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Research Article

High-Temperature, Noncatalytic Oxidation of Polyethylene to a Fermentation Substrate Robustly Utilized by *Candida maltosa*

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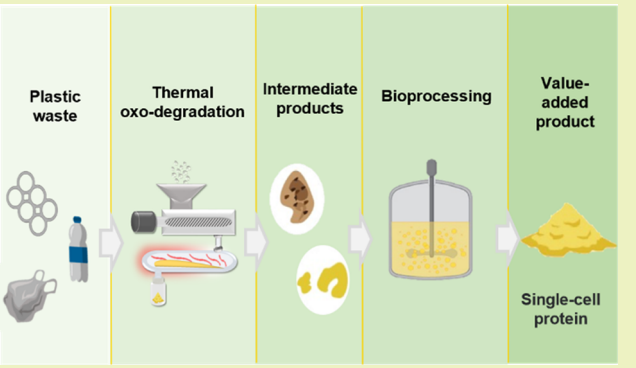
ABSTRACT: This study applies thermal oxo-degradation (TOD) of plastics to produce substrates suitable for uptake and utilization by some microbial species. At moderate temperatures (500 °C) in a noncatalytic, oxidative environment, TOD rapidly deconstructs high-density polyethylene (HDPE) producing a mixture of hydrocarbons, alcohols, aldehydes, and carboxylic acids. Three yeast species were examined for their ability to utilize TOD as the sole carbon source. *Candida maltosa* showed growth similar to that observed on glucose. Growth of *Scheffersomyces stipitis* was observed but at a greatly reduced level relative to glucose. Growth of *Saccharomyces cerevisiae* was negligible. For *C. maltosa* and *S. stipitis*, the added oxygen functionality of TOD products dramatically expedites and improves microbial utilization of the degraded plastic product over that of the pyrolysis product, offering a novel process for funneling waste plastics into microbial metabolic pathways.



KEYWORDS: *plastic, bioprocessing, polyethylene, thermal oxo-degradation, pyrolysis, Candida maltosa*

INTRODUCTION

Environmental degradation of nonbiodegradable plastic by natural processes is estimated to take thousands of years.[1] Given this time scale, virtually all nonbiodegradable plastic disposed in the environment still exists.[2] Efforts to recycle plastics have been largely unsuccessful.[2] As a result, plastics will continue to accumulate in the environment unless new approaches to managing them are developed.[3]

Currently, plastic recycling is limited to mechanical processing into equivalent products (primary recycling) or conversion into products of lower properties (secondary recycling).[4] However, polymer degradation is evident after just a few cycles, requiring large amounts of virgin plastic to be blended with the recycled plastic to preserve desirable properties.[5] Complete deconstruction of the polymers through tertiary recycling techniques such as pyrolysis and gasification converts the original polymers into shorter fragments to reuse as platform chemicals. The current state-of-the-art method for tertiary recycling of plastics is pyrolysis to a wide range of hydrocarbons. Pyrolysis of high-density polyethylene (HDPE), the most common type of plastic, at temperatures between 400 and 600 °C and vapor residence times of a few seconds produces both oil and wax fractions. Liquid products typically have carbon lengths of C6−C20 and can be used as fuel or precursors in the hydrocarbon value chain.[6−8] While waxes have applications as lubricants,[9] costly further refining of the wax is required for fuel production.[10] Narrowing the product distribution has proved difficult.[11,12] Catalytic pyrolysis can increase the selectivity toward the oil fraction,[13] yet this method raises additional challenges. The presence of contaminants in mixed plastic waste streams can deactivate the catalyst, necessitating catalyst regeneration.[14] Additionally, the endothermic nature of pyrolysis reactions leads to large thermal requirements and operating costs.[15,16] Consequently, development of new upcycling technologies is critical to promote the valorization of waste plastics.[17]

Microbial conversion of plastics (e.g., polyethylene, polypropylene, and polystyrene) has the potential to expand and diversify the range of valuable products that can be made from plastics wastes.[18−22] Although some microorganisms can directly break the carbon−carbon bonds in synthetic polymers, some degree of oxidation must generally precede microbial conversion of plastics[23] and the most abundant types of plastics remain recalcitrant to microbial degradation. Prominent exceptions are plastics, in which the monomer contains

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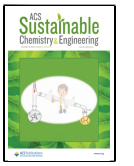

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oxygen in its structure. For example, polyethylene terephthalate (PET)—with two ester moieties per monomer—can be degraded by several microorganisms via PET hydrolases.[24,25] Notably, valorization of waste PET into valuable platform chemicals, such as fiber reinforced biobased monomers, has shown promise.[25]

In nature, plastic decomposition proceeds through the two-step process of thermally or photochemically induced oxidation into alcohols, aldehydes, and carboxylic acids with subsequent microbial catabolism of the oxygenated products.[26,27] However, the time scale for this natural oxidation and utilization is far too long for any meaningful degradation. The abiotic process of oxo-degradation is the rate-limiting step for plastic degradation in the natural environment.[28] Modest increases in temperature above the ambient environment are expected to dramatically increase the incorporation of oxygenated moieties among the depolymerization products of plastics.[29,30] This suggests that the rate of the overall process could be increased by harnessing oxo-degradation at temperatures higher than ambient, increasing the availability of oxygenated substrates for microbial utilization.[31] Focusing on polyethylene, previous work has shown that oxo-degradation at temperatures above 350 °C produced oxygenated volatile products as quickly as five seconds.[32] This process, known as thermal oxo-degradation (TOD), overcomes the environmental rate-limiting step. Because TOD involves exothermic partial oxidation reactions that supply energy to the endothermic carbon-bond cracking, less energy is needed to sustain the reaction.

Hybrid processing of plastics, in which thermal or catalytic depolymerization converts plastic into a substrate suitable for bioconversion into a single product, was introduced more than a decade ago. Kenny et al.[33] depolymerized PET into terephthalic acid that served as the substrate for bacterial production of a biodegradable plastic. Byrne et al.[34] demonstrated the ability of a bacterial consortia to biodegrade pyrolysis-treated polyethylene over the course of 5 days, while Guzik et al.[35] achieved bioconversion of pyrolysis-treated PE to polyhydroxyalkanoate in 2 days. Chemical oxidants such as ozone[36] or additives with oxygen functionality[22] have been used to improve bioconversion rate of wax produced from plastic pyrolysis. The addition of oxygen functionality also improves solubility of plastics in growth media, facilitating accessibility of the carbon source to microorganisms.[37,38] A 4 h catalytic oxidation of PE pyrolysis wax introduced carboxyl groups into the wax to improve bioconversion.[39] In a recent paper by Sullivan et al.,[38] plastic depolymerization occurred through metal-catalyzed chemical oxidation in a batch system with time scales of 2−5 h, converting 34.2 mol % of HDPE into carboxylic acids used for bacterial bioconversion. Relative to catalytic oxidation, air is attractive as an abundant and low-cost oxidizing agent. We hypothesize that at sufficiently high temperatures, air can rapidly and efficiently add oxygen functionality to plastic fragments, producing a substrate serving as the sole carbon and energy source for microorganisms.

Yeasts are attractive microorganisms for utilizing plastic-derived substrates for several reasons, including the ease of growing them compared to filamentous fungi, their resistance to phage contamination compared to bacteria, and their range of potential products. These characteristics have made yeast suitable for multiple industrial bioprocesses, including production of single-cell protein (SCP).[40] While sugars are the conventional carbon source for yeast growth, the metabolism of noncarbohydrate hydrocarbons in yeast is widely documented. Alkane utilization has been observed in at least 180 species, including *Candida maltosa* and *Scheffersomyces stipitis*,[41] and other studies have demonstrated alkene utilization by *C. maltosa*.[42] Other *Candida* strains metabolize aldehydes,[43] while *Saccharomyces cerevisiae* can metabolize alcohols, albeit at very low levels.[44] Both short-chain and long-chain fatty acid metabolism has been extensively studied and engineered for biofuel and biochemical production in yeasts such as *S. cerevisiae* and *Yarrowia lipolytica*.[45,46]

The goal of this study is to thermally oxo-degrade HDPE into oxygenated compounds suitable as both carbon and energy sources for rapid microbial utilization, as evident by the production of yeast biomass. The TOD process was used to convert HDPE into a mixture of fatty alcohols, acids, aldehydes, and straight-chain hydrocarbons, which was used as the sole carbon source for microbial growth. *C. maltosa* and *S. stipitis* were selected for this task from a yeast survey that tested consumption of model compounds representative of some of the major components in the mixture of HDPE TOD product.[47] Importantly, through the introduction of oxygen into the thermal degradation process, microbial utilization was significantly enhanced while offering processing benefits. This novel approach to utilizing waste plastics serves as a platform for future research in the production of macronutrients for animal or human consumption.

EXPERIMENTAL SECTION (MATERIALS AND METHODS)

Pyrolysis and TOD. A stainless steel tubular reactor was constructed to continuously process waste plastic under either inert (N_2) or oxidative conditions. The reactor, illustrated in Figure S1, consisted of three zones: a gas preheat zone, a plastics devolatilization zone, and a vapor cracking/oxidation zone. Product vapors were recovered in a staged collection system consisting of a shell and tube condenser operated at 105 °C to collect higher boiling point compounds, called stage fraction 1 (SF1); a heated in-line, 10 μm sintered stainless steel filter to collect aerosols, termed stage fraction 2 (SF2); and a second shell and tube condenser operated at −25 °C to collect lower boiling point products, termed stage fraction 3 (SF3). Due to their similar boiling points, the products of SF1 and SF2 were collectively referred to as wax, reflecting the solidification of these fractions upon cooling to room temperature. In contrast, SF3 remained liquid upon cooling and is henceforth referred to as "liquid". After passing through a glass-wool filter to collect any remaining aerosols, the gas stream was analyzed via MicroGC (Varian 4900) and a drum gas meter to determine yield and concentrations of carbon monoxide (CO), carbon dioxide (CO_2), light alkanes, and light alkenes.

Experiments were conducted using injection-grade HDPE plastic pellets (ranging from 3 to 5 mm in diameter) procured from Advanced Production Systems (Ohio, U.S.). Characterization of the HDPE feedstock is provided in the Supporting Information. HDPE pellets were fed into the reactor at 125 g h^{-1}. For TOD experiments, the incoming gas mixture of air and nitrogen was preheated to 525 °C. The gas-flow controllers were set at 1.2 standard liters per minute (SLPM) of air and 1.3 SLPM of nitrogen to achieve an equivalence ratio (ER) of 0.05. This equivalence ratio, which is the air-to-fuel mass ratio employed divided by the stoichiometric air-to-fuel mass ratio required for complete combustion, is suitable for partial oxidation of plastics to long-chain oxygenated products.[48] The devolatilization zone, where solid plastics are melted, depolymerized, and devolatilized, was set to 500 °C. The gas mixture and volatilized products entered the vapor cracking/oxidation zone, operated at 425 °C, where the volatile compounds undergo secondary cracking and oxidation to smaller product molecules. Total mass fed for each experiment was

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160 g. Tests were conducted in triplicate. Total mass closure was greater than 85 wt % on a fed plastic basis. The yields of products were calculated by

$$Y_{\text{product}} = \frac{m_{\text{product}}}{m_{\text{fed plastic}}} \quad (1)$$

where Y_{product} is the mass yield of product (wax, liquid, or noncondensable gases (NCGs)), $m_{\text{product}}$is the mass of the product, and $m_{\text{fed plastic}}$ is the mass of the fed plastic. Fed plastic is the only reactant accounted for in yield calculations; oxygen addition is not accounted for in the mass of the reactants.

Because TOD reaction rates are higher than for conventional (nonoxidative) pyrolysis, the gas preheater and devolatilization zones were set at 600 and 525 °C, respectively, for pyrolysis experiments to produce comparable cracking and molecular weight reduction as achieved in TOD experiments.[49] The nitrogen gas-flow controller was set to 2.5 SLPM, and air was excluded from the system. Only pyrolysis wax was collected for comparison against TOD wax, as preliminary tests indicated that TOD wax was the preferred substrate. No provisions were included in the collection of volatile liquid products from pyrolysis. Thus, only pyrolysis wax and gas yields were calculated, with the liquid yield calculated by difference.

Product Characterization. TOD and pyrolysis waxes were analyzed using gel permeation chromatography (GPC), Fourier-transform infrared spectroscopy (FTIR), gas chromatography−mass spectrometry (GC-MS), elemental analysis, and titrated for total acid number (TAN), in accordance with standard plastic pyrolysis wax analysis.[50,51] These analytical methods determined the molecular weight distribution, product carbon numbers, and oxygen functionality of the TOD and pyrolysis products. Additional information on product characterization methods can be found in the Supporting Information.

Microbial Utilization. To determine the viability of TOD and pyrolysis products as microbial carbon sources, three representative yeast species were evaluated: *C. maltosa* NRRL Y-17677, *S. stipitis* NRRL Y-7127, and *S. cerevisiae* NRRL Y-12632. These yeasts were selected based on a previous survey of utilization of compounds found in TOD substrates.[47] *S. cerevisiae* was included as a control because it is a common industrial yeast species.

C. maltosa, *S. stipitis*, and *S. cerevisiae* were grown in Difco Yeast Nitrogen Base (YNB) minimal media without amino acids (5 g L^{-1} ammonium sulfate and basic salts and minerals) with an initial pH of 5.6 ± 0.2. The wax (SF1 and SF2) and liquid (SF3) fractions from TOD of HDPE and waxes from pyrolyzed HDPE were tested in 250 mL baffled shake flasks at a 0.5% w/v concentration in 50 mL of YNB. Negative controls (no inoculation) for each stage fraction were included, as well as a 0.5% w/v glucose positive control (inoculated) for each organism. Inoculations were carried out with aliquots from a 2% (w/v) glucose starter culture. These aliquots were washed three times with YNB without amino acids to avoid carryover of a residual carbon source. All conditions were assessed in triplicates with a starting OD_{600} of 0.1. All flasks were incubated at 30 °C with 250 rpm agitation. The orbital motion and disturbances caused by the baffles supported oxygenation of the medium. Small samples (1 mL) were taken aseptically at time increments of 8 h from each flask. Growth was monitored by measuring the OD_{600} of these samples. The no cell controls serve as blanks for the growth curves.

Elemental Analysis of Residual Wax. The TOD wax fractions were combined and incubated in triplicate in YNB media without inoculation and inoculated with *C. maltosa*, following the same protocol used for microbial utilization. The elemental analysis used in product characterization was performed before and after 72 h incubation for all samples. Wax was removed from flasks after inoculation through centrifugation and subsequent drying in a desiccant chamber to remove growth media.

Microscopy. To microscopically image yeast cells in TOD culture, a *C. maltosa* culture was prepared in a 250 mL baffled flask with 50 mL of YNB without amino acids and 0.5% w/v TOD waxes. The culture was inoculated to a starting OD600 of 0.1 with a washed preculture as was done for utilization tests. The flask was incubated for 48 h at 30 °C with 250 rpm agitation. After the incubation, a sample of the liquid culture was observed under a Zeiss Axioscope Imager with a Differential Interference Contrast configuration at Iowa State University's High Resolution Microscopy Facility.

RESULTS AND DISCUSSION

Increased Oxygen Functionality during TOD versus Pyrolysis. We hypothesized that introduction of oxygen during TOD would nonselectively deconstruct HDPE to produce a mixture of alkanes, alkenes, aldehydes, alcohols, and carboxylic acids. By operating at low equivalence ratios and moderate temperatures, the primary product was expected to be liquids and/or waxes versus NCG such as light hydrocarbons and carbon dioxide and water.

Mass yields from TOD of HDPE at 500 °C and pyrolysis of HDPE at 600 °C are reported in Figure 1. In the absence of oxygen, pyrolysis of HDPE cracks the carbon backbone into smaller hydrocarbons without producing an oxygen functionality. Higher yields of the wax (46 wt %) in comparison to the liquid (21 wt %) are consistent with previous pyrolysis studies at reaction temperatures of 600 °C and short vapor residence times.[52] The components of the highest concentration in the NCG product were propane/propylene and ethylene at 9.5 and 9.4 wt %, respectively, on a fed plastic basis.

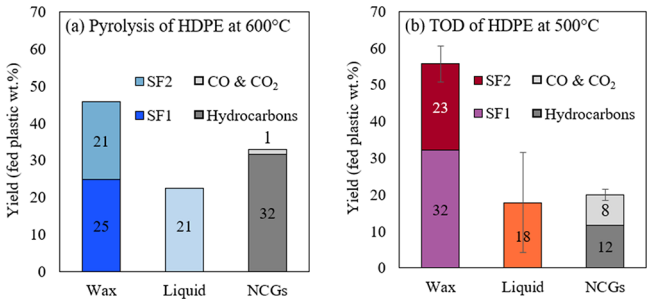

Figure 1. Product distribution from pyrolysis of HDPE at 600 °C (a) and thermal oxo-degradation of HDPE at 500 °C (b). Yield is on a fed plastic basis with the error bars for (a) representing the sample standard deviation from triplicate tests. Noncondensable gases (NCGs) are separated into light hydrocarbons and carbon oxides. The error bar for the TOD wax yield is the sample deviation of combined wax SF1 and SF2 yield. Liquid yield for pyrolysis of HDPE was calculated by difference. The pyrolysis test was performed only once.

For TOD trials, the yields of wax and liquid were 56 and 18 wt %, respectively. The sample standard deviation for the combined wax SF1 and SF2 fractions was ±5 wt %. The liquid yield had greater variation for the three trials with a standard deviation of ±14 wt %, which was attributed to losses during handling of these highly volatile, low molecular weight products. NCGs were only measured for two out of the three trails, with an average mass yield of 20 wt %. Carbon dioxide and propane and/or propylene were present in the highest amounts in the NCGs, at 5.5 and 4.2 wt %, respectively, with lesser amounts of carbon monoxide, methane, ethane, ethylene, butane, butene, and hydrogen detected (all gas yields are included in the Electronic Supporting Information, Section S6).

Even with the addition of oxygen, the relatively low reaction temperatures should favor the production of wax versus NCGs and shorter chain (<C14) hydrocarbons (and their respective

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Table 1. Elemental Analysis, Total Acid Number (TAN), and Carbonyl Indices of TOD and Pyrolysis Products[a]

	carbon (wt %)	hydrogen (wt %)	oxygen (wt %)	TAN (mg KOH g^{-1} sample)	carbonyl index
TOD					
wax SF1	82.8 ± 0.6	13.4 ± 0.2	**2.1 ± 0.5**	**2 ± 1**	**1.1 ± 0.3**
wax SF2	83 ± 1	13.6 ± 0.3	**2.4 ± 0.9**	**3 ± 1**	**1.1 ± 0.4**
liquid				**4 ± 2**	**1.4 ± 0.6**
pyrolysis					
wax SF1	84 ± 1	14.0 ± 0.3	**0.11 ± 0.19**	**below detection limit**	**0.3 ± 0.1**
wax SF2	85.47 ± 0.02	14.1 ± 0.2	**0 ± 0**	**below detection limit**	**0.3 ± 0.1**

[a]Elemental analysis determined that through partial oxidation reactions, oxygen is added onto the cracked backbone of HDPE. Note: the liquid of TOD is excluded due to sample devolatilization at room temperature which skewed oxygen results during elemental analysis. Oxygen content was determined to be significantly different between TOD wax and pyrolysis wax (p-value = 0.0001). Total acid number (TAN) was greater in TOD wax SF2 and liquid indicating greater acidity in these fractions than in TOD wax SF1. As expected, pyrolysis fractions did not contain sufficient acid functionality for detection (lower limit of detection for instrument <0.05 mg KOH g^{-1} sample). Error was determined by standard deviation of two measurements. Values in bold indicate statistically significant differences between TOD products and pyrolysis products.

oxidized analogs) that are liquid at room temperature. However, it is difficult to compare yields directly to conventional plastic pyrolysis as oxygen increases the rate of decomposition and cracking reactions.[31,53]

It is well documented that pyrolysis wax from HDPE contains long-chain aliphatic hydrocarbon compounds. As shown in Table 1, elemental analysis showed more oxygen in the TOD wax (SF1:2.1 wt %, SF2:2.4 wt %) than the pyrolysis wax (SF1:0.11 wt %, SF2:0 wt %, p-value = 0.0001). During TOD, partial oxidation reactions add oxygen as various functional groups onto fragmented polyethylene carbon chains, demonstrated by the large increase of oxygen in SF1 and SF2 fractions compared to the starting HDPE feedstock, which was determined to have negligible oxygen content. As TOD waxes contain molecules of high carbon numbers, the mass contribution of one oxygen atom to the compound compared to the long chain of carbon results in a seemingly low oxygen content. However, the mass percentage of oxygen of a C40 alcohol would be no more than 2.8 wt %. Thus, 2.1 and 2.4 wt % would suggest that most of the wax molecules have at least one oxygen functionality.

Table 1 also compares total acid numbers (TAN) and carbonyl indices for pyrolysis and TOD products. Supporting the results of the elemental analysis, both TAN and carbonyl indices indicate significantly more oxygen in the products of TOD than the products of pyrolysis as carboxylic acids were not detected in the pyrolysis waxes (the lower limit of detection for the instrument is 0.05 mg KOH g^{-1} samples). Other studies showed that HDPE degraded through natural processes in the environment required two years of exposure to reach similar carbonyl indices.[54,55] Clearly, even small amounts of oxygen can quickly oxidize plastics during TOD.

Additionally, FTIR spectra showed increased oxygen functionality added in TOD products (ESI Figure S3). Carbonyl peaks at wavenumbers of approximately 1700 cm^{-1} are evident in the spectra of all TOD samples. Hydroxyl group stretching at 3300 cm^{-1} indicates that TOD added hydroxyl functionality to the SF2 wax and liquid. The presence of carbonyl and hydroxyl groups is expected in TOD products as previous research proposed that the weak O−O bond in hydroperoxides, the primary HDPE oxidation product, is broken to form hydroxyl and alkoxyl radicals that can further react to produce carboxylic acids, esters, and aldehydes.[56] Pyrolysis products did not show evidence of hydroxyl stretching of carbonyl peaks in the FTIR spectra (see ESI Figure S4).

From Figure 2, GPC spectra from pyrolysis wax and TOD SF1 wax are similar, having major peaks at around 600 Da

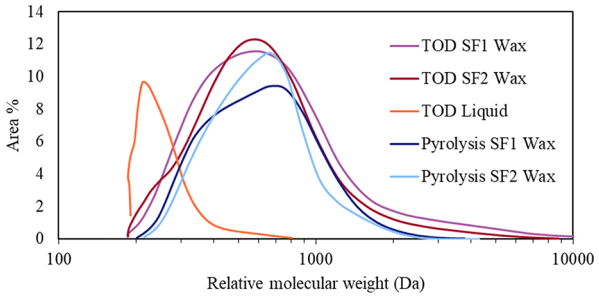

Figure 2. GPC spectra for products of the TOD and pyrolysis. Most compounds in TOD and pyrolysis waxes and molecular weights below 1000 Da, while compounds with much lower molecular weights were detected in the liquid of TOD. The column used in the GPC had a detection range of 190−25,000 Da.

(Da). The molecular weight (MW) of polyethylene oligomers is expected to affect their suitability for microbial metabolization; bacterial utilization of fragments of polyethylene smaller than 800 Da was previously described for *Acinetobacter*, while higher MW fragments were not utilized.[57]

GC-MS detected hundreds of alkanes, alkenes, alkadienes, alcohols, and aldehydes with carbon lengths less than 35 (<500 Da) in the TOD products as expected for nonselective cracking of the polymer chain (see ESI S6). Triplet peaks are evident at 2.5 min intervals, representing the alkane, alkene, and alkadiene for increasing carbon numbers. Between these triplets, oxygenated product peaks were identified, including alcohols, aldehydes, and carboxylic acids. This phenomenon is shown in the GC-MS chromatogram in Figure S5 for the TOD SF2 wax. No products were present in amounts greater than 1 wt % on the basis of HDPE feedstock. The presence of oxygenated products was confirmed through semiquantitative analysis with ^{13}C NMR (see ESI Section S6) and TAN/FTIR, which detected long-chain aldehydes, acids, and alcohols. Table S2 lists compounds identified through GC-MS present in the TOD wax fractions and the TOD liquid fraction. Molecular weight distributions of the TOD waxes indicate numerous constituents larger than 500 Da, which GC-MS is unable to detect, given their low vapor pressures and high boiling points.[58] Future research will utilize multidimensional gas chromatography with high-temperature columns to

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improve compound identification and quantification in wax and liquid products.

TOD Wax Products Show Microbial Utilization Superior to Pyrolysis Products. We hypothesized that oxygenated plastic depolymerization products would be more suitable for yeast bioconversion because addition of oxygen functionality to hydrocarbons is the first step in their metabolism by fungi,[59] and it would improve their solubility in aqueous media.[37,38] This hypothesis was supported by the growth of *C. maltosa* (Figure 3a) and *S. stipitis* (Figure 3b) on

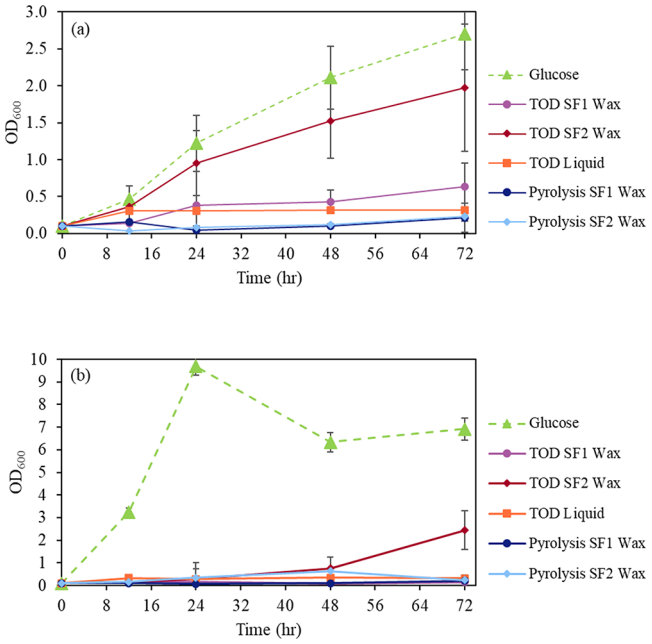

Figure 3. (a) Growth curve of *C. maltosa* on TOD SF2 wax is not statistically different from that on glucose (pairwise *p*-value of 0.06). (b) Growth curves of *S. stipitis* showed that TOD SF2 wax was a viable carbon source, reaching an OD_{600} of 2.4 after 72 h of incubation. TOD SF1 shows an increase in OD_{600} at 48 h, but it is negligible compared OD_{600} recorded in no cell controls. Error bars represent the standard deviation of three replicates in separate flasks.

the TOD wax as the sole source of carbon, while neither of these microorganisms showed evidence of growth on pyrolysis wax. These two species were used in this study because prior work characterized their substrate range and showed their ability to utilize hydrocarbons, fatty acids, and fatty alcohols, similar to the ones in the TOD composition, as their sole carbon source.[47]

C. maltosa showed superior growth on TOD SF2 wax (final OD_{600} of 2.0) compared to the other HDPE-derived substrates (final OD_{600} of 0.64 for TOD SF1 wax, 0.32 for TOD liquid, 0.22 for pyrolysis SF1 wax, and 0.24 for pyrolysis SF2 wax; all with *p*-value <0.05 when compared to TOD SF2) as shown in Figure 3. Remarkably, the growth of *C. maltosa* on TOD SF2 wax was statistically indistinguishable from growth on glucose, with a pairwise *p*-value of 0.06. Previous work showed that *C. maltosa* could achieve comparable growth to glucose with 1-tetradecanol as the sole carbon source,[47] and this work now shows it can do the same with a complex mixture of hydrocarbons and other organic molecules.

The microbial growth observed is indicative of substrate utilization because the TOD and pyrolysis fractions were the only available carbon source in their respective culture conditions. An analysis of the molecular weight distribution of the TOD substrates before and after microbial utilization provided insights into which of the molecules in the TOD mixture were consumed. The gel permeation chromatographs shown in Figure 4 show that the smaller molecules in the

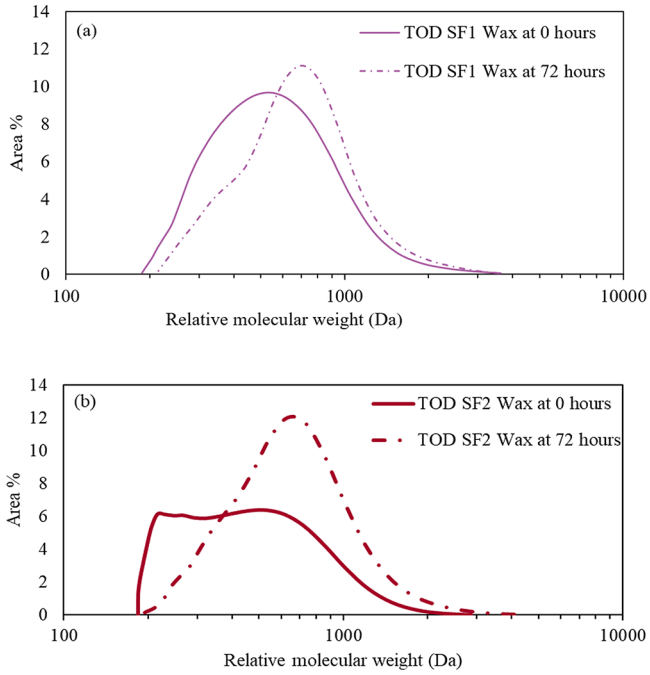

Figure 4. Differences in molecular weight distributions of TOD wax fractions SF1 (a) and SF2 (b) before and after 72 h as the carbon source for *C. maltosa* growth.

mixture were preferred by *C. maltosa*. These molecules, in the range of 340−600 Da for TOD SF1 and 190−340 Da, would be funneled into the central carbon metabolism through various enzymatic reactions and serve as the building blocks for the lipids, proteins, DNA, and other molecules that compose the cells.

The solubility of the components of the TOD and pyrolysis fractions could be a contributing factor to the preferential utilization of TOD products. The bulk of the tested substrates remained in the solid form in aqueous media, possibly creating a mass transfer limitation to microbial growth. The pyrolysis fractions were composed only of hydrocarbons, which are less soluble than the fatty acids, alcohols, and aldehydes present in the TOD fractions due to the oxidative nature of the process.[60,61] The added oxygen functionalities in TOD fractions could be dually beneficial for microbial growth from both metabolic and mass transfer points of view.

The oxygen composition of the TOD wax was measured before and after the 72 h *C. maltosa* culture and in noninoculated media. The results (Table 2) show a significant decrease in oxygen consumption after incubation, even in the noninoculated media. This indicates that oxygenated molecules from the TOD wax can go into solution abiotically, supporting the mass transfer argument. The oxygen concentration of residual TOD wax in media with and without *C. maltosa* did not significantly change.

In addition, some wax was found in the media as microscopic particles, which were often closely surrounded by yeast (see Figure 5). This suggests that yeast was able to

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Table 2. Oxygen Content of Starting TOD Wax and TOD Wax Incubated in Media with and without *C. Maltosa* for 72 h[a]

	oxygen wt %			
carbon source	TOD wax SF1		TOD wax SF2	
wax	2.25 ± 0.02	A	3.2 ± 0.2	A
wax in media for 72 h, no yeast culture	0.7 ± 0.7	B	2.6 ± 0.2	B
wax in media for 72 h with yeast culture	0.8 ± 0.7	B	2.4 ± 0.05	B

[a]Mass percentage of oxygen of starting TOD wax, TOD wax stirred in media without a carbon source for 72 h, and residual TOD wax after used as a carbon source for *C. maltosa* for 72 h for a single sample of TOD wax. Values not connected by the same letter are significantly different (p-value <0.05 in Student's t test).

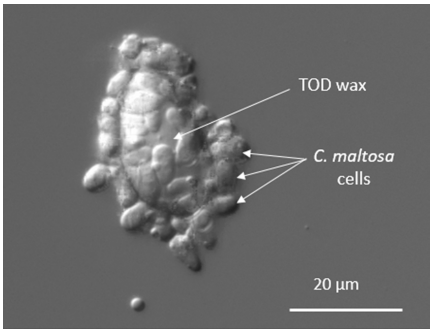

Figure 5. Microscopic image of *C. maltosa* cells associated with a TOD wax particle.

directly feed on the insoluble waxy substrate, although solubilization of plastic-derived compounds likely facilitated their microbial uptake.

The impressive growth on the TOD SF2 wax fraction indicates that oxidative pyrolysis is superior to conventional pyrolysis in the production of substrates for microbial conversion of plastics. However, this is clearly microorganism-dependent: *S. stipitis* achieved significantly slower growth than *C. maltosa* (Figure 3b). On TOD SF2 wax, *S. stipitis* required 56 h to reach on OD_{600} of 1, vs only 24 h for *C. maltosa*. *C. maltosa* has been shown to outperform *S. stipits* in utilizing alcohols and alkanes, which are a large portion of the TOD composition, and therefore, it is not surprising that *C. maltosa* would show better growth in TOD. Nevertheless, both yeasts reached comparable final OD_{600} given sufficient time. *S. cerevisiae* achieved negligible growth on HDPE-derived substrates produced by either TOD or pyrolysis (see Figure S6 in the ESI), justifying our use of nonconventional oleaginous yeast species in our experiments.

The liquid product from the TOD of HDPE was unusable by any of the microorganisms as a carbon source. Microbial utilization of a substrate requires the presence of the appropriate metabolic pathways but also can be suppressed by molecular toxicity, which can vary drastically among organisms.[62] However, this liquid has alternative applications, such as precursor in production of fuels and chemicals.[63−65]

None of the yeast species were able to utilize a pyrolysis-derived substrate as the sole carbon source. Since TOD and pyrolysis cracked HDPE to similar molecular weight distributions, it is clear that addition of oxygen functionality played a crucial role in their utilization.

The main purpose of this study was to demonstrate the ability of TOD to rapidly deconstruct plastic waste into a product suitable for bioconversion to microbial biomass. It is well known that oxidation is a precursor to microorganism utilization of polyolefins, which use β-oxidation to increase biodegradability of carbon sources.[66] This study demonstrates that high-temperature oxidation of plastic in air without recourse to catalysts can produce a substrate suitable for microbial utilization. TOD produces alcohols, aldehydes, and carboxylic acids which can enter the β-oxidation catabolic pathway.[66] Oxidation mechanisms for LDPE and PP are similar to HDPE given their common status as polyolefins and would thus behave similarly under TOD conditions.[30] Thermal depolymerization of other plastics and wastes would be accelerated by the addition of oxygen. Thus, abiotic deconstruction via TOD is both rapid and potentially feedstock agnostic, while biotic synthesis can funnel diverse substrate molecules into microbial biomass from yeast.

Yeasts have long been used as a direct food source for human consumption or as animal feed due to their rich nutritional value.[67] The *C. maltosa* and *S. stipitis* strains used have not been reported to be pathogenic, suggesting their possible use as SCP.[68] Other researchers also assert that *C. maltosa* can be used for SCP production.[68,69] Production of SCP from carbon thought not to be valuable (e.g., waste plastic) can increase the resiliency of global food production systems and divert waste from landfills.[21] The current findings provide the foundation for the extension of the microorganisms employed for SCP production and for waste plastic valorization. Thus, one can envision a pathway for the production of macronutrients from thermally oxo-degraded plastics.

CONCLUSIONS

TOD of plastic offers a viable route to utilize waste plastic streams. As microbial growth was observed in media containing thermally oxo-degraded HDPE without supplemental carbon, a pathway to breakdown waste plastic for biological upgrading to microbial biomass is possible. Selecting the microorganisms based on their utilization of model compounds representative of TOD products gave way to substantial growth, which is imperative for industrial applications. Unlike other efforts at upcycling (or recycling) HDPE, the coupling of thermochemical and biological processes allows for a nonselective deconstructive process, while ultimately yielding a single product. A more thorough characterization of products from TOD using analytical techniques such as multidimensional gas chromatography would allow for greater understanding of bioconversion pathways. Additionally, the optimization of TOD process variables could produce higher yields of desirable compounds for microbial growth. Future work should explore the TOD of mixed wastes providing additional streams to valorize and optimize this approach to producing food from waste.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acssuschemeng.3c05918.

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Additional experimental details, methods, and results, including feedstock characterization, detailed gas yields, FTIR spectra, ^{13}C NMR spectra, GC-MS identified compounds, and *S. cerevisiae* growth curves (PDF)

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$^{\perp}$J.L.B. and E.R.-O. contributed equally. C.P., M.B., L.J., R.S., and R.B. originated the idea, with J.L.B., E.R.-O., and C.P. conceptualizing the manuscript. J.L.B. and C.P. developed the TOD and pyrolysis methodology and performed TOD and pyrolysis data curation and analysis. E.R.-O. developed the organism selection, carbon utilization, tolerance test, and microscopy methodology. E.R.-O. performed biological data curation and analysis. J.L.B., E.R.-O., and C.P. wrote the original draft with reviewing and editing completed by all authors.

Notes

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ABBREVATIONS

TOD, thermal oxo-degradation

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