SHORT COMMUNICATION

Waste Viscose for Optically pure Lactic acid Production

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Abstract

Recycling of textile fibers is a mandatory step in the life-cycle of fabrics to avoid incineration or landfilling. After fiber-to-fiber recycling, cellulose fiber saccharification opens the door to the production of biochemical building blocks through microbial fermentation. This study documents for the first time the production of optically pure D-lactic acid (D-LA), one of the precursors of polylactic acid (PLA), from post-consumer waste viscose. Enzymatic hydrolysis was applied to white and colored waste viscose to produce two glucose-rich solutions that were used as carbon source for the genetically modified *Pediococcus acidilactici* ZP26. Afterward, batch and fed-batch cultivations were conducted with very similar results for the control and both waste viscose hydrolysates (white and colored). The strain was able to produce D-LA without any signs of inhibition by possibly toxic compounds present in the recycled textiles (additives, dyes, and other contaminants). The highest D-LA concentration and yield was reached in the batch reactors with colored waste viscose at 42.4 g/L and 0.97 g/g, respectively. In the fed-batch cultivations, the best result was achieved with the white waste solution, with 59.5 g of D-LA (40.8 g/L), and a yield on total glucose of 0.70 g/g. Notwithstanding the need for more research in the utilization of saccharified waste viscose as carbon source for bioprocesses, this is an important proof of concept of an application of streams from tertiary recycling of cellulosic fibers in the production of platform biochemicals.

Graphical Abstract
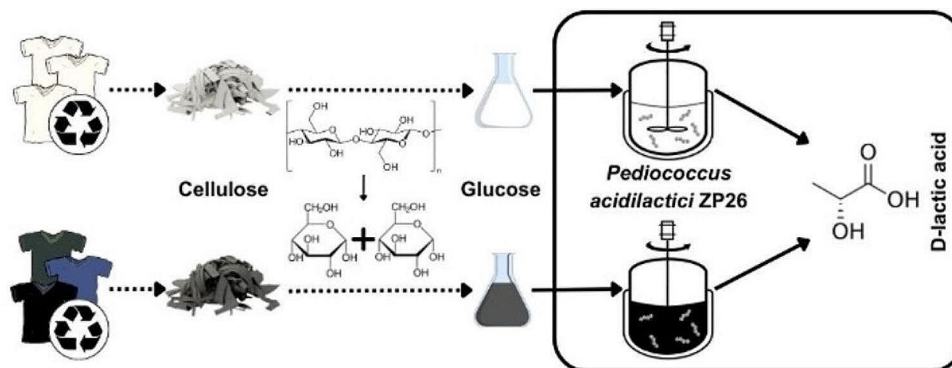
Highlights

- Optically pure lactic acid was produced with glucose-rich solutions originated from white and colored post-consumer waste viscose.
- *Pediococcus acidilactici* ZP26 produced D-lactic acid from both white and colored waste viscose solutions.
- The D-lactic acid yields and volumetric production rates were very similar between both waste viscose solutions and the control.

- This process can be potentially used for the production of polylactic acid (PLA) fibers, which can be reapplied in the textile industry.

Introduction

The textile industry's environmental impact is vast, burdened by its large emissions and waste generation, heavy use of polluting chemicals and pesticides, and excessive consumption of natural resources [1]. In attempts to address the problem of waste generation, several initiatives to develop recycling processes for waste textiles have been established. These initiatives include repurposing waste textiles as wiping cloths, producing stuffing material for furniture and insulation, and lately, fiber-to-fiber recycling to produce new textile fibers [2]. Nevertheless, repurposing strategies and continued usage decrease the quality of the fibers and these need to be disposed of, eventually. The hydrolysis of cellulosic fibers to monomeric sugars and its application as carbon source in microbial bioprocesses is one of the alternatives to avoid incineration or landfilling.

Cellulosic fibers (natural or man-made) are biobased, and account for more than a third of the global fiber production [3]. Therefore, cellulosic waste textiles can be considered an abundant raw material to be used in biorefinery processes to obtain renewable chemicals and/or fuels [4]. Although fiber-to-fiber recycling is a preferable first approach because it maximizes the recovered value of the fibers, tertiary recycling methods are necessary to avoid incineration or landfilling of waste textiles when fiber-to-fiber recycling is unfeasible. Cellulosic waste textiles suitable for tertiary recycling would include cellulose recovered from fiber blends, damaged cotton textiles, and man-made cellulosic fibers such as viscose [4].

The prospect of using waste viscose as a raw material for tertiary recycling methods is especially interesting. Viscose is the most widely produced man-made cellulosic fiber (80% market share), with a production that achieved 5.8 million tons in 2021. Initiatives to recycle cellulosic waste textiles generally focus on cotton, as it is the most abundant cellulosic fiber on the market; examples of companies developing fiber-to-fiber recycling technologies include Lenzing and Ioncell [1–3, 5–7]. While it is possible to recycle waste cotton into man-made cellulosic fibers, regenerating waste viscose into a functional fiber is a much greater challenge. The low degree of polymerization of viscose fibers, which is decreased even further by wear and tear, makes them unfit for fiber regeneration [9, 10]. However, the low degree of polymerization and the more accessible supramolecular structure of viscose compared to cotton makes valorization of the waste through saccharification an attractive option

[8]. Saccharifying waste viscose can be done through acid or enzymatic hydrolysis at high yields, each method with its distinct advantages: acid hydrolysis is significantly faster while enzymatic hydrolysis produces a biocompatible broth without the need for an additional purification step [9, 10].

Glucose obtained from the hydrolysis of waste viscose has been used to produce value-added products such as ethanol and biogas [9]. However, hydrolyzed glucose can be used in other valorization pathways that can benefit the textile industry. This would allow for the replacement of virgin raw materials by recycled ones, thus reducing waste generation. One example is the conversion of glucose to hydroxymethylfurfural (HMF), which is a precursor for a variety of valuable polymers used in the textile industry [11–13]. Another attractive possibility is to use the glucose as feedstock for lactic acid (LA) fermentation. LA has plenty of industrial applications in sectors such as food and beverages, cosmetics, medicine, and the chemical industry as precursor of other chemical compounds [14]. LA is also the building block for the production of the polymer polylactic acid (PLA), which has been identified as a promising alternative to polyester (PET) [15, 16] – currently the most produced textile polymer, with more than 99.9% of its production being fossil-based [3]. PLA is, in contrast to PET, renewable, biodegradable and industrially compostable. It is possible to produce PLA fibers with comparable properties to regular PET fibers while using the same processing equipment. Additionally, PLA fibers have also been shown to possess properties superior to PET fibers in some instances, such as improved breathability and thermal insulation as well as producing more comfortable polycotton fiber blends compared to equivalent PET-cotton blends [15, 17]. Different PLA mechanical properties can be achieved by controlling the ratio of each LA monomer – D-LA and L-LA – during polymerization [18]. These can be advantageous in the development of new materials and blends to be used, for example, in the textile industry.

In the present study, the genetically modified lactic acid bacterium *Pediococcus acidilactici* ZP26 is used for the production of D-LA from enzymatically hydrolyzed waste viscose. Optically pure D-LA is less often produced naturally, and equally as interesting as L-LA for the polymerization into PLA. Therefore, D-LA-producing organisms and processes are of interest for the biopolymer industry [19]. Genetically modified *P. acidilactici* ZP26 and *P. acidilactici* TY112 were engineered from the same parent strain (*P. acidilactici* DQ2 [20]) to produce optically pure D-LA and

L-LA, respectively, with very similar results between both [21, 22]. The production of the two separate enantiomers facilitates the production process of tailored PLA polymers, comparatively with processes that have a racemic LA mixture as the final product [16, 18]. The purpose of this work was to demonstrate the conversion of saccharified waste viscose into D-LA by *P. acidilactici* ZP26, effectively transforming low-value textile waste into a raw material of great potential for the textile industry. Batch and fed-batch cultivations were performed to evaluate if D-LA production was affected by the utilization of complex hydrolysates from both white and colored textiles. The benefit of investigating the feasibility of such a process would be two-fold, as it would decrease the environmental impact of the textile industry by decreasing the amount of waste generated while also reducing the need for virgin, fossil, raw materials.

Materials and Methods

Microorganism and Inoculum Preparation

Pediococcus acidilactici ZP26 (Chinese General Microorganisms Collection Center (CGMCC), registration number 8665), engineered from corn stover slurry wild-type isolate *P. acidilactici* DQ2, by knockout of the *ldh* gene [22]. The organism was stored at -80 °C in 15% (v/v) glycerol, in 1.5 mL aliquots, which were used for inoculum preparation. *P. acidilactici* ZP26 was grown on simplified Man-Rogosa-Sharp (MRS) medium: 10.0 g yeast extract, 10.0 g peptone, 5.0 g sodium acetate, 2.0 g ammonium citrate dibasic, 2.0 g dipotassium phosphate, 0.58 g magnesium sulfate heptahydrate, 0.25 g manganese sulfate monohydrate in 1 L of demineralized water [22]. The inoculum was prepared with 0.5% (v/v) of cryopreserved culture, supplemented with 20 g/L glucose, with a solution prepared and autoclaved separately, 0.6 g of $CaCO_3$ per g of glucose as pH buffer, and grown by 12 h at 42 °C, and 150 rpm. All solutions and materials were autoclaved at 121 °C for 20 min.

Waste Viscose and Enzyme Collection and Preparation

The waste viscose used in this study consisted of post-consumer waste viscose textiles obtained from a Swedish and a Finnish textile sorting facility (Siptex, Malmö, Sweden; Lounais-Soumen Jätehuolto, Turku, Finland). The textiles were sorted based on fiber type and color manually and using near-infrared light into one white and one colored fraction of 100% viscose, before delivery. Non-viscose components, such as buttons, seams, and decorative details were removed and the textiles were subsequently shredded using a textile shredder with a 170×107 mm chamber and 15 mm blades (Filamaker, Kaufungen, Germany) until the textile pieces were approximately 10×10 mm. The shredded waste viscose was assumed to contain 100% cellulose. The enzyme blend used in this study was Cellic CTec 2 (Novozymes, Bagsværd, Denmark).

Viscose Hydrolysis

27 kg of de-ionized water was added to a stirred tank reactor with a working volume of 40 L, equipped with a heating jacket, and heated to 50℃. Upon reaching 50℃, the pH of the water was adjusted to 5 by the addition of sulfuric acid (72%) (Chem-Lab NV, Zedelgem, Belgium) and sodium hydroxide (50%) (Merck KGaA, Darmstadt, Germany). Upon reaching pH 5, 1.4 kg of waste viscose, corresponding to a solids loading of 5%, was added to the reactor and the contents were mixed. The mixture was allowed to combine for 30 min after which the pH was controlled and adjusted back to pH 5. Cellic CTec 2 (Novozymes, Bagsværd, Denmark) corresponding to 0.15 g enzyme blend/g dry waste viscose was added to the reactor. Cellic CTec 2 has an approximate activity of 200 FPU/g enzyme. The hydrolysis was conducted for 96 h during which the pH and temperature were regularly monitored and adjusted. The buildup of partially digested textiles was occasionally removed from the impeller and returned to the bulk of the reactor. After the reactor had run for 96 h, the contents were evacuated and strained to separate the liquid product from the unhydrolyzed solid residue. The procedure for the hydrolysis was the same for both white and colored waste viscose. The hydrolysates were concentrated by vacuum evaporation with a Büchi Rotavapor R-153, coupled with a Büchi B-721 vacuum controller and V-512 vacuum pump. Solutions were sterilized at 121 °C for 20 min before being stored at 4 °C until further use. Samples were collected from white and colored waste viscose hydrolysates for further HPLC analyses.

Preliminary Study on Shake Flasks

Cultivations were performed in duplicate in a Kuhner ShakerX orbital shaker at 42 °C, 150 rpm for 48 h. 100 mL non-baffled shake flasks were used, with an initial working volume of 55 mL. This comprised of 5.5 mL inoculum (grown as previously described), 15 mL were simplified MRS medium, and carbon source adjusted to each chosen condition, up to an initial concentration of 50 g/L of glucose. Any remaining volume was topped up with sterile deionized water to ensure a total working volume of 55 mL. Three experimental conditions were tested for both the white and colored waste viscose, by varying the amount of

glucose supplied in the form of the viscose solution equivalent to 100%, 66%, and 33% of the total glucose present. The remaining glucose topped up from a 500 g/L sterile glucose solution, where required. A control experiment was performed in duplicate with 50 g/L of commercially available glucose. For buffering, 0.6 g $CaCO_3$ per g of glucose was added to each flask.

Samples were withdrawn throughout the cultivation to measure offline pH, sugar consumption, and lactic acid production.

Bioreactor Cultivations

The non-aerated cultivations were run in duplicate in 2 L Labfors bioreactors (Infors HT, Switzerland), at 150 rpm, 42 °C, with a start working volume at 0.5 L, 10% (v/v) inoculum and 50 g/L of glucose from the three different carbon sources. Simplified MRS medium was used and pH was automatically controlled at 5.5 with NaOH 2 M. 1% amyloglucosidase from *Aspergillus niger* was added at the beginning of each cultivation, to prevent biomass flocculation [21, 23]. Control experiments were run with glucose, supplemented as a 500 g/L solution prepared and autoclaved separately. Experiments were run for both white and colored waste viscose hydrolysates. Bioreactors were run in batch and fed-batch mode, for 48 h. Fed-batch cultivations were fed continuously with 0.8 L of each respective carbon source solution to the same concentration (approximately 90 g/L), starting at 12 h of cultivation, with a feeding rate comparable with the glucose consumption rate, at 3.3 g/h. Samples were withdrawn for offline pH measurements and HPLC analyses.

Sampling and Analyses

All samples were centrifuged at 12,000×g for 5 min, filtered with 0.2 μm syringe filters, and stored at -20 °C for chromatographical analysis. HPLC analysis was conducted in an Agilent Technologies HPLC, model 1260 Infinity II, comprised of a module with an isocratic solvent pump, autosampler, column oven, UV detector, and RI detector. Sugars (cellobiose, glucose, xylose, arabinose), organic acids (succinic acid, lactic acid, formic acid, acetic acid, levulinic acid), ethanol and furans (HMF and furfural) were analyzed on a Hi-Plex H (Agilent Technologies, CA, USA) column at 65 °C, using 5 mM sulphuric acid as eluent at 0.6 mL/min. Lactic acid isomers were identified on a Supelco Astec CLC-D (Merck, Darmstadt, Germany) column run at room temperature, with 5 mM copper sulfate in demineralized water as eluent at 1.0 mL/min.

Calculations

For the shake flask experiments, D-LA productivity (q_P) and glucose consumption rate (q_G) were determined considering the total extent of the cultivations (48 h). For the bioreactor cultivations, D-LA productivity (q_P) and glucose consumption rate (q_G) were determined for each time frame specified on Table 2, to evaluate the evolution of this parameter over time. For this parameter, volume changes resultant from any addition or sampling were considered. The yield of D-LA on consumed glucose ($Y_{consumed}$) was calculated with the produced D-LA over the consumed glucose at the end of the respective process. To complement, the yield of D-LA on total glucose available for consumption (Y_{total}) was calculated with the final amount of D-LA produced over the total glucose added to the cultivation.

Results

Viscose Saccharification

The enzymatic hydrolysis of the waste viscose resulted in two glucose solutions, a yellow for the white waste viscose fraction and a heavily colored (dark blue) for the colored fraction. The focus of this work was the test of the viscose hydrolysate solutions as carbon source for D-LA production. Therefore, no further optimization or analysis of the viscose saccharification step was considered. Only glucose was detected in the chromatographical analysis, at approximately 20 g/l before vacuum evaporation. After the concentration of the solutions, the white waste viscose hydrolysate had a glucose concentration of 81 g/L, while the colored waste viscose hydrolysate had 94 g/L.

Shake Flask Cultivations

Shake flask tests were conducted with a total of 50 g/L of sugars, with different percentages of white or colored waste viscose hydrolysate (33%, 66%, 100%), against a glucose control. These tests served as the base for the following bioreactor experiments. The results are presented in Fig. 1; Table 1.

Perhaps surprisingly, D-LA concentrations, productivity, and yields reached higher values in the waste viscose hydrolysates than in the control, notably when using the colored solution. The average D-LA concentration in the control was 13.7±0.79 g/L, while the averages obtained for the final D-LA concentration were 26.2±5.5 g/L and 36.1±4.4 g/L for the white and colored solutions, respectively. D-LA productivity (0.22±0.02 g/(L.h)) and yields (0.21±0.01 g/g on consumed glucose and 0.19±0.02 g/g

Fig. 1 D-LA and glucose concentrations in different percentages of (a and c) white waste viscose and (b and d) colored waste viscose hydrolysates
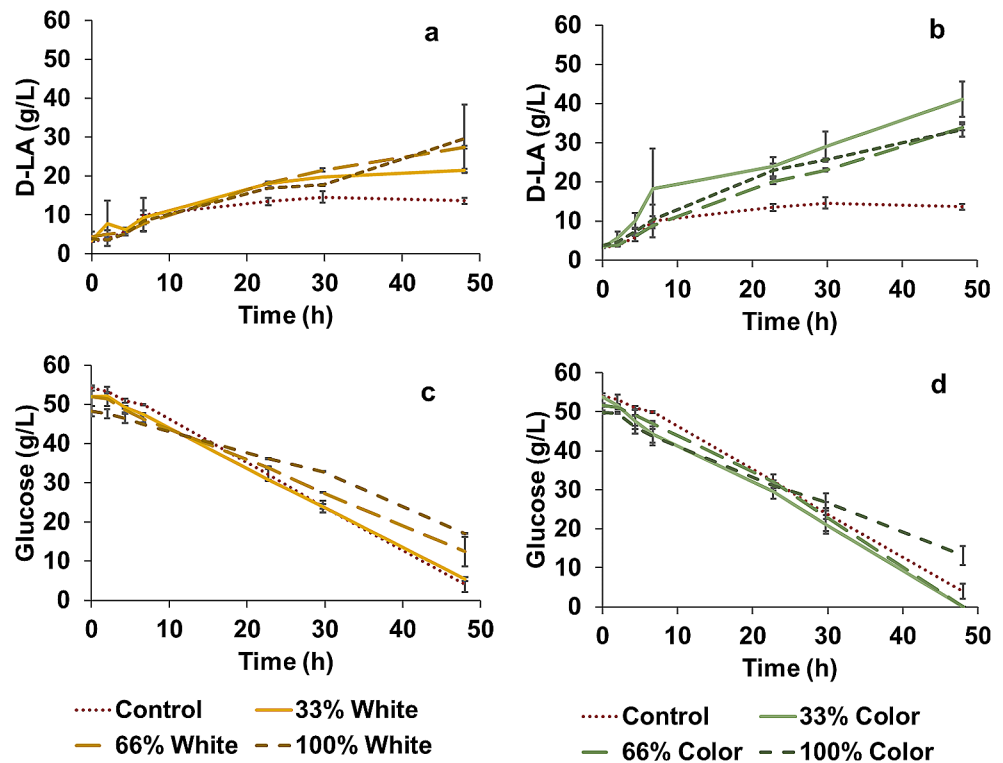

Table 1 D-LA final concentration (g/L), D-LA productivity (q_P, g/(L·h)), glucose uptake rate (q_G, g/(L·h)), and yield of D-LA on consumed glucose ($Y_{consumed}$, g/g) and on total glucose added (Y_{total}, g/g) for different percentages of white and colored waste viscose hydrolysates

Condition	D-LA (g/L)	q_P (g/(L·h))	q_G (g/(L·h))	$Y_{consumed}$ (g/g)	Y_{total} (g/g)
Control	13.7	0.22	1.04	0.21	0.19
33% White	21.4	0.38	0.97	0.39	0.35
66% White	27.4	0.48	0.82	0.59	0.44
100% White	29.6	0.54	0.65	0.82	0.53
33% Colored	41.1	0.79	1.12	0.70	0.70
66% Colored	33.9	0.63	1.08	0.59	0.59
100% Colored	33.4	0.62	0.77	0.81	0.60

on total glucose added) in the control were below the overall average – 0.52 ± 0.19 g/(L.h), 0.59 ± 0.23 g/g and 0.49 ± 0.17 g/g, respectively. In comparison, the glucose uptake rate (1.04 ± 0.01 g/(L.h)) of the control experiments was within the total average (0.92 ± 0.18 g/(L.h)).

Looking at the results from the cultivations with white waste viscose (Table 1), D-LA concentrations, productivity, and yields increased proportionally ($R^2 > 0.9$) with the amount of waste viscose solution added. The glucose uptake rate, however, decreased by almost 40% in the flasks with a higher amount of white viscose solution (0.65 ± 0.03 g/(L.h)), when compared to the control.

In the flasks with colored solution, the highest D-LA concentration, D-LA productivity, and glucose intake rate were obtained for the flasks with 33% of the carbon source coming from colored waste viscose – 41.1 ± 4.48 g/L, 0.79 ± 0.10 g/(L.h) and 1.12 ± 0.00 g/(L.h), respectively. However, contrarily to the trend observed in the white viscose experiments, the respective results decreased with the increase of colored waste viscose solution supplemented to the medium, and no clear proportional relation was observed. More noteworthy are the yields of D-LA obtained with 33% and 66% colored solution, that reached 0.70 ± 0.09 g/g and 0.59 ± 0.01 g/g, with the same value for both the yield on consumed glucose and total glucose added, since these were the only two conditions in which full glucose consumption was observed.

The D-LA yields in the shake flasks with 100% hydrolysate on consumed glucose (0.82 ± 0.25 g/g for white and 0.81 ± 0.11 g/g for colored) and on total glucose added (0.53 ± 0.17 g/g for white and 0.60 ± 0.04 g/g for colored) were amongst the highest obtained in these preliminary trials. Despite the residual glucose observed in this condition, it was chosen to use the maximum concentration of waste hydrolysate (50 g/L) as a benchmark for the following bioreactor cultivations.

Bioreactor Cultivations

Figure 2; Table 2 show the results obtained in the bioreactor cultivations regarding D-LA production and glucose consumption.

Table 2 D-LA final concentration (g/L), D-LA productivity (q_P, g/(L·h)), glucose uptake rate (q_G, g/(L·h)) calculated at 12 h, 24 h, and 48 h of fermentation, and D-LA yield on consumed glucose ($Y_{consumed}$, g/g) and on total glucose added (Y_{total}, g/g), for each condition tested

Condition		D-LA (g/L)	q_P (g/(L·h)) 12 h	24 h	48 h	q_G (g/(L·h)) 12 h	24 h	48 h	$Y_{consumed}$ (g/g)	Y_{total} (g/g)
Batch	Control	37.2	2.41	1.61	[1]	2.48	1.75	[1]	0.95 [2]	
	White	39.1	2.41	1.61	[1]	2.65	1.75	[1]	0.93 [2]	
	Colored	42.4	3.19	1.69	[1]	3.45	1.78	[1]	0.97 [2]	
Fed-batch	Control	32.8	3.05	1.42	0.65	3.38	1.54	0.79	0.92	0.50
	White	40.8	2.87	1.50	0.83	2.93	1.78	1.00	0.87	0.71
	Colored	39.8	2.54	1.18	0.81	2.64	1.55	1.00	0.86	0.57

[1]D-LA productivity and glucose uptake rate were not calculated for the batch condition because no more D-LA was produced when glucose was exhausted after 24 h of cultivation.

[2]$Y_{consumed}$ and Y_{total} are the same because the glucose added was fully consumed.

In the batch reactors (Fig. 2 (a) and (c)) D-LA production followed the profile of glucose consumption, with yields above 0.9 g/g for the three conditions. Since glucose was fully consumed at the end of the batch cultivations, the yields on total glucose added are the same as on glucose consumed. As expected, D-LA productivity and glucose uptake rate were higher in the first 12 h of fermentation in all conditions studied. Nevertheless, glucose was exhausted at around 27 h for all batch conditions. Results were higher for the reactors with colored hydrolysate, with the final D-LA concentration reaching 42.4 ± 1.25 g/L, with 3.19 ± 0.06 g/(L.h) of D-LA productivity and 3.45 ± 0.11 g/(L.h) of glucose consumption rate at 12 h, with the yield at 0.97 ± 0.00 g/g. To study the influence of the increase of hydrolysate added to the bioreactor, fed-batch cultivations were conducted.

The feed was initiated at 12 h in the fed-batch reactors (Fig. 2, (b) and (d)), ensuring that there was no substrate limitation until the end of the fermentation. The feed rate was calculated to maintain the glucose concentration below the starting concentration (50 g/L), which removed any possibility for substrate inhibition [22].

The final concentrations of D-LA were higher in the reactors fed with waste viscose solutions (40.8 ± 0.09 g/L for the white and 39.8 ± 0.12 g/L for the colored) than for the control (32.8 ± 1.59 g/L). This was a consequence of a steeper decrease on the glucose consumption rate, and consequently on the D-LA productivity, in the control. Although the control presented the highest D-LA yield on consumed glucose in the fed-batch cultivations at 0.92 ± 0.04 g/g, it also presented the lowest yield on total glucose added. This is also related to the highest residual glucose concentration at 29.2 ± 0.93 g/L.

The causes for the differences between the three conditions are not yet clear, and a more in-depth study is necessary. Nevertheless, some hypotheses will be discussed in the discussion section. Importantly, these results demonstrate that waste viscose hydrolysate can be successfully used for the production of optically pure lactic acid (D-LA, >99%) with *P. acidilactici* ZP26.

Discussion

The purpose of this work was to show for the first time the production of optically pure lactic acid from viscose hydrolysates from either white or colored post-consumer waste textiles. *P. acidilactici* ZP26 is a strain resistant to inhibitory compounds originating in lignocellulosic pretreatment, e.g. organic acids, furans, and aldehydes [24, 25]. On another range of inhibitory compounds, dyes and other additives added in the textile industry have been proven toxic to

Fig. 2 (a) D-LA and (c) residual glucose amounts in batch reactors with white and colored waste viscose hydrolysates against control experiments; (b) D-LA and (d) residual and added glucose amounts in fed-batch reactors with white and colored waste viscose hydrolysates against control experiments
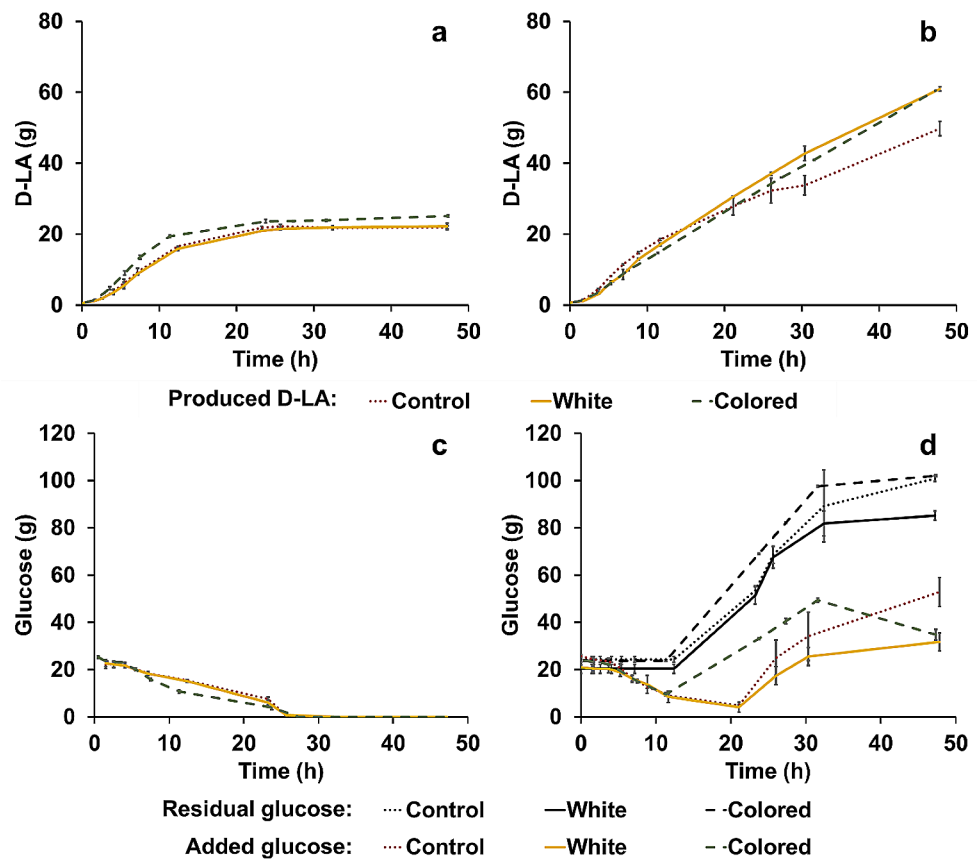
living organisms [26–28]. However, the extensive range of alternative, sometimes toxic, compounds found in post-consumer use textiles adds layers of complexity to the integration of these resources as raw materials in a bioprocess.

The waste viscose hydrolysates produced for this test were obtained through enzymatic hydrolysis of shredded post-consumer textiles. Optimal enzymatic hydrolysis efficiency was not the aim of this study, to redirect the attention to the utilization of the sugars in the solutions. The hydrolysis was followed by water removal by vacuum evaporation to concentrate the solutes in the mixtures. Water removal also led to the concentration of possible microbial inhibitors used for textile dyeing and finishing or accumulated through the use of the garments. The use of concentrated hydrolysates decreased the dilution of the fermentation broth by the feed and differences between each hydrolysate and the control would be more evident.

The good results obtained for D-LA production were not correlated with microbial growth because both solutions had particles and colors that affected the possible methods for biomass quantification. Nevertheless, it is known that *P. acidilactici* ZP26 reaches low biomass concentrations and continues to produce D-LA during the stationary phase of growth [21, 22]. *P. acidilactici* ZP26 has also been shown to produce exopolysaccharides (EPS), which form cell aggregates that deviate sugar away from the production of D-LA. Although amyloglucosidase was not added to the shake flask experiments, biomass flocculation was not clear because of the addition of calcium carbonate as a pH buffering agent. Nonetheless, EPS formation might be part of the reason for the lower yields obtained in the shake flasks, compared to the bioreactor cultivations. As it was shown in previous work [21], the addition of amyloglucosidase to the bioreactors prevented biomass flocculation by hydrolysis of the EPS and reduced the EPS to monomers accessible to the microorganisms to maximize D-LA production. This is clear in the yields above 0.9 g/g in the batch cultivations. Since D-LA and EPS are the only fermentation products known to this organism, it is not clear why the yields on consumed glucose decreased in the fed-batch cultivations. Since the composition of the EPS produced by *P. acidilactici* ZP26 has not been studied yet, a possible hypothesis is the incomplete hydrolysis of the EPS, therefore hindering the utilization of these sugars to produce D-LA. Further studies on this matter are important to strategize the limitation of EPS formation and assure its complete hydrolysis and conversion of the sugars to D-LA.

The results obtained in the shake flask and bioreactor experiments show that *P. acidilactici* ZP26 was able to homofermentatively produce D-LA using glucose in white or colored waste viscose hydrolysates. As previously mentioned, the trials performed in shake flasks served as

preliminary trials to assess if there was any major inhibition by any of the hydrolysates, and therefore redirect the study performed with bioreactors. As there were no clear signs of inhibition in the batch cultivations in shake flasks, the hydrolysates were used as sole carbon source in the cultivations in bioreactors.

The positive results obtained in the shake flask experiments anticipated positive results in the batch cultivations in bioreactors. In the batch reactors, the maximum D-LA productivity, at 12 h of cultivation, and yield were comparable with data obtained for the same cultivation stage (exponential production phase) in other control cultivations from previous work (2.54 g/(L.h) and 0.82 g/g, respectively) [29]. These results for maximum D-LA productivity and overall yield are also in the same range of values obtained with other D-LA producers such as *Lactobacillus delbrueckii* ssp. *delbrueckii* fed with orange peel waste hydrolysate supplemented with corn steep liquor – 2.35 g/(L.h) and 0.92 g/g [30]; *Sporolactobacillus nakayamae* fed with crystallized sugar – 2.16 g/(L.h) and 0.94 g/g [31]; and a *Lactobacillus delbrueckii* strain isolated from commercial yogurt fed with molasses and corn steep liquor – 2.35 g/(L.h) and 0.88 g/g [32]. Although D-LA productivity decreases after 12 h, as documented in Table 2, the numbers encourage further exploration and optimization of this process.

Upon analysis of Table 2, it is not possible to observe a clear trend of inhibition or enhancement of the production with any of the carbon sources. Some of the increased results obtained in the cultivations fed with viscose hydrolysates might be due to mechanisms for stress response of *P. acidilactici*. One of the mechanism for stress response in lactic acid bacteria is an increase in the energy metabolism [33]. In the case of homofermentative lactic acid bacteria such as *P. acidilactici* ZP26, this would probably be translated into higher D-LA production to provide ATP to the cell via glycolysis. Regardless, this is merely a hypothesis, and more studies are required to understand behaviour of *P. acidilactici* ZP26 and other interesting microorganisms in cultivations with viscose hydrolysate.

For this proof of concept, the main goal was to observe the profiles of D-LA with the three different carbon sources. Considering the differences shown in the shake flask cultivations, it would be interesting to assess if there is less EPS accumulation when using the waste viscose hydrolysates and if glucose is redirected to D-LA production without or with less need for enzymes to break these polymers. It will also be very important to obtain hydrolysates with less water content to maximize D-LA production and study the effects of higher concentrations of dyes, additives, and other contaminants [26, 27] with less limitation by dilution of the culture broth.

Nevertheless, these results open the door to new applications of glucose-rich solutions obtained from saccharification of waste viscose, and possibly other types of textiles. Research on the hydrolysis of cellulose fibers from textiles to monomeric glucose has been conducted to find ways to close the circle of life of textile fibers. As procedures for this saccharification start to emerge, possible applications for these glucose-rich solutions need to be explored. Renewable energy produced from natural sources (e.g., ethanol and biogas) is a possible application that is currently being explored [4, 9]. Most of the research is done on cotton textiles given its abundance, but waste viscose produced better results in studies on ethanol production because of the lower crystallinity of its fibers [4, 8]. Other microbial utilization strategies have been looked at for this type of waste material, such as the production of biogas and succinic acid [4, 34, 35]. Reinforcing the importance of studies in this field, a recent study has demonstrated the production of L-LA with *Weizmannia coagulans*, through simultaneous saccharification and fermentation (SSF) of pre-consumer textile blends of viscose and synthetic fibers [36].

From an environmental and bioprocess perspective, the study of textile additives and dyes in textile waste is a challenge because of the variety of complex compounds that are used in various combinations and concentrations [28]. The step following the bioprocessing of textile wastes, i.e. downstream for product purification, poses also a series of challenges and opportunities that have not been addressed yet. Nevertheless, the first approach for the expansion of the financed studies in this field is to prove that there are organisms, like *P. acidilactici*, that can produce biochemicals (e.g., optically pure LA) that can be turned into value-added products (e.g., PLA).

Conclusions

D-LA was produced with glucose-rich solutions obtained from the enzymatic hydrolysis of waste viscose, white and colored. Shake flask trials, and bioreactors in batch and fed-batch modes were conducted, achieving a maximum average of 60 g (approximately 40 g/L) of D-LA in the fed-batch cultivations with white and colored waste viscose hydrolysates. D-LA production and glucose consumption profiles in the cultivations with hydrolysates as carbon source were comparable to those of the control, and yields on consumed glucose were all above 0.8 g/g. This study documents the production of optically pure D-LA from post-consumer textile waste which, after purification, is one of the possible building blocks for the synthesis of PLA fibers.

This field of research has attracted a lot of attention recently and more studies are required to understand the

range and impact of dyes, additives, and other textile contaminants in all steps of the process: pretreatment, saccharification, fermentation, and product purification and separation. This knowledge will be essential to conduct feasibility studies for scale-up production.

Declarations

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