H_{32}N_4O$	-	-	331.2488
22	34.3	-	$C_{17}H_{37}NO_2$	288.2904	244	-
Feces						
23	3.2	282	$C_{17}H_{26}N_2O_8$	387.1733	-	-

24	35.2	-	$C_{24}H_{34}O_2$	-	-	353.2473
25	35.3	-	$C_{16}H_{30}O_3$	-	-	269.2137
26	43.8	-	$C_{39}H_{63}N_9O_5$	738.5051	-	-
27	44.6	-	$C_{37}H_{72}O_9$	661.5252	-	-
28	45.7	-	$C_{42}H_{73}N_9O_5$	784.5837	-	-

MF: molecular formula; RT: retention time

3.4 PE biomineralization by *G. mellonella*

The formation of CO_2 is recognized as a good indicator of polymer mineralization. To evaluate whether PE is biomineralized into CO_2 by *G. mellonella* larvae, we performed aerobic biodegradation assay based on the analytical quantification of CO_2 produced by PE-fed larvae and control groups during 8 days. At the final of the experiment, the control and PE-fed groups produced 124.8 ± 2.33 and 120.2 ± 2.72 mg of CO_2, respectively (Figure 5). Our results indicate that PE consumption by the larvae did not affect the CO_2 production. However, it remains to be clarified if PE is not biomineralized into CO_2 as a final metabolite or if as soon as CO_2 is produced, it is eliminated by insect enzymes, such as carbonic anhydrase[35]. Considering the physiological role of carbonic anhydrases in the pH and ions regulation for the survival of insects and that *G. mellonella* is sensitive to atmospheres enriched with CO_2 [36], the last suggestion is feasible. Although *T. molitor* larvae has been showed to completely convert polystyrene into CO_2 during biodegradation process[37], this is the first time biomineralization is studied in *G. mellonella*.

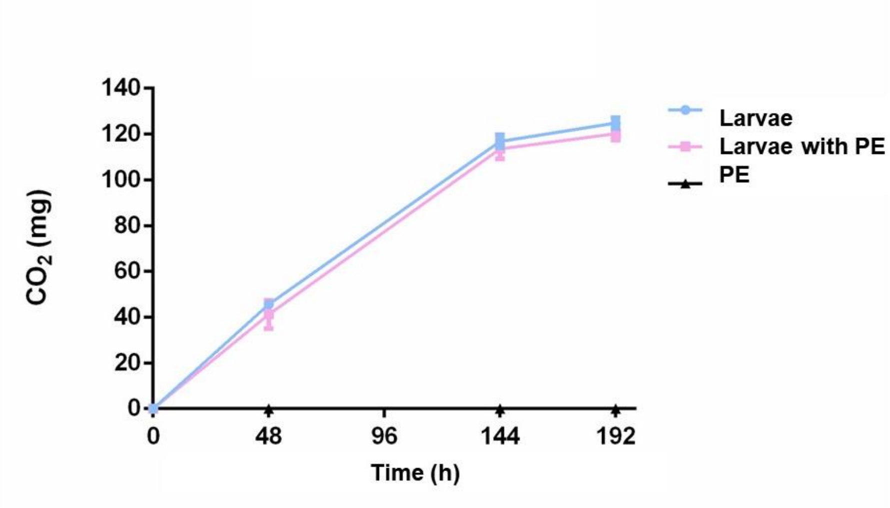


Figure 5. PE biomineralization analyses by *G. mellonella* larvae: cumulative CO_2 measurement (mg) in relation to time (h), where the pink line represents the mean for the bioreactor with larvae in presence of PE for feeding, the blue line represents the mean for the bioreactor with larvae without food (used as control 1) and the black line the bioreactor only PE (control 2) in the absence of larvae.

3.5 PE-feeding *G. mellonella* larvae microbiome

We identified the intestinal bacterial community in PE and laboratorial diet-fed larvae using high-throughput sequencing (HTS) of partial amplification of *16S rRNA* gene (V4 region) (Figure 6). A total of 438,505 raw reads were obtained, with 390,761 reads remaining after removing undesired sequences, resulting in 15 phyla, 21 classes, 42 orders, and 85 families. A total of 1213 OTUs were observed and most (99.8%) *16S rRNA* gene sequences were classified at the order level. For a few OTUs it was possible to assign the taxonomy at a genera level (9,6%). Three phyla presented relative abundances greater than 1%, which accounted up to 99.9% of the bacterial microbial community and were present in all evaluated samples. To PE-fed larvae gut samples the results was: *Firmicutes* dominated, with the highest relative abundance (54.5%), followed by *Proteobacteria* (34.75%), and *Actinobacteria* (10.16%). These sequences belonged mainly to four orders: *Lactobacillales*

(42.18%), *Enterobacteriales* (37.75%), *Bacillales* (12.4%) and *Corynebacteriales* (10.16%) (Table 2).

Lactobacillus are associated with normal microbiota in insects, it may explain the decreased in larvae PE-fed. A study showed the microbiota dominated by one *Enterococcus* taxon, using *G. mellonella* skin, feces, a fat body, and a hemolymph as sample to perform high throughput *16S rRNA* gene sequencing[38]. Other study, conducted by Montazer et al., (2020) verified three bacterial species isolate from body extracts of *G. mellonella* larvae (*Lysinibacillus fusiformis*, *Bacillus aryabhattai*, and *Microbacterium oxydans*) and evaluate the ability to utilize the LDPE as a sole carbon source[39].

Thus, the authors observed changes at PE surfaces and loss weight of this plastic when this material was incubated with these microorganisms. Cassone and coworkers (2020) identified the genus *Acinetobacter* (isolate gut *G. mellonella* larvae) and described its involving in the PE biodegradation. It was performed an cultive, where the plastic was only carbon source during 1 year, indicating that after short-term exposure, the intestinal microbiome of *G. mellonella* is intricately associated with PE biodegradation *in vivo*[40]. Lou and collaborators (2020) showed the influence at some gut microbiota of *G. mellonella* larvae on PE and PS biodegradation. The authors describe that *Bacillus cereus* and *Serratia marcescens* species were significantly associated to the PS and PE diets; in which *S. marcescens* showed high superiority in the PE-fed gut microbiome[41]. *Pseudomonas*, *Geobacillus* and *Propionibacterium* were also significantly related to the PS-fed group when bran was the control diet. Still, others studies show that microbiota gut of *G. mellonella* larvae may be responsible for PE degradation, which is the case of *Aspergillus flavus* as a PE degrading fungus[42] and *Enterobacter* spp. D1[43]. Thereby, our results indicated PE feed selects a microbiota that is potentially plastic degrading, which may be related to the PE degradation process using *G. mellonella* larvae.

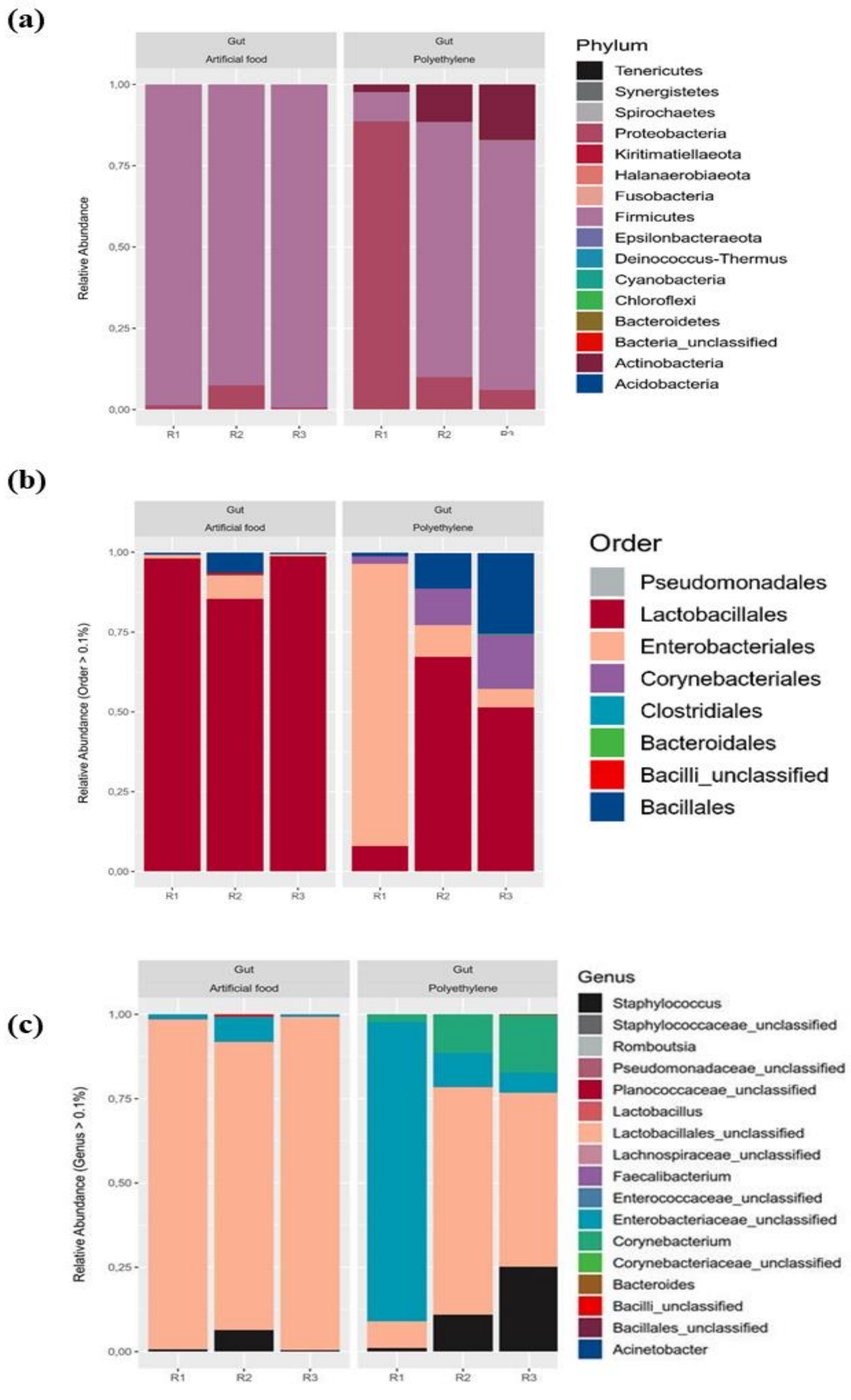


Figure 6. Diversity microbiome *G. mellonella* larvae by sequencing *16 rRNA* data. Relative Abundance according (a) Phylum. (b) Order. (c) Genus. All samples using samples from *G. mellonella* (i) larvae fed with laboratorial diet or artificial food (control) all life and (ii) fed with PE for 7 days.

Table 2: Diversity microbiome *G. mellonella* larvae by sequencing *16 rRNA* datas showing: Out, class, order, family, genus and values to the relative abundance (%) and standard deviation (%) by the samples from gut *G. mellonella* (i) larvae fed with laboratorial diet (control) all life and (ii) fed with PE for 7 days.

						Control		**PE**	
Otu	**Phylum**	**Class**	**Order**	**Family**	**Genus**	**Abundance (%)**	**SD (%)**	**Abundance (%)**	**SD (%)**
Otu0002	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	*Enterobacteriaceae*_unclassified	3,08%	3,05%	34,75%	38,06%
Otu0026	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Enterococcaceae*	*Enterococcaceae*_unclassified	0,18%	0,06%	0,13%	0,01%
Otu0001	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Lactobacillales*_unclassified	*Lactobacillales*_unclassified	93,83%	6,15%	42,05%	25,02%
Otu0003	*Firmicutes*	*Bacilli*	*Bacillales*	*Staphylococcaceae*	*Staphylococcus*	2,46%	2,74%	12,26%	9,77%
Otu0005	*Actinobacteria*	*Actinobacteria*	*Corynebacteriales*	*Corynebacteriaceae*	*Corynebacterium*	0,00%	0,00%	10,16%	5,98%
Otu0010	*Firmicutes*	*Bacilli*	*Bacillales*	*Bacillales*_unclassified	*Bacillales*_unclassified	0,00%	0,00%	0,14%	0,00%
Otu0009	*Firmicutes*	*Bacilli*	*Bacilli*_unclassified	*Bacilli*_unclassified	*Bacilli*_unclassified	0,67%	0,00%	0,00%	0,00%
Otu0007	*Firmicutes*	*Bacilli*	*Bacillales*	*Planococcaceae*	*Planococcaceae*_unclassified	0,00%	0,00%	0,00%	0,00%

4. Conclusion

Our study shows that the larval stage directly influences in the PE amount that *G. mellonella* larvae can ingest. By optimizing it, the highest rate of PE consumption by a single larva per day (17.22 mg ± 1.29) was reached using 130 mg-animals. We advanced by using a series of physical-chemical techniques to analyze other body samples, such as hemolymph, cocoon and feces, in order to understand in a more holistic view, as well as by innovative metabolomic studies what happens with PE after been ingested by the larvae and how this substrate is used by the animal for its metabolism. We showed, for the first time, that PE-related substances are present in insect hemolymph and cocoon. We proposed that part of the mechanically fragmented polymer is (i) metabolized and absorbed by larvae, then the PE metabolites undergo hemolymph flow and are secreted by silk glands, becoming part of the cocoon composition, as well as excreted in feces; and another part is (ii) eliminated in larvae feces as PE polymer. At this time, it would be important to perform our tests with controls in a situation of hunger and/or with a low-nutrient diet, this to confirm that these changes come from a diet with PE. In especial, SEM-FEG images of cocoon produced by laboratorial diet-fed larvae (control group) showed a more compact structure than those from PE-fed larvae, an unprecedented information. Considering that PE is nutritionally poorer than laboratorial diet, it is expected that these animals present lesser compact cocoon structure, lesser body weight and prolonged time to be turn into pupae compared to the control group (laboratorial diet-fed larvae). Our study also demonstrated that a PE feeding selects a distinct microbiota, which possibly is related to or may enhance the larvae PE degrading activity. Therefore, this study contributes in the understanding of this particular mechanism of the nature, specifically, and how physiology and fitness of the *G. mellonella* larvae are affected by PE ingestion, and to assist future biotechnological process for plastic breakdown and waste management.

Acknowledgment

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CAPÍTULO IV

Nesse capítulo serão abordados experimentos realizados na padronização de ensaios para estudo da biodegradação de PE por larvas do inseto *G. mellonella*.

O nosso grupo de pesquisa, BACMEA (Bacteriologia e Modelos Experimentais Alternativos) é pioneiro no estado do Rio Grande do Sul na criação laboratorial de *G. mellonella*, bem como, na implementação deste animal como um hospedeiro experimental alternativo. Assim, utilizamos as larvas desse inseto como um modelo alternativo *in vivo* para estudar processos infecciosos bacterianos e para avaliar a eficácia e toxicidade de moléculas ativas *in vitro* (MORELO; TRENTIN, 2016, PINTO et al., 2020). Interessantemente, durante a criação laboratorial, observou-se que essas larvas eram capazes de comer plásticos e isopor, como mostrado na Figura 1, despertando o interesse do grupo no entendimento deste processo. Assim, este é o primeiro trabalho desenvolvido pelo nosso grupo de pesquisa com enfoque na biodegradação de plásticos.

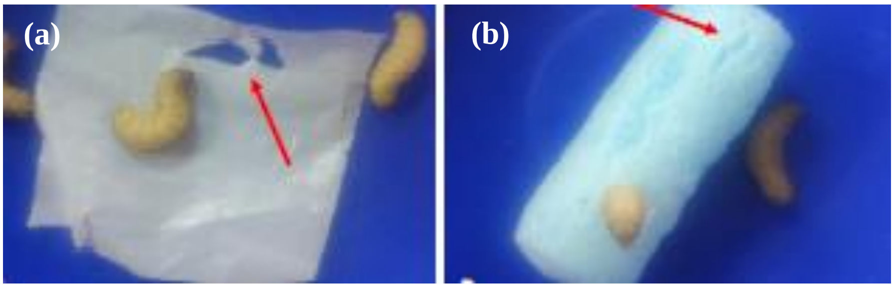


Figura 1: Larvas de *G. mellonella* comem sacolas plásticas (a) e isopor (b). Fotos obtidas após 8 h de exposição.

O primeiro relato sobre a capacidade de larvas de *G. mellonella* em degradar sacolas plásticas e filmes de PE foi publicado por Bombelli, Howe e Bertocchini em 2017. Os autores mostraram que aproximadamente 100 larvas conseguiam consumir até 92 mg dos filmes de PE, em um intervalo de tempo de apenas 12 h. A fim de eliminar a hipótese de que o sistema mastigatório das larvas seria o único responsável por esse processo, os autores conduziram uma série de experimentos utilizando um homogenato (ou pasta) dessas larvas. Após 14 h de contato do homogenato com a superfície do PE, houve uma perda de massa de 13% em relação ao peso inicial do plástico, o que corresponde a uma taxa de 0,23 mg cm^{-2} h^{-1}, e que é considerada muito superior quando comparada a taxas de biodegradação relatadas em estudos anteriores utilizando micro-organismos. Além disso, análises de FTIR demonstram que quando os filmes de PE eram tratados com a pasta de larva, uma banda em 3.350 cm^{-1} era visualizada, o que os autores reportaram como corresponde a presença de etilenoglicol, um metabólito de degradação do plástico. Baseado neste achado, os autores concluíram que as larvas de *G. mellonella* são capazes de biodegradar filmes de PE, e enfatizaram o fato de que futuros estudos deveriam determinar se a ação degradadora de PE é advinda do próprio animal ou dos micro-organismos intestinais.

Portanto, nosso grupo de pesquisa utilizou como suporte essa metodologia, com a intenção de reproduzir os experimentos de Bombelli e colaboradores (2017) e a partir daí estudar os processos de biodegradação e o envolvimento da microbiota neste processo. Neste capítulo são apresentadas todas as tentativas experimentais realizadas para estudar a biodegradação de filmes de PE com homogenatos de larvas de *G. mellonella*, bem como a padronização de ensaios que serão desenvolvidos após o retorno das atividades presenciais de bancada na Universidade.

Nesse capítulo serão apresentados os experimentos que não foram incluídos nos artigos científicos, mas que foram fundamentais para a padronização das metodologias utilizadas neste estudo. Desse modo, o procedimento experimental, bem como os resultados, será apresentado de forma conjunta para facilitar a leitura do capítulo.

1 MATERIAL

1.1 *G. mellonella*

Larvas de *G. mellonella* foram cultivadas em laboratório e durante todo o ciclo de vida as larvas foram mantidas a 28ºC. Previamente aos experimentos as larvas eram alimentadas com dieta laboratorial, a qual é composta por farinha de trigo (14,7%), farelo de trigo grosso (14,7%), gérmen de trigo (14,7%), leite em pó (29,4%), mel líquido (8,83%), glicerol (8,83%) e açúcar mascavo (8,3%). Ao atingirem o peso ideal para os experimentos, grupos de larvas foram então alimentadas com PE ou com dieta artificial (grupo controle).

1.2 PE

Foi utilizado PE de baixa densidade (PEBD - PB 608 Brasken). Filmes de PEBD foram produzidos com espessura de 0,06 mm, a fim de mimetizar sacolas plásticas, e área variou de acordo com o experimento. Essas amostras foram obtidas através de colaboração estabelecida com o Laboratório de Organometálicos e Resinas da Pontifícia Universidade Católica de Rio Grande do Sul (PUCRS).

2 PADRONIZAÇÃO E REPRODUTIBILIDADE DE ESTUDOS ANTERIORES

2.1 CONSUMO DE PE POR LARVAS VIVAS

Para que fosse possível o entendimento de como as larvas de *G. mellonella* reagem a uma alimentação exclusiva de PE, foi realizado um experimento preliminar. Então foram utilizados dois grupos com 88 larvas, os quais receberam alimentação distinta:(i) alimentação exclusiva com filmes de PE e (ii) alimentação exclusiva com dieta laboratorial. O peso inicial dessas larvas variou entre 180 mg e 190 mg e os dois grupos foram mantidos a uma temperatura de 28 ºC. Após um período de 7 dias foram avaliados o número de larvas vivas (nesse momento não foram incluídas a quantidade de pupas formadas durante esse processo, para que fosse possível realizar uma comparação entre as diferentes alimentações em relação as larvas vivas) e o peso final das larvas com essas diferentes alimentações.

Portanto, como resultado, observa-se na Figura 2a, que 84% dos animais alimentados com dieta laboratorial não se mantiveram em seu estágio larval após 7 dias. Isso ocorreu devido a rápida evolução desses insetos ao estágio de pupa, quando comparadas com o grupo de larvas que foram alimentadas exclusivamente com PE. Em contraste, Kundungal et al., (2018) observaram que larvas de *Achroia grisella* alimentadas exclusivamente com LDPE, conseguiram completar seu ciclo de vida mais rápido (16 dias), quando comparadas as larvas alimentadas com cera de abelha (28 dias). Outro estudo de Yang et al., (2018) mostrou larvas de *Tenebrio molitor* alimentadas com PEBD ou EPS (poliestireno expandido), durante 20 dias não conseguiam completar seu ciclo de vida, em comparação com o grupo de larvas alimentadas com farelo, um produto comum na agricultura. O processo de pupação pode ser utilizado como uma indicação para descrever a saúde e o desenvolvimento das larvas (YANG et al., 2018). Portanto, esses resultados indicam que larvas de *T. molitor* em jejum ou alimentando-se apenas com PEBD ou EPS careceram dos nutrientes necessários para completar seu ciclo de vida. Nossos resultados, com larvas de *G. mellonella* mostram que de fato há uma privação de nutrientes, o que acarreta uma demora para o processo de pupação se desenvolver na larva. Em contrapartida, seria necessário realizar um experimento que indicasse a massa crítica de pupação, isso pois esse processo de pupação também pode ser acelerado por condições de: (i) estresse ao animal e (ii) exposição a xenobióticos.

Além disso, das larvas restantes alimentadas com dieta laboratorial, menos de 1% apresentou perda de peso (Figura 2b), enquanto o grupo de larvas alimentadas com PE, que mantiveram em 50% os animais em estágio larval, foi observada maior diminuição de peso. Esse grupo de larvas perdeu aproximadamente 23,47% de seu peso inicial (Figura 2b). Isso está de acordo com os relatos da literatura que mostram que quando um animal possui uma dieta exclusiva com o plástico, naturalmente há uma redução em seu peso corporal. Wu, Tao e Wang (2018) demonstraram que larvas de *Tenebrio molitor*, quando alimentadas com diferentes tipos de plásticos (PVC, PEBD e PS) durante 30 dias, perdem até 37% de massa corporal. Urbanek et al., (2019) mostraram que larvas de *T. molitor* podiam perder até 20% do seu peso em relação ao peso inicial, após 21 dias de alimentação restrita com PS. Os autores ainda afirmam que por esse motivo, as larvas de *T. molitor*não eram capazes debiodegradar o PS. No entanto, vale ressaltar que para a validação do processo de biodegradação de plásticos por larvas de diferentes espécies, é preciso realizar análises que caracterizem o polímero para avaliar mudanças em sua estrutura físico-química e em seu peso molecular.

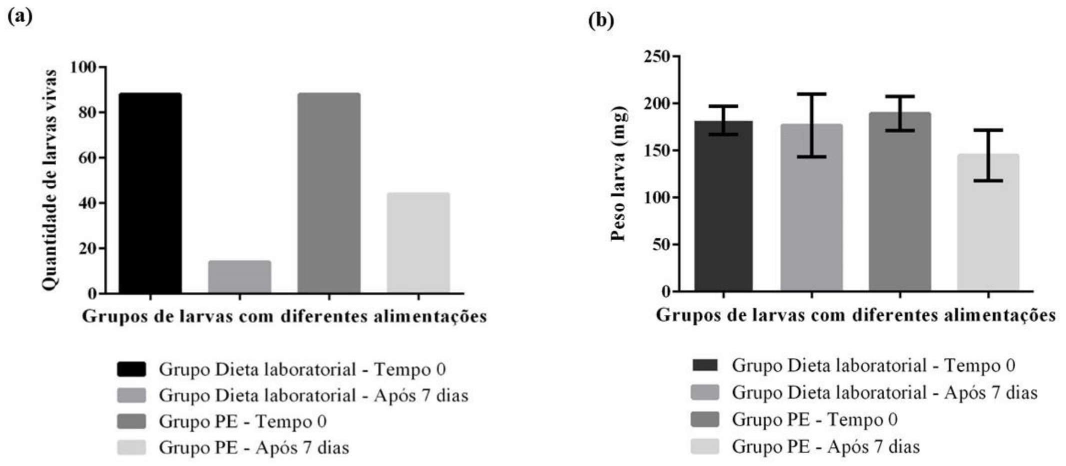


Figura 2: Efeito do consumo de PE por larvas de *G. mellonella*: (a) Número de larvas vivas após alimentação com (i) PE por 7 dias e (ii) dieta laboratorial. Amostra inicial por grupo: 88 larvas. (b) Peso das larvas após alimentação com (i) PE por 7 dias e (ii) dieta laboratorial.

2.2 REPRODUÇÃO DO ENSAIO DESCRITO POR BOMBELLI E COLABORADORES (2017)

2.2.1 Experimento com homogenato de larvas de *G. mellonella*

A fim de eliminar a hipótese que o sistema mastigatório da larva de *G. mellonella* seria o único responsável pelo consumo de PE, foi realizado um experimento com o homogenato da larva de acordo com protocolo descrito por Bombelli e colaboradores (2017). Para isso, foram utilizadas cerca de 3 larvas (170-190 mg) para um filme de PE de 4,0 cm², com uma alimentação de (i) dieta laboratorial ou (ii) PE durante 7 dias. O experimento ocorreu em 24 ha 28 ºC, sem trocas do homogenato. Essa pasta não passou por processos de filtração para a eliminação de micro-organismos. Esse ensaio incluiu um grupo controle (filme de PE sem o homogenato de larva) e foi realizado em quadruplicata.

No entanto, a primeira tentativa de reproduzir o protocolo estabelecido previamente (Bombelli et al., 2017) não foi bem-sucedida. A Figura 3a demonstra a superfície do filme de PE, com área de 4 cm², recoberto com o homogenato de 3 animais. Nota-se que há vários espaços vazios no filme, além de uma dificuldade para realizar a maceração do animal. Os resultados de análise gravimétrica (Figura 3b) obtidos a partir desse ensaio não mostraram diminuição da massa do filme de PE após um contato de 24 h com a pasta de larvas e foram considerados inconclusivos devido a não homogeneidade do recobrimento do filme com a pasta.

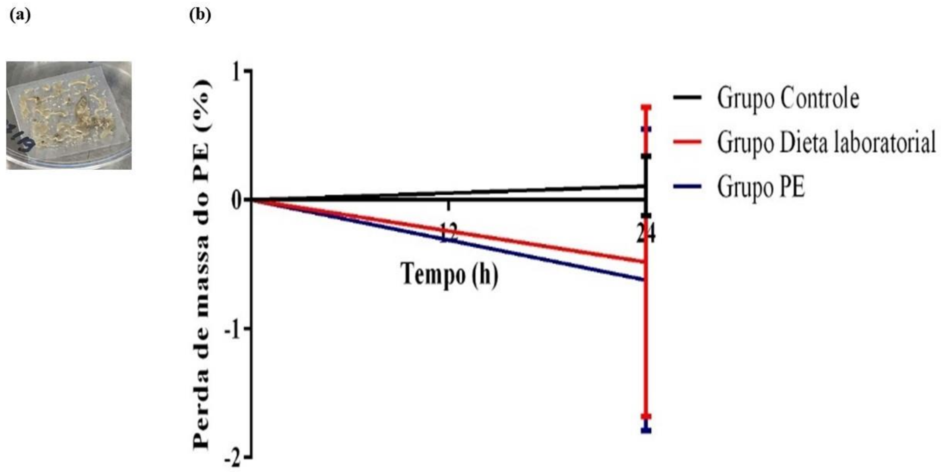


Figura 3: Reprodução do ensaio descrito por Bombelli e colaboradores (2017): (a) Homogenato de 3 larvas sobre o filme PE de 4,0 cm². (b) Percentual da variação do peso do filme do PE de 4,0 cm² após 24 h em contato com um homogenato de larvas (i) alimentadas

com dieta laboratorial (linha vermelha) ou (ii) alimentadas com PE (linha azul), em comparação com o grupo controle (linha preta).

Posteriormente passamos a controlar a quantidade de homogentato depositado sobre o filme do PE. Aproximadamente 450 mg de pasta foram adicionadas sobre filmes de plástico com área de 2,5 cm², de modo que toda a superfície do PE ficasse coberta com a pasta (Figura 4a). Esse homogenato de larvas foi realizado com grupos de larvas com alimentação de (i) dieta laboratorial ou (ii) PE durante 7 dias A cada 2 h foram realizadas as trocas dessa pasta por pastas frescas (de modo a deixar um ambiente mais suscetível a uma possível ação enzimática), completando 14 h de experimento. Nesses intervalos, o filme do PE era lavado com água destilada e pesado em balança analítica. Ao final das 14 h, o filme de PE foi lavado com água e álcool 70% e deixado secar *overnight* para posterior pesagem do material. Esse ensaio também incluiu um grupo controle (filme de PE sem o homogenato de larva) que passou por todo procedimento das demais amostras, ou seja, lavagens e pesagem. O experimento foi realizado em duplicata.

O resultado do porcentual de perda de massa do filme de PE que ficou em contato com o homeganato de larva, assim como o controle, está exposto na Figura 4b. Pode-se verificar que não houve diminuição do peso do filme do PE ao final de 26 h de experimento, ao contrário do resultado encontrado por Bombelli e colaboradores (2017) que em 14 h de experimento, utilizando um homogenato de *G. mellonella* encontrou uma perda de peso dos filmes de PE de 13%, quando comparado com seu peso inicial.

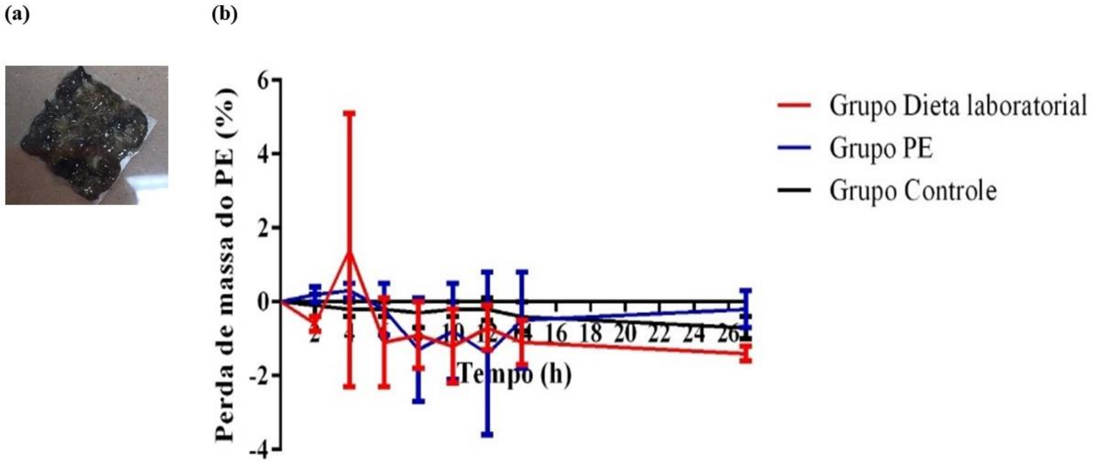


Figura 4: Reprodução do ensaio descrito por Bombelli e colaboradores (2017): (a) Homogenato de 450 mg de larvas sobre filme de PE de 2,5 cm². (b) Percentual da variação do peso do filme do PE de 2,5 cm² após 26 h em contato com um homogenato de larvas (i) alimentadas com dieta laboratorial (linha vermelha) ou (ii) alimentadas com PE (linha azul), em comparação com o grupo controle (linha preta).

Importantemente Ren et al., (2019) destacam que apenas a análise gravimétrica não é suficiente para afirmar se há um processo de biodegradação de plástico, e por isso, os autores sugerem que outras análises físico-químicas sejam incluídas. A Figura 5 mostra os espectros de FTIR obtidos a partir de amostras de filmes de PE que ficaram em contato com o homogenato de larvas alimentadas previamente com (i) PE durante 7 dias (Figura 5 a e b - duplicata) e (ii) dieta laboratorial (Figura 5 c e d - duplicata). Além disso, foram avaliados também um grupo controle, ou seja, filme de PE que não ficou em contato com o homogenato, indicado na Figura 5. Observa-se uma similaridade nos picos encontrados nas duplicatas, o que indica a reprodutibilidade das amostras. Em todas as amostras, é possível observar picos característicos do PE (em 1377 cm^{-1}, que corresponde a grupos funcionais de CH_3 e em 720 cm^{-1}, que corresponde a grupos funcionais de ligações C-H). Isso faz sentido, uma vez que a estrutura química do filme de PE é representada por ligações de C-H e C-C. Além disso, em todas as amostras aparecem grupos funcionais de C=O (1746 cm^{-1}), $=CH_2$ (1463 cm^{-1}), C-O (1164 cm^{-1}), lactose (1118 cm^{-1}) e estiramento C-O de álcool e éter alifático (1095 cm^{-1}). Para as amostras relacionadas ao grupo do homogato de larvas alimentadas com PE, aparece também o pico em 876 cm^{-1}, referente ao grupo funcional carbonato de cálcio. Já para o grupo de um homogenato de larvas alimentadas com dieta laboratorial aparece o pico 970 cm^{-1}, atribuído a modos vibracionais de proteínas fosforiladas.

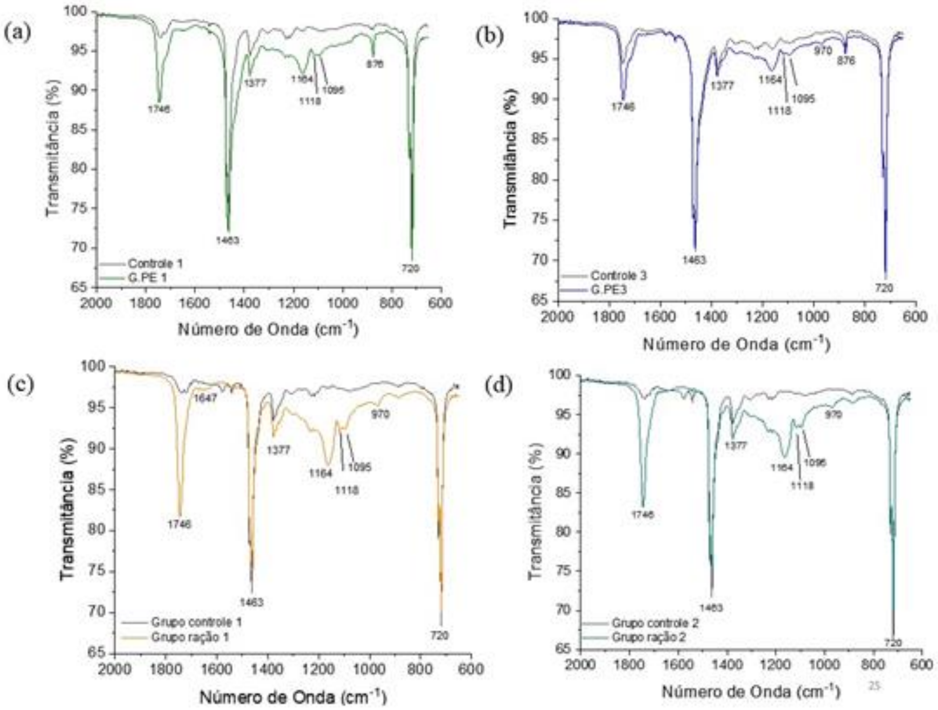
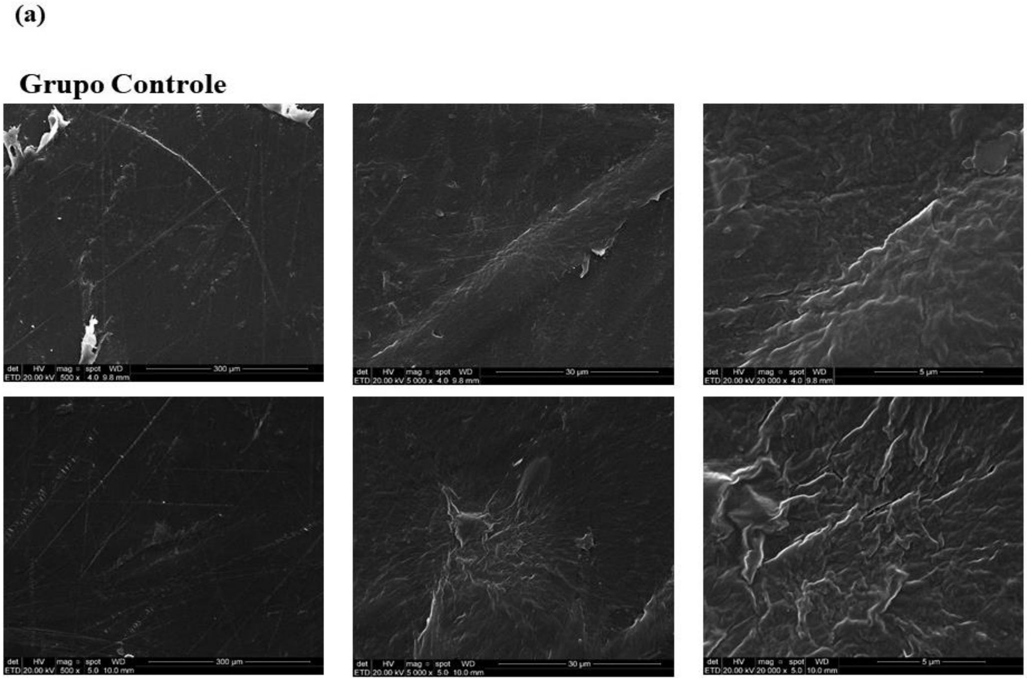


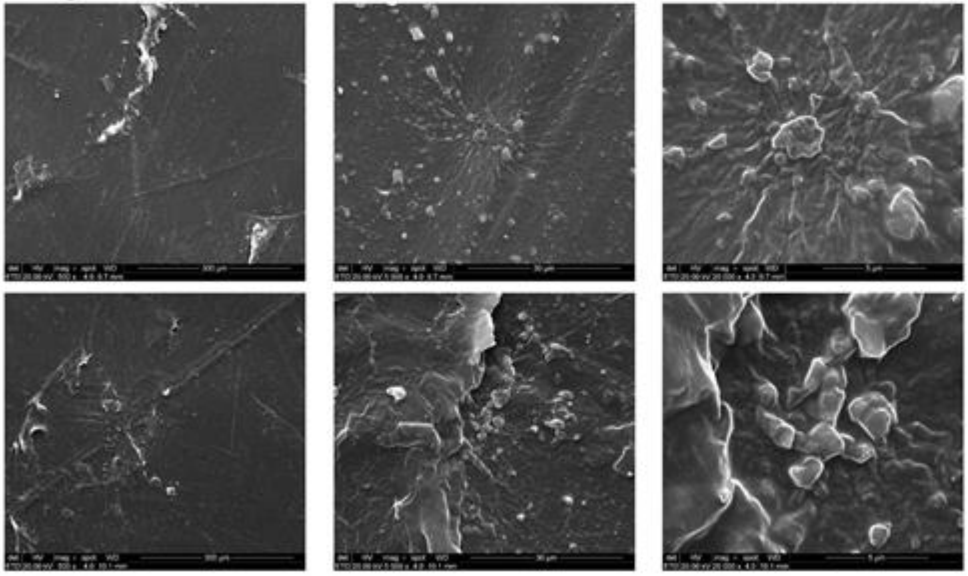
Figura 5: Espectros de FTIR de filmes de PE expostos a homogenatos de larvas durante 26 h. (a) e (b) Filmes de PE expostos a homogenato de larvas alimentadas com PE durante 7 dias: a linha verde indica o grupo PE de uma amostra, a linha azul indica o grupo PE de outra amostra e a linha preta indica o grupo controle (filme de PE sem contato com homogenato de larva). e (c) e (d) Filmes de PE expostos a homogenato de larvas alimentadas com dieta laboratorial (ração): a linha laranja indica o grupo dieta laboratorial de uma amostra, a linha verde indica o grupo o grupo dieta laboratorial de uma amostra e a linha preta indica o grupo controle (filme de PE sem contato com homogenato de larva).

Esses resultados indicam grupos funcionais não existentes na composição do filme do PE, o que poderia então revelar a presença de metabólitos de degradação na superfície do filme de PE. Bombelli et al., (2017) também encontraram metábolitos de degradação (etileno glicol) por FTIR em filmes de PE após a exposição dessas amostras a um homogenato de larvas. No entanto, Weber e colaboradores (2017) questionaram a validade dos resultados encontrados por Bombelli e colaboradores (2017), uma vez que os picos de 3.300 cm^{-1}, indicativos de etilenoglicol, encontrado na superfície do PE poderiam ser devido a presença de resquícios da pasta da larva e não da biodegradação do material. Para isso, Weber et al. (2017) depositaram gema de ovo e carne de porco moída em filmes de PE, para a realização da análise de FTIR. A autora relatou a absorção em torno de 3.300 cm^{-1}(geralmente atribuída às vibrações de alongamento N-H das proteínas). Além disso, foram demonstrados absorbâncias em 1.744, 1.651 e 1.545 cm^{-1}(indicios para lipídios e proteínas). Vale pontuar que Weber e colaboradores (2017) realizaram uma lavagem apenas com água, assim como realizado por Bombelli et al. (2017). Considerando estas observações, julgamos necessário a análise microscópica da superfície dos filmes de PE nas amostras conduzidas em nosso trabalho para verificar se existiam resíduos da pasta na superficie do plástico. Os resultados obtidos por MEV-FEG estão expostos na Figura 6 a, b, c, d e.



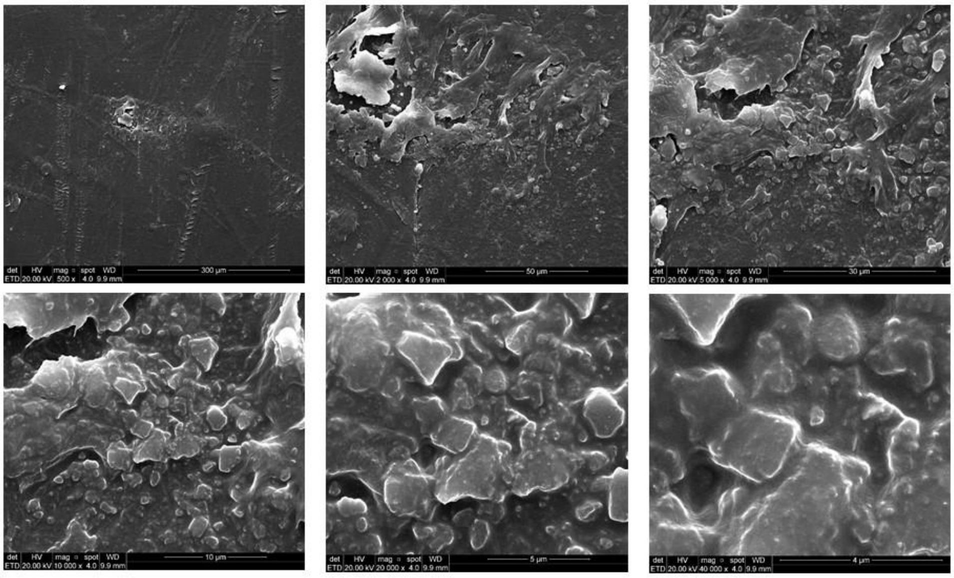
(b)

Grupo PE 1



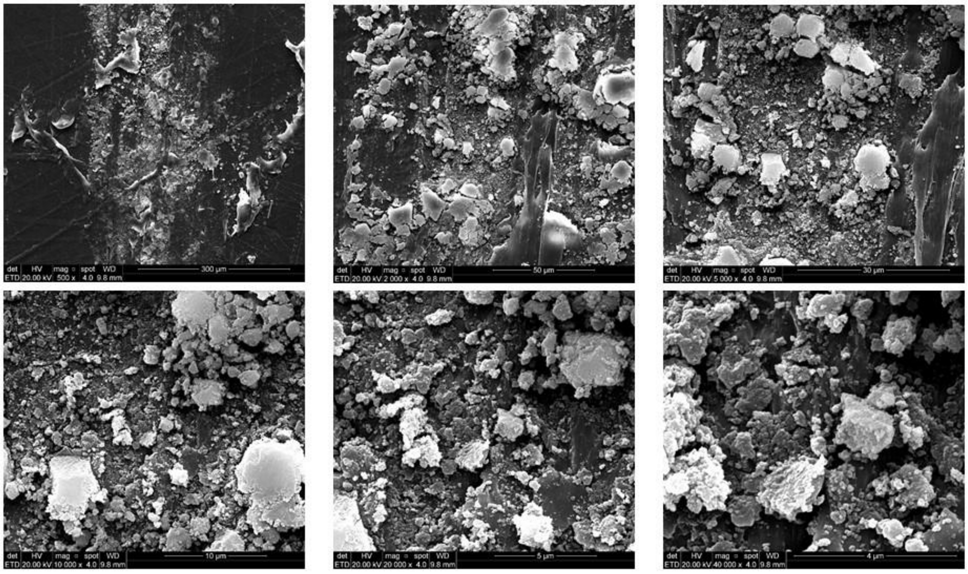
(c)

Grupo PE 2



(d)

Grupo Dieta laboratorial 1



(e)

Grupo Dieta laboratorial 2

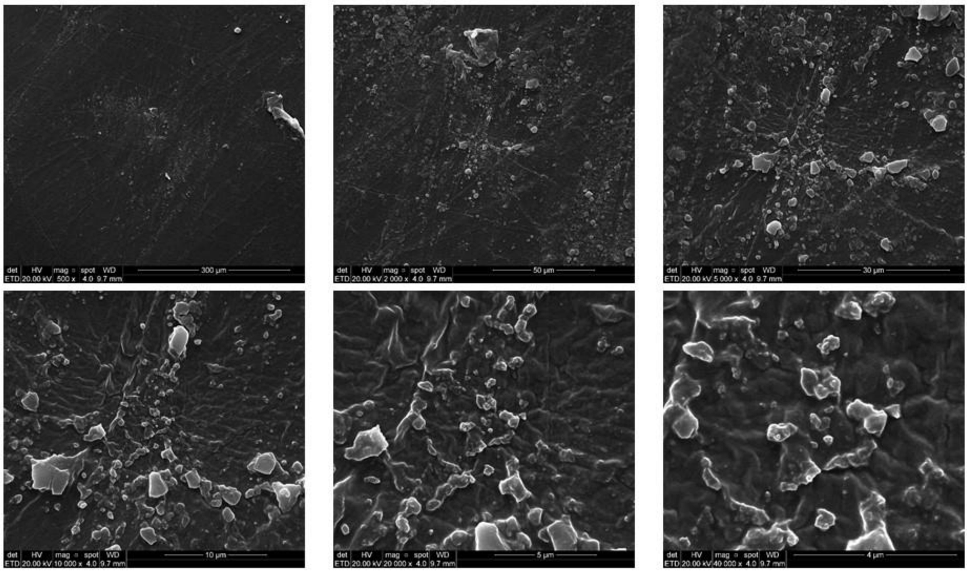


Figura 6: Imagens de MEV-FEG de filmes de PE após 26 h de contato com homogenato de larvas. (a) Controle, ou seja, filme de PE em contato com homogenato de larvas. (b) e (c) Filmes de PE que ficaram expostos ao homogenato de larvas alimentadas com PE por 7 dias (duplicata), (d) e (e) Filmes de PE expostos ao homogenato de larvas alimentadas com dieta laboratorial (duplicata).

Visualiza-se claramente a presença de resíduos, que possivelmente são de origem orgânica, nas imagens dos filmes de PE que receberam a pasta de larvas, o que revela que a lavagem realizada posteriormente ao tratamento das amostras não foi o suficiente para a remoção de sujidades e pode ter interferências nas análises gravimétricas e de FTIR.

2.2.2 Experimento em solução PBS com homogenato de larvas

Uma vez que o homogenato de larvas utilizado como escrito anteriormente muitas vezes ressecava entre uma troca e outra, otimizamos a condição experimental a fim de favorecer a atividade de uma possível ação enzimática. Para tanto, o pH intestinal das larvas de *G. mellonella* foi medido (pH ~ 7.0) e o filme de PE foi mantido em contato com a pasta de larvas misturada a uma solução PBS com pH 7, estéril. Então, para essa análise foram realizados macerados de larva de *G. mellonella* (130 mg) divididas em dois grupos: (i) alimentadas durante 7 dias com PE e (ii) alimentadas com dieta laboratorial. Desse modo, com auxílio de gral e pistilo, foi realizada uma pasta de larva e 450 mg dessa pasta foi depositada em tubo falcon, com adição de 2 mL de solução PBS pH 7, estéril. Dois grupos dessa pasta foram anteriormente autoclavadas: (i.a) pasta autoclavada do grupo de larvas alimentadas com PE e (ii.a) pasta autoclavada do grupo de larvas alimentadas com dieta laboratorial, a fim de desnaturar possíveis proteínas e enzimas presentes no homogenato. Então foram pesados os filmes de PE com área de 2,5 cm², e esses foram depositados nos tubos falcon que continham: (i) pasta de larvas previamente alimentada com PE, (i.a) pasta de larvas previamente alimentas com PE e autoclavada, (ii) pasta de larvas alimentas com dieta laboratorial e (ii.a) pasta de larvas alimentadas com dieta laboratorial e autoclavada. O grupo controle consistiu em 2 mL da solução PBS, sem a adição do homogenato de larvas, e o filme de PE, as amostras desse experimento são observadas na Figura 7a. Todos os grupos foram mantidos a 28ºC e 120 rpm e as trocas das pastas se deram a cada 36 h, com lavagem dos filmes de PE com água e medida do peso. O ensaio teve duração total de 180 h, sendo que no último ponto houve a lavagem de todos os grupos (inclusive do controle) com solução SDS 2% durante 8 h, além de álcool 70% e água destilada. e posterior pesagem dos filmes de PE. Todos os ensaios foram realizados em triplicata.

Como resultado, nota-se que neste caso houve uma maior área de contato dos filmes de PE com as pastas de larvas, o que poderia facilitar a biodegradação. Uma vez que no ensaio mostrado anteriormente (item 2.2.1) as trocas de pasta ocorreram a cada 2 h, conforme metodologia reportada por Bombelli e colaboradores (2017), e nós não observamos indícios de biodegradação dos filmes de PE que ficaram em contato com o homogenato de larvas, intervalos maiores para as trocas da pasta (24 h) foram realizados, para que uma possível ação enzimática fosse conduzida por um tempo maior. No estudo conduzido por Billen et al., (2020), que também objetivou reproduzir o experimento descrito por Bombelli et al. (2017), os filmes de LDPE foram deixados em contato com um homogenato de larvas de *G. mellonella* por tempos diferentes (24 h e 48 h) a 30 °C. Os autores não realizaram a substituição da pasta em nenhum intervalo de tempo, ao contrário de Bombelli et al. (2017), com a justificativa de evitar danos mecânicos no substrato do LDPE, como sugerido por Weber et al. (2017).

Os resultados obtidos por gravimetria são apresentados na Figura 7b. Novamente observa-se que não houve perda de massa dos filmes de PE expostos aos diferentes homogenatos de larvas. Semelhantemente, Billen et al., (2020) mostram que após 48 horas de incubação dos homogenatos com os filmes de PE e subsequente lavagem e secagem, não houve perda de peso detectável do polímero. Da mesma forma, em outro experimento conduzido pelos mesmos autores, em ambiente controlado com oxigênio e umidade relativa de 100%, não resultou em perda de massa observável após 20 h. Os autores então levam a hipótese de que a microbiota do intestino da larva poderia ser diferente das larvas utilizadas por Bombelli et al. (2017), ou ainda que as perdas de massa detectadas anteriormente pelo grupo de pesquisa de Bombelli, foram de fato um resultado de desgaste mecânico, ao invés de biodegradação verdadeira, como também foi sugerido por Weber et al. (2017).

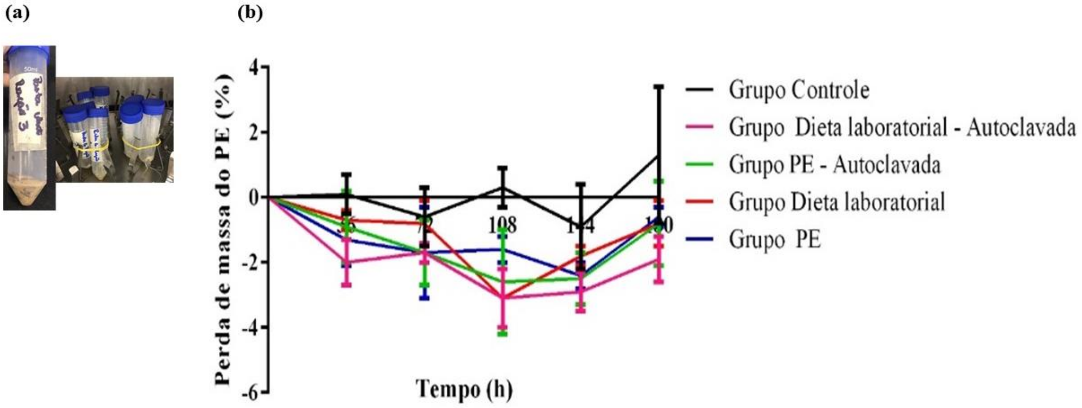


Figura 7: Experimento em solução PBS com homogenato de larvas de *G. mellonella* durante 180 h. (a) Em tubos tipo Falcon, o homogenato preparado com 450 mg de larvas

está em solução PBS pH 7, estéril na presença de filmes de PE de 2,5 cm². (b) Percentual da variação do peso do filme do PE de 2,5 cm² após 180 h em contato com um homogenato de larvas em solução PBS pH 7 estéril: (i) larvas alimentadas com dieta laboratorial – homogenatos autoclavado (linha rosa), (ii) larvas alimentadas com PE por 7 dias – homogenatos autoclavado (linha verde), (iii) larvas alimentadas com dieta laboratorial (linha vermelha) ou (iv) alimentadas com PE (linha azul), comparando com o grupo controle, filme de PE sem contato com o homogenato (linha preta).

Os filmes de PE também foram submetidos à análise por FTIR. Os resultados estão demonstrados na Figura 8. A Figura 8a apresenta o espectro dos filmes de PE que ficaram em contato com o homogenato de larvas previamente alimentadas com dieta laboratorial (ração). Observa-se picos em 1085 cm^{-1} (estiramento C-O de grupos álcoois e éter etílico) e em 832 cm^{-1} (ligações C=C, de alcenos), quando essa pasta não foi previamente autoclavada. Estes picos podem também ser associados a resíduos orgânicos na superfície do filme do PE, como comentado anteriormente. Os resultados da análise de FTIR do filme de PE que passou por esse tratamento, mostram picos em 1747 cm^{-1} (estiramento de C=O de ésteres) e em 1279 cm^{-1} (estiramento de C-O de ácido carboxílico). Esses resultados não são conclusivos, pois os resultados da análise de FTIR realizada no grupo controle (PE em contato com a solução PBS, sem o homogenato de larvas) mostram a presença dos mesmos picos, indicando que a alteração na superfície do filme de PE possa ser devido ao processo de lavagem com o detergente SDS. Além disso, a Figura 8b apresenta o espectro dos filmes de PE que ficaram em contato com o homogenato de larvas previamente alimentadas com PE por 7 dias. É observado um pico em 1745 cm^{-1} (estiramento de C=O de ésteres) no grupo controle, o que revela que esse pico é devido à solução PBS ou a resíduos do detergente de SDS utilizado após a remoção da pasta. Na Figura 8b ainda, observa-se picos em 1273 cm^{-1} (estiramento de C-O de ácido carboxílico), 1092 cm^{-1} (estiramento C-O de grupos álcoois e éter etílico) e em 824 cm^{-1} (ligações C=C, de alcenos). Billen et al., (2020) também realizou análise de FTIR em filmes de PE expostos ao homogenato de larvas. Os autores reportam que os resultados foram inconclusivos pois os picos na região de 1750-1600 cm^{-1}observada na amostra de PE em contato direto com o homogenato, podem ser devido a carbonilas resultantes da biodegradação ou podem ser atribuídos a mudanças no homogenato, sendo assim, uma provável mudança na estrutura química da a própria biomassa, sugerindo que o resíduo estava presente no filme de PE durante a análise.

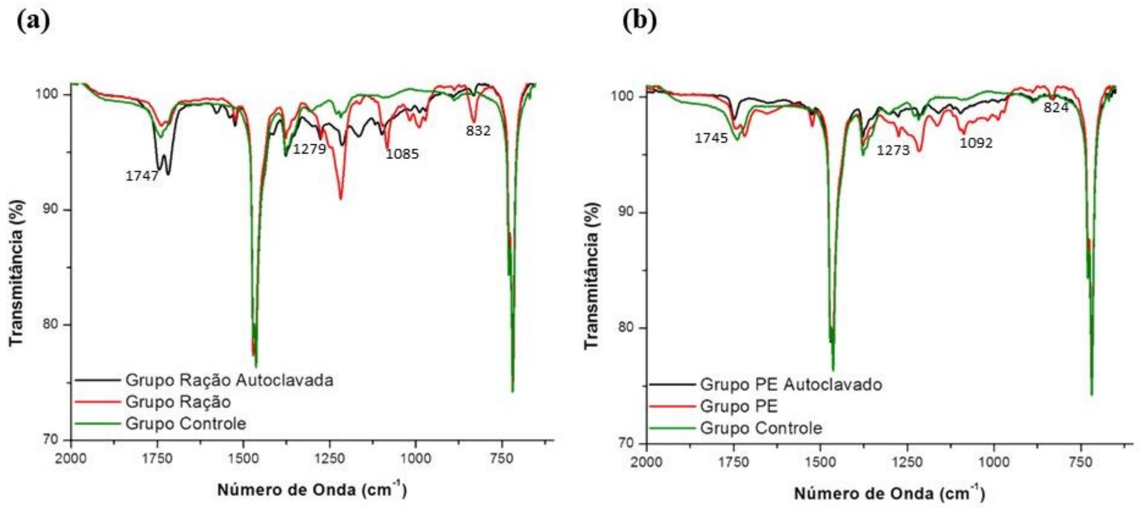


Figura 8: Espectros de FTIR de filmes de PE expostos a homogenatos de larvas em solução PBS pH 7. (a) Filmes de PE expostos a homogenato de larvas alimentadas com dieta laboratorial (ração): linha preta indica o homogenato que sofreu processo prévio de autoclavagem, linha vermelha indica um homogenato de larvas alimentadas com dieta laboratorial e a linha verde indica o controle (filme de PE sem contato com homogenato de larva); (b) Filmes de PE expostos a homogenato de larvas alimentadas com PE por 7 dias: a linha preta indica o homogenato que sofreu processo prévio de autoclavagem, a linha vermelha indica um homogenato de larvas alimentadas com PE por 7 dias e a linha verde indica o controle (filme de PE sem contato com homogenato de larva).

2.2.3 Experimento em câmera úmida com homogenato de larvas

Para essa análise foram realizados macerados de larva de *G. mellonella* (130 mg) divididas em dois grupos: (i) alimentadas durante 7 dias com PE e (ii) alimentadas com dieta laboratorial. Com auxílio de gral e pistilo, foram realizadas pastas de larvas e 450 mg dessa pasta foi depositada em ambos os lados dos filmes de PE com área de 2,5 cm², a fim de manter a pasta em contato com toda a superfície do PE. Foram realizados testes com (i) pasta de larvas alimentada com PE, (i.a) pasta de larvas alimentas com PE e autoclavada, (ii) pasta de larvas alimentas com dieta laboratorial e, (ii.a) larvas alimentadas com dieta laboratorial e autoclavada. O grupo controle consistiu apenas do filme de PE, sem adição da pasta. Os filmes cobertos com pasta foram armazenados em placas de Petri estéreis, as quais foram mantidas em câmera úmida. Isso para deixar o homogenato com condições biológicas favoráveis para a biodegradação do PE e ao mesmo tempo, não interferir quimicamente na estrutura do polímero (Figura 9a). A cada 24 h eram realizadas as trocas das pastas por pastas frescas, havendo a necessidade de lavagens com água destilada. Os pesos dos filmes de PE foram controlados a cada troca de pasta. O ensaio teve duração de 120 h, sendo que no último ponto houve a lavagem de todos os grupos (inclusive do controle) com solução SDS 2% por 8 h, além de álcool 70% e água destilada, e posterior pesagem dos filmes de PE. Todos os ensaios foram realizados em triplicata.

Os resultados da percentagem da perda de massa do PE, em relação ao seu peso inicial quando em contato com uma pasta de larvas estão expostos na Figura 9b. Observa-se então, que não houve variação do peso do filme de PE ao longo do experimento, assim como relatado para os outros experimentos com homogenatos previamente apresentados.

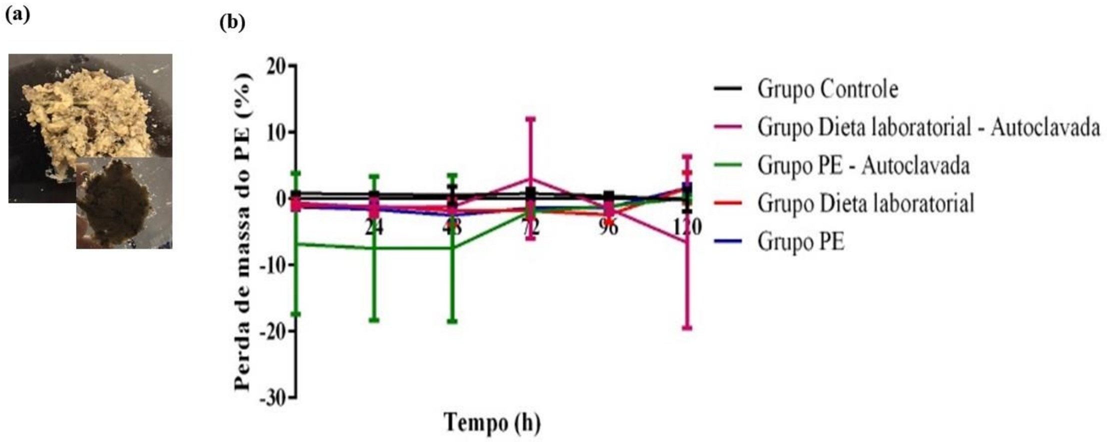


Figura 9: Experimento em câmera úmida com homogenato de larvas de G. mellonella durante 120 h: (a) Homogenato de 900 mg de larvas em contato com filmes de PE de 2,5 cm² matidos em placa de Petri dentro de câmera úmida. (b) Percentual da variação do peso do filme do PE de 2,5 cm² após 120 h em contato com homogenato de larvas em câmera úmida estéril: (i) larvas alimentadas com dieta laboratorial – homogenatos autoclavado (linha rosa), (ii) larvas alimentadas com PE por 7 dias – homogenatos autoclavado (linha verde), (iii) larvas alimentadas com dieta laboratorial (linha vermelha) ou (iv) larvas alimentadas com PE (linha azul), comparando com o grupo controle (linha preta) (filme de PE sem contato com homogenato de larva).

A Figura 10 mostra alguns grupos funcionais presentes na superfície do polímero, identificados por análise de FTIR após o contato com os diferentes tipos de homogenato de larvas. Observa -se na Figura 10a picos encontrados para os filmes de PE que ficaram em contato com um homogenato de larvas previamente alimentadas com dieta laboratorial (ração) e a Figura 10b mostra os picos encontrados para os filmes de PE que ficaram em contato com o homogenato de larvas previamente alimentadas com PE por 7 dias. A presença de dois picosem 1749 cm^{-1} (estiramento C=O de ésteres) e em 1161 cm^{-1} (estiramento C-O de éster) é observada no espectro de todos os grupos. O que novamente indica que não houve biodegradação detectável do filme do PE por um homogenato de larvas de *G. mellonella* com diferentes alimentações.

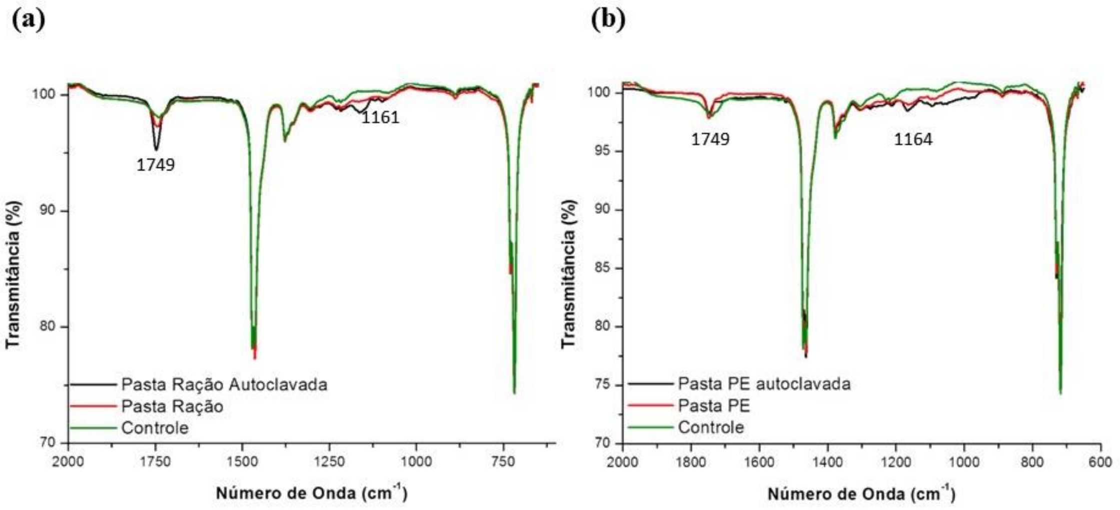


Figura 10: Espectros de FTIR de filmes de PE expostos a homogenatos de larvas em câmera úmida. (a) Filmes de PE expostos a homogenato de larvas alimentadas com dieta laboratorial (ração): linha preta indica o homogenato que sofreu processo prévio de autoclavagem, linha vermelha indica homogenato de larvas alimentadas com uma dieta laboratorial e a linha verde indica o controle (filme de PE sem contato com homogenato de larva); (b) Filmes de PE expostos a homogenato de larvas alimentadas com PE por 7 dias: linha preta indica o homogenato que sofreu processo prévio de autoclavagem, linha vermelha indica homogenato de larvas alimentadas com PE por 7 dias e a linha verde indica o controle (filme de PE sem contato com homogenato de larva).

2.3 RESPIROMETRIA COM HOMOGENATO DE LARVAS E LARVAS VIVAS

A quantificação de CO_2 para a avaliação do metabólito final produzido na biodegradação aeróbia de PE foi realizada no Laboratório Organometálicos e Resinas na Pontifícia Universidade Católica de Rio Grande do Sul (PUCRS). A análise de respirometria ocorreu por meio de titulação. O gás de CO_2 gerado pela possível biodegradação do PE acumula-se no interior do biorreator (Erlenmeyer) e reage com solução alcalina de KOH 0,36 N contida em recipiente acoplado ao biorreator, como mostra o esquema na Figura 11. Dessa forma, o CO_2 foi quantificado de modo analítico por meio de titulação com HCl 0,25N.

A taxa de biodegradação foi quantificada utilizando as equações químicas 1, 2 e 3 e as equações numéricas 4, 5 e 6.

$$C + O_2 \rightarrow CO_2 \tag{1}$$

$$2\ KOH + CO_2 \rightarrow K_2CO_3 + H_2O \tag{2}$$

$$KOH + HCl \rightarrow KCl + H_2O \tag{3}$$

$$\text{mg de } CO_{2\text{produzido}} = \left(\frac{M_{KOH} \cdot V_{KOH}}{M_{HCl}} - (V_b)\right) . \frac{M_{HCl}}{2} . 44\,\frac{g}{mol} \tag{4}$$

$$\text{mg de } CO_{2\text{teórico}} = \frac{y . 44\frac{g}{mol}}{12\frac{g}{mol}} \tag{5}$$

$$\%\ \text{Biodegradação} = \frac{\text{mg } CO_2\text{produzido}}{\text{mg } CO_2\text{teórico}} \tag{6}$$

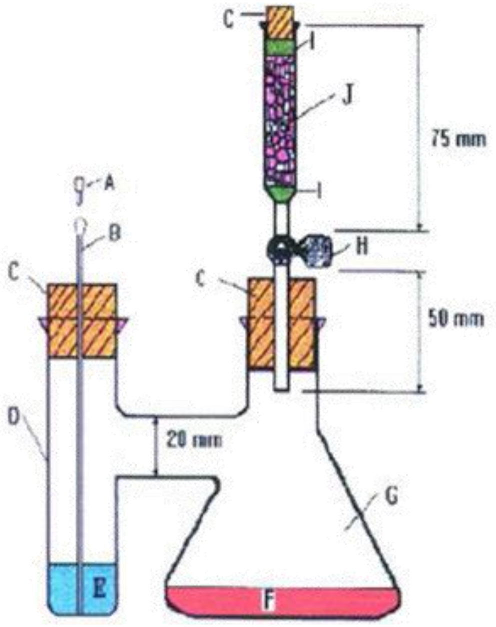


Figura 11: Esquema do biorreator com adaptação ISO 17556 (2012), onde C- Rolha de silicone; D- Braço lateral; E- Solução de KOH 0,36 M; F- Amostra; G- Erlenmeyer de 250 ml (Recipiente da amostra); H- Válvula; I- Algodão (camada de suporte); J- Cal sodada (Filtro de CO_2)

Nos experimentos com larvas vivas, foram utilizados quatro grupos: (i) em um biorreator foi adicionado PE (pesado previamente) e 10 larvas vivas, alimentadas previamente com dieta laboratorial; (ii) em um biorreator foi adicionado PE (pesado previamente) e 10 larvas vivas, alimentadas previamente com PE, por 7 dias, (iii) controle 1: com 10 larvas alimentadas com dieta laboratorial, sem a presença de PE e (iv) controle 2: com 10 larvas alimentadas durante 7 dias com PE, sem a presença de PE. Já para os experimentos com homogenato de larvas, foram utilizados os seguintes grupos: (i) em um biorreator um homogenato de 10 larvas sobre um filme de PE, com peso conhecido e área de 2,5 cm². Essas larvas foram alimentadas previamente com dieta laboratorial, denominada como grupo ração + PE, (ii) em um biorreator um homogenato de 10 larvas, essas larvas foram alimentadas previamente com uma dieta laboratorial, denominada como grupo ração controle, (iii) em um biorreator um homogenato de 10 larvas sobre um filme de PE com peso conhecido e área de 2,5 cm². Essas larvas foram alimentadas previamente com filmes de PE por 7 dias, denominada como grupo PE + PE.

Os sistemas foram mantidos a 28ºC e as análises de quantificação de CO_2ocorreram em tempos diferentes: para os ensaios com larvas vivas, o experimento ocorreu em 7 dias, sendo que nos tempos de 1 e 7 dias se realizou a titulação. Já o ensaio contendo um homogenato de larva ocorreu durante 29 dias, com intervalos de 0, 1, 7, 10, 16, 26 e 29 dias.

Os resultados para os ensaios de respirometria utilizando larvas vivas realizados em Erlenmeyer como biorreatores não ocorreram de forma satisfatória. Isso pois, como mostra a Figura 12, as larvas exploraram o biorreator e não consumiram o PE fornecido. Pode-se observar que alguns animais conseguiram escapar do compartimento, outras ainda caíram na solução de KOH e outras consumiram a rolha do biorreator, que também é feita de um material polimérico, o que tornou inviável a interpretação desses resultados.

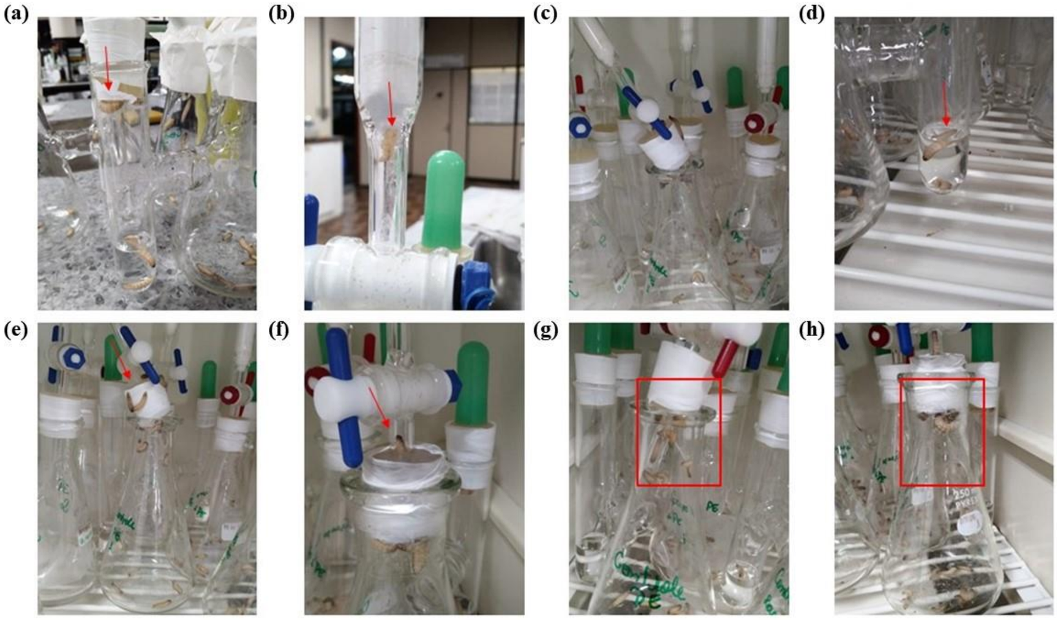


Figura 12: **Experimento de respirometria com larvas vivas.** (a) (b) (d) (e) (f) Biorreator com larvas em localização inadequada para a realização do experimento, indicada pelas setas em vermelho. (c) Desacoplamento da rolha do biorreator (g) (h) Biorreator com larvas em

localização inadequada para a realização do experimento, indicada por quadrados em vermelho

Sendo assim, o experimento foi realizado de forma satisfatória utilizando dessecadores como biorreator, A metodologia, os resultados e a discussão desses resultados são demonstrados no Capítulo III desse presente estudo. Já os resultados para a avaliação da biomineralização de PE com homogenatos de larvas está disposta na Tabela 1, que apresenta os resultados de quantificação em mg de CO_2 cumulativos obtidos por essa análise e na Tabela 2 estão presentes os resultados da porcentagem de biomineralização.

Tabela 1: Medida de CO_2 cumulativo (mg) no experimento de respirometria realizado com homogenato de larvas de *G. mellonella*.

Dias de experimento	Grupo Controle*	Grupo Ração + PE**	Grupo PE + PE***
0	0	0	0
1	13,2	8,448	5,808
7	83,424	76,296	69,696
10	137,28	107,976	107,712
16	191,598	146,564	161,744
26	223,608	178,288	220,066
29	233,31	194,568	252,648

*Grupo controle: homogenato de larva sem a presença de filmes de PE.

**Grupo Ração + PE: filme de PE que ficou exposto ao homogenato de larvas previamente alimentadas com dieta laboratorial (ração).

***Grupo PE + PE: filme de PE que ficou exposto ao homogenato de larvas previamente alimentadas com PE.

Tabela 2: Percentual de biomineralização no experimento de respirometria realizado com homogenato de larvas

Dias de experimento	Grupo Ração + PE**	Grupo PE + PE***
0	0	0
1	-0,427	-0,699
7	-0,641	-1,298
10	-2,634	-2,796
16	-4,048	-2,823
26	-4,047	-0,335
29	-3,482	1,828

**Grupo Ração + PE: filme de PE que ficou exposto ao homogenato de larvas previamente alimentadas com dieta laboratorial (ração).

***Grupo PE + PE: filme de PE que ficou exposto ao homogenato de larvas previamente alimentadas com PE.

Esses resultados estão apresentados na forma de gráfico (Figura 13), onde observa-se que a geração do CO_2 nas diferentes amostras foi similar, incluindo a amostra controle (somente homogenato de larvas, sem adição do filme de PE). Isso indica que a produção desse gás não é referente a biomineralização do polímero em seu processo final de biodegradação, mas sim devido a liberação de CO_2 pela microbiota da larva, uma vez que foi notado o desenvolvimento de micro-organismos a partir da pasta de larva. Por isso, esses resultados preliminares são insatisfatórios para a avaliação do produto de biomineralização de PE utilizando larvas de *G. mellonella*.

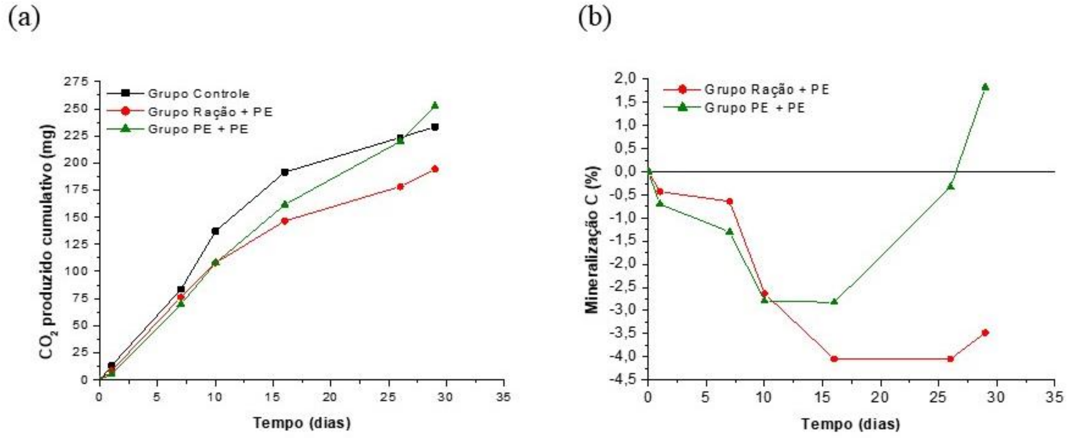


Figura 13: Ensaio de respirometria desenvolvido com homogenato de larvas. (a) Medida de CO2 cumulativo (mg) em relação aos dias de experimento, totalizando 29 dias: a linha preta representa o grupo controle (sem adição do filme de PE), a linha vermelha representa o grupo ração + PE (filme de PE que ficou exposto ao homogenato de larvas previamente alimentadas com dieta laboratorial) e a linha verde representa o grupo PE+ PE (filme de PE que ficou exposto ao homogenato de larvas previamente alimentadas com PE por 7 dias). (b) Percentual de biomineralização: linha vermelha representa o grupo ração + PE (filme de PE que ficou exposto ao homogenato de larvas previamente alimentadas com dieta laboratorial) e a linha verde representa o grupo PE+ PE (filme de PE que ficou exposto ao homogenato de larvas previamente alimentadas com PE por 7 dias).

2.4 PADRONIZAÇÃO DE COQUETEL ANTIMICROBIANO PARA REDUZIR/ELIMINAR MICROBIOTA DAS LARVAS DE *G. mellonella*

A fim de eliminar e/ou reduzir a microbiota das larvas de *G. mellonella* foi padronizado um coquetel antimicrobiano. Para isso, as larvas foram privadas de alimentação por 4 h e então, submetidas a injeção na última *proleg*, duas vezes com intervalo de 12 h de10 µL do coquetel antimicrobiano: 2 mg/mL de ampicilina, 2 mg/mL de canamicina, 2 mg/mL de polimixina B, 2 mg/mL de neomicina, 1 mg/mL de vancomicina, previamente diluídos em água estéril e 1 mg/mL de voriconazol previamente diluído em metanol, conforme protocolo adaptado por Kong et al., 2019. A injeção foi realizada com seringa Hamilton de 10 µL e controles com água estéril sem antimicrobianos foram incluídos. Após 24 h do tratamento com antimicrobianos, as larvas foram imergidas em etanol 70% por 1 min e enxaguadas duas vezes com solução salina estéril, a fim de eliminar a microbiota externa presente no corpo da larva. Foram avaliadas (i) todo o conteúdo corporal, através de um homogenato e (ii) o intestino da larva. O material foi adicionado em tubos eppendorf estéreis contendo 1 ml de solução PBS pH 7, estéril. Os tubos foram vortexados por 1 min e foi realizada uma diluição seriada para permitir a contagem de colônias por ml. A eficácia na redução/eliminação da microbiota foi avaliada por cultura em meio ságar-sangue, para permitir o desenvolvimento de colônias bacterianas, e ágar extrato de levedura-peptona-dextrose (YPD), para permitir o desenvolvimento de colônias fúngicas, após 24 hs de incubação a 28 °C.

Os resultados do plaqueamento em ágar sangue e ágar YPD são expostos na Figura 14, e a contagem das unidades formadoras de colônias por ml estão dispostas na Tabela 3. O coquetel antimicrobiano foi eficiente na ação de redução e/ou eliminação da microbiota larval e intestinal, isso pois houve uma redução significativa quando analisamos as médias de UFC/ml do grupo de amostras que foram tratadas com esse coquetel antimicrobiano em relação a UFC/ml do grupo de amostras que foram tratas com água estéril, utilizadas como um controle.

Esses resultados são interessantes uma vez que a partir deles conseguimos avaliar a rota de ingestão de PE por larvas de *G. mellonella* (proposta no item de discussão geral) na ausência da microbiota. Assim, nossas perspectivas são a retomada de alguns experimentos, como a análise físico-química dos sítios corporais da larva com alimentações distintas (PE ou dieta laboratorial), incluindo grupo tratados com o coquetel antimicrobiano já padronizado. Com esses futuros resultados, esperamos maior suporte para avaliar o papel da microbiota de larvas de *G. mellonella* na biodegradação do PE.

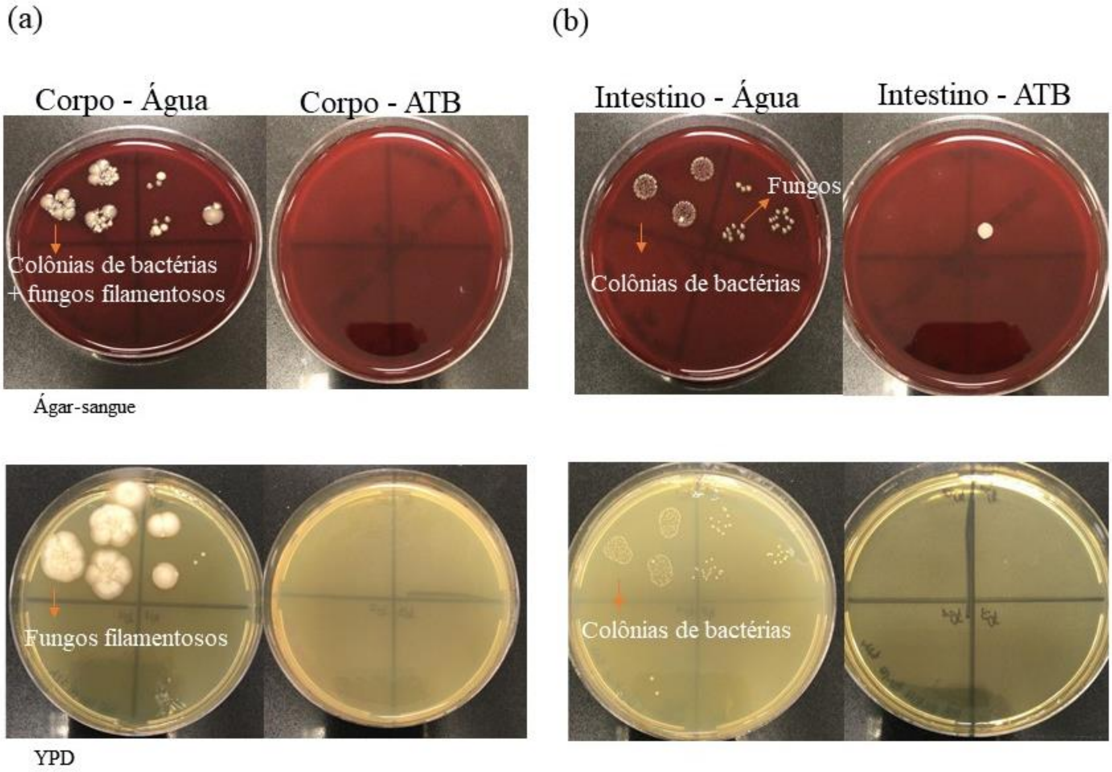


Figura 14: Determinação da presença de micro-organismos cultiváveis em sítios corporais das larvas de *G. mellonella* tratadas com (i) coquetel antimicrobiano e (ii) com água estéril (controle): (a) plaqueamento do homogenato total das larvas em meios de cultura ágar sangue e ágar YPD e incubação por 24 h a 28 °C e (b) plaqueamento do conteúdo intestinal das larvas em meios de cultura ágar sangue (contendo uma colônia de micro-organismo não identificado, em um “ponto” branco) e ágar YPD e incubação por 24 h a 28 °C.

Tabela 3: Resultados em UFC/ml do plaqueamento do corpo e do conteúdo intestinal de larvas de *G. mellonella* após tratamento com água destilada ou com coquetel antimicrobiano e tempo de incubação de 24 h

Tratamento	Meio	Amostra	Média (UFC/mL)	Desvio Padrão
Água	Ágar-sangue	Corpo	6330	5770
		Intestino	6330	5770
	YPD	Corpo	7000	2650
		Intestino	50000	3610
ATB	Ágar-sangue	Corpo	0	0
		Intestino	200	100
	YPD	Corpo	33,3	57,7
		Intestino	167	115

2.5 ANÁLISES FISÍCO-QUÍMICAS

Essas análises foram realizadas em amostras de filmes de PE tratadas com um homogenato de larvas, que previamente, foram mantidas com diferentes alimentações (item 2.2).

2.5.1 Espectroscopia de Infravermelho com Transformada de Fourier (FTIR)

Foi utilizado o equipamento Perkin Elmer Instruments Spectrum One FT-IR Spectrometer (USA) e a aquisição do espectro foi realizada com o acessório de amostras UATR (acessório de refletância atenuada total universal) no intervalo de número de onda de 2000 a 600 cm^{-1} disponível no Laboratório de Organometálicos e Resinas (LOR) da PUCRS.

2.5.2 Microscopia Eletrônica de Varredura com Emissão de Campo (MEV-FEG)

As amostras foram metalizadas com ouro e as imagens de MEV- FEG foram obtidas em equipamento FEI Inspect F50 no modo de elétrons secundários (SE) disponível no Laboratório Central de Microscopia e Microanálise (LabCEMM) da PUCRS.

3 DISCUSSÃO GERAL

O Capítulo I desse trabalho traz informações importantes sobre as características físico-químicas dos termoplásticos mais utilizados no mundo, abordando também dados importantes sobre o gerenciamento de resíduos desses materiais, mostrando a importância do estudo de novas metodologias, como (bio)degradação, para esse tipo de tratamento. Além disso, são apresentadas técnicas utilizadas para detecção dessa degradação plástica. Assim, conseguimos compreender esse processo, o qual ocorre através de 4 etapas (discutidas com detalhamento nesse Capítulo I), e também elucidar como é realizada a detecção desse procedimento em materiais plásticos.

Importantemente, o Capítulo II explora a capacidade de larvas de inseto em ingerir e biodegradar diferentes tipos de plásticos. Foi possível avaliar a relevância desse assunto, tendo em vista a ascensão de artigos com tema publicados nesses últimos anos. Além disso, nota-se que as técnicas estão cada vez mais sofisticadas de modo a responder perguntas importantes: (i) quais metabólitos são gerados a partir da biodegradação de plásticos por larvas de insetos, (ii) quais enzimas estão envolvidas nesse processo e (iii) qual o papel da microbiota nessa biodegradação.

Ainda no Capítulo II, pode-se observar que há 13 estudos que relatam a biodegradação de plásticos por larvas de *G. mellonella*. Dentre esses trabalhos, 6 deles relatam a importância da microbiota intestinal desse animal no processo de metabolização do plástico (Tabela II e III, Capítulo II). No entanto, verifica-se que não há consenso sobre espécies e gêneros de micro-organismos que podem auxiliar na biodegradação do plástico, tampouco das enzimas e sobre a etapa de ação na cascata de degradação. No entanto, sabe-se que a ação microbiana não poderia ser totalmente essencial para essa atividade, ou seja, o animal através de seu próprio organismo possui capacidade de ingerir e biofragmentar o polímero (KONG et al., 2019). Neste ponto, a ação de uma possível microbiota das glândulas salivares de *G. mellonella* ainda precisa ser avaliada para o melhor compreendimento sobre a ação desse sítio corporal na biodegradação de PE (PEYDAEI et al., 2020). Por isso, mostra-se importante a elucidação de como ocorre esse processo e a continuidade de trabalhos com essa temática e assim, o Capítulo III desse trabalho mostra experimentos conduzidos por nosso grupo de pesquisa, a fim de confirmar a biodegradação de PE por larvas de *G. mellonella* e compreender como esse processo ocorre.

No Capítulo IV são abordadas as metodologias utilizadas para padronização da utilização de larvas de *G. mellonella* para biodegradação desse plástico. Portanto, houve a tentativa de reprodutibilidade da metodologia descrita no trabalho pioneiro sobre a biodegradação de PE por larvas de *G. mellonella,* o qual fazia uso de homogenatos de larvas. As tentativas de reprodução desta técnica, seguida de diversas etapas de otimização, não forneceram resultados condizentes com os relatados no trabalho de Bombelli e colaboradores (2017). Este trabalho sofreu fortes críticas pela comunidade científica visto que seus dados de FTIR não eram suficientes para comprovar o processo de biodegradação do plástico pelas larvas (Weber et al., 2017) e pela falta de reprodutibilidade dos ensaios (Billen et al., 2020), como foi relatado.

Desse modo, a estratégia foi modificada e passamos a trabalhar com larvas vivas e com a análise de sítios corporais da larva, como a hemolinfa, o casulo, e as fezes para compreender o que ocorre com o polímero após a ingestão pelas larvas, e a partir disso, constatar uma biodegradação do plástico. No Capítulo III dessa dissertação está apresentada a metodologia utilizada para a proposta de uma rota de digestão do PE por larvas de *G. mellonella*. Nesse capítulo, pode-se avaliar que um grupo de larva com uma alimentação com PE, por 7 dias, possui metabólitos com estruturas ricas em ligações C-H-N que não existem nas análises para um grupo de larvas alimentadas com uma dieta laboratorial. Além disso, observa-se mudanças nas estruturas físico-químicas (através de análises de LC-MS, FTIR, XPS e MEV-FEC-EDS) dos casulos e das fezes de larvas alimentadas com PE, por 7 dias, em relação ao grupo de larvas com uma dieta laboratorial, o que sugere novamente uma biodegradação do polímero. No entanto, essa biodegradação parece ocorrer de forma parcial, uma vez que análises de FTIR, EDS e microscopia ótica revelam que as fezes do animal que passou por uma alimentação de PE, excreta esse polímero em sua forma inteira (sem quebras na cadeia polimérica). Portanto, a proposta para a rota de digestão do PE utilizando larvas de *G. mellonella* está apresentada na Figura 1.

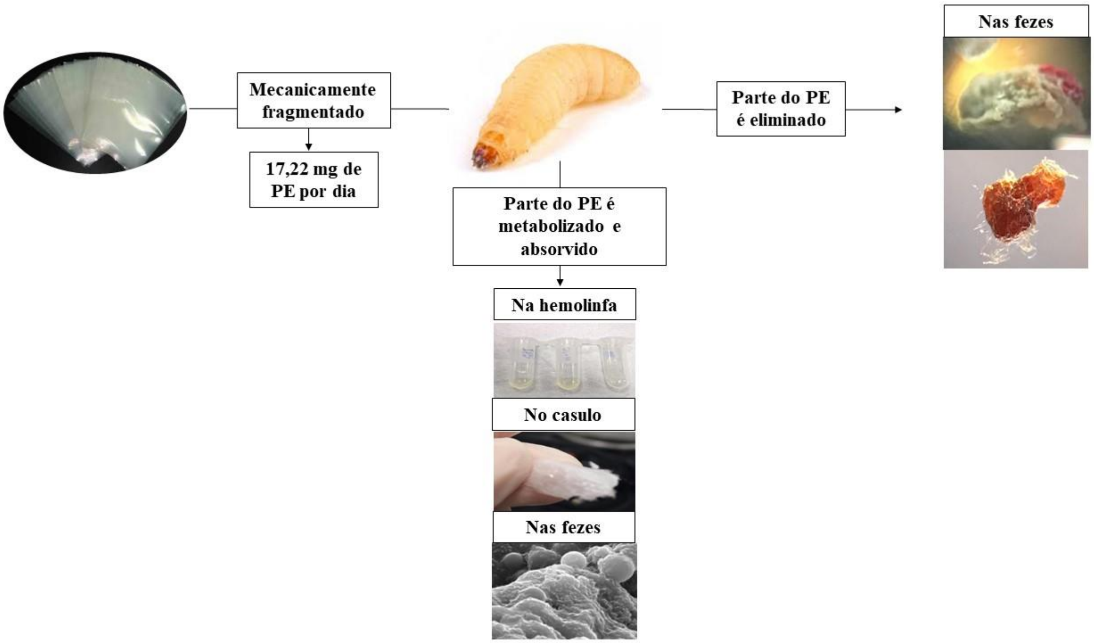


Figura 1: Esquema proposto sobre o processo de biodegradação de PE por larvas de *G. mellonella* a partir dos resultados obtidos no presente estudo.

De acordo com os nossos resultados e de outros dois estudos: Kong et al., (2019) e LeMoine et al., (2020), sugerimos um sinergismo entre o organismo de larva de *G. mellonella* e sua microbiota para a degradação do PE. No entanto, a cascata de degradação não é bem compreendida. Em um estudo realizado por Kong e colaboradores (2019), foi verificada a capacidade das larvas de *G. mellonella* em realizar a degradação de hidrocarbonetos de cadeia longa usando técnicas de genoma e transcriptoma de alta qualidade. Como resultado, os autores propõem que esses hidrocarbonetos sejam, em primeira instância, despolimerizados ou hidrolisados em ácidos graxos de cadeia longa pelas próprias enzimas do animal. O microbioma intestinal desempenha um papel complementar na realização da curta degradação dos ácidos graxos da cadeia.

Ainda nesse estudo, a análise por GC-MS mostrou pirrolidina, palmidrol e 9-octadecenamida como ácidos graxos de cadeia longa, no grupo de larvas alimentadas com cera de abelha junto à microbiota intestinal. Esses metabólitos não foram encontrados nas fezes desse grupo de larvas e isso não foi afetado no grupo de larvas sem a microbiota intestinal. Isso indica que os alcanos foram metabolizados em ambas as condições: com e sem a microbiota intestinal. Esses achados mostram uma abordagem metabólica semelhante para a biodegradação do PE.

Por outro lado, no modelo hipotético proposto por LeMoine e colaboradores (2020), há um processamento precoce do LDPE pela microbiota intestinal de *G. mellonella*, usando enzimas-chave (como CYP, ADH, ALDH) para quebrar cadeias de alcano em aldeídos e depois em carboxilatos. Mais tarde, esses ácidos graxos são armazenados e também podem sofrer oxidação beta e o ciclo de Krebs para produzir energia metabólica. Portanto, podemos concluir que não há consenso sobre como ocorrem os primeiros estágios de degradação: por enzimas do próprio animal ou microbianas. Assim, mais estudos são necessários para demonstrar o papel da microbiota das larvas na biodegradação do plástico e como a cascata ocorre nas larvas de *G. mellonella*. Isso indica a necessidade de estudos com maiores detalhamentos para esse processo, e assim, indicamos a continuidade do presente trabalho, com perspectivas exploradas no item 5.

4 CONCLUSÕES

- Dados da literatura mostram a importância de novas metodologias para o tratamento de resíduos plásticos pois os procedimentos usualmente utilizados para o gerenciamento desses resíduos possuem limitações;

- Foram compilados em um artigo de revisão crítica 46 estudos, publicados até dezembro de 2020, que avaliam a capacidade de 9 diferentes espécies de larvas de inseto em consumir e/ou biodegradar plásticos;

- Uma única larva de *G. mellonella* com peso de 130 mg consegue consumir 17,22 mg de PE por dia e, apesar das larvas perderem peso com essa alimentação, elas conseguem realizar metamorfose para estágio de pupa e mariposa;

- Metodologias utilizando homogenato de larvas de *G. mellonella* para o estudo da biodegradação de PE trazem resultados inconclusivos na determinação desse processo;

- Distintos sítios corporais de larvas de *G. mellonella* alimentadas com PE apresentam, características físico-químicas que não foram encontradas nos sítios corporais de larvas alimentadas com dieta laboratorial, o que sugere a metabolização desse plástico;

- A análise de respirometria (produção de CO_2) não demonstra o processo de biomineralização aeróbica do PE por larvas de *G. mellonella*, no entanto o animal pode capturar esse gás equilibrar o pH intestinal;

- Foi proposta uma rota de digestão de PE por larvas de *G. mellonella*;

- A análise do sequenciamento de região do gene *rRNA 16S* bacteriano indica que a alimentação com PE promove mudanças na microbiota em comparação ao grupo de larvas alimentadas com dieta laboratorial.

5 PERSPECTIVAS

Como perspectivas deste estudo, tem-se:

- Quantificar quanto do PE ingerido por larvas de *G. mellonella* é eliminado nas fezes. Este dado fornecerá a quantidade de PE não biodegradado e, por diferença, a taxa de metabolização de PE por larvas de *G. mellonella*.

- Análisar com técnicas de LC-MS, FTIR e MEV-FEG-EDS as amostras de sítios corporais de larvas tratadas com o coquetel de antimicrobianos (com redução/eliminação de microbiota) e alimentadas com (i) dieta laboratorial e (ii) polietileno por 7 dias. Assim será possível evidenciar o papel da microbiota na biodegrdação de PE por larvas de *G. mellonella*.

- Ampliar o número de amostras em novo experimento de sequenciamento metagenômico do gene *16S rRNA*, para que nossos resultados tenham maior significância em termos de quantidades de amostras;

- Publicar os artigos de revisão (Capítulo I e Capítulo II) e artigo original (Capítulo III).

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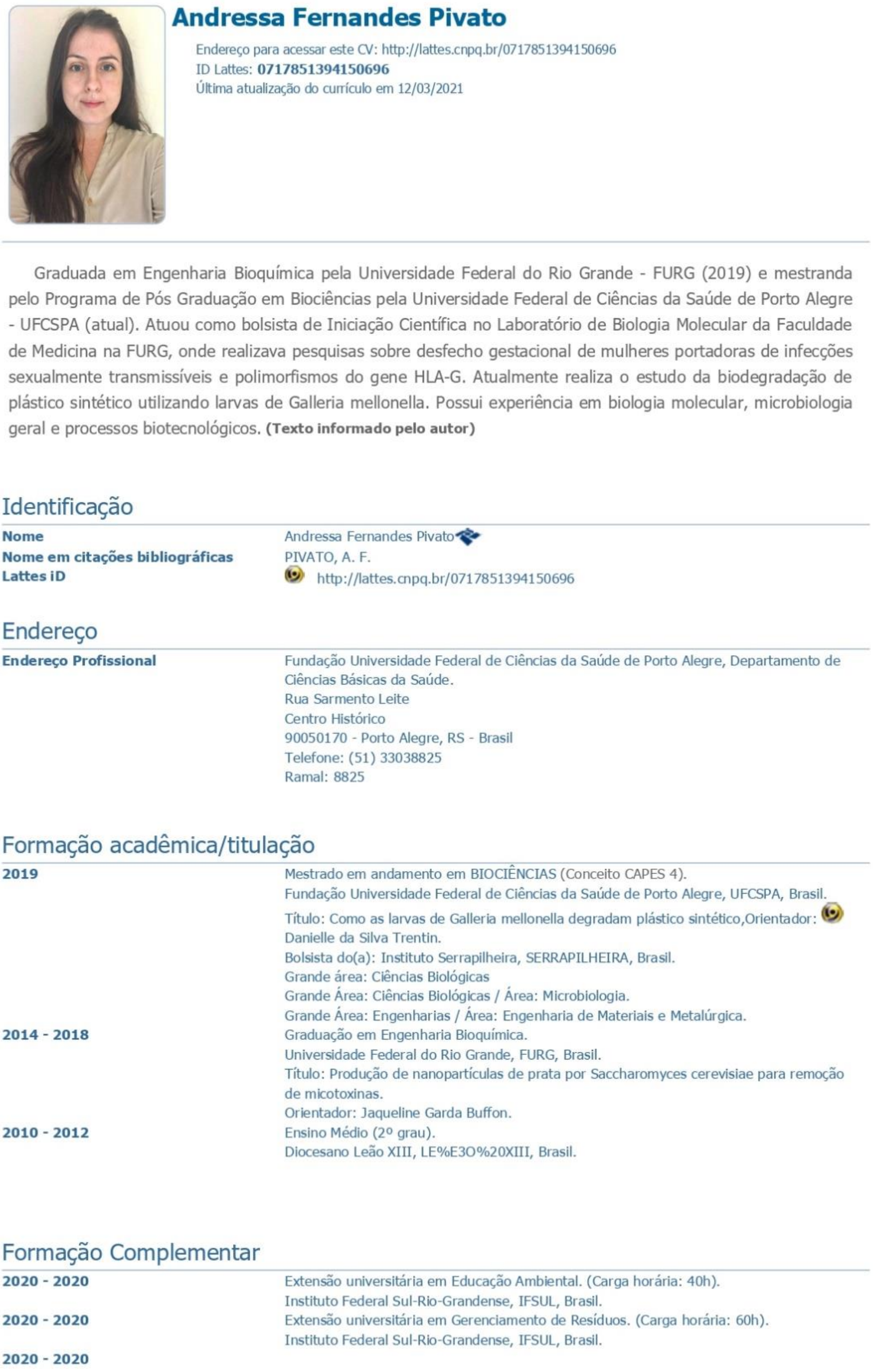
ANEXO I: Currículo lattes da aluna Andressa Pivato



Andressa Fernandes Pivato

Endereço para acessar este CV: http://lattes.cnpq.br/0717851394150696
ID Lattes: **0717851394150696**
Última atualização do currículo em 12/03/2021

Graduada em Engenharia Bioquímica pela Universidade Federal do Rio Grande - FURG (2019) e mestranda pelo Programa de Pós Graduação em Biociências pela Universidade Federal de Ciências da Saúde de Porto Alegre - UFCSPA (atual). Atuou como bolsista de Iniciação Científica no Laboratório de Biologia Molecular da Faculdade de Medicina na FURG, onde realizava pesquisas sobre desfecho gestacional de mulheres portadoras de infecções sexualmente transmissíveis e polimorfismos do gene HLA-G. Atualmente realiza o estudo da biodegradação de plástico sintético utilizando larvas de Galleria mellonella. Possui experiência em biologia molecular, microbiologia geral e processos biotecnológicos. **(Texto informado pelo autor)**

Identificação

Nome	Andressa Fernandes Pivato
Nome em citações bibliográficas	PIVATO, A. F.
Lattes iD	http://lattes.cnpq.br/0717851394150696

Endereço

Endereço Profissional	Fundação Universidade Federal de Ciências da Saúde de Porto Alegre, Departamento de Ciências Básicas da Saúde. Rua Sarmento Leite Centro Histórico 90050170 - Porto Alegre, RS - Brasil Telefone: (51) 33038825 Ramal: 8825

Formação acadêmica/titulação

2019	Mestrado em andamento em BIOCIÊNCIAS (Conceito CAPES 4). Fundação Universidade Federal de Ciências da Saúde de Porto Alegre, UFCSPA, Brasil. Título: Como as larvas de Galleria mellonella degradam plástico sintético,Orientador: Danielle da Silva Trentin. Bolsista do(a): Instituto Serrapilheira, SERRAPILHEIRA, Brasil. Grande área: Ciências Biológicas Grande Área: Ciências Biológicas / Área: Microbiologia. Grande Área: Engenharias / Área: Engenharia de Materiais e Metalúrgica.
2014 - 2018	Graduação em Engenharia Bioquímica. Universidade Federal do Rio Grande, FURG, Brasil. Título: Produção de nanopartículas de prata por Saccharomyces cerevisiae para remoção de micotoxinas. Orientador: Jaqueline Garda Buffon.
2010 - 2012	Ensino Médio (2º grau). Diocesano Leão XIII, LE%E3O%20XIII, Brasil.

Formação Complementar

2020 - 2020	Extensão universitária em Educação Ambiental. (Carga horária: 40h). Instituto Federal Sul-Rio-Grandense, IFSUL, Brasil.
2020 - 2020	Extensão universitária em Gerenciamento de Resíduos. (Carga horária: 60h). Instituto Federal Sul-Rio-Grandense, IFSUL, Brasil.
2020 - 2020	

	Extensão universitária em Planejamento Ambiental. (Carga horária: 40h). Instituto Federal Sul-Rio-Grandense, IFSUL, Brasil.
2020 - 2020	Analisador de Carbono Orgânico Total: Princípios e Aplicações. (Carga horária: 1h). Shimadzu do Brasil Comércio, SBC, Brasil.
2020 - 2020	How to write a Review paper. (Carga horária: 2h). Fundação Universidade Federal de Ciências da Saúde de Porto Alegre, UFCSPA, Brasil.
2020 - 2020	BIOSSEGURANÇA E PESQUISA EM TEMPOS DA COVID-19. (Carga horária: 2h). Fundação Universidade Federal de Ciências da Saúde de Porto Alegre, UFCSPA, Brasil.
2020 - 2020	Princípios de Microdureza. (Carga horária: 1h). Shimadzu do Brasil Comércio, SBC, Brasil.
2020 - 2020	Você conhece os ensaios necessários para atendimento da Diretiva RoHS?. (Carga horária: 1h). Shimadzu do Brasil Comércio, SBC, Brasil.
2020 - 2020	Módulo 1 - Introdução a Espectrometria de Massas. (Carga horária: 3h). Sociedade Brasileira de Espetrometria de Massas, BRMASS, Brasil.
2020 - 2020	Transforme sua pesquisa com illumina Next Generation Sequencing (NGS). (Carga horária: 2h). Fundação Universidade Federal de Ciências da Saúde de Porto Alegre, UFCSPA, Brasil.
2020 - 2020	Primeiros Socorros da Liga de Emergência e Trauma na modalidade EAD. (Carga horária: 16h). Fundação Universidade Federal de Ciências da Saúde de Porto Alegre, UFCSPA, Brasil.
2020 - 2020	Fundamentos de LCMS - Módulo I. (Carga horária: 1h). Shimadzu do Brasil Comércio, SBC, Brasil.
2020 - 2020	Princípios e Aplicações de Cromatografia Gasosa - Módulo I. (Carga horária: 1h). Shimadzu do Brasil Comércio, SBC, Brasil.
2020 - 2020	Tecnologias de Caracterização de Polímeros e Compósitos. (Carga horária: 1h). Shimadzu do Brasil Comércio, SBC, Brasil.
2020 - 2020	Fundamentos de ICPMS. (Carga horária: 1h). Shimadzu do Brasil Comércio, SBC, Brasil.
2020 - 2020	Curso Básico de HPLC - Módulo II. (Carga horária: 1h). Shimadzu do Brasil Comércio, SBC, Brasil.
2020 - 2020	Otimizando sua pesquisa através dos ensaios de Multiplex com o Luminex 200. (Carga horária: 1h). Merck Sharp & Dohme Farmacêutica, MSD, Brasil.
2019 - 2019	Sequenciamento de Nova Geração. (Carga horária: 3h). Fundação Universidade Federal de Ciências da Saúde de Porto Alegre, UFCSPA, Brasil.
2019 - 2019	Comunicação com Foco Organizacional. (Carga horária: 20h). SESI - Departamento Regional do Espírito Santo, SESI, Brasil.
2018 - 2018	Extensão universitária em II Trilha Empreendedora. (Carga horária: 40h). Universidade Federal do Rio Grande, FURG, Brasil.
2018 - 2018	Extensão universitária em Comunicação Efetiva. (Carga horária: 12h). SESI - Departamento Regional do Espírito Santo, SESI, Brasil.
2018 - 2018	Extensão universitária em Redação Administrativa. (Carga horária: 10h). SESI - Departamento Regional do Espírito Santo, SESI, Brasil.
2018 - 2018	Oratória. (Carga horária: 4h). Universidade Federal do Rio Grande, FURG, Brasil.
2018 - 2018	Criação e atualização do Currículo Lattes. (Carga horária: 3h). Universidade Federal do Rio Grande, FURG, Brasil.
2018 - 2018	Engenharia de Segurança. (Carga horária: 4h). Universidade Federal do Rio Grande, FURG, Brasil.
2018 - 2018	Visita Técnica do II Fórum MACando. (Carga horária: 12h). Universidade Federal do Rio Grande, FURG, Brasil.
2017 - 2017	Gestão de Qualidade nos Processos Produtivos. (Carga horária: 2h). Universidade Federal do Rio Grande, FURG, Brasil.
2017 - 2017	Visita Técnica Cervejaria Brasserie 35. (Carga horária: 2h). Universidade Federal do Rio Grande, FURG, Brasil.
2017 - 2017	Visita Técnica Três Tentos Agroindustrial. (Carga horária: 2h). Universidade Federal do Rio Grande, FURG, Brasil.
2016 - 2016	Curso de Noções Básicas de CAD. (Carga horária: 12h). Universidade Federal do Rio Grande, FURG, Brasil.
2016 - 2016	CURSO DE EXCEL. (Carga horária: 9h). Universidade Federal do Rio Grande, FURG, Brasil.
2014 - 2014	Segurança do Trabalho no Laboratório. (Carga horária: 4h). Universidade Federal do Rio Grande, FURG, Brasil.
2007 - 2011	Inglês. Athus Franchising, ATHUS, Brasil.

Atuação Profissional

Fundação Universidade Federal de Ciências da Saúde de Porto Alegre, UFCSPA, Brasil.

Vínculo institucional	
2019 - Atual	Vínculo: Bolsista, Enquadramento Funcional: Estudante de mestrado em biociências, Carga horária: 40, Regime: Dedicação exclusiva.
Outras informações	Bolsista de mestrado pelo Programa de Pós Graduação em Biociências, desenvolvendo o projeto intitulado "Como as larvas de Galleria mellonella degradam plástico sintético", com a orientação da professora Drª Danielle Trentin.

Universidade Federal do Rio Grande, FURG, Brasil.

Vínculo institucional	
2018 - 2018	Vínculo: Bolsista, Enquadramento Funcional: Monitora Voluntária, Carga horária: 12
Outras informações	Bolsista de monitoria voluntária na disciplina de Bioquímica I, para o curso de Engenharia Bioquímica na Universidade Federal de Rio Grande (FURG), sob a orientação de Susan Hartwing Duarte.
Vínculo institucional	
2018 - 2018	Vínculo: Bolsista, Enquadramento Funcional: Monitora Voluntária, Carga horária: 12
Outras informações	Bolsista de monitoria voluntária na disciplina de Microbiologia I, para o curso de Engenharia Bioquímica na Universidade Federal de Rio Grande (FURG), sob a orientação de Susan Hartwing Duarte.
Vínculo institucional	
2017 - 2018	Vínculo: Bolsista, Enquadramento Funcional: Iniciação Científica, Carga horária: 12
Outras informações	Bolsista de Iniciação Científica pelo CNPq, no Laboratório de Biologia Molecular da Faculdade de Medicina (FAMED) na Universidade Federal do Rio Grande (FURG).
Vínculo institucional	
2017 - 2018	Vínculo: Colaborador, Enquadramento Funcional: Integrante do Diretório Acadêmico, Carga horária: 12
Outras informações	Integrante do Diretório Acadêmico da Engenharia Bioquímica, atuando como 1ª Tesoureira, na Universidade Federal de Rio Grande (FURG).
Vínculo institucional	
2017 - 2017	Vínculo: Bolsista, Enquadramento Funcional: Bolsista/Monitor, Carga horária: 12
Outras informações	Bolsista de pesquisa no projeto 0406/2016 - Prevalência de Infecções Sexualmente Transmissíveis (IST) na Placenta de Colostro de Gestantes e Incidência de IST no Cordão Umbilical de Neonatos Atendidos no Hospital Universitário (HU-FURG), Rio Grande - RS da unidade FAMED - Faculdade de Medicida, na Universidade Federal do Rio Grande (FURG), sob a coordenação de Ana Maria Barral de Martinez, no laboratório de Biologia Molecular.
Vínculo institucional	
2017 - 2017	Vínculo: Bolsista, Enquadramento Funcional: Monitor, Carga horária: 4
Outras informações	Monitora Voluntária (PQA) na Escola de Química e Alimentos (EQA) na disciplina de Fundamentos de Engenharia Bioquímica I, sob a orientação da professora Michele da Rosa Andrade Zimmermann de Souza.
Vínculo institucional	
2017 - 2017	Vínculo: Bolsista, Enquadramento Funcional: Monitor, Carga horária: 4
Outras informações	Monitora Voluntária (PQA) na Escola de Química e Alimentos (EQA) na disciplina de Reatores Bioquímicos I, sob a orientação da professora Michele da Rosa Andrade Zimmermann de Souza.
Vínculo institucional	
2016 - 2017	Vínculo: Bolsista, Enquadramento Funcional: Bolsista Voluntário, Carga horária: 12
Outras informações	Bolsista de pesquisa no projeto 0154/2017 - Prevalência de Infecções Sexualmente Transmissíveis (IST) na Placenta de Colostro de Gestantes e Incidência de IST no Cordão Umbilical de Neonatos Atendidos no Hospital Universitário (HU-FURG), Rio Grande - RS da unidade FAMED - Faculdade de Medicida, na Universidade Federal do Rio Grande (FURG), sob a orientação de Vanusa Pousada da Hora, no laboratório de Biologia Molecular.

Schutter, SCHUTTER, Brasil.

Vínculo institucional	
2018 - 2018	Vínculo: Estagiária, Enquadramento Funcional: Auxiliar laboratorial, Carga horária: 40
Outras informações	Auxiliar de Analista de Laboratório para a execução de análises físico-químicas do grão, farelo e óleo de soja (análises de fibras, umidade, cinzas, proteínas, gorduras, acidez, pH, entre outras). Auxilio na execução de análises físico-químicas em fertilizantes (matéria-prima e mistura) e na elaboração de laudos técnicos acerca da qualidade dos produtos avaliados.

Projetos de pesquisa

2019 - Atual	Como as larvas de Galleria mellonella degradam plástico sintético? Descrição: A eliminação de resíduos plásticos é uma importante questão ambiental que a nossa sociedade enfrenta hoje. Polietileno (PE), presente em sacos plásticos e

embalagens, é um material inerte amplamente resistente à biodegradação e representa uma ameaça ecológica crescente para a vida selvagem terrestre e marinha. O primeiro relato de consumo de PE utilizando larvas de Galleria mellonella foi reportada em 2017, no entanto, não está claro se a atividade provém de enzimas presentes no corpo das larvas ou da atividade enzimática da microbiota. Esta proposta visa entender o processo de degradação natural do plástico por G. mellonella, o que poderia fornecer soluções para gerenciar o problema deste resíduo. Para responder a essa grande questão, propomos análises culturais e metagenômicas da microbiota, análises físico-químicas do PE e em sítios corporais da larva para comprovar biodegradação deste polímero..
Situação: Em andamento; Natureza: Pesquisa.
Alunos envolvidos: Graduação: (1) / Mestrado profissional: (1) / Doutorado: (5) .

Integrantes: Andressa Fernandes Pivato - Integrante / Danielle da Silva Trentin - Coordenador / Adriana Seixas - Integrante / Janira Prichula - Integrante / Gabriel Vieira Soares - Integrante / Denise Bretan da Silva - Integrante / Gabriela Messias Miranda - Integrante.

2016 - 2018

Prevalência de Infecções Sexualmente Transmissíveis (IST) na Placenta e Colostro de Gestantes e Incidência de IST em Cordão Umbilical de Neonatos Atendidos no Hospital Universitário (HU-FURG) em Rio Grande
Descrição: As Infecções Sexualmente Transmissíveis (IST) estão entre os problemas de saúde pública mais frequentes em todo o mundo. As mulheres têm maiores índices de prevalência e consequências mais sérias do que os homens frente às IST, com consequente risco de transmissão vertical da infecção. Estudos têm demonstrando que essas IST podem ser transmitidas de maneira não-sexual, como pela via transversal pela placenta e/ou colostro e/ou sangue do cordão umbilical. Este modo de transmissão não-sexual pode ter um impacto importante sobre as estratégias de controle, vacinação e manejo clínico de mulheres infectadas antes ou durante a gravidez, porém ele é pouco estudado. Dessa forma, esse projeto tem como objetivo estimar a prevalência desses agentes de IST na biópsia da placenta e no colostro de gestantes atendidas no Hospital Universitário Dr. Miguel Riet Corrêa Jr. HU/FURG na cidade do Rio Grande e a incidência daqueles agentes e desse composto no sangue do cordão umbilical dos neonatos dessas gestantes e os fatores de risco associados..
Situação: Concluído; Natureza: Pesquisa.
Alunos envolvidos: Graduação: (5) / Doutorado: (4) .

Integrantes: Andressa Fernandes Pivato - Integrante / Michele Tornatore - Integrante / Ana Maria Barral de Martinez - Coordenador / Vanusa Pousada da Hora - Integrante.

Projetos de extensão

2018 - 2018

Uso da ferramenta estatística aplica à apresentação de dados experimentais - 2ª Edição
Descrição: Os discentes de graduação do curso de Engenharia de Alimentos e Engenharia Bioquímica da FURG apresentam em seus Quadros de Sequência Lógica as disciplinas Probabilidade e Estatística. No entanto, há uma demanda por parte destes estudantes em como utilizar programas estatísticos, ou ainda como tratar os dados experimentais e apresentá-los, seja em trabalhos técnico-científicos na academia, como relatórios, ou apresentação em eventos e similares, ou em estágios supervisionados no âmbito industrial ou em institutos de pesquisas. Neste sentido, a realização deste curso vem ao encontro desta necessidade, suprindo através de estudos de casos com dados reais e integrando a utilização de um programa computacional para tratamento estatístico, apresentação e discussão destes dados. A análise estatística de dados experimentais é uma abordagem aplicada em diferentes disciplinas na graduação da engenharia. Portanto, este curso de ensino visa possibilitar aos alunos um maior suporte e familiarização com o tema. Além disso, a realização desta ação promove uma atividade optativa extracurricular, através de um curso de ensino e formação acadêmica aos discentes que necessitam de uma carga horária em atividades complementares para integralização de seus cursos de graduação, nos atuais Quadros de Sequência Lógica..
Situação: Concluído; Natureza: Extensão.
Alunos envolvidos: Graduação: (10) / Doutorado: (2) .

Integrantes: Andressa Fernandes Pivato - Integrante / Luisa Sala - Integrante / Janaína Fernandes de Medeiros Burkert - Coordenador.

2017 - 2018

Grupo de Trabalho Tutorial em Engenharia Bioquímica
Descrição: O Grupo de Trabalho Tutorial em Engenharia Bioquímica tem por objetivo integrar docentes, discentes e servidores técnico-administrativos, para o planejamento e execução de atividades de ensino, pesquisa e extensão, que contribuirão para a formação profissional qualificada no curso de Engenharia Bioquímica..
Situação: Concluído; Natureza: Extensão.
Alunos envolvidos: Graduação: (8) / Mestrado acadêmico: (5) / Doutorado: (3) .

Integrantes: Andressa Fernandes Pivato - Integrante / Allana Arcos Comitre - Integrante /

Susan Hartwing Duarte - Coordenador / Lucielen Oliveira dos Santos - Integrante / Duna Joanol da Silveira Mastrantonio - Integrante / Bruna Abel Packowski - Integrante / Giuliana Fernandez - Integrante.

Idiomas

Português	Compreende Bem, Fala Bem, Lê Bem, Escreve Bem.
Espanhol	Compreende Razoavelmente, Fala Pouco, Lê Razoavelmente, Escreve Pouco.
Inglês	Compreende Bem, Fala Razoavelmente, Lê Bem, Escreve Bem.

Prêmios e títulos

2020	Destaque na categoria mérito popular no evento: V Biosciences Meeting ? Crossing Borders, Universidade Federal de Ciências da Saúde de Porto Alegre.
2018	Destaque da II Trilha Empreendedora - (re)link, FURG.

Produções

Produção bibliográfica

Trabalhos completos publicados em anais de congressos

1. OLIVEIRA, F. K. ; **PIVATO, A. F.** ; COMITRE, A. A. ; SIBAJA, K. V. M. ; SANTOS, L. O. ; BUFFON, J. G. . Remoção de aflatoxina B1 por nanopartículas de prata sintetizadas em meio fermentado do cultivo de Saccharomyces cerevisiae. In: 7° Simpósio de Segurança Alimentar, 2020. 7° Simpósio de Segurança Alimentar, 2020.

Resumos expandidos publicados em anais de congressos

1. LUQUEZ, K. S. ; Tornatore, M. ; **PIVATO, A. F.** ; Jardim, F. F. ; KRAUS, F. ; Avila, E. C. ; Martinez, A. M. B. ; Gonçalves, C. V. ; HORA, V. P. . OCORRÊNCIA DE INFECÇÃO VIRAL E DISTÚRBIOS HIPERTENSIVOS NA GRAVIDEZ EM UMA POPULAÇÃO DO EXTREMO SUL DO BRASIL. In: XVII Mostra de Produção Universitária, 2018, Rio Grande. 27ª Congresso de Iniciação Científica - 17ª MPU, 2018.

2. COMITRE, A. A. ; **PIVATO, A. F.** ; MASTRANTONIO, D. J. S. ; FERREIRA, C. F. J. ; PACKWSKI, B. A. ; HARTWING, S. D. ; SANTOS, L. O. . PERCEPÇÃO DOS ESTUDANTES DE ENGENHARIA BIOQUÍMICA SOBRE ATIVIDADES EXTRACURRICULARES REALIZADAS PELO GRUPO DE TRABALHO TUTORIAL EM ENGENHARIA BIOQUÍMICA. In: XVII Mostra de Produção Universitária, 2018, Rio Grande. 27ª Congresso de Iniciação Científica, 2018.

3. **PIVATO, A. F.**; Tornatore, M. ; LUQUEZ, K. S. ; Martinez, A. M. B. ; Gonçalves, C. V. ; HORA, V. P. . RELATO DE ABORTO ENTRE AS PARTURIENTES ATENDIDAS EM UM HOSPITAL UNIVERSITÁRIO DO EXTREMO SUL DO BRASIL. In: XVII Mostra de Produção Universitária, 2018, Rio Grande. 27ª Congresso de Iniciação Científica - 17ª MPU, 2018.

4. OLIVEIRA, F. K. ; **PIVATO, A. F.** ; COMITRE, A. A. ; SANTOS, L. O. ; BUFFON, J. G. . EFEITO DA PRESENÇA DO ÍON DE PRATA NO CULTIVO DE SACCHAROMYCES CEREVISIAE. In: VI Semana Integrada de Inovação, Ensino, Pesquisa e Extensão, 2018, Pelotas. XXVII Congresso de Iniciação Cientifica - CIC, 2018.

5. COSTA, R. M. ; COMITRE, A. A. ; FERREIRA, C. F. J. ; **PIVATO, A. F.** . PERCEPÇÃO DOS ESTUDANTES, CONHECIMENTO PRÉVIO E GERAÇÃO DE DEMANDA POR ESTUDO NA DISCIPLINA DE INTRODUÇÃO DE BIOPROCESSOS INDUSTRIAIS. In: XVI Mostra de Produção Universitária, 2017, Rio Grande. 26ª Congresso de Iniciação Científica, 2017.

6. **PIVATO, A. F.**; LUQUEZ, K. S. ; MARTINS, D. E. ; SANTANA, C. S. ; MATINEZ, A. M. B. ; POUSADA, V. H. ; Tornatore, M. ; Gonçalves, C. V. . PREMATURIDADE E BAIXO PESO NEONATAL ENTRE PARTURIENTES ATENDIDAS NO EXTREMO SUL DO BRASIL. In: XVI Mostra de Produção Universitária, 2017, Rio Grande. 26ª Congresso de Iniciação Científica, 2017.

7. Pinheiro, M. M. ; Tornatore, M. ; **PIVATO, A. F.** ; Jardim, F. F. ; Avila, E. C. ; Quaresma. A. S. ; Gonçalves, C. V. ; Martinez, A. M. B. . PROJETO IST/PLACENTA, SANGUE DE CORDÃO E COLOSTRO, BANCO DE DNA E EFICÁCIA DE EXTRAÇÃO. In: XVI Mostra de Produção Universitária, 2016, Rio Grande. 26ª Congresso de Iniciação Científica, 2016.

Apresentações de Trabalho

1. **PIVATO, A. F.**. Consumo de degradação de plásticos por larvas de insetos. 2020. (Apresentação de Trabalho/Outra).

2. **PIVATO, A. F.**. Como as larvas de Galleria mellonella degradam plástico sintético. 2019. (Apresentação de Trabalho/Outra).

3. **PIVATO, A. F.**; TRENTIN, D. S. . How Galleria mellonella larvae is capable to degrade synthetic plastic?. 2019. (Apresentação de Trabalho/Outra).

4. **PIVATO, A. F.**; Tornatore, M. ; LUQUEZ, K. S. ; Martinez, A. M. B. ; Gonçalves, C. V. ; HORA, V. P. . RELATO DE ABORTO ENTRE AS PARTURIENTES ATENDIDAS EM UM HOSPITAL UNIVERSITÁRIO DO EXTREMO SUL DO BRASIL. 2018. (Apresentação de Trabalho/Congresso).

5. **PIVATO, A. F.**; Tornatore, M. ; LUQUEZ, K. S. ; MARTINS, D. E. ; SANTANA, C. S. ; Martinez, A. M. B. ; Gonçalves, C. V. ; HORA, V. P. . PREMATURIDADE E BAIXO PESO NEONATAL ENTRE PARTURIENTES ATENDIDAS NO EXTREMO SUL DO BRASIL. 2017. (Apresentação de Trabalho/Congresso).

Produção técnica

Entrevistas, mesas redondas, programas e comentários na mídia

1. **PIVATO, A. F.**. Estágio: o relato da trajetória de acadêmicos. 2018. (Programa de rádio ou TV/Mesa redonda).

Demais tipos de produção técnica

Eventos

Participação em eventos, congressos, exposições e feiras

1. ?Kombucha: o que é, como produzir e quais são seus benefícios??. 2020. (Outra).
2. 1º Seminário Virtual de Engenharia de Alimentos do Mato Grosso do Sul. 2020. (Seminário).
3. A importância da vacinação em saúde pública e as dificuldades e oportunidades de desenvolver vacinas no Brasil. 2020. (Outra).
4. Aplicação das Ferramentas da Qualidade na Produção de Alimentos. 2020. (Outra).
5. Drosophila RS - online. 2020. (Encontro).
6. I Congresso Digital de Nanobiotecnologia e Bioengenharia. 2020. (Congresso).
7. I HealthTech Conference Online. 2020. (Outra).
8. III Seminário de Internacionalização da UFCSPA - "Como podemos melhorar o mundo juntos?. 2020. (Seminário).
9. III Seminário do Dia do Químico. 2020. (Seminário).
10. I International Online Congress of Food Science and Tecnologyy. 2020. (Congresso).
11. I SIMPÓSIO DE BIOQUÍMICA E BIOLOGIA MOLECULAR. 2020. (Simpósio).
12. I WORKSHOP DE BIOTECNOLOGIA DA UNIVERSIDADE FEDERAL DA BAHIA. 2020. (Outra).
13. Nanotecnologia e suas aplicações - IV Semana Acadêmica de Engenharia de Bioprocessos e Biotecnologia. 2020. (Outra).
14. One health e o impacto da química neste processo.Consumo de degradação de plásticos por larvas de insetos. 2020. (Outra).
15. Saúde Mental na Pandemia. 2020. (Outra).
16. V Biosciences Meeting ? Crossing Borders.Larvas que comem plástico: isto é possível?. 2020. (Encontro).
17. Vermicompostagem - IV Semana Acadêmica de Engenharia de Bioprocessos e Biotecnologia. 2020. (Outra).
18. V SIES - Simpósio de Ecologia e Sustentabilidade. 2020. (Simpósio).
19. V Workshop do Grupo de Pesquisa Processos e Produtos na Indústria Química e de Alimentos. 2020. (Outra).
20. 9 Encontro do Núcleo de Estudos em Microbiologia Aplicada.Como as larvas de Galleria mellonella degradam plástico sintético. 2019. (Encontro).
21. Congresso da UFCSPA: conectando saúde e sociedade. 2019. (Congresso).
22. IV Encontro de Biociências.How Galleria mellonella larvae is capable to degrade synthetic plastic?. 2019. (Encontro).
23. Carreira do Engenheiro: uma Experiência Internacional na BRF. 2018. (Outra).
24. Compostos Bioativos e Alimentos Funcionais. 2018. (Outra).
25. IV Simpósio de Engenharia Bioquímica e Bioprocessos. 2018. (Simpósio).
26. Produção de Cerveja. 2018. (Outra).
27. VI Semana Acadêmica da Escola de Química e Alimentos.Cerveja: comparação entre as produções artesanais. 2018. (Oficina).
28. VI Semana Acadêmica da Escola de Química e Alimentos. 2018. (Outra).
29. Competências Interculturais Desenvolvidas em um Período d. 2017. (Oficina).
30. Engenharia Genética. 2017. (Outra).
31. I FÓRUM AMBIENAL. 2017. (Outra).
32. II Congresso de AutoAvaliação. 2017. (Congresso).
33. III Ciclo de Palestras da Engenharia Bioquímica. 2017. (Outra).
34. Justiça Ambiental. 2017. (Oficina).
35. Recrutamento para Empresas. 2017. (Outra).
36. Saúde Mental na Vida Acadêmica. 2017. (Outra).
37. VII Ciclo de Palestras Específica para Engenharia de Alimentos. 2017. (Outra).
38. A importância do trabalho em grupo na Universidade. 2016. (Outra).
39. I Bio Dia Engenharia. 2016. (Outra).
40. III Simpósio de Engenharia Bioquímica e Bioprocessos. 2016. (Simpósio).
41. IV Semana Acadêmica da Escola de Química e Alimentos. 2016. (Outra).
42. Oficina de Criação e Atualização do Currículo Lattes. 2016. (Oficina).
43. Você no Controle de suas Emoções: Na Conquista Dos Sonhos e Objetivos. 2016. (Outra).
44. Workshp Propriedade Intelectual em Ambientes de Inovação. 2016. (Outra).
45. I Ciclo de Palestras da Engenharia Bioquímica. 2015. (Outra).
46. II Ciclo de Palestras da Engenharia Bioquímica. 2015. (Outra).
47. Toxicologia Forense. 2015. (Outra).
48. II Simpósio de Engenharia Bioquímica e Bioprocessos. 2014. (Simpósio).

Organização de eventos, congressos, exposições e feiras

1. **PIVATO, A. F.**. 9 Encontro do Núcleo de Estudos em Microbiologia Aplicada. 2019. (Outro).
2. **PIVATO, A. F.**. Acolhida da Engenharia Bioquímica. 2018. (Outro).
3. **PIVATO, A. F.**. IV Simpósio de Engenharia Bioquímica. 2018. (Outro).
4. **PIVATO, A. F.**. VI Semana Acadêmica da Escola de Química e Alimentos. 2018. (Outro).
5. **PIVATO, A. F.**. 22ª Semana Aberta da FURG. 2017. (Exposição).
6. **PIVATO, A. F.**. 15ª Mostra de Produção Universitária. 2016. (Congresso).
7. **PIVATO, A. F.**. Feira de Adoção - Adote um Cão Universitário. 2015. .
8. **PIVATO, A. F.**. 14ª Mostra de Produção Universitária. 2015. (Congresso).