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Preliminary Investigation of Upcycling Polylactic Acid 3-D Printing Waste to Candidate Single-Cell Protein Feedstock

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Abstract: Polylactic acid (PLA) is the most common extrusion-based distributed 3-D printing material. Unfortunately, large amounts of PLA are wasted from failed prints, support materials, material changes for multi-color printing, and poorly designed iterative prototypes. To overcome this waste challenge, distributed recycling with recyclebot technology is used to convert 3-D printing waste back into filament. This process can only be repeated five times before serious mechanical degradation of the resulting materials is observed. To overcome these challenges at the end-of-life of PLA 3-D printing material, this preliminary study explores a new approach that uses hydrolysis of PLA to create a candidate single-cell protein (SCP) feedstock that can be converted to human-edible food after required safety validation. Three concentrations of sodium hydroxide (NaOH) are tested for their ability to perform hydrolysis on PLA at room temperature using readily accessible equipment and chemicals. The solution is then neutralized, and yeast is grown in an open-source bioreactor, dried, and quantified to determine the preliminary yield of SCP. The results show a clear positive correlation between PLA degradation efficiency with higher NaOH concentration and yeast biomass production. The average performance of the 0.33 g NaOH/g PLA treatment resulted in an 8.5-fold yeast biomass increase. In summary, the current bench-scale process has proven technically viable and may be an economically justified method of yeast production on the household scale using PLA waste as a starting material. The dominant cost is energy, not reagents, which also lends the positive early results to future safety investigation using a scaled-up bioreactor.

Keywords: yeast; polylactic acid; PLA; upcycling; recycling; waste recovery; plastic

1. Introduction

One of the first truly successful examples of open-source hardware development [1] was the release of the self-replicating rapid prototyper (RepRap) 3-D printer [2], as it jump-started the consumer additive manufacturing (AM) industry.[3] Both DIY RepRaps and their commercial derivatives quickly scaled to a market growing by multi-millions of printers per year [4] as distributed manufacturing using 3-D printing is highly profitable, even at the household level [5,6]. At this point, a desktop 3-D printer can be economically justified to make a single scientific tool [7,8] or, after a short payback by printing one consumer good per week [9]. The RepRap community not only made headway on AM designs themselves (e.g., increasing print speed and accuracy while reducing costs aggressively) [10,11] but also on AM materials. The RepRap project initially used polycaprolactone as the printing material but explicitly planned to switch to polylactic acid (PLA) because it could be synthesized by fermentation from corn or potato starch, enabling a self-replicating source of build materials alongside the self-replicating

printer [12,13]. The initial plan materialized as PLA is the predominant filament material used in material extrusion (MEX) 3-D printing (more commonly referred to as fused filament fabrication (FFF), which is the non-trademarked version of fused deposition modelling (FDM))-based desktop 3-D printing [14,15]. PLA is relatively easy to print for beginners, and desktop 3-D printing generally has an excellent ecological balance sheet [16].

Despite these good properties, a substantial amount of waste is still created when 3-D printing PLA, primarily from failed prints, support materials, material changes for multi-color printing, or poorly designed iterative prototypes [17,18]. For example, a study on a commercial FDM found the waste-to-materials ratio averaged 34.6%, with failed prints accounting for about 55% of total material waste, and the remainder being support structures [19]. This type of waste spurred the development of an open-source recyclebot, a single screw extruder used for converting plastic waste into 3-D printing filament [20]. The latest version of a RepRapable Recyclebot has many components 3-D printed, costs less than $700 in materials, produces filament at 0.4 kg/h, and can fabricate recycled waste plastic into filament for ~2.5 cents/kg—roughly 1/1000th the cost of commercial filament [21]. The use of recyclebots provides a path to a circular economy [22], particularly when they are solar photovoltaic (PV)-powered [23]. Recyclebots led the way to the concept of distributed recycling and additive manufacturing (DRAM) that enables a new path to a circular economy, as environmental harm is cut for transportation, packaging and storage because products can be 3-D printed on demand from plastic waste [24,25]. This use of integrating AM into the circular economy allows for a new life for discarded plastic products [26]. It is clear from life cycle analysis (LCA) studies that the DRAM approach is better for the environment [27,28], and gets better the more cycles the plastic goes through [29].

Unfortunately, PLA degrades with each melt/solidification cycle needed in such recycling. Hidalgo-Carvajal et al. report that PLA from a known commercial source can withstand up to 3 re-extrusion cycles without significant degradation (only 8.9% molecular weight loss), while PLA from mixed/unknown sources is limited to 2 cycles (2.4% loss) [30]. Similarly, Lee et al. found PLA can be mechanically recycled up to approximately 5 times before significant degradation occurs, such as a 38.7% decrease in tensile strength [31]. Cruz Sanchez et al. also evaluated PLA degradation over five recycling cycles using four different recycling process chains, finding progressive mechanical property degradation [25]. Each cycle increases brittleness and difficulty to process, and thus, inconsistent filament quality. Thus, although mechanical recycling with recyclebots and similar devices extends the lifetime of PLA, it is not fully circular and after ~5 re-extrusions, another approach is needed rather than discarding, burning or landfilling the PLA.

To overcome these challenges at the end of life for PLA 3-D printing filament material, this preliminary study explores a new approach to use hydrolysis of PLA and an open-source bioreactor to convert PLA into candidate single-cell protein (SCP) feedstock requiring safety validation. Specifically, this study investigates converting PLA into Saccharomyces cerevisiae (baker's yeast). Three concentrations of sodium hydroxide (NaOH) are tested for their ability to perform hydrolysis on PLA at low temperatures using readily accessible equipment and chemicals. This solution is then neutralized, and yeast is grown, dried, and quantified to determine the preliminary yield of SCP. The results are discussed in terms of technical and economic viability, and future safety validation work will be recommended.

2. Methods

2.1. Experimental

PLA plastic waste that had been 3-D printed, undergoing one melt-solidification cycled was selected. The post 3-D printed PLA plastic waste was shredded using the Filabot Reclaimer (Vermont, USA) for consistency of 1 mm^2. Three concentrations of sodium hydroxide (NaOH) were made by mixing 2 g, 4 g, and 5 g with 15 g of PLA and 89 mL of water in three separate glass jars, respectively. Thus, the mass ratios investigated were: 0.13, 0.27, and 0.33 g NaOH/g PLA, respectively. Each jar was sealed with a lid to minimize evaporation and external factors. After 1 week, a portion of the PLA plastic waste had degraded, as seen in Figure 1a, and was added to an open-source bioreactor (Pioreactor, Waterloo Canada) for experiments. The yield after the first step was determined by weighing the remaining non-degraded plastic and subtracting that weight from the initial sample weight using a Malama digital scale (BC, Canada) with 0.01 g accuracy. Prior to adding the dissolved PLA plastic waste, its pH was adjusted to 7.0 manually using approximately 2–4 mL of lactic acid ($C_3H_6O_3$) (Royal Command, USA) via syringe to create optimal conditions for Saccharomyces cerevisiae yeast growth.

To begin the bioreactor experiment, a pioreactor was fully sterilized using isopropyl alcohol. Next, after separating the degraded and non-degraded PLA using a strainer, the degraded PLA and neutralized NaOH-$C_3H_6O_3$ mixture were placed in the pioreactor. The pioreactor was increased to an operating temperature of 30 °C using the heater and temperature sensors. Next, 0.05 g of Fleischmann's Quick-Rise baker's yeast (Canada) was introduced into the system for 24 h, maintaining a stirring speed of 500 rpm, a temperature of 30 °C, and a pH of

7.0. After the completion of the experiment, the bioreactor vessel was detached and placed in refrigeration overnight to halt the reaction and facilitate yeast settling, as seen in Figure 1b. After approximately 12 h, the yeast was extracted from the vessel by filtering the resulting liquid through a 0.2-micron filter. Post-filtration, the yeast-laden filter papers were air-dried until thoroughly dried. The resulting yeast yield was accurately measured using the digital scale, accounting for the weight of the filter papers. The experiments were repeated three times for the highest yielding concentration.

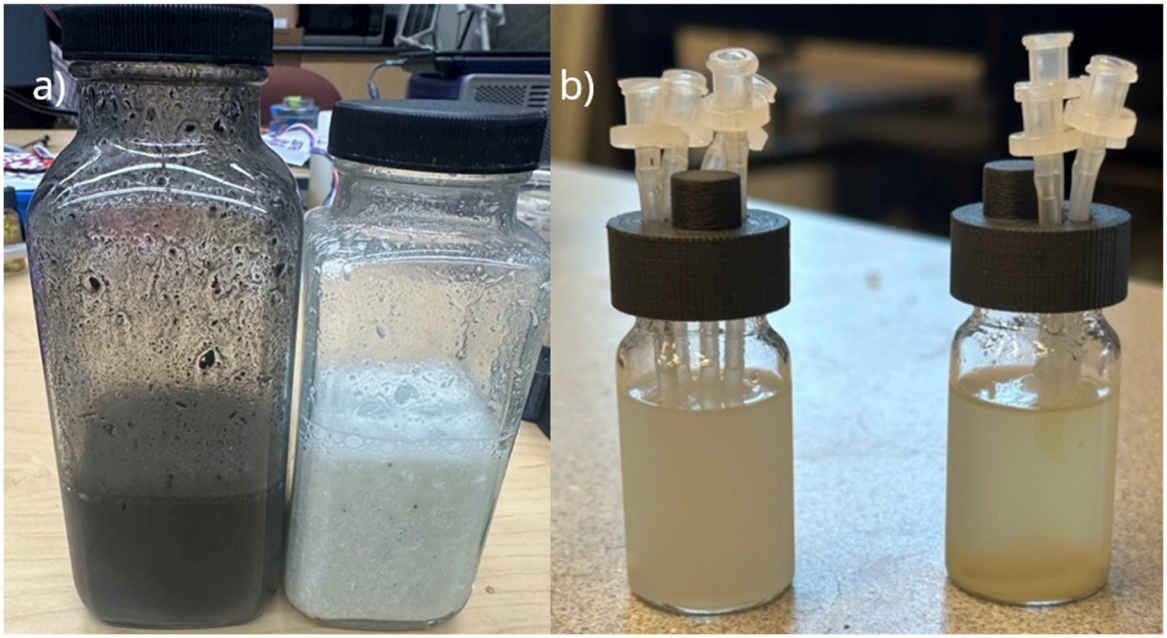

Figure 1. Photographs of the PLA waste hydrolysis and Saccharomyces cerevisiae yeast cultivation stages. (**a**) sealed glass jars containing examples of 15 g of dissolving PLA after one week of room-temperature hydrolysis; (**b**) PLA hydrolysate following 24-h yeast cultivation in the pioreactors.

2.2. Economic Analysis

To assess the economic viability of this process, it is necessary to consider the cost of each input against the market value of the yeast biomass produced. The principal inputs are: PLA plastic waste, sodium hydroxide, lactic acid, and energy. The inoculum cost is negligible: 0.05 g of Fleischmann's Quick-Rise baker's yeast. Baker's yeast at retail small-packet prices is $0.09/g [32]. PLA 3D-printing filament currently retails at approximately $20–30/kg [33], which places the cost of the 15 g sample at around $0.30–0.45. If the PLA feedstock is sourced as post-consumer 3-D-printing waste, however, which is consistent with the framing of this study (distributed household-level SCP production), the material and labor costs can be treated as zero. Sodium hydroxide is available for $4/kg [34], meaning that the 5 g of NaOH used per run costs approximately $0.06. Food-grade lactic acid, purchased for $1.00/L and used for pH adjustment (2–4 mL per run), adds a further negligible cost ($0.004). The pioreactor unit draws up to 15 W during active operation [35] (heating to 30 °C, stirring at ~500 rpm, and sensor monitoring). Over the 24-h cultivation period, total electricity consumption is therefore approximately 0.36 kWh per run. Refrigeration of the vessel overnight takes place in a standard laboratory refrigerator already in continuous operation, which contributes to negligible marginal energy cost. At a Canadian residential electricity rate of ~$0.15/kWh[36], this amounts to approximately $0.054 per run in pioreactor energy costs.

3. Results

The hydrolysis of PLA plastic waste using NaOH showed clear dose-dependent degradation patterns. When shredded PLA waste was treated with varying concentrations of NaOH, the degradation efficiency increased significantly with higher base concentrations. The lowest concentration (2 g NaOH) dissolved only 0.13 g NaOH/g PLA of the original plastic sample, achieving 20% degradation efficiency and leaving 12 g of material in solution. Treatment with 0.27 g NaOH/g PLA improved performance considerably by dissolving 5.23 g of PLA (34.9% degradation efficiency) and reducing the solid waste to 9.77 g. The highest concentration tested (0.33 g NaOH/g PLA) proved most effective and dissolved 10.2 g of the original plastic (68% degradation efficiency) with 4.8 g of undissolved material remaining. These results are summarized in Figure 2. Figure 2a shows the PLA degradation efficiency as a function of

NaOH concentration. Figure 2b clearly shows the mass of degraded PLA (dissolved material) and undissolved PLA remaining after one week of treatment, demonstrating dose-dependent degradation patterns.

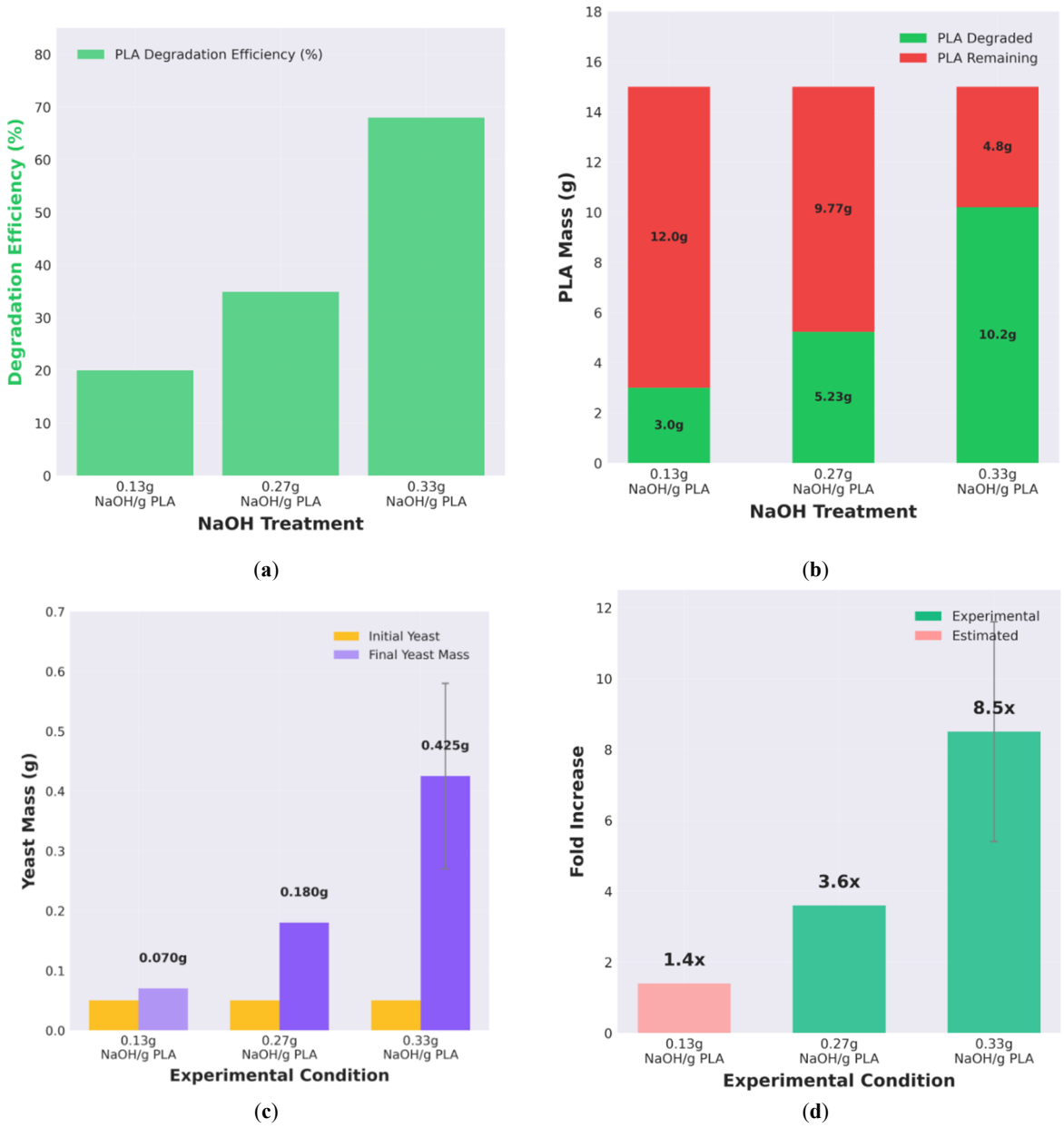

Figure 2. Bar graphs display (**a**) degradation efficiency percentage, (**b**) total degraded PLA mass, and remaining undissolved PLA mass for 0.13, 0.27, and 0.33 g NaOH/g PLA treatments applied to 15 g PLA samples, (**c**) displays the yeast production (initial and final) based on NaOH concentrations, and (**d**) shows the numerical fold increase of yeast at the three concentrations.

Yeast cultivation in PLA-degraded medium demonstrated substantial biomass production that correlated directly with PLA degradation efficiency, as shown in Figure 1c. Starting with 0.05 g of baker's yeast in approximately 89 mL of medium, final dried yeast yields varied significantly based on NaOH treatment concentration. The 0.27 g NaOH/g PLA treatment, which achieved 34.9% PLA degradation efficiency, produced 0.18 g of dried yeast biomass from the initial 0.05 g inoculum. As shown in Figure 1d, this represented a 3.6-fold increase in yeast mass, which corresponds to 0.13 g of net biomass production and a 260% yield increase over the 24-h cultivation period. The 0.33 g NaOH/g PLA treatment, achieving 68% PLA degradation efficiency, demonstrated the highest yeast production with considerable variability between replicates. The first replicate yielded 0.58 g of dried yeast (11.6-fold increase, 1.060% yield increase), while the second replicate produced 0.27 g (5.4-fold increase, 440% yield increase). The average performance of the 0.33 g NaOH/g PLA treatment was 0.42 g final yeast mass, which represents an 8.5-fold biomass increase and 750% yield increase.

A clear positive correlation emerged between PLA degradation efficiency and yeast biomass production. The 0.33 g NaOH/g PLA treatment produced 136% more yeast biomass than the 0.27 g NaOH/g PLA NaOH condition, indicating that more extensive plastic degradation provided enhanced substrate availability for yeast metabolism.

4. Discussion

The results demonstrate that alkaline hydrolysis of PLA waste can support yeast biomass production, with the best-performing run yielding 0.42 g of dried yeast from a 0.05 g inoculum. Thus, it is technically viable to convert PLA 3-D printing waste to SCP using a low-temperature process that could be household scale to match distributed manufacturing using desktop 3-D printers.

To analyze whether this process is a practical pathway for waste remediation, biomass recovery, or both depends on its cost relative to the value it generates. The best-performing condition (5 g NaOH, 15 g PLA) yielded an average of 0.42 g of dried yeast from an initial 0.05 g inoculum, which represents a net biomass gain of approximately 0.37 g per run. As can be seen in Table 1, on the microscale, yeast can be generated from PLA waste at a profit. The total cost of materials is $0.0285, and with electricity, a run in a pioreactor costs $0.0825. The experiment creates $0.0333 of yeast and thus a total value of $0.0378.

Several important caveats can change the framing of the economics of the process. First, it is clear that the process is not optimized by the error bars in Figure 1d. Further process optimization would be expected to increase yield using the same equipment. Second, the process cannot only be SCP production, but PLA waste remediation, with SCP as a valuable co-product. If a tipping fee or avoided landfill cost can be attributed to processing waste plastic—set at about $135 per tonne for general garbage disposal in Ontario [37] the economics improve. Processing 15 g of PLA waste per run would attract a tipping fee equivalent of approximately $0.002 per run, which takes the value up to $0.038. Second, a continuous-flow or fed-batch system processing hundreds of grams of PLA could reduce energy cost per gram of yeast. This is the most important factor that would make this distributed process economic. Third, lactic acid—the primary hydrolysis product of PLA degradation by NaOH—is itself a bulk chemical ($41/L) [38]. If a portion of the dissolved PLA remains as sodium lactate in the effluent, this represents an additional value stream, although future work is needed to recover it.

Table 1. Per-run cost and value summary (best result: 5 g NaOH, 15 g PLA, 0.42 g mean yeast yield) at the small-scale.

Item	Quantity	Unit Price	Cost per Run ($CAD)
PLA (waste scenario)	15 g	$0/g (waste)	$0.0000
NaOH	5 g	$0.004/g	$0.0200
Lactic acid	4 mL	$1.00/L	$0.0040
Yeast inoculum	0.05 g	$0.09/g	$0.0045
Cost of materials no energy	$	$	$0.0285
Energy (pioreactor 24 h + refrigeration)	0.36 kWh	$0.15 CAD/kWh	$0.0540
Total cost (with energy)	$	$	$0.0825
Value of yeast (retail)	0.37 g net	$0.09/g	$0.0333
Total value of all yeast recovered (retail)	0.42 g	$0.09/g	$0.0378

At the extremely small-scale of this experiment, this process is not economically viable. It would be expected that the scaling up of this process to a large bioreactor would decrease unit energy costs, which make up more than half of the costs. Large-scale open-source bioreactors are needed with the same capabilities as the pioreactor for such promising future work.

After PLA has been mechanically recycled and has reached end-of-life, it can be biodegraded [39,40] and thus industrially composted [41,42]. The conditions for industrial composting are not available everywhere [40], and it involves centralized collection [42]. The process produces low-quality compost, and the yields are extremely low; therefore, it is seen as one of the worst options from a climate perspective [41]. Another option is chemical recycling, where the PLA is converted back to monomers of L-lactide via catalytic depolymerization, achieving up to 99% yield using a zinc catalyst at 180 °C under vacuum [43]. Similarly, Ellis et al. have demonstrated continuous flow chemical recycling of PLA to 92% conversion to lactide with 92–97% selectivity for L-lactide at 150–170 °C using tetrahydrofuran as solvent and a tin catalyst [44]. Both of these processes recovered L-lactide that is pure enough and can be repolymerized without further purification, demonstrating a true polymer-monomer-polymer circular loop. Unfortunately, these processes demand high temperatures, vacuum, and careful selection of solvents and metal catalysts [45]. In addition, purification and isolation of the monomers can be challenging and reduce yield [45]. Chemical recycling of waste plastics faces technological barriers across the entire recycling chain, including high energy inputs, the need for toxic chemicals, and the challenge of handling contaminated or mixed waste streams at

an industrial scale. [46] From an environmental perspective, chemical recycling is low impact but not as low-impact as mechanical recycling; it is, however, much better than (i) composting (water use and ecotoxicity), (ii) incineration for energy recovery, or (iii) the worst landfilling [47]. Currently, it is not viable at the household scale, unlike the recyclebot mechanical recycling. Lastly, another option in the literature is to blend PLA with another waste to make a composite material with useful properties. For example, PLA has been blended with wood to make wood-plastic composites [48,49], which can also be applied to waste wood (e.g., furniture manufacturing waste [50]). PLA biocomposites reinforced with natural fibers (e.g., kenaf, hemp, flax, jute, and sisal) produce composites that are biodegradable, renewable, and can substitute petroleum-based products [51]. Composites extend the life of PLA further, but often demand coupling agents (compatibilizer) [52] to achieve acceptable mechanical strength because of poor interfacial adhesion between PLA and the natural fillers. These materials can swell when exposed to water, and thus some applications outdoors and in wet environments are limited [52]. PLA biocomposites have inherent limitations, including brittleness, low heat resistance, and high flammability (that may demand flame retardants that further compromise mechanical properties [53].

5. Limitations and Future Work

This work had several limitations. It is just a preliminary study to investigate the technical and economic viability of household-level-scale SCP production. It relied on only a single solvent, which is, however, already commonly used in homes (e.g., degreaser, stain remover, soap making). The technical sophistication of this process may be challenging, but is eased substantially by pH adjustment with an open-source bioreactor. Future work can investigate degrading PLA with other common materials, such as acetic acid, which is incompatible [54], to determine if it is a better method. Similarly, this study also only investigated one type of SCP production. Further work could look at other microorganisms that could digest hydrolyzed PLA, as well as the safety and taste of the resultant food.

For this last limitation, it should be clarified that for most locations the regulatory status of a food ingredient depends not only on the identity of the final substance, but also on how it is produced, what it is made from, and what residues or by-products may be carried over. Saccharomyces cerevisiae is broadly recognized as safe when grown on conventional substrates like sugar. PLA hydrolysate as used in this experiment, however, is a novel feedstock that has never been part of yeast's history of safe use, which changes the regulatory viewpoint in several ways: i) the substrate is a synthetic polymer, not a traditional agricultural feedstock, ii) alkaline hydrolysis with NaOH introduces sodium lactate, residual NaOH, and potentially unreacted oligomers into the growth medium, iii) PLA may contain additives (e.g., residual catalysts, colorants, or plasticizers) and iv) contaminants picked up during the 3-D printed part's fabrication or use. The yeast itself may bioaccumulate any contaminants present in the hydrolysate.

Using the U.S. FDA as an example, under the FDA's framework, when a previously recognized-as-safe food is produced by a materially different process or from a non-traditional source, the GRAS (Generally Recognized as Safe) status does not automatically transfer. This is explicitly addressed in 21 CFR 170.30, which defines GRAS and notes that general recognition requires the same quality of evidence that would support a food additive petition, applied to the specific substance and specific manufacturing process [55]. To make this pathway a viable FDA-approved method of generating yeast for human edible consumption, peer-reviewed safety data must be published. If the input were restricted to virgin, single-source, additive-free PLA from a documented food-contact-grade supplier, the testing burden would be small, but for the approach proposed here, there is substantial future work because of contaminant uncertainty. The key research gap is not proof of yeast growth shown here, but proof that the resulting biomass is safe, nutritionally useful, reproducible, contaminant-controlled, and acceptable to humans or as a feedstock for other animals (e.g., chicken or fish food). Table 2 outlines the necessary steps summarized from [56–67].

As can be seen from the 55 required sub-studies needed to convert the proposed PLA-waste into human edible food or animal feed, this work is at a preliminary stage. Several points of clarification emerge, however. First, the animal feed pathway is far more tractable as a first target, particularly for non-food-producing animals (e.g., pet food or food for ornamental fish) where edible-tissue residue testing is avoided. Second, the human food pathway should be considered only after PLA substrate restriction, process maturation, and complete contaminant characterization demonstrate that the residual contaminant profile is comparable to conventionally produced yeast. This is primarily a problem, which has been discussed before to make AM recycling easier, because all current PLA filament suppliers do not disclose their recipes [68]. If this were to be done, the effort needed for experiments in section D. (Heavy metal & filament additive screen), would be substantially reduced. If, after screening a few more organisms that are already human edible, like the Saccharomyces cerevisiae used here, and the initial battery

of tests A, B, and C, were completed, it would be in the best interest of filament suppliers to consider making their methods and materials open to enable a more rapid diffusion of this solution. There would be a competitive advantage to whichever filament supplier opens their PLA materials for this extended application.

Table 2. Outline of future work to meet safety standards for PLA-waste-derived SCP.

Task Number	Test Category	Specific Test	Human Food Requirement	Animal Feed Requirement	Key Method/Standard	Why Required
A. Regulatory & identity						
1	Regulatory classification	Pre-submission consultation	Required—FDA GRAS notice, food additive petition, or novel food submission (HealthCanada/EFSA)	Required—FDA/CVM consultation, AAFCO definition check, or CFIA novel feed review	FDA GRAS 21 CFR 170 Subpart E; CFIA novel feeds; EFSA novel food guidance	Establishes a legal pathway before any investment in testing
2	Strain identification	Species + strain ID by ITS/WGS sequencing	Required to genus, species, strain	Required to genus, species; strain preferred	ITS rDNA sequencing; whole-genome sequencing	Confirms organism identity; rules out pathogenic/ toxigenic strains
3	Strain safety history	QPS/GRAS status check; absence of acquired antimicrobial resistance	Required—must demonstrate safe-use history or QPS-equiv.	Required but threshold lower	EFSA QPS list; literature review	Saccharomyces cerevisiae has QPS status; non-Saccharomyces strains need more data
4	Viable vs. inactivated status	Plate counts, viability assay	Typically inactivated for food use	Either acceptable; declare on label	USP/ISO microbiology methods	Affects regulatory category and shelf stability
B. Compositional characterization						
5	Proximate analysis	Moisture, ash, crude protein, true protein, fat, fiber, carbohydrates	3–5 independent lots (also used for reproducibility)	3 independent lots minimum	AOAC official methods	Establishes nutritional identity and label claims
6	Amino acid profile	All 20 amino acids, including tryptophan	Required, with digestibility correction (DIAAS or PDCAAS)	Required with species-specific digestibility	AOAC 994.12; UPLC	Determines protein quality for both uses
7	Fatty acid profile	Full FA spectrum	Required	Recommended	GC-FID	Nutritional value; oxidative stability
8	Nucleic acid content	Total RNA + DNA quantification	Critical—must demonstrate <2 g/day RNA contribution for adults	Recommended; species-specific limits	Fluorometric or HPLC assay	Excess purines → uric acid → gout risk in humans; less critical in animals
9	Cell wall composition	β-glucan, mannan, chitin quantification	Required if claimed	Required if claiming functional benefit	Enzymatic assays	Affects digestibility and immunomodulation claims
10	Vitamin profile	B-complex, ergosterol, vitamin D2	Required if claimed	Recommended	HPLC; LC-MS	Supports nutritional claims
11	Mineral profile	Macro + trace minerals by ICP-MS	Required	Required	ICP-MS after microwave digestion	Nutritional value + heavy metal screen
C. Process residue chemistry (PLA-specific)						
12	Residual lactic acid/lactate	Quantification in final biomass	Required with safety limit	Required	HPLC; ion chromatography	Direct PLA hydrolysis product
13	Residual lactide/PLA oligomers	LC-MS detection of unreacted PLA	Required	Required	LC-HRMS	Process completeness indicator
14	Residual NaOH/sodium	Total sodium, alkalinity, pH	Required—sodium load matters for food	Required	Titration; flame photometry	Process neutralization verification
15	Sodium lactate	Quantification	Declare on label	Declare	HPLC	Major neutralization by-product

Table 2. *Cont*.

Task Number	Test Category	Specific Test	Human Food Requirement	Animal Feed Requirement	Key Method/Standard	Why Required
D. Heavy metal & filament additive screen						
16	Heavy metals panel	As, Cd, Pb, Hg, Cr, Ni, Sn, Sb, Al, Ba	Required—meet Codex/FDA food limits	Required—meet EU Dir. 2002/32/EC feed limits	ICP-MS after microwave digestion	PLA filaments can contain metal additives, pigments, fillers
17	Extended metals (if metal-filled PLA suspected)	Cu, Zn, Fe, Ti, Co, Mn, V	Required	Required	ICP-MS/ICP-OES	Metal-filled PLA filaments documented to contain these
18	Pigment/dye screen	Targeted analysis for known filament colorants	Required	Required	LC-MS targeted methods	Colored filaments contain undisclosed organic pigments
19	Plasticizers	Phthalates (DEHP, DBP, BBP, DINP, DIDP, DNOP)	Required—food contact limits apply	Required	GC-MS	Possible PLA additives
20	Bisphenols	BPA, BPS, BPF	Required	Recommended	LC-MS/MS	Possible contamination from blended filaments
21	Flame retardants	Brominated FRs, organophosphate FRs	Required	Required	GC-MS; LC-MS	Some specialty filaments contain FRs
22	UV stabilizers/antioxidants	Targeted panel	Required	Recommended	LC-MS	Filament additives
23	PAHs	EU 15 + 1 PAH panel	Required	Required	GC-MS/MS	Combustion/contamination markers
24	Non-target organic screen	GC-HRMS and LC-HRMS suspect screening	Strongly recommended	Recommended	HRMS with library matching	Catches unexpected filament additives
E. Microbiological safety						
25	Total aerobic count	Plate count	$<10^4$ CFU/g typical	$<10^5$ CFU/g typical	ISO 4833	General hygiene indicator
26	Yeasts and molds (contamination)	Plate count on selective media	Required	Required	ISO 21527	Cross-contamination indicator
27	Salmonella	Absence in 25 g	Absent in 25 g	Absent in 25 g	ISO 6579	Mandatory zero-tolerance pathogen
28	E. coli	Quantification	<10 CFU/g typical	$<10^2$ CFU/g typical	ISO 16649	Fecal contamination indicator
29	Listeria monocytogenes	Absence in 25 g	Required if RTE food	Recommended	ISO 11290	RTE food pathogen
30	Coliforms/Enterobacteriaceae	Plate count	Required	Required	ISO 21528	Process hygiene indicator
31	Bacillus cereus	Quantification	Required for dried products	Recommended	ISO 7932	Spore-forming pathogen in dried foods
32	Staphylococcus aureus	Quantification	Required	Recommended	ISO 6888	Toxin-producing pathogen
33	Sulfite-reducing clostridia	Quantification	Required for some categories	Recommended	ISO 15213	Spore-formers
34	Environmental monitoring	Zone 1–4 swabs of bioreactor/drying	Required under preventive controls	Required under 21 CFR 507	Swab + culture	FSMA/PCAF compliance
F. Mycotoxins & microbial toxins						
35	Aflatoxin panel	B1, B2, G1, G2	Required	Required	LC-MS/MS or ELISA	EU/FDA limits in foods and feeds
36	Ochratoxin A	Quantification	Required	Required	LC-MS/MS	Storage mold contamination
37	Other mycotoxins	DON, ZEN, fumonisins, T-2/HT-2, citrinin	Required based on substrate	Required	LC-MS/MS multi-mycotoxin panel	Standard feed contaminant suite
38	Yeast-derived toxins	Strain-specific metabolite screen	Required for non-QPS strains	Required for non-QPS strains	LC-HRMS metabolomics	Only if non-Saccharomyces
G. Allergenicity						
39	Allergenicity assessment	Bioinformatic comparison to known allergens; sera IgE binding	Required for novel proteins	Not required	COMPARE database; ELISA	EFSA/FDA novel food guidance
40	Yeast allergen cross-reactivity	Known yeast allergen markers	Recommended	Not required	Immunoassay	Yeast-sensitive consumer protection

Table 2. *Cont*.

Task Number	Test Category	Specific Test	Human Food Requirement	Animal Feed Requirement	Key Method/Standard	Why Required
			H. Toxicology			
41	In vitro genotoxicity	Ames test, micronucleus, chromosomal aberration	Required—full battery	Not required	OECD 471, 487, 473	Standard novel food/additive battery
42	90-day repeated-dose oral toxicity (rodent)	Subchronic toxicity	Required	Not standard; replaced by targeted study	OECD 408	Detects subchronic systemic toxicity
43	Reproductive/developmental toxicity	For chronic human use	Required for some categories	Not standard	OECD 414, 416	Required for substantial human exposure
44	Chronic toxicity/carcinogenicity	If high chronic exposure	Case-by-case	Not standard	OECD 451, 453	Triggered by exposure level
45	Human tolerance/clinical study	Short-term human feeding trial	Often required for novel proteins	Not applicable	GCP-compliant clinical protocol	EFSA/FDA novel food expectation
			I. Target-species animal trials (feed pathway)			
46	Palatability/acceptance	Short feeding trial, target species	Not applicable	Required	Species-specific protocol	Confirms voluntary intake
47	In vivo digestibility	Apparent digestibility coefficients	Not applicable	Required by target species	NRC/standard methods	Establishes nutritional value
48	Tolerance trial	14–28 day dose-range study, target species	Not applicable	Required	Low/intended/high inclusion	Detects acute adverse effects
49	Performance/efficacy trial	8–12 week growth study	Not applicable	Required for feed claims	Species-specific protocol	Demonstrates feed efficacy
50	Edible tissue residue	Meat, milk, eggs, fish contaminant transfer	Not applicable	Required for food-producing animals	ICP-MS + targeted LC/GC-MS	Protects human food chain
			J. Stability & manufacturing			
51	Shelf-life/stability	Real-time + accelerated stability	Required	Required	ICH guidelines adapted	Establishes expiry/use-by
52	Water activity	Aw measurement	Required for dried products	Required	Aw meter	Microbial growth predictor
53	Process validation	Sterilization, harvest, drying kill steps	Required under FSMA	Required under 21 CFR 507	Validation protocols	Preventive controls compliance
54	Mass balance/contaminant transfer	Tracking contaminants input → product	Required	Required	Multi-point sampling	Process safety verification
55	Supplier/feedstock acceptance	PLA waste source qualification	Required—restrict to known sources	Required—restrict to known sources	Supplier qualification program	Reduces additive uncertainty at source

6. Conclusions

In summary, the current bench-scale process has proven technically viable method of producing an SCP feedstock and may be an economically justified method of yeast production on the household scale using PLA waste as a starting material. The dominant cost is energy, not reagents, which also lends the positive early results to future investigation using a scaled-up bioreactor. Future work should focus on increasing volumetric productivity, integrating the process within a broader biorefinery context where PLA depolymerization and waste credits offset the energy burden, and exploring the necessary safety experiments to fulfill the potential of converting AM waste to food.

Author Contributions

L.D.: methodology, data curation, writing—original draft preparation; writing—reviewing and editing, visualization, investigation. J.M.P.: conceptualization, methodology, data curation, writing—original draft preparation; writing—reviewing and editing; supervision. All authors have read and agreed to the published version of the manuscript.

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The authors have no conflicts of interest.

Use of AI and AI-Assisted Technologies

During the preparation of this work, the authors used Anthropic Opus 4.7 to improve the clarity, grammar, and flow of the manuscript. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

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