Co-Fermentation of Food Waste and Municipal Sludge from the Saudi Arabian Environment to Improve Lactic Acid Production by *Lactobacillus rhamnosus* AW3 Isolated from Date Processing Waste

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**Abstract:** Food waste and municipal sludge were used as the substrates for the biosynthesis of lactic acid in a batch fermentor. The probiotic bacterial strain *Lactobacillus rhamnosus* AW3 isolated from date processing waste was used to produce lactic acid in a batch fermentor. Co-fermentation enhanced the biosynthesis of lactic acid and decreased substrate inhibition more than mono-substrate fermentation. A maximum yield of $28.4 \pm 0.87$ g/L of lactic acid was obtained through co-fermentation of food waste and municipal sludge at an optimized ratio of 2:0.5. Lactic acid production was improved by the supplementation of fructose, peptone, and sodium dihydrogen phosphate at pH 5.5 after 48 h fermentation. This production was approximately three-fold higher than that during mono-fermentation of food waste. The tested bacterial strains were obtained from the Microbial Type Culture Collection (MTCC). Lactic acid showed potent antimicrobial activity against pathogenic organisms, such as *Bacillus subtilis* MTCC 5981 (14 mm), *Staphylococcus aureus* MTCC 737 (20 mm), *Pseudomonas aeruginosa* MTCC 424 (24 mm), *Enterobacter aerogenes* MTCC111 (19 mm), *Escherichia coli* MTCC 443 (18 mm), *Penicillium chrysogenum* MTCC 5108 (19 mm), and *Aspergillus niger* MTCC 282 (19 mm). The antimicrobial properties of lactic acid have significant potential to inhibit the growth of pathogenic bacteria and fungi and improve probiotic properties. The lactic acid extracted from *L. rhamnosus* AW3 decreased the pH value of soil ($p < 0.01$) and increased the availability of soil phosphorus ($p < 0.01$). These findings demonstrate the bioconversion of food waste and municipal sludge into lactic acid, and the recycling of food wastes in urban areas to enhance soil nutrients.

**Keywords:** food waste; municipal solid sludge; co-fermentation; *Lactobacillus rhamnosus*; lactic acid; waste recycling

1. Introduction

Biomass valorization is the process of converting biomass into highly useful materials including fuels and chemicals. Many valorization methods have shown significant promise in meeting various industrial demands. In recent years, interest has increased in recycling various types of waste, including food waste, due to large-scale generation and the application of organic substances to numerous uses, such as, biogas, animal feed, and compost [1]. The FAO estimated that about one-third of food generated for human consumption was wasted or lost globally, and the average waste generation was about 1.3 billion tons per year [2]. The amount of generated waste varies widely among the human population based on the types of food used for consumption. Food waste generation has increased continuously and waste accounts for 32% of the total food produced globally [3]. Food waste in the
global food supply chain has been extensively analyzed by food supply chain experts. The global food supply chain varies widely depending on types of food waste, post-harvest waste, and behavioral changes of consumers [4]. In European Union, food waste generation increased every year and it changed in each stage of food supply chain. At household level, 25% of food waste generated in the food supply chain and this percentage increased as 40% during postharvest and processing stage. In food supply chain another 40% food waste generated during the retail and consumer levels [3].

In Spain and, more broadly, Europe, the food industry is a major industrial sector and its generated waste is aerobically digested for biogas production [5]. In North America, including United States, Mexico, and Canada, an estimated food loss and waste was about 170 million tons. In the United States of America, the estimated food loss and waste was 30–50% based on the types of food waste generated. In India, the annual production of food and vegetable wastes reaches 5.6 million tons. Vegetable and fruit wastes contain simple carbohydrates, and high solid and moisture contents increase the availability of nutrients to microorganisms. Depending on the characteristics of food and vegetable waste and on the existing market demand, the most relevant valorization options are production of exopolysaccharides, enzymes, extraction of various bioactive compounds, synthesis of biopolymers, bioplastics, and production of biofuels [6].

Food waste is one of the major environmental problems, with waste and losses generated at every stage of the food supply chain. There are many waste management methods for the safe disposal of food waste, however these have various problems such as environmental pollution, toxic by-products, and high costs. Recently, the pyrolysis method has been recommended for the utilization of food waste for the production of novel products. This method is used for the production of biochar, syngas, and bio-oil [7]. Food supply chain begins with the agriculture and livestock sector, which generated various by-products. This early stage of supply chain produces food waste and food loss in the form of very low-quality products and products with no commercial value. Food waste generation continues even at the final stage (bad storage or preservation). These substrates are highly suitable to be treated by anaerobic digestion because of high moisture content and solid and simple carbohydrates [8]. Numerous organic waste recycling methods have been reported, however, none of these methods can eliminate the whole food waste problem in modern cities. In urbanized areas, high transportation costs, scattered food waste generation sources, and low selling prices of various regenerated products are impediments to the waste recycling process [9]. Hence, the generation of high value-added products from food wastes is highly desirable.

Food waste is produced during food processing, production, retail, wholesale, and consumption. Food waste consists of 5–10% proteins, 10–40% lipids (w/w), and 30–60% starch [2,10]. The use of food waste as a feedstock in fuel, material, and chemical production has been proposed and demonstrated to eliminate nutrient-rich food waste [11]. Food waste is used in generic fermentation, and by fungi such as Aspergillus oryzae and Aspergillus awamori in submerged fermentation [12]. Fungal hydrolysis has a large number of social and environmental advantages including no unpleasant smell or air pollution and low energy intensity compared to the traditional method food waste recycling process. This process is useful for the production of various bio-based value-added products. In addition, it allows recycling of various types of food wastes using hydrolysate as a feedstock for the fermentation process. Pleissner et al. [13] used Halomonas boliviensis for the production of polyhydroxybutyrate. Mixed food waste hydrolysate has also been used as the nutrient medium for the cultivation of Chlorella pyrenoidosa for algal biomass production [9]. Generally, the low concentration of end products and prolonged fermentation was not advantageous. However, Kwan et al. [14] reported that the production of lactic acid from food waste as a bulking agent was profitable due to the high lactic acid yield and short fermentation time. Lactic acid has various uses in the beverage and food sector, in addition to the chemical and pharmaceutical industries, and its polymerization abilities are an advantage in the formulation of polymer poly(lactic acid) [15]. Solid-state fermentation has been performed using starter culture with the addition of enzymes [16], a single microorganism [17], or isolated indigenous bacterial/fungal strains [18].
Lactic acid can be derived from various natural sugars and applied in the production of many value-added products and various chemicals [19]. Food waste (FW) contains simple sugars, which have significant potential for use as a simple medium for lactic acid production. The production of poly(lactic acid) by lactic acid contributes about 35% of the total bioplastic market due to its favorable material performance and eco-friendly approach [20]. Lactide is an intermediate product of poly(lactic acid) with numerous applications as surfactants, printing toners, coatings, adhesives, and polymer additives [21]. In recent years, these two products have been derived from corn and sugar beet [22]. Food wastes such as brewer’s spent grains, wheat bran, corn stalks, coffee mucilage, whey, and kitchen waste have been used as the feedstock for the production of lactic acid [23]. Bioconversion processes, such as direct fermentation, open fermentation, simultaneous fermentation and hydrolysis, and fermentation and enzymatic hydrolysis have been reported using various lactic-acid-producing bacteria [17]. Food wastes are a mixture of various residues, and the heterogeneity of the biomass leads to uncertain results when increasing the scale of production of lactic acid. Several investigations have shown that production of lactic acid can be achieved via fermentation of food waste using various microbial consortia at higher temperatures [24]. Most previous studies have reported low yields of lactic acid. However, the acidification and hydrolysis processes have been improved by synergistic properties of various microorganisms. Recently, co-fermentation of food waste with wastewater sludge was used for the production of lactic acid and achieved lactic acid stabilization at room temperature and at alkaline pH [25]. To reduce the production cost of lactic acid, various types of biomass, such as potato peel, fruit and vegetable wastes, and municipal solid waste, have been used to improve the yield [26–28]. Due to its high yield and organic content, food waste has been used as a suitable substrate for lactic acid production [29]. The anaerobic process has four steps: Hydrolysis, acidogenesis, acetogenesis, and methanogenesis. Lactic acid production is achieved by acidogenesis and hydrolysis. Process parameters such as pH, inoculums, C/N ratio, substrate, and temperature significantly influence lactic acid production [28,29]. An acidic pH range (4–5) effectively promotes the hydrolysis of various food wastes for the production of lactic acid [28]. In the present investigation, the impact of environmental and nutrient factors on lactic acid fermentation in a batch bioreactor was studied. Furthermore, the influence of lactic acid on soil characteristics was analyzed.

2. Materials and Methods

2.1. Substrate

Food waste (FW) was obtained from municipal food wastes at Riyadh, Saudi Arabia. The food wastes consisted of meat, chicken bone, bread, egg, lobster shell, and rice. It was crushed with a mechanical blender into small pieces to enhance composting after the manual separation of bones from the FW using forceps. The final substrate slurry was filtered using a sieve with 1 mm thickness and stored at 4 °C in a refrigerator. Municipal sludge (MS) was collected from a wastewater treatment facility at Riyadh. The FW and MS mixture was completely sterilized for 30 min at 121 °C to eliminate indigenous microbial flora from the food and sludge. The prepared slurry was applied to the reactors for the fermentation process. The physico-chemical properties of the substrate were analyzed.

2.2. Characterization of Lactobacillus Strain

Lactobacilli were isolated from the date effluent. Wastewater was collected from a date processing facility at Riyadh, Saudi Arabia. The sample was serially diluted and plated on Man–Rogosa–Sharpe (MRS) agar (Himedia, Mumbai, India) medium. The morphology of the bacterial isolate was studied by incubating on MRS agar medium for 48 h. Gram-staining and biochemical characteristics were studied. To identify the strain in molecular level using forward and reverse primers, 16S rDNA sequence analysis was performed [30].
2.3. Batch Fermentation

Municipal solid sludge was previously collected from the wastewater treatment facility at Riyadh, Saudi Arabia. After the sample was incubated for 24 h at room temperature, the clear supernatant was discarded. Food waste and the concentrated sludge were mixed in a ratio of 4:1 based on volatile suspended solids. Tap water was added to maintain total COD at 44,864 ± 376 mg/L. Batch fermentation was carried out in the reactors and the working volume was fixed at 1 L. The schematic presentation of a batch fermentor is provided in the Supplementary Figure S1. The inoculum was prepared by culturing Lactobacillus strains in MRS broth by incubating at 37 °C under shaking condition (150 rpm for 18 h). It was stirred at 120 rpm at 35 °C. The pH of the culture medium was adjusted to be 7.0 using 5 M hydrochloric acid or 5 M sodium hydroxide [31]. The batch fermentation experiment was performed for 72 h. A 2.5 L vessel was equipped with in- and outflow of filtered air, alkali, and inoculum ports. Culture medium was pumped into the fermentor up to a volume of 1 L. To control the pH of the medium, alkali was added continuously. The medium was mixed continuously with the use of a blade. Sampling was conducted using a sample hole. The vessel was agitated with a constant speed (220 rpm). Then, lactic acid production (g/L), total sugar (g/L), consumption of substrate, and biomass (g/L) was analyzed. Lactic acid content was analyzed using HPLC (Shimadzu Corporation, Tokyo, Japan) using a Shim-pack Fast-OA column. The chromatography column was operated at pH 2.2 and 0.005 N H₂SO₄ was used as the mobile phase. The column temperature was set as 30 °C and the flow rate was adjusted to 0.8 mL/min. A refractive index was used to determine the compound. The amount of carbohydrate content was evaluated using the phenol–sulfuric acid method as described by Dubois et al. [32]. Reduction of the sugar level of the fermented medium was performed by 3,5-dinitro salicylic acid method [33]. Briefly, the reagent was prepared by mixing 3,5-dinitro salicylic acid (0.63%), Rochelle salts (18.2%), phenol (0.5%), sodium hydroxide (0.5%), and sodium bisulfite (0.5%). To this reagent, 0.1 mL sample was added and kept in a boiling water bath for 5 min. The sample was cooled, and the absorbance was read at 540 nm using a UV-vis spectrophotometer.

2.4. Monitoring Lactic Acid Bacteria

The viability of lactic acid bacteria (LAB) was analyzed during the fermentation process using Man–Rogosa–Sharpe (MRS) agar medium. This medium consists of (g/L) 5 g yeast extract, 10 g beef extract, 20 g peptone, 20 g glucose, 14 g agar, 2 g CaCO₃, 0.25 g MnSO₄·4H₂O, 0.58 g MgSO₄·7H₂O, 5 g CH₃COONa, 2 g diammonium citrate, 2 g K₂HPO₄, and 1 mL Tween-80. The pH of the culture medium was adjusted to 6.5 ± 0.2. The medium was sterilized for 15 min at 121 °C. Samples were withdrawn periodically from the fermentor and overlaid with the MRS agar medium. Plates were incubated for 48 h at 37 °C in a temperature-controlled incubator. Experiments were performed in triplicate and average cell counts were considered for analysis.

2.5. Optimum FW and MS Sample

FW and MS samples were mixed at various proportions to determine the optimum concentration for lactic acid production. Six different ratios of FW and MS samples (0:1, 1:0, 0.5:1; 1:0.5, 1:1, 1:1.5, 1.5:1, and 2:0) were selected based on the dry weight of the sample. The culture medium was not supplemented with any other nutrients, pH maintenance, or autoclaving. About 10% (v/v) inoculum was applied for batch fermentation. The saccharification process was initiated by the addition of amylase and cellulases. After the addition of these enzymes, the fermentation process was started. The sample was stirred at 150 rpm and incubated at 37 °C for two days. Each experiment was performed in duplicate and an average value was used for data processing.

2.6. Effect of pH on Lactic Acid Production

Lactic acid production was performed in a batch fermentor and the experiment was conducted using 14 fermentation reactors. Each fermentation unit was prepared with 1000 mL culture medium.
and stirred mechanically at 125 rpm. This fermentor (2.5 L) contained 1 L of fermentation medium. The medium pH was controlled automatically between 4.5 and 7.0 by the addition of 10 M NaOH or 2 M hydrochloric acid. The temperature of the fermentor was maintained at 30 ± 2 °C. Samples were withdrawn every 24 h and lactic acid content was analyzed by the HPLC method.

2.7. Effect of Carbon and Nitrogen Sources on Lactic Acid Production

Lactic acid production in the medium containing additional carbon sources (1%) was studied. Carbon sources such as xylose, starch, lactose, maltose, sucrose, fructose, and glucose were incorporated. Nitrogen sources such as ammonium chloride, ammonium sulphate, peptone, yeast extract, beef extract, and casein were added at the 0.5% level. The effect of various phosphate sources (0.1%, \( \text{w/v} \)) on lactic acid production was studied. Phosphate ions such as di-potassium hydrogen phosphate, potassium dihydrogen phosphate, di-sodium hydrogen phosphate, and sodium di-hydrogen phosphate were incorporated in the bioreactor.

2.8. Effect of Metal Ions on Lactic Acid Production

Metallic ions play significant roles in activating various metabolic enzymes. Ions such as Mn\(^{2+}\), Mg\(^{2+}\), Cu\(^{2+}\), and Fe\(^{3+}\) have been reported to influence lactic acid production \([34,35]\). Cu\(^{2+}\) ions also positively influence lactic acid production. During the fermentation process, the presence of copper ions inhibits the consumption of D-lactic acid to pyruvate. To analyze the influences of various metals on lactic acid production, the substrate was enriched with Mn\(^{2+}\), Mg\(^{2+}\), Cu\(^{2+}\), Fe\(^{3+}\), Ca\(^{2+}\), and Co\(^{2+}\) added to the culture medium at 0.01% concentration. The fermentor without any metal ions was considered a blank.

2.9. Antimicrobial Activity of Lactic Acid

Antimicrobial activity was determined using the disc diffusion method for various pathogenic bacteria, including Bacillus subtilis MTCC 5981, Staphylococcus aureus MTCC 737, Pseudomonas aeruginosa MTCC 424, Enterobacter aerogenes MTCC111, E. coli MTCC 443, Penicillium chrysogenum MTCC5108, and Aspergillus niger MTCC 282. The culture obtained from the cultivation of the Lactobacillus strain in the batch fermentor for 48 h at 37 °C was harvested and the sample was centrifuged at 10,000× g for 10 min. The cell-free culture supernatant was further neutralized with NaOH (1 M) and the final pH was maintained at 7.0 and the sample was filtered using a 0.2 μm membrane filter. Culture media such as Mueller Hinton agar and Potato Dextrose Agar were prepared according to the manufacturer’s instructions (Himedia, Mumbai, India). A sterile 6 mm (Himedia, Mumbai, India) disc was kept on the solid agar medium and then cell-free extract (25 μL) was loaded on the disc. The culture plates were incubated for 24 h and 72 h, respectively, for bacteria and fungi.

2.10. Application of Lactic Acid in Soil Amendment

One kilogram of soil was preincubated for 7 days at 25 °C at 35–45% water-holding capacity of as described by Tejada \([36]\) with few modifications. After 7 days of incubation, lactic acid was added at four different concentrations: T1 (0.25%, \( \text{w/v} \)), T2 (0.5%, \( \text{w/v} \)), T3 (0.75%, \( \text{w/v} \)), and T4 (1%, \( \text{w/v} \)). Lactic acid was not incorporated into the control experiment. Soil was incubated for five weeks under dark conditions at 25 °C. A sample was withdrawn every 7 days and used for biochemical analysis. The pH of the soil was determined after extraction with distilled water using a pH meter. The soil sample (1 gm) was treated with extraction buffer (10 mL) containing 0.1 N \( \text{H}_2\text{SO}_4 \) for 1 h and centrifuged at 10,000× g for 10 min. The supernatant was filtered, and lactic acid content was analyzed. Soil phosphorus content was determined as described by Olsen et al. \([37]\). Soil dehydrogenase activity was measured as described by Tabatabai \([38]\). p-nitrophenyl-\( \beta \)-D-glucopyranoside was used as the substrate for the determination of \( \beta \)-glucosidase activity \([39]\).
2.11. Statistical Analysis

Experiments were performed in triplicate and the data are expressed as mean ± standard deviation. One-way analysis of variance was used to analyze the significance and \( p < 0.05 \) was considered statistically significant.

3. Results and Discussion

3.1. Characterization of Lactobacillus Strains and Production of Lactic Acid

A total of seven Lactobacillus colonies from date processing waste were isolated and recovered from Rogosa agar plates and MRS agar plates as Lactobacillus species based on the catalase test and Gram staining. Each of the seven bacterial isolates was Gram positive and rod shaped and showed a negative reaction towards citrate and catalase tests. The Lactobacillus strains survived in 3% bile salts and utilized most of the tested carbohydrates. The morphological and biochemical characteristics of Lactobacillus are presented in Table 1. Microscopic observation showed that 100% of LAB was rod-shaped and to achieve taxonomic classification, 16S rDNA analysis was undertaken. The isolate AW3, identified as L. rhamnosus AW3, has potent lactic acid producing capacities. Bacteria from the genera Lactobacillus, Enterococcus, and Lactococcus produce types of lactic acids and have been used to enhance bioavailability and reduce mycotoxins [40]. Recent findings showed that probiotic Lactobacillus strains can be widely used to remove toxins such as fumonisins, trichotheceines, aflatoxins, and mycotoxins during harvest and storage [41]. These LAB species isolated from various food products can be effectively utilized in the formulation of functional foods to manage the growth of pathogenic bacteria and indirectly control various diseases that may affect consumers [42].

The lactic acid-producing ability of Lactobacillus rhamnosus has been described previously and used in various substrates. Rivas et al. [43] used corn steep liquor and spent yeast cells for the production of lactic acid. L-lactic acid production of Lactobacillus rhamnosus using cassava powder was reported by Wang et al. [44]. Recently, Chen et al. [45] used a mixed culture of Lactobacillus rhamnosus and Bacillus coagulans to optimize the medium for the production of lactic acid.

Table 1. Biochemical characteristics of lactic acid bacteria isolated from date processing waste.

| Biochemical Test                  | AW1 | AW2 | AW3 | AW4 | AW5 | AW6 | AW7 |
|-----------------------------------|-----|-----|-----|-----|-----|-----|-----|
| Gram staining                     | +   | +   | +   | +   | +   | +   | +   |
| Shape                             | Rod | Rod | Rod | Rod | Rod | Rod | Rod |
| Citrate utilization test          | –   | –   | –   | –   | –   | –   | –   |
| Catalase test                     | –   | –   | –   | –   | –   | –   | –   |
| Bile salt hydrolysis              | +   | +   | +   | +   | +   | +   | +   |
| Growth at temperatures            |     |     |     |     |     |     |     |
| 10 °C                             | +   | +   | +   | +   | +   | +   | +   |
| 20 °C                             | +   | +   | +   | +   | +   | +   | +   |
| 40 °C                             | –   | –   | –   | –   | –   | –   | –   |
| Glucose hydrolysis                | +   | +   | +   | +   | +   | +   | +   |
| Sucrose hydrolysis                | +   | +   | +   | +   | +   | +   | +   |
| Lactose hydrolysis                | +   | +   | +   | +   | +   | +   | +   |
| Dextrose hydrolysis               | +   | +   | +   | +   | +   | +   | +   |
| Xylose hydrolysis                 | +   | +   | +   | +   | +   | +   | +   |
| D-arabinose                       | –   | –   | –   | –   | –   | –   | –   |
| Mannose                           | +   | +   | +   | +   | +   | +   | +   |
| A-cellulose                       | –   | –   | –   | –   | –   | –   | –   |
| D-raffinose                       | +   | +   | +   | +   | +   | +   | +   |
| Sorbitol                          | +   | +   | +   | +   | +   | +   | +   |

Positive results, +; Negative results, –.
The physico-chemical factors and nutrient contents of FW and MS are described in Table 2. The culture medium was found to be rich in soluble proteins, soluble carbohydrate, suspended solids, and volatile suspended solids. The increased availability of simple carbohydrate from the food waste favored lactic acid production. FW contains various simple sugars that are useful for the production of lactic acid by various bacteria [6]. The batch fermentation system is useful for the production of lactic acid because its technical simplicity. However, fed-batch production of lactic acid has been reported previously to improve yield [46]. The fed-batch fermentation system offers various advantages such as reducing substrate inhibition and improving productivity [47]. Batch fermentation was carried out for 72 h, and lactic acid yield, sugar consumption, and biomass are described in Table 3. The maximum production of lactic acid was $28.3 \pm 0.22$ g/L and the biomass yield was $24.2 \pm 0.13$ g/L after 72 h. Lactic acid production attained $26.5 \pm 0.15$ g/L within 48 h fermentation and increased to $28.2 \pm 0.23$ g/L. In previous research, *Lactobacillus rhamnosus* B103 has been used for the production of lactic acid [48].

### Table 2. Physico-chemical factors and nutrient content of food waste and municipal sludge.

| Parameter                  | Food Waste          | Municipal Sludge     | Mixture            |
|----------------------------|---------------------|----------------------|--------------------|
| pH                         | 6.3 ± 0.12          | 6.5 ± 0.04           | 6.7 ± 0.13         |
| Total suspended solid (mg/L)| 190489 ± 427        | 16489 ± 579          | 45972 ± 1487       |
| Volatile suspended solid (mg/L) | 187681 ± 698       | 11952 ± 397          | 42087 ± 720        |
| Total COD (mg/L)           | 190275 ± 387        | 15486 ± 296          | 44864 ± 376        |
| Soluble COD (mg/L)         | 70142 ± 302         | 6359 ± 46.9          | 9736 ± 104         |
| Soluble protein (mg/L)     | 5984 ± 31.5         | 4085 ± 56.5          | 4622 ± 3.6         |
| Soluble carbohydrate (mg/L)| 53830 ± 2.4         | 36280 ± 109.2        | 45392 ± 284.4      |

### Table 3. *Lactobacillus* strains isolated from palm effluent and lactic acid yield in a batch reactor.

| *Lactobacillus* Strains | Lactic Acid Yield (g/L) |
|-------------------------|-------------------------|
|                         | 24 h            | 48 h            | 72 h            |
| AW1                     | 23.1 ± 0.209     | 23.6 ± 0.217    | 23.9 ± 0.209    |
| AW2                     | 20.98 ± 0.17     | 21.9 ± 0.208    | 20.52 ± 0.11    |
| AW3                     | 22.9 ± 0.12      | 26.4 ± 0.209    | 26.9 ± 0.1      |
| AW4                     | ND              | 23.8 ± 0.14     | 24.3 ± 0.11     |
| AW5                     | 22.3 ± 0.22      | 25.6 ± 0.211    | 26.2 ± 0.1      |
| AW6                     | 23.3 ± 0.201     | 24.8 ± 0.212    | 24.7 ± 0.204    |
| AW7                     | ND              | 23.1 ± 0.201    | 24.2 ± 0.13     |

Batch fermentation has been recommended for industrial production of lactic acid, however substrate inhibition of lactic acid synthesis is the major disadvantage [49]. Hence, a feeding technique has been recommended to eliminate the inhibition of the substrate in the microbial environment. This strategy allows control of the growth rate and maximization of the lactic acid yield in batch culture. In the present analyses, all lactic acid producing organisms were classified from the genus *Lactobacillus* and cultured in a batch fermentor. In previous research, the probiotic *L. lactis* was isolated from various flours such as sorghum, wheat, and quinoa [50–52]. The strain *Lactobacillus rhamnosus* has been isolated from kidney beans, sorghum, amaranth flour, wheat, and sourdough [53–55]. Lactic acid production has been carried out in test tubes (15 mL) previously in aerobic conditions and lactic acid yield variation was reported. In *Lactobacillus* strains such as *Lactobacillus delbrueckii* ssp. bulgaricus CECT 5038 and CECT 4005, the lactic acid yield was lower, however the case of *L. delbrueckii* ssp. bulgaricus CECT 5036 showed maximum yield in micro-aerobic conditions [56]. To improve the silage process, *Lactobacillus* strains such as *L. plantarum, L. brevis*, and *L. paracasei* were used to reduce the concentrations of various pathogenic organisms [57].
3.2. Batch Fermentation of Lactic Acid in Relation with Other Factors

The *Lactobacillus* strain was cultured for 72 h and lactic acid production was assayed. Total sugar content of the fermentor declined after 72 h of incubation. Biomass production reached a maximum up to 72 h (4.2 ± 0.13 g/L wet weight) and a marginal decrease was observed after 96 h (3.73 ± 0.11 g/L) (Table 4). The pH of the culture medium was initially adjusted to 5.5. Generally, lactic acid bacteria synthesize lactic acid in a wide pH range (pH 4.0–6.0). Lactobacilli produced maximum lactic acid at a low pH value (pH 4.0) [58]. Carbohydrate content of the medium continuously declined from 63 ± 4.2 to 10 ± 1.1 after 96 h. The culture medium pH was 5.5 and carbohydrate content declined more rapidly than in previous studies. In previous research, a decreased carbohydrate level was reported at a higher pH (6.0) due to high bacterial activity and acid feedback inhibition [28,58]. In the current study, protease and α-glucosidase activity during the fermentation process was tabulated (Table 5).

Both enzyme activities were low at lower pH values and at pH 6.5 enzyme activities were maximum (97 ± 3.1 and 11.2 ± 1.03 U/mL after 48 h). These findings indicate increased microbial activity at higher pHs. α-glucosidase activity was used to indirectly measure the processes of methanogenesis, acidogenesis, and hydrolysis. However, when the pH value increased in the fermentor above 7.0, protease (40 ± 0.18 U/mL) and α-glucosidase (7.5 ± 0.8 U/mL) activity decreased after 72 h. Protease production was detected from pH 4.0 to pH 7.0 and maximum enzyme production was detected at pH 8.0 (97 ± 3.2 U/mL). α-glucosidase reached a maximum in an alkaline pH range (pH 8.0 to pH 10.0) and this enzyme activity was very low in an acidic medium.

### Table 4. Analysis of nutrients and lactic acid production at various fermentation periods.

| Fermentation Period (h) | Total Sugar (g/L) | Biomass (g/L) | Lactic Acid (g/L) |
|-------------------------|-------------------|---------------|-------------------|
| 12                      | 63 ± 4.2          | 1.3 ± 0.2     | 21.3 ± 1.11       |
| 24                      | 54 ± 1.9          | 2.15 ± 0.9    | 21.7 ± 0.13       |
| 36                      | 43 ± 2.2          | 3.4 ± 0.2     | 22.8 ± 1.2        |
| 48                      | 21 ± 1.8          | 3.6 ± 0.1     | 26.5 ± 1.15       |
| 60                      | 20 ± 3.3          | 4.1 ± 0.2     | 28.2 ± 0.23       |
| 72                      | 14 ± 1.2          | 4.2 ± 0.13    | 28.3 ± 1.22       |
| 84                      | 13 ± 1.3          | 3.91 ± 0.12   | 22.5 ± 1.2        |
| 96                      | 10 ± 1.1          | 3.73 ± 0.11   | 20.3 ± 1.1        |

### Table 5. Protease and α-glucosidase activity.

| pH   | Protease Activity (U/mL) | α-Glucosidase Activity (U/mL) |
|------|--------------------------|-------------------------------|
|      | 48 h                     | 72 h                          |
|      | 48 h                     | 72 h                          |
| 4.5  | 32 ± 2.2                 | 34 ± 1.3                      | 1.5 ± 0.31        | 1.9 ± 0.2 |
| 5.0  | 45 ± 1.3                 | 40 ± 0.9                      | 4.9 ± 0.18        | 5.3 ± 0.13|
| 5.5  | 51 ± 0.5                 | 59 ± 1.1                      | 5.7 ± 1.1         | 5.6 ± 0.32|
| 6.0  | 92 ± 4.1                 | 73 ± 1.8                      | 6.2 ± 0.73        | 6.8 ± 0.49|
| 6.5  | 97 ± 3.1                 | 80 ± 1.5                      | 11.2 ± 1.03       | 13.6 ± 0.23|
| 7.0  | 41 ± 1.3                 | 45 ± 1.5                      | 10.3 ± 0.56       | 8.1 ± 0.12|
| 7.5  | 40 ± 0.18                | 37 ± 0.9                      | 7.5 ± 0.8         | 6.3 ± 0.15|

3.3. Effect of FW and MS Ratios on Lactic Acid Production

Table 3 shows the production of lactic acid by co-fermentation of FW and MS at 0:1, 1:0, 0.5:1, 1:0.5, 1:1, 1:1.5, 1.5:1, and 2:0.5 concentrations. Fermentation was performed for 48 h and lactic acid was stable at this fermentation period. The mono-fermentation of MS yielded less lactic acid (21.3 g/L), followed by the FW:MS ratio of 0.5:1 (22.5 g/L) after 24 h fermentation. The highest lactic acid concentration of 28.4 g/L was obtained when the FW and MSS ratio was 2:0.5. Co-fermentation of FW and MS enhanced lactic acid yield. These findings indicate that fermentation of FW with MS at a ratio of 2:0.5 was optimal for lactic acid productivity (Table 6). During the fermentation process, fatty acids were generated.
from the FW due to hydrolysis and these fatty acids decreased the pH value during the fermentation process [59]. Mixing of FW with an optimal level of MS enhanced lactic acid production. In recent years, co-fermentation of FW with other wastes has been extensively studied as a better option for energy recycling and the waste-handling process [60]. Wu et al. [61] reported that FW co-fermented with a de-oiled grease trap enhanced the yield of biogas. Co-digestion of FW with primary sludge enhanced the lactic acid yield by about 97% [62]. A 2:0.5 ratio of FW with MS enhanced lactic acid conversion by three-fold compared to mono-fermentation. Waste-activated sludge (WAS) and FS have been used to increase lactic acid production. Valorization of these wastes has attracted much attention in recent years [63]. The synergistic property of WAS and food waste was previously studied by Wu et al. [61] and Edwards et al. [64]. An optimized level of FW and MS was found to enhance lactic acid production. In addition, an optimal ratio of WAS and FW has also been reported to enhance lactic acid production. The ratio of these wastes varied based on the nutrient content and physical nature of the components [65].

Table 6. Co-fermentation of food waste and municipal sludge at various ratios.

| FW:MS Ratios | Dry Weight of FW | Dry Weight of MS | Lactic Acid Yield (g/L) |
|--------------|------------------|------------------|------------------------|
|              |                  |                  | 24 h | 48 h |
| 0:01         | 0                | 15               | 21.3 | 23.2 |
| 1:00         | 15               | 0                | 24.5 | 25.9 |
| 0.5:1        | 5                | 10               | 22.5 | 23.3 |
| 0.01:0.5     | 10               | 5                | 24.9 | 25.4 |
| 1:01         | 7.5              | 7.5              | 24.3 | 25.2 |
| 0.5:1.5      | 6                | 9                | 22.7 | 23.5 |
| 1.5:1        | 9                | 6                | 25.4 | 26.3 |
| 02:00.5      | 12               | 3                | 27.3 | 28.4 |

3.4. Effect of pH on Lactic Acid Production

In this study, pH adjustment was not undertaken for blank experiments, and the analyzed pH range was between 4.5 and 7.0. However, these ranges may vary widely based on the type of substrate and organism used for the composting process. Tang et al. [29] used FW for lactic acid production using indigenous microbiota and the pH between 5.0 and 6.0 was tested for lactic acid production. The blank experiment yielded 22.9 ± 0.31 g/L. As shown in Figure 1, the culture medium in weak alkaline conditions enhanced lactic acid yield more than in a strong alkaline environment. Maximum lactic acid production was observed after 48 h at pH 5.5. However, lactic acid yield decreased at higher pH ranges (6.0–7.0). It was observed that increasing the pH value undermined the production of lactic acid after 24 h. Akao et al. [66] used garbage for the production of lactic acid in unsterile conditions and reported enhanced product yield at pH 5.5 in thermophilic conditions. In the case of lactic acid fermentation, temperature and pH of the medium overlapped in the bio-process. During the process of fermentation, volatile fatty acids from the FW reduced the pH of the culture medium. At higher temperatures, a low pH value was found to be optimum to enhance the product yield [67]. Volatile fatty acids were generated from food wastes and these inhibited anaerobic digestion [59]. Mixing of an appropriate concentration of FW with MS can result in a favorable pH to maximize lactic acid production. Sophora flavescens residues and food wastes at a ratio of 1:1.5 was found to be optimal to maintain pH during the fermentation process [65].
3.5. Nutrient Sources on Lactic Acid Production

Carbon sources were found to influence lactic acid production and the results are shown in Figure 2a. *Lactobacillus* sp. showed various fermentation characteristics with respect to carbon sources. When the bioreactor was treated with glucose, fructose, and maltose, the selected bacterial strain metabolized these carbon sources and converted them into 27.4 ± 0.4 to 29.8 ± 0.56 g/L lactic acid. This might be mainly due to acidification of the fermentation medium and the various *Lactobacillus* carbohydrates using the homofermentation pathway. In previous research, a co-culture of engineered *Lactococcus lactis* and *Lactobacillus delbrueckii* revealed the conversion of sugar into lactic acid [68]. In *L. coryniformis*, high levels of lactic acid production have been achieved with glucose supplementation [69]. Enhanced lactic acid production in a packed bed reactor has been previously achieved in a medium containing sucrose as the carbon source in *Lactobacillus delbrueckii* [70]. In the current study, FW and sludge supplemented with nitrogen sources enhanced lactic acid production. Figure 2b reveals the influence of nitrogen sources on lactic acid production. Inorganic nitrogen and complex nitrogen sources, namely peptone, yeast extract, ammonium sulphate, and beef extract, significantly influenced lactic acid production. Among the nitrogen sources, peptone was utilized by the strain for maximum lactic acid production. Types of organic biomass such as molasses enhanced bacterial cell growth because of high nitrogen content and calcium. The available calcium content in the molasses buffers the enzymatic action [71]. Nitrogen implied either in catabolic processes or anaerobic processes is mainly available in the form of inorganic compounds, peptides, and amino acids. These sources can be generally supplemented in the form of ammonium sulphate, yeast extract, urea, and peptone [72]. The tested phosphates regulated lactic acid production. Lactic acid yield was high in the medium containing potassium dihydrogen phosphate (27.5 ± 1.1 g/L) and sodium di-hydrogen phosphate (28.3 ± 0.8 g/L). Dibasic ions, such as di-potassium hydrogen phosphate (28.7 ± 0.82 g/L), and monobasic ions, such as sodium di-hydrogen phosphate (28.91 ± 0.57 g/L), enhanced lactic acid production. These metal ions served as the co-factor for enzyme production. Mineral elements and ions provided in the form of various salts and vitamins available in yeast extract and other natural nitrogen sources act as cofactors in various enzymatic reactions [73].
3.6. Antimicrobial Properties of Crude Extract from L. rfhamnosus AW3

The cell-free sample obtained from the batch fermentation experiment was evaluated for antibacterial and antifungal properties; results are presented in Table 7. The crude sample containing approximately 34.2 g/L lactic acid with various final pH values clearly revealed antibacterial and antifungal properties against all of the tested bacterial and fungal pathogens. Antimicrobial activity was higher in the Lactobacillus strain cultured at pH 5.5 than that cultured at pH 6.0. The zone of inhibition was maximized against P. aeruginosa MTCC 424 (24 mm), S. aureus MTCC 737 (20 mm), and E. aerogenes MTCC111 (19 mm), whereas a smaller clear zone was observed with pH 6.0 (15, 14, and 15 mm zones, respectively). Antibacterial activity was also detected against E. coli MTCC 443 (18 mm) and B. subtilis MTCC 5981 (13 mm). Fungi such as A. niger MTCC 282 (19 mm) and P. chrysogenum MTCC5108 (19 mm) showed almost the same level of activity against the L. rfhamnosus AW3 stain maintained at pH 5.5. At pH 6.0, antimicrobial potency of the sample decreased by about 28% compared to the pH value 5.5. Probiotic Lactobacillus strains produce proteolytic enzymes and antimicrobial compounds and vitamins [74]. The preservative role of these Lactobacillus strains is mainly due to the production of various bioactive secondary metabolites including antifungal compounds, bacteriocins, lactic acids, hydrogen peroxide, acetoic, and hydrogen peroxide [75]. The inhibition activity of lactic acid varies among Lactobacillus species. Pelaez et al. [76] reported the inhibitory activity of acetic and lactic acid produced by Lactobacillus species. In recent years, the antimicrobial potential of Lactobacillus has been reported by Manhanzva et al. [77] and Meleh et al. [78]. In addition, Zhao et al. [79] studied the correlation of the colonization of the bacterial flora and biological properties of bacteria from the genus Lactobacillus.
Table 7. Antimicrobial activity of lactic acids from *L. rhamnosus* AW3 extracted from a batch fermentor.

| Microorganism                  | Zone of Inhibition (mm) at Various pH Values |
|-------------------------------|---------------------------------------------|
|                              | 4.5 | 5   | 5.5 | 6   | 6.5 |
| B. subtilis MTCC 5981         | 14  | 13  | 13  | 10  | 11  |
| S. aureus MTCC 737           | 16  | 17  | 20  | 14  | 15  |
| P. aeruginosa MTCC 424       | 20  | 19  | 24  | 15  | 15  |
| E. aerogenes MTCC 111        | 18  | 18  | 19  | 15  | 14  |
| E. coli MTCC 443             | 14  | 15  | 18  | 17  | 18  |
| P. chrysogenum MTCC 5108     | 10  | 14  | 19  | 18  | 17  |
| A. niger MTCC 282            | 13  | 12  | 19  | 19  | 18  |

3.7. Lactic Acid Induced Changes in Soil Chemical and Biological Properties

Lactic acid was applied to the soil at various concentrations and resulted in a significant decrease in pH values (*p* < 0.01). Lactic acid in the soil medium enhanced the pH value of the soil and the soil pH reached the pH value of control. The T1 soil reached a maximum value within 14 days of incubation, whereas T4 reached a maximum value after 28 days (Table 8). The decrease in the soil pH for several weeks was mainly due to the acidity of the soil sample [80]. The applied lactic acid was degraded continuously in the soil. After seven days of treatment lactic acid concentration decreased by about 75% in T1, whereas this decrease was more than 60% in the case of T2–T4 (Table 9). The continuous degradation of lactic acid was mainly due to the activity of microorganisms in the soil and this trend was described previously [81]. Treatment of the soil sample with lactic acid enhanced P solubilization, which was caused by the sudden decrease in the soil lactic acid. The applied lactic acid improved P availability, hence allowing it to be used when required by plants. Activity of soil enzymes such as dehydrogenase and acid phosphomonoesterase was improved during the first two weeks. These two enzymes from the soil sample indirectly measure microbial activity of the soil sample. Dehydrogenase enzyme activity was found to be maximized after 14 days of treatment. However, no significant change was found in β-glucosidase activity and lactic acid did not induce this enzymatic activity.

Table 8. Influence of lactic acid on the pH profile of the soil. Lactic acid was added at various concentrations and the pH was tested for 35 days.

| Treatment (Days) | pH         |
|-----------------|------------|
|                 | T1         | T2         | T3         | T4         |
| 0               | 7.3 ± 0.3  | 6.7 ± 0.14 | 6.3 ± 0.21 | 5.9 ± 1.1  |
| 7               | 7.9 ± 0.21 | 7.1 ± 0.34 | 6.6 ± 0.24 | 6.1 ± 2.2  |
| 14              | 7.7 ± 0.12 | 7.3 ± 0.11 | 6.9 ± 0.12 | 6.3 ± 1    |
| 21              | 7.72 ± 0.1 | 7.6 ± 0.16 | 7 ± 0.15   | 6.4 ± 0.59 |
| 28              | 7.73 ± 0.17| 7.73 ± 0.1 | 7.1 ± 0.31 | 6.9 ± 0.32 |
| 35              | 7.73 ± 0.15| 7.73 ± 0.15| 7 ± 0.22   | 7 ± 0.9    |

Table 9. Lactic acid content of soil samples at various incubation times. Lactic acid was added at various concentrations and its quantity was determined by the HPLC method for 35 days.

| Treatment (Days) | Lactic Acid (%) |
|-----------------|-----------------|
|                 | T1   | T2   | T3   | T4   |
| 0               | 100 ± 0 | 100 ± 0 | 100 ± 0 | 100 ± 0 |
| 7               | 46 ± 2.4 | 50 ± 1.5 | 64 ± 3.1 | 71 ± 3.1 |
| 14              | 32 ± 1.9 | 28 ± 3.1 | 56 ± 1.1 | 45 ± 1.1 |
| 21              | 18 ± 2.1 | 22 ± 1.4 | 33 ± 2.3 | 37 ± 3.1 |
| 28              | 8 ± 1.2  | 13 ± 0.9 | 25 ± 1.1 | 39 ± 2.2 |
| 35              | 0 ± 0    | 0 ± 0   | 0 ± 0   | 0 ± 0   |
4. Conclusions

Lactic acid was produced from food waste (FW) and municipal sludge (MS) in a batch fermentor. *Lactobacillus rhamnosus* AW3 isolated from date processing effluent showed an efficient conversion of wastes to lactic acid. Co-fermentation of FW and MS for lactic acid production was carried out with a three-fold increase in the overall yield (28.4 ± 0.87 g/L). Using a batch fermentor with an optimal ratio of FW and MS (2:0.5 ratio) stabilized lactic acid production. Lactic acid yield was improved by nutrient supplements and an optimal pH (5.5) after two days of fermentation. The lactic acid extracted from *L. rhamnosus* AW3 decreased the pH value of the soil and increased the availability of soil phosphorus, thus improving soil fertility. These findings are useful in the bioconversion of FW and MS into lactic acid and food waste recycling in urban areas.

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References

1. Lin, C.S.K.; Koutinas, A.A.; Stamatelatou, K.; Mubofu, E.B.; Matharu, A.S.; Kopsahelis, N.; Pfaltzgraff, L.A.; Clark, J.H.; Papanikolaou, S.; Kwan, T.H.; et al. Current and future trends in food waste valorization for the production of chemicals, materials and fuels: A global perspective. *Biofuels Bioprod. Biorefining* **2014**, *8*, 686–715. [CrossRef]

2. FAO. *Global Food Losses and Food Waste—Extent, Causes and Prevention*; FAO: Rome, Italy, 2011.

3. Morales-Polo, C.; Cledera-Castro, M.D.M.; Moratilla-Soria, B.Y. Reviewing the anaerobic digestion of food waste: From waste generation and anaerobic process to its perspectives. *Appl. Sci.*** **2018**, *8*, 1804. [CrossRef]

4. Parfitt, J.; Barthel, M.; Macnaughton, S. Food waste within food supply chains: Quantification and potential for change to 2050. *Philos. Trans. R. Soc. B* **2010**, *365*, 3065–3081. [CrossRef] [PubMed]

5. Morales-Polo, C.; del Mar Cledera-Castro, M.; Hueso-Kortekaas, K.; Revuelta-Aramburu, M. Anaerobic digestion in wastewater reactors of separated organic fractions from wholesale markets waste. Compositional and batch characterization. *Energy and environmental feasibility. Sci. Total Environ.* **2020**, *726*, 138567. [CrossRef] [PubMed]

6. Esparza, I.; Jiménez-Moreno, N.; Bimbela, F.; Ancín-Azpilicueta, C.; Gandia, L.M. Fruit and vegetable waste management: Conventional and emerging approaches. *J. Environ. Manag.* **2020**, *259*, 120816. [CrossRef]

7. Kim, S.; Lee, Y.; Lin, K.Y.A.; Hong, E.; Kwon, E.E.; Lee, J. The Valorization of Food Waste via Pyrolysis: A Review. *J. Clean. Prod.* **2020**, *259*, 120816. [CrossRef]

8. Morales-Polo, C.; Cledera-Castro, M.D.M.; Moratilla Soria, B.Y. Biogas production from vegetable and fruit markets waste—Compositional and batch characterizations. *Sustainability* **2019**, *11*, 6790. [CrossRef]

9. Kwan, T.H.; Lin, C.S.K.; Chan, K.M. Application of Food Waste Valorization Technology in Hong Kong. In *Renewable Resources for Biorefineries*; Lin, C.S.K., Luque, R., Eds.; The Royal Society of Chemistry: London, UK, 2014; pp. 93–116.

10. Pleissner, D.; Lam, W.C.; Sun, Z.; Lin, C.S. Food waste as nutrient source in heterotrophic microalgae cultivation. *Bioresour. Technol.* **2013**, *137*, 139–146. [CrossRef]

11. Lin, C.S.K.; Pfaltzgraff, L.A.; Herrero-Davila, L.; Mubofu, E.B.; Abderrahim, S.; Clark, J.H.; Koutinas, A.A.; Kopsahelis, N.; Stamatelatou, K.; Dickson, F.; et al. Food waste as a valuable resource for the production of chemicals, materials and fuels. Current situation and global perspective. *Energy Environ. Sci.* **2013**, *6*, 426–464. [CrossRef]
12. Pleissner, D.; Kwan, T.H.; Lin, C.S. Fungal hydrolysis in submerged fermentation for food waste treatment and fermentation feedstock preparation. *Bioreour. Technol.* **2014**, *115*, 48–54. [CrossRef] [PubMed]

13. Pleissner, D.; Lam, W.C.; Han, W.; Lau, K.Y.; Cheung, L.C.; Lee, M.W.; Lei, H.M.; Lo, Y.; Ng, W.Y.; Sun, Z.; et al. Fermentative polyhydroxybutyrate production from a novel feedstock derived from bakery waste. *Biomed. Res. Int.* **2014**, *2014*, 8. [CrossRef] [PubMed]

14. Kwan, T.H.; Pleissner, D.; Lau, K.Y.; Venus, J.; Pommeret, A.; Lin, C.S.K. Techno economic analysis of a food waste valorization process via microalgal cultivation and co-production of plasticizer, lactic acid and animal feed from algal biomass and food waste. *Bioreour. Technol.* **2015**, *198*, 292–299. [CrossRef] [PubMed]

15. Abdel-Rahman, M.A.; Sonomoto, K. Opportunities to overcome the current limitations and challenges for efficient microbial production of optically pure lactic acid. *J. Biotechnol.* **2016**, *236*, 176–192. [CrossRef] [PubMed]

16. Wang, J.; Chang, Q.; Yu, M.; Niu, R.; Wu, C.; Wang, Q. Production of L-lactic acid from food waste and *Sophora flavescens* residues. *Procedia Environ. Sci.* **2016**, *31*, 122–126. [CrossRef]

17. Pleissner, D.; Demichelis, F.; Mariano, S.; Fiore, S.; Navarro Gutierez, I.; Scheneider, R.; Venus, J. Direct production of lactic acid based on simultaneous saccharification and fermentation of mixed restaurant food waste. *J. Clean. Prod.* **2017**, *143*, 615–623. [CrossRef]

18. Al-Dhabi, N.A.; Esmail, G.A.; Ghilan, A.-K.M.; Arasu, M.V. Composting of vegetable waste using microbial consortium and biocatalytic efficiency of *Streptomyces* sp. Al-Dhabi 30 isolated from the Saudi Arabian environment for sustainable agriculture. *Sustainability* **2019**, *9*, 6845. [CrossRef]

19. Department of Energy (DOE). *Top Value Added Chemicals from Biomass: Results of Screening for Potential Candidates from Sugars and Synthesis Gas*; Department of Energy (DOE): Washington, DC, USA, 2004; Volume 1.

20. Allied Market Research (AMR). *Polylactic Acid (PLA) Market—Global Opportunity Analysis and Industry Forecast 2014–2020*; Allied Market Research (AMR): Portland, OR, USA, 2015.

21. Vink, E.T.; Davies, S. Life cycle inventory and impact assessment data for 2014 Ingeo™ polylactide production. *Ind. Biotechnol.* **2015**, *11*, 167–180. [CrossRef]

22. Castro-Aguirre, E.; Iniguez-Franco, F.; Samsudin, H.; Fang, X.; Auras, R. Poly (lactic acid)—Mass production, processing, industrial applications, and end of life. *Adv. Drug Deliv. Rev.* **2016**, *107*, 333–366. [CrossRef]

23. Venus, J. Utilization of renewables for lactic acid fermentation. *Biotechnol. J.* **2006**, *1*, 1428–1432. [CrossRef]

24. Kim, M.S.; Na, J.G.; Lee, M.K.; Ryu, H.; Chang, Y.K.; Triolo, J.M.; Yun, Y.M.; Kim, D.H. More value from food waste: Lactic acid and biogas recovery. *Water Res.* **2016**, *96*, 208–216. [CrossRef]

25. Li, X.; Chen, Y.; Zhao, S.; Chen, H.; Zheng, X.; Luo, J.; Liu, Y. Efficient production of optically pure L-lactic acid from food waste at ambient temperature by regulating key enzyme activity. *Water Res.* **2015**, *70*, 148–157. [CrossRef] [PubMed]

26. Liang, S.B.; McDonald, A.G.; Coats, E.R. Lactic acid production with undefined mixed culture fermentation of potato peel waste. *Waste Manag.* **2014**, *34*, 2022–2027. [CrossRef] [PubMed]

27. Dreschke, G.; Probst, M.; Walter, A.; Pümpel, T.; Walde, J.; Insam, H. Lactic acid and methane: Improved exploitation of biowaste potential. *Bioreour. Technol.* **2015**, *176*, 47–55. [CrossRef] [PubMed]

28. Wu, Y.Y.; Ma, H.L.; Zheng, M.Y.; Wang, K.J. Lactic acid production from acidogenic fermentation of fruit and vegetable wastes. *Bioreour. Technol.* **2015**, *191*, 53–58. [CrossRef]

29. Tang, J.L.; Wang, X.C.; Hu, Y.S.; Zhang, Y.M.; Li, Y.Y. Lactic acid fermentation from food waste with indigenous microflora: Effects of pH, temperature and high OLR. *Waste Manag.* **2016**, *52*, 278–285. [CrossRef]

30. Naser, S.M.; Dawyndt, P.; Hoste, B.; Gevers, D.; Vandemeulebroeke, K.; Cleenwerck, I.; Vancanneyt, M.; Swings, J. Identification of lactobacilli by pheS and rpoA gene sequence analyses. *Int. J. Syst. Evol. Microbiol.* **2007**, *57*, 2777–2789. [CrossRef]

31. Kanpiengjai, A.; Reantrakoonchait, W.; Pratanaph, R.; Pathom-aree, W.; Lumyong, S.; Khanongnuch, C. High efficacy bioconversion of starch to lactic acid using an amyloytic lactic acid bacterium isolated from Thai indigenous fermented rice noodles. *Food Sci. Biotechnol.* **2014**, *23*, 1541–1550. [CrossRef]

32. Dubois, M.; Gilles, K.A.; Hamilton, J.K.; Rebers, P.A.; Smith, F. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* **1956**, *28*, 350–356. [CrossRef]

33. Miller, G.L. Use of dinitrosalicic acid reagent for determination of reducing sugar. *Anal. Biochem.* **1959**, *31*, 426–428. [CrossRef]

34. Yuan, Y.; Peng, Y.; Jin, B.; Wang, B.; Wang, S. Fermentation and dewaterability of waste activated sludge under alkaline conditions: Effect of Mg(OH)2. *China Environ. Sci.* **2014**, *34*, 1790–1796.
35. Liu, X.; Wang, J.; Duan, L.; Song, Y.; Hu, X.; Wei, J. Enhancing the production of butyric acid from sludge fermentation with an emphasis on zinc, cobalt, cuprum, ferrum and manganese. *Environ. Earth Sci.* 2015, 73, 5057–5066. [CrossRef]

36. Tejada, M. Evolution of soil biological properties after addition of glyphosate, diflufenican and glyphosate+diflufenican herbicides. *Chemosphere* 2009, 76, 365–373. [CrossRef] [PubMed]

37. Olsen, S.R.; Cole, C.V.; Watanabe, F.S.; Dean, L.A. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. *USDA Circ.* 1954, 939, 1–19.

38. Tabatabai, M.A. Soil Enzymes. In *Methods of Soil Analysis. Part 2. Microbiological and Biochemical Properties*; Weaver, R.W., Angle, S., Bottomley, P., Bezdicek, D., Smith, S., Tabatabai, A., Wollum, A., Eds.; Soil Science Society of America: Madison, WI, USA, 1994; pp. 775–833.

39. Rodriguez-Morgado, B.; Jiménez, P.C.; Moral, M.T.; Rubio, J.P. Effect of l-lactic acid from whey wastes on enzyme activities and bacterial diversity of soil. *Biol. Fert. Soil.* 2017, 53, 389–396. [CrossRef]

40. Deepthi, B.V.; Somashekaraiah, R.; Poornachandra Rao, K.; Deepa, N.; Dharanesha, N.K.; Girish, K.S.; Sreenivasa, M.Y. *Lactobacillus plantarum* MY56 ameliorates fumonisin B1-induced hepatorenal damage in broilers. *Front. Microbiol.* 2017, 8, 2317. [CrossRef]

41. Poornachandra Rao, K.; Deepthi, B.V.; Rakesh, S.; Ganesh, T.; Achar, P.; Sreenivasa, M.Y. Antiaflatoxigenic potential of cell-free supernatant from *Lactobacillus plantarum* MY544 against *Aspergillus parasiticus*. *Probiot. Antimicrob. Protein.* 2017, 11, 55–64. [CrossRef]

42. Batista, A.L.D.; Silva, R.; Cappato, L.P.; Ferreira, M.V.S.; Nascimento, K.O.; Schmiele, M.; Esmerino, E.A.; Balthazar, C.F.; Silva, H.L.A.; Moraes, J.; et al. Developing a symbiotic fermented milk using probiotic bacteria and organic green banana flour. *J. Funct. Food.* 2017, 28, 242–250. [CrossRef]

43. Rivas, B.; Moldes, A.B.; Domínguez, J.M.; Parajo, J.C. Development of culture media containing spent yeast cells of *Debaryomyces Hansenii* and corn steep liquor for lactic acid production with *Lactobacillus rhamnosus*. *Int. J. Food Microbiol.* 2004, 97, 93–98. [CrossRef]

44. Wang, L.; Zhao, B.; Liu, B.; Yang, C.; Yu, B.; Li, Q.; Ma, C.; Xu, P.; Ma, Y. Efficient production of L-lactic acid from cassava powder by *Lactobacillus rhamnosus*. *Bioresour. Technol.* 2010, 101, 7895–7901. [CrossRef]

45. Chen, H.; Chen, B.; Su, Z.; Wang, K.; Wang, B.; Wang, Y.; Si, Z.; Wu, Y.; Cai, D.; Qin, P. Efficient lactic acid production from cassava bagasse by mixed culture of *Bacillus coagulans* and *Lactobacillus rhamnosus* using stepwise pH controlled simultaneous saccharification and co-fermentation. *Ind. Crop. Prod.* 2020, 112175. [CrossRef]

46. Kuo, Y.C.; Yuan, S.F.; Wang, C.A.; Huang, Y.J.; Gou, G.L.; Hwang, W.S. Production of optically pure L-lactic acid from lignocellulosic hydrolysate by using a newly isolated and d-lactate dehydrogenase gene-deficient *Lactobacillus paracasei* strain. *Bioresour. Technol.* 2015, 198, 651–657. [CrossRef] [PubMed]

47. Villadsen, J. Innovative technology to meet the demands of the white biotechnology revolution of chemical production. *Chem. Eng. Sci.* 2007, 62, 6957–6968. [CrossRef]

48. De Lima, C.J.B.; Coelho, L.F.; Silva, G.P.; Alvarez, G.M.; Contiero, J. Lactic acid production by new *Lactobacillus casei* fermentation using di... *J. Funct. Food.* 2006, 41, 1451–1454. [CrossRef]

49. Ding, S.; Tan, T. L-Lactic acid production by *Lactobacillus casei* fermentation using different fed-batch feeding strategies. *Process Biochem.* 2006, 41, 1289–1301. [CrossRef]

50. Rodriguez, L.R.; Vera Pingitore, E.; Rollan, G.; Cocconcelli, P.; Fontana, C.; Saavedra, L.; Vignolo, G.; Hébert, E. Biodiversity and technological–functional potential of lactic acid bacteria isolated from spontaneously fermented quinoa sourdoughs. *J. Appl. Microbiol.* 2016, 120, 1289–1301. [CrossRef]

51. Madoroba, E.; Steenkamp, E.T.; Theron, J.; Scheirlinck, I.; Eugene Cloete, T.; Huys, G. Diversity and dynamics of bacterial populations during spontaneous sorghum fermentations used to produce ting, a South African food. *Syst. Appl. Microbiol.* 2011, 34, 227–234.

52. Ercolini, D.; Pontonio, E.; De Filippis, F.; Minervini, F.; La Storia, A.; Gobbetti, M.; Di Cagno, R. Microbial ecology dynamics during rye and wheatsourdough preparation. *Appl. Environ. Microbiol.* 2013, 79, 7827–7836. [CrossRef]

53. Sáez, G.D.; Hébert, E.M.; Saavedra, L.; Zárate, G. Molecular identification and technological characterization of lactic acid bacteria isolated from fermented kidney beans flours (*Phaseolus vulgaris* L. and *P. coccineus*) in northwestern Argentina. *Food Res. Int.* 2017, 102, 605–615. [CrossRef]
54. Ruiz Rodriguez, L.; Vera Pingitore, E.; Rollan, G.; Martos, G.; Saavedra, L.; Fontana, C.; Hebert, E.; Vignolo, G. Biodiversity and technological potential of lactic acid bacteria isolated from spontaneously fermented amaranth sourdoughs. *Lett. Appl. Microbiol.* 2016, 63, 147–154. [CrossRef]

55. De Vuyst, L.; Van Kerrebroeck, S.; Harth, H.; Huys, G.; Daniel, H.M.; Weckx, S. Microbial ecology of sourdough fermentations: Diverse or uniform? *Food Microbiol.* 2014, 37, 11–29. [CrossRef]

56. Bustamante, D.; Tortajada, M.; Ramón, D.; Rojas, A. Production of D-Lactic Acid by the Fermentation of Orange Peel Waste Hydrolysatde by Lactic Acid Bacteria. *Fermentation* 2020, 6, 1. [CrossRef]

57. Fijałkowska, M.; Przemieniecki, S.W.; Purwin, C.; Lipiński, K.; Kurowski, T.P.; Karwowska, A. The effect of an additive containing three *Lactobacillus* species on the fermentation pattern and microbiological status of silage. *J. Sci. Food Agric.* 2020, 120, 174–1184. [CrossRef] [PubMed]

58. Itoh, Y.; Tada, K.; Kanno, T.; Horiuichi, J.I. Selective production of lactic acid in continuous anaerobic acidogenesis by extremely low pH operation. *J. Biosci. Bioeng.* 2012, 114, 537–539. [CrossRef] [PubMed]

59. Guendouz, J.; Buffiere, P.; Cacho, J.; Carrere, M.; Delgenes, J.P. High-solids anaerobic digestion: Comparison of three pilot scales. *Water Sci. Technol.* 2008, 9, 1757–1763. [CrossRef] [PubMed]

60. Li, J.; Wei, L.; Duan, Q.; Hu, G.; Zhang, G. Semi-continuous anaerobic codigestion of dairy manure with three crop residues for biogas production. *Bioresour. Technol.* 2014, 156, 307–313. [CrossRef] [PubMed]

61. Wu, L.J.; Kobayashi, T.; Kuramochi, H.; Li, Y.Y.; Xu, K.Q. Improved biogas production from food waste by co-digestion with de-oiled grease trap waste. *Bioresour. Technol.* 2016, 201, 237–244. [CrossRef]

62. Red Com, R.; Engelberth, A.S. Identifying conditions to optimize lactic acid production from food waste co-digested with primary sludge. *Biochem. Eng. J.* 2016, 105, 205–213.

63. Li, X.; Zhang, W.; Xue, S.; Lai, S.; Li, J.; Chen, H.; Liu, Z.; Xue, G. Enrichment of D-lactic acid from organic wastes catalyzed by zero-valent iron: An approach for sustainable lactate isomerization. *Green Chem.* 2017, 19, 928–936. [CrossRef]

64. Edwards, J.; Othman, M.; Crossin, E.; Burn, S. Anaerobic co-digestion of municipal food waste and sewage sludge: A comparative life cycle assessment in the context of a waste service provision. *Bioresour. Technol.* 2017, 223, 237–249. [CrossRef]

65. Zhang, W.; Li, X.; Zhang, T.; Li, J.; Lai, S.; Chen, H.; Gao, P.; Xue, G. High-rate lactic acid production from food waste and waste activated sludge via interactive control of pH adjustment and fermentation temperature. *Chem. Eng. J.* 2017, 328, 197–206. [CrossRef]

66. Akao, S.; Tsuno, H.; Horie, T.; Mori, S. Effects of pH and temperature on products and bacterial community in-lactate batch fermentation of garbage under unsterile condition. *Water. Res.* 2007, 41, 2636–2642.

67. Zhang, P.; Chen, Y.; Zhou, Q. Waste activated sludge hydrolysis and short-chain fatty acids accumulation under mesophilic and thermophilic conditions: Effect of pH. *Water. Res.* 2009, 43, 3735–3742. [CrossRef] [PubMed]

68. Sahoo, T.K.; Jayaraman, G. Co-culture of *Lactobacillus delbrueckii* and engineered *Lactococcus lactis* enhances stoichiometric yield of D-lactic acid from whey permeate. *Appl. Microbiol. Biotechnol.* 2019, 103, 5653–5662. [CrossRef] [PubMed]

69. Yanez, R.; Alonso, J.L.; Parajo, J.C. D-Lactic acid production from waste cardboard. *J. Chem. Technol. Biotechnol.* 2005, 80, 76–84. [CrossRef]

70. Rangaswamy, V.; Ramakrishna, S.V. Lactic acid production by *Lactobacillus delbrueckii* in a dual reactor system using packed bed biofilm reactor. *Lett. Appl. Microbiol.* 2008, 46, 661–666. [CrossRef]

71. Dumbrepatil, A.; Adsul, M.; Chaudhari, S.; Khire, J.; Gokhale, D. Utilization of molasses sugar for lactic acid production by *Lactobacillus delbrueckii* subsp. delbrueckii mutant Uc-3 in batch fermentation. *Appl. Environ. Microbiol.* 2008, 74, 333–335. [CrossRef]

72. Nancib, N.; Nancib, A.; Boudjelal, A.; Benslimane, C.; Blanchard, F.; Boudrant, J. The effect of supplementation by different nitrogen sources on the production of lactic acid from date juice by *Lactobacillus casei* subsp. *rhamnosus*. *Bioresour. Technol.* 2001, 78, 149–153. [CrossRef]

73. Fitzpatrick, J.J.; O’Keeffe, U. Influence of whey protein hydrolysate addition to whey permeate batch fermentations for producing lactic acid. *Process Biochem.* 2001, 37, 183–186. [CrossRef]

74. Oliveira, R.B.P.; Oliveira, A.L.; Gloria, M.B.A. Screening of lactic acid bacteria from vacuum packaged beef for antimicrobial activity. *Braz. J. Microbiol.* 2008, 39, 368–374. [CrossRef]

75. De Vuyst, L.; Leroy, F. Bacteriocins from lactic acid bacteria: Production, purification, and food applications. *J. Mol. Microbiol. Biotechnol.* 2007, 13, 194–199. [CrossRef]
76. Pelaez, A.M.L.; Catano, C.A.S.; Yepes, E.A.Q.; Villarroela, R.R.G.; De Antoni, G.L.D.; Giannuzzi, L. Inhibitory activity of lactic and acetic acid on *Aspergillus flavus* growth for food preservation. *Food Control* 2012, 24, 177–183. [CrossRef]

77. Manhanzva, M.T.; Abrahams, A.G.; Gamieldien, H.; Froissart, R.; Jaspan, H.; Jaumdally, S.Z.; Barnabas, S.L.; Dabee, S.; Bekker, L.G.; Gray, G.; et al. Inflammatory and antimicrobial properties differ between vaginal *Lactobacillus* isolates from South African women with non-optimal versus optimal microbiota. *Sci. Rep.* 2020, 10, 1–13. [CrossRef] [PubMed]

78. Meleh, H.U.; Choo, S.; Desa, M.N.M.; Chew, S.Y.; Rangasamy, P.; Hassan, H.; Than, L.T.L. Isolation and safety characterisation of lactobacilli strains with antimicrobial properties as potential probiotics for human use. *LWT* 2020, 131, 109796. [CrossRef]

79. Zhao, G.; Cui, M.; Wang, M.; Chen, W.; Li, J.; Yao, Y. The correlation between colonization and the biological properties of *Lactobacillus* sp. *Food Biosci.* 2020, 36, 100613. [CrossRef]

80. Chen, Y.P.; Rekha, P.D.; Arun, A.B.; Shen, F.T.; Lai, W.; Young, C.C. Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. *Appl. Soil Ecol.* 2006, 34, 33–41. [CrossRef]

81. Siotto, M.; Sezenna, E.; Saponaro, S.; DegliInnocenti, F.; Tosin, M.; Bonomo, L.; Mezzanotte, V. Kinetics of monomer biodegradation in soil. *J. Environ. Manag.* 2012, 93, 31–37. [CrossRef]