RSC Advances

PAPER

View Article Online
View Journal | View Issue

Cite this: *RSC Adv.*, 2016, **6**, 92420

Biologically synthesized crude calcium lactate as a substrate for propylene glycol production

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Received 17th August 2016
Accepted 20th September 2016

DOI: 10.1039/c6ra20722g

www.rsc.org/advances

Lactic acid (LA) can be obtained easily through fermentation of saccharides, and is an extremely useful building block for the synthesis of valuable chemicals, including propylene glycol (PG). Catalysis is one of the key elements in this platform approach. The present study focuses on PG synthesis *via* catalytic hydrogenation of lactate ions. In the conventional biological method, separation and downstream processing of pure LA account for up to 50% of production costs. Therefore, we propose using a less pure solution of lactate ions as a feedstock. To this end, we have developed a new method for the treatment of post-fermentation broth containing calcium lactate, obtained from sugar beet pulp. Activated carbon was used for the purification of the post-fermentation broth, which was acidified to pH 2–3. Hydrogenation of LA into PG was performed over supported metallic catalysts, based mainly on ruthenium, under mild conditions. The yield of PG was satisfactory and the PG water solution obtained could be used in concentrated form as a component in antifreeze.

1. Introduction

Lactic acid (LA) is one of the primary platform chemicals and can be used to synthesize a wide variety of useful products.[1] By virtue of the unique presence of both hydroxyl and carboxylic acid groups, 2-hydroxypropanoic acid can participate in a wide variety of chemical reactions, including esterification, condensation, polymerization, reduction and substitution.[2] Oxygenated chemicals, such as propylene glycol (PG), propylene oxide, acrylic acid and acrylate esters, or other chemical intermediates (including lactate ester plasticizers), can be made from LA, although further efforts are needed to increase the efficiency and cost-effectiveness of the process of LA production. Many studies have been conducted into the possibility of green production of LA derivatives, with one promising avenue being their production from renewable biomass.[1]

Catalytic transformation of LA into PG over metal based heterogeneous catalysts is of great interest to industry. The production of PG from lactate was first reported by Adkins *et al.* in the 1930s and 1940s,[3–5] who converted neat ethyl lactate into PG over RANEY® Ni with yields exceeding 80% at very high pressures (>25 MPa). Broadbent *et al.*[6] conducted the first catalytic hydrogenation of free LA using unsupported rhenium black as a catalyst. They achieved 84% PG after 8 h at 150 °C and 27 MPa. Ford[7] and Carnahan *et al.*[8] used supported ruthenium-based catalysts to directly hydrogenate LA at low temperature under high pressure hydrogen. Zhang *et al.*[9] identified ruthenium on activated carbon as an extremely active catalyst for the reduction of LA at temperatures of 100–170 °C under H_2 pressures of 7–14 MPa. Selectivity to PG reached 90–95% under optimal reaction conditions. Jang *et al.*[10] performed liquid-phase catalytic hydrogenation of LA (50 mL, 1 M, 110–170 °C, 6–9 MPa) over various metals (Ag, Co, Cu, Ni, Pt and Ru) supported on SiO_2. Of the catalysts tested, Ru/SiO_2 provided the highest PG yield, which increased with pressure. In further studies,[11] liquid-phase catalytic hydrogenation of LA (50 mL, 1 M, 110–170 °C, 6–9 MPa) was performed over Ru on various carbon supports (Vulcan XC-72, ketjenblack, CNTs, CNFs, graphite). It was found that the PG yield increased with pressure and depended strongly on the type of support used. Of the catalysts tested, Ru on ketjenblack showed the highest activity.

Copper is another metal with good activity for the reduction of LA to PG. Numerous studies have demonstrated that LA can be reduced to PG on Cu–Si catalysts under mild conditions.[12–17] Cortright *et al.*[12] showed that it was possible to produce PG with high yields *via* the vapour-phase hydrogenation of biomass-derived LA over Cu/SiO_2 catalysts. Copper-containing catalysts (copper chromite, copper-zinc hydroxysilicate and copper hydroxysilicate) exhibit high activity, due to the formation of stable, highly-dispersed particles of metallic copper during preliminary reduction.[13–15] Copper–silicon catalysts have been found to have the highest selectivity for PG, at ~75%.

The first stage of our investigations into the catalytic reduction of LA in water phase at Lodz University of Technology was the synthesis of biological LA solutions, in which the

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concentrations of acid were around 0.5 M. We tested many catalysts, based on Cu, Ni, Pd, Ru and Rh. Each variant of the process was completed in less than 4 hours under mild temperature conditions ($T = 130$ °C) and hydrogen pressure ($p = 3.5$ MPa). Wet impregnation of the supports (support = C, SiO_2, Al_2O_3) was performed in aqueous solutions of metal salt (M = Cu, Ni, Pd, Ru, and Rh) to obtain 5% M/support systems. Before testing, the catalysts were activated in a stream of H_2 at 300 °C for 2 h, to produce active metallic phase. The ruthenium catalysts showed the best catalytic properties for the reduction of LA to PG (5% Ru/C: $X_{LA} = 94.10\%$, $S_{PG} = 87.25\%$; 5% Ru/SiO_2: $X_{LA} = 66.39\%$, $S_{PG} = 81.62\%$; 5% Ru/Al_2O_3: $X_{LA} = 81.24\%$, $S_{PG} = 82.31\%$). Commercial 5% Ru/C (Sigma-Aldrich, CAS 206180) catalyst produced the highest percentage of LA ($X_{LA} = 97.78\%$) and showed the best selectivity for PG ($S_{PG} = 93.25\%$). For these reasons, it was decided to use the commercial 5% Ru/C catalyst in further research.

Advanced and integrated hydrogenation technology enables production of PG from LA with good yields.[18,19] However, it is a material-intensive process (requiring 1.25 kg LA/1.00 kg PG), so it is important to reduce costs and increase efficiency. The use of less pure LA as a feedstock offers one way to do this. In the conventional biological method of pure LA production, the separation and purification stages account for up to 50% of production costs.[20] The sulphur-containing amino acids present in post-fermentation broths irreversibly poison metal-based catalysts (including ruthenium catalysts). Non-sulphur amino acids partially but reversibly poison the catalysts.[21] It is therefore necessary to develop new methods for treating post-fermentation broths containing calcium lactate, which could reduce the cost of the conventional industrial method of pure LA production.

Lactic acid can be obtained either by fermentation of renewable carbohydrates or by chemical synthesis.[22] Both methods are used in commercial production.[23] Due to the depletion of resources and the accumulation of environmentally hazardous chemicals, the manufacture of LA from fossil fuels is now widely considered unsustainable. Even though fermentation could replace chemical synthesis, the costs involved must be reduced.[24] Low-cost cellulosic materials, such as industrial wastes, agricultural waste and forestry waste, have received much attention as possible feed stocks to substitute edible starch material, and have been recommended as cost-effective alternatives for large scale fermentation.[19,25,26] These substrates also show promise in terms of their chemical characteristics, levels of contaminants, possible fermentation rates, LA yields, their by-products and ability to be fermented with little or no pre-treatment.[2]

Because of its low lignin content, the sugar beet pulp (SBP) proposed in this paper for use as a substrate in the production of PG does not require any special biomass preparation. Substrate type is also one of the major causes of problems connected with downstream purification. The nature of the substrate may determine the degree of conversion, or which specific reagents and microbes are involved in the biological process.[2] Fermentation broth contains a number of impurities as part of its cell mass, such as residual sugars, colour, nutrients and other organic acids. These impurities must be removed from the broth in order to achieve purer LA.[25] The classical route for the recovery of LA from fermentation broth is based on the precipitation of calcium lactate. The filtered salt is then treated with sulphuric acid, leading to free acid and $CaSO_4$. Additional purification steps are conducted to produce commercial grade LA.[27]

In this study, we propose an approach to improve the yield of lactate ions from post-fermentation broths, which can then be catalytically reduced to PG. Two different fermentation processes are considered: separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF). A mixture of fermentative sugars was obtained by enzymatic hydrolysis of SBP. Simultaneous saccharification and fermentation proved particularly effective as a way of increasing the concentration of calcium lactate in the post-fermentation broths. Since it was known from the literature that potassium and calcium lactate salts cannot be converted directly to PG, but that they can be converted by the simple addition of sulphuric acid into free acid (which is then hydrogenated),[9,28] a simple method was found to release lactate ions from the broth. The broth was also purified of constituents containing sulphur and nitrogen heteroatoms. Catalytic reduction of the purified water solution of LA over 5% Ru/C catalyst produced PG under mild pressure and temperature conditions ($p = 3.5$ MPa, $T = 130$ °C).

2. Materials and methods

2.1. Biological material

In both SHF and SSF processes, six lactic acid bacteria (LAB) strains (five from the Polish Collection of Microorganisms PCM and one from the American Type Culture Collection) were used: *Lactococcus lactis* PCM 2379, *Lactobacillus acidophilus* PCM 2510, *Lactobacillus delbrueckii* PCM 490, *Lactobacillus plantarum* PCM 2675, *Lactobacillus brevis* PCM 488, *Lactobacillus plantarum* ATCC 8014. Four environmental isolates were also used: *Lactobacillus plantarum* R, *Lactobacillus plantarum* HII, *Lactobacillus plantarum* AXD and *Lactobacillus plantarum* AXG, isolated from sugar beet and self-fermented grass. The environmental isolates were identified using molecular methods based on 16S rRNA gene sequencing and their accession numbers were made available in the NCBI GenBank database: KT751286, KT751287, KT751284, KT751285.

2.1.1. Preparation of inoculum. The LAB strains were activated and propagated on SBP hydrolysate. The medium was supplemented with yeast extract (4.0 g L^{-1}), ammonium citrate (2.0 g L^{-1}), beef extract (8.0 g L^{-1}), peptone K (10.0 g L^{-1}), dipotassium phosphate (2.0 g L^{-1}), sodium acetate (5.0 g L^{-1}), magnesium sulphate (0.2 g L^{-1}) and manganese sulphate (0.05 g L^{-1}), then sterilized at 120 °C. The LAB cultures were incubated for 48 h at 37 °C. At least three cultivation passages were conducted prior to each process.

2.2. Raw material

Sugar beet pulp hydrolysate was produced in a semi-industrial reactor (3 m^3) at the Sugar Factory in Dobrzelin (Poland). The

biomass was saccharificated for 20 h at 50 °C using a mixture (1 : 1) of two multi-enzyme preparations made by Novozymes: Viscozyme® and Ultraflo® Max (3 L/100 kg of SBP dry weight). The resulting medium contained glucose (13.11 g L^{-1}), fructose (4.92 g L^{-1}), xylose (0.65 g L^{-1}), rhamnose (2.37 g L^{-1}), raffinose (22.53 g L^{-1}), galactose (14.11 g L^{-1}) and arabinose (10.83 g L^{-1}). Enzyme activity was halted at the end of the process by 30 minutes of incubation at 37 °C.

2.3. Lactic acid fermentation

2.3.1. Separate hydrolysis and fermentation (SHF). Separate hydrolysis and fermentation was carried out in 100 mL Erlenmeyer flasks in 50 mL hydrolysate supplemented with yeast extract (4.0 g L^{-1}), ammonium citrate (2.0 g L^{-1}), beef extract (8.0 g L^{-1}), Peptone K (10.0 g L^{-1}), dipotassium phosphate (2.0 g L^{-1}), sodium acetate (5.0 g L^{-1}), magnesium sulphate (0.2 g L^{-1}) and manganese sulphate (0.05 g L^{-1}). The sterilized medium was inoculated with LAB monocultures and incubated for 48 h at 37 °C. Sterile calcium carbonate ($CaCO_3$) suspension was added in selected trials to stabilize the pH.

2.3.2. Simultaneous saccharification and fermentation (SSF). Simultaneous saccharification and fermentation was carried out in 100 mL Erlenmeyer flasks filled with SBP (6 g dry weight), water and $CaCO_3$. Prior to the process, the SBP was suspended in warm water to obtain a dry matter concentration of around 12% (w/v). After supplementation with nitrogen and mineral compounds (yeast extract, ammonium citrate, beef extract, peptone K, dipotassium phosphate, sodium acetate, magnesium sulphate, manganese sulphate) the suspension was sterilized at 120 °C. Hydrolysis was initiated after the addition of a 1 : 1 mixture of Ultraflo Max and Viscozyme (0.1 mL/10 g SPB dry weight). The process was continued for 10 h at 37 °C, after which the medium was inoculated with LAB strains (without enzyme denaturation). The fermentation process was conducted for 48 h in the case of monocultures, and for 96 h in the case of mixed cultures (second inoculations were made after the first 48 h).

2.4. Lactic acid recovery and purification

The samples were purified on activated carbon (ERCARBON GE, 2.5 g/50 mL) or on a mixture of activated carbon and silica (m_{Cakt} = 2.5 g/50 mL; m SiO_2 (SiO_2, POCH Gliwice SA) = 2.5 g/50 mL). The solid adsorbent was weighed and added to a metered volume of a liquid biological sample. The mixture was stirred vigorously for 1 minute at 10 °C. The supernatant was then separated by filtration and subjected to catalytic hydrogenation. The results indicated that the sample had been successfully purified.

2.5. Propylene glycol synthesis

Hydrogenation of LA was performed in a 50 mL autoclave (Parr Company) at 130 °C and under 3.5 MPa of H_2 pressure. The reactions were conducted with equal amounts of catalyst (m_{cat} = 0.5 g). The mixture was stirred at 500 rpm. The autoclave was flushed with Ar, then flushed again with H_2 and pressurized with H_2 to 3.5 MPa. The temperature was gradually raised to 130 °C at a heating rate of 20 °C min^{-1}. The reaction was sustained for 4 hours. The reaction conditions were optimized for a 5% Ru/C catalyst (Sigma-Aldrich, CAS 206180), used for the reduction of 20 mL water solution of commercial LA (0.5 M, POCh Gliwice SA, p.p.a.). The biological LA samples, after purification on activated carbon (or a mixture of activated carbon and silica), were either catalytically reduced or first diluted with deionized water to a concentration of LA 0.5 M and then reduced over 5% Ru/C. Following the reaction, the autoclave was cooled to room temperature. The reaction mixture was then filtered and analyzed using HPLC and GC-FID techniques.

2.6. Media analysis

The concentration of lactate ions was measured spectrophotometrically using a D-/L-Lactic Acid Assay Kit (K-DLATE) (Megazyme). To improve the solubility of the calcium lactate, the probes were heated to 80 °C for 15 minutes.

An HPLC system (LaChrome, Merck-Hitachi) coupled to a variable wavelength UV (210 nm) detector was used to determine the concentration of LA. The reactant was separated on an Agilent ZORBAX SB-C18 column, with the use of mobile phase acetonitrile/phosphate buffer = 12 : 88 v/v (pH = 4.5, C = 0.01 M).

To assess the concentration of free LAs in the fermented hydrolysates, they were first passed through a centrifugal ultrafilter with a cut-off mass of 3 kDa. Samples (50 μl) were then transferred into Eppendorf tubes and evaporated to dryness in a vacuum centrifuge. Next, the free amino acids were converted into a phenylthiocarbamide (PTC) derivative. The derivatives were dissolved in amino acid solvent, added in portions of (200 mL). Samples (5 μl) were analyzed using high pressure liquid chromatography (HPLC) on a PicoTag 3.9 × 150 mm column (Waters, USA). Quantitative calibration was performed using four concentrations of amino acid standards (Pierce Company, USA).

The concentration of PG was determined using the GC-FID technique (Hewlett Packard 5890A; Restek RTX 5 column). The operating conditions for GC-FID analysis were: injection port temperature 70 °C; FID detector temperature 250 °C; column oven temperature 150 °C; temperature increase 15 °C min^{-1}; carrier gas He (Linde, 99.999%, flow rate 30 mL min^{-1}); injection volume 1 μl.

The trials in each experiment were repeated in triplicate. The mean values are presented.

3. Results and discussion

3.1. Fermentation processes and calcium lactate formation

Any sugar can be used as a carbon source for the microbial production of LA.[26,29] Sugar in its pure forms, such as glucose, sucrose and lactose, starchy materials from potatoes, tapioca, wheat, barley, carrot, and waste materials containing sugar, including molasses, whey, sugarcane bagasse, cassava bagasse or sugar beet, coffee husks and pulp, apple pomace, oilcakes or wheat/rice bran, may all be suitable for this purpose. Sugar beet pulp hydrolyzate has been shown to be a useful substrate for LA

Table 1 Production of lactic acid in SHF processes

	Production of lactate ions [g L^{-1}]	
	without $CaCO_3$	with $CaCO_3$
Lb. delbrueckii PCM 490	6.89 ± 1.38	11.96 ± 0.19
L. lactis PCM 2379	5.54 ± 0.02	13.44 ± 1.75
Lb. plantarum HII	7.01 ± 0.23	12.60 ± 0.37
Lb. acidophilus PCM 2510	7.62 ± 0.44	12.48 ± 0.65

fermentation conducted in SHF mode using *Lb. delbrueckii* PCM 490, *Lb. lactis* PCM 2379, *Lb. plantarum* HII and *Lb. acidophilus* PCM 2510 strains (Table 1.). However, owing to the inhibitory effects of low pH on cell growth and LA productivity, an agent should be added to maintain a constant pH.[1] In our experiments, the addition of $CaCO_3$ doubled the LA yield, which reached values of 11.96–14.44 g L^{-1} (Table 1.). This corresponded to 0.13–0.16 M lactate concentrations. However, even higher concentrations of lactate ions are preferable for catalytic purposes.[26]

Whether the production of LA and its derivatives is economical depends on many factors, among the most important of which are the cost of the raw material and fermentation efficiency.[19,29] Many processing costs can be reduced by using SSF. This procedure helps to increase the yield and productivity of LA. Especially in the case of lignocellulosic materials, SSF processes have advantages over SHF. Consumable sugars, such as glucose, released by lytic enzymes, are gradually converted into the end product by microorganisms.[24] Carbon catabolite repression and enzyme glucose inhibition are thereby minimized.[30,31] Moreover, Hofvendahl[32] showed that SSF requires lower quantities of enzymes to produce LA, thus reducing production costs further. It was therefore decided to ferment the SBP (in the presence of $CaCO_3$) simultaneously with enzymatic hydrolysis (Table 2). This resulted in a fourfold increase in the production of lactate ions, while using around a third of the amount of enzymes.

The main barrier to use of lignocellulose is the composition of the carbohydrates in its hydrolyzates. Hydrolysis leads to the release of hexoses and pentoses. However, most commercial LAB strains used by industry lack the ability to assimilate pentose.[33] Using more than one fermenting strain can improve the efficiency of the process by 10–30%.[34] The use of mixed cultures has been shown to substantially improve LA yield from fermented corn stover[35] and date juice.[34] This can be explained by greater utilization of different carbon sources and by reduced nutrient limitation.[36] In our study, the concentration of lactate ions increased by 10–12% after the second fermentation step using mixed cultures (Table 3).

3.2. Lactic acid recovery and purification

Downstream processing of LA broth is extremely complicated and involves a number of steps.[37] Pure LA has been commercially produced using strong acids, cation-exchange resins and solid catalysts.[25,38] Musahino Chemical Laboratory in Japan, CCA Biochemical BV in the Netherlands and Natureworks LLC are among the majority of large-scale LA manufacturers which have switched to fermentation technologies based on a number of downstream treatments, including precipitation, conventional filtration, acidification, carbon adsorption and evaporation.[2] The separation and purification of LA from fermentation broth adds significantly to production costs.[1,25] The usual alternatives are: (1) precipitation of cations salts; (2) liquid–liquid extraction with simultaneous lactate salt formation using a strong base and back-extraction of LA with water; (3) simultaneous acidification and esterification with alcohol followed by back-extraction with water; (4) direct removal of LA from the broth using advanced separation methods, such as adsorption, membrane separations and ion exchange.[37]

Two methods of LA recovery are generally employed. In the first, the clarified fermented liquor is concentrated to 32%, well above crystallization point, and then acidified using sulphuric acid to obtain crude LA. Alternatively, the crude calcium salt which precipitates from the concentrated fermented liquor can be crystallized, filtered, dissolved and then acidified using sulphuric acid.[39] The filtered fermented broth contains impurities such as residual sugar compounds, colour and other organic acids, which can be removed by reactive extraction, adsorption, electrodialysis or esterification-hydrolysis with distillation.[39,40]

Esterification can be performed efficiently through reactive distillation, using cation exchange resin columns acting as both packing and catalyst. However, the columns need to be fed with pre-purified LA. In the case of reactive extraction, adsorption, electrodialysis or conventional precipitation–filtration, on the other hand, calcium lactate washing treatments are necessary.[39] The route involving quantitative precipitation of salts from the broth and separation of the solid phase requires the calcium lactate to be washed, to remove impurities retained during

Table 2 Production of lactic acid in SFF processes conducted with monocultures

LAB strains	Lactate ions [g L^{-1}]	Lactate ions [mol L^{-1}]
Lb. delbrueckii PCM 490	49.16 ± 1.14	0.546 ± 0.013
L. lactis PCM 2379	43.45 ± 0.41	0.482 ± 0.005
Lb. plantarum HII	47.11 ± 2.15	0.523 ± 0.024
Lb. acidophilus PCM 2510	45.18 ± 1.95	0.502 ± 0.022

Table 3 Production of lactic acid in SFF processes conducted with mixed cultures

LAB strains	Lactate ions [g L^{-1}]	Lactate ions [mol L^{-1}]
Lb. delbrueckii PCM 490 *Lb. plantarum* ATCC 8014	56.42 ± 2.87	0.626 ± 0.032
L. lactis PCM 2379 *Lb. plantarum* AXD	51.71 ± 2.71	0.574 ± 0.030
Lb. plantarum HII *Lb. brevis* PCM 488	55.57 ± 1.85	0.617 ± 0.021
Lb. acidophilus PCM 2510 *Lb. plantarum* AXG	50.35 ± 2.29	0.559 ± 0.025

Table 4 Quantitative analysis of amino acids in the biological sample, before and after purification using activated carbon. Lactic fermentation was performed in SBP hydrolyzate using *Lactobacillus delbrueckii* PCM 490

	Amino acid content [mmol L^{-1}]	
Amino acid	Before purification	After purification
Asp	23.43 ± 1.93	b.d.l.
Glu	47.93 ± 4.37	b.d.l.
Ser	11.70 ± 1.24	b.d.l.
Gly	40.30 ± 3.96	b.d.l.
His	20.44 ± 2.01	b.d.l.
Arg	26.24 ± 3.02	b.d.l.
Thr	1.10 ± 0.1	b.d.l.
Ala	113.42 ± 10.75	13.4 ± 1.55
Pro	159.54 ± 14.44	8.9 ± 0.9
Tyr	87.47 ± 7.97	11.3.± 0.98
Val	48.96 ± 5.62	b.d.l.
Met[a]	18.08 ± 2.04	b.d.l.
Cys[a]	28.89 ± 2.51	b.d.l.
Leu	85.08 ± 8.46	2.4 ± 0.19
Phe	47.62 ± 4.95	4.9 ± 0.50
Lys	14.07 ± 1.38	b.d.l.

[a] Sulphur-containing amino acids b.d.l. – below detection limit.

precipitation. Although some of the impurities are removed with the washes, there is also significant loss of calcium lactate.[39]

In our investigations, it was decided to use activated carbon (ERCARBON GE) or a mixture of activated carbon (Ecocarbon GE) and silica (POCH Gliwice SA) as adsorbents.[26] These effectively removed impurities containing heteroatoms and significantly increased the concentration of lactic ions. Table 4 shows the results of quantitative analysis of amino acids after lactic fermentation in a solution with SBP hydrolyzate. Results for the same solution after purification on activated carbon are also presented. As can be seen, the concentration of total amino acids, particularly those containing sulphur atoms in the amino acid structure, is significantly lower without purification. It can be concluded that the presence of compounds, such as amino acids, which contain heteroatoms N and S in the crude fermentation media inhibits the activity of ruthenium catalysts for the reduction of lactate ions.

The effect of different types and amounts of activated carbon and silica on the composition of the post-fermentation media will be in the subject of a subsequent article. Despite recent progress, fermentative production of LA is still costly, mainly due to the complex and diverse purification steps required.[41]

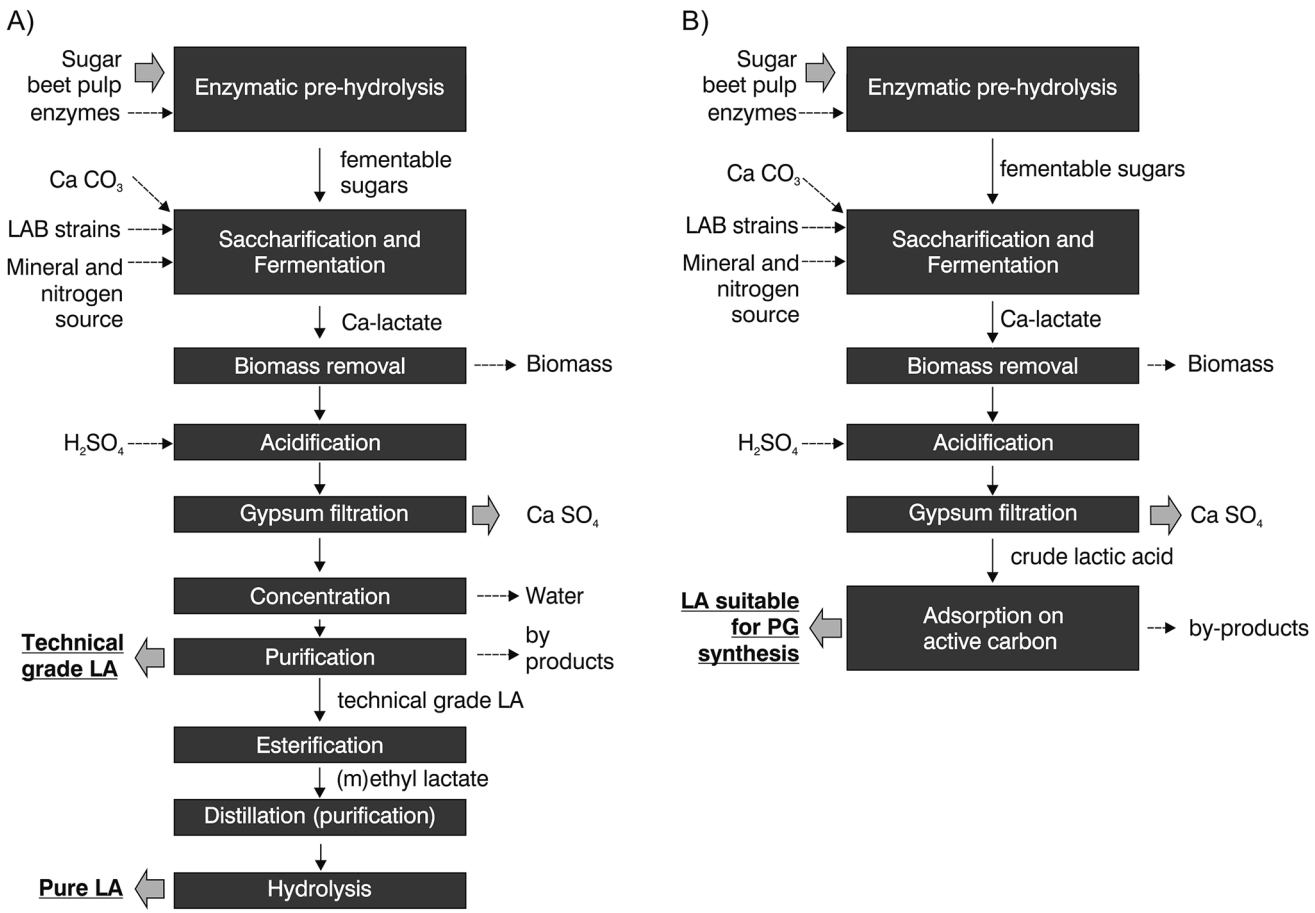

Fig. 1 Simplified block scheme for the fermentative production of LA: (A) – classical procedure (adapted from Dusselier[41]); (B) – proposed procedure for PG synthesis.

Given the increasing use of biobased chemicals, it is important to address this bottleneck. With these factors being considered, the possibility of using SBP for LA fermentation can now be examined (Fig. 1).

Low-lignin SBP biomass does not require special pretreatment. As shown by this study, enzymatic saccharification of SBP can best be achieved through the SSF process. Once the lactate salts reach the required concentration, the medium is filtered (for cell removal) and is sulphuric acid added. In this way, free LA and low-value insoluble salts (mainly $CaSO_4$) are formed as by-products. Following the removal of the gypsum, the liquid is further purified and concentrated to obtain technical grade LA. The impure LA can be esterified and subjected to further downstreaming for use in food or solvents.[41] Catalytic reduction of lactate ions does not require the pure LA to be isolated from the broth nor concentration of the solution. After efficient fermentation, the amounts of unused sugars in the broth are very low, and they do not compete in the reduction reaction. However, all impurities, including sulphur and nitrogen atoms, should be removed, as these can potentially poison the metallic catalysts. Therefore, a simple method for the treatment of postfermentation media with activated carbon (or with a mixture of activated carbon and silica) is proposed (Fig. 1B).

Table 5 Influence of the addition of H_2SO_4 to 0.1 M calcium lactate solution on the conversion of lactate ions and selectivity to propylene glycol[a]

pH of solution	X [%]	S [%]
7.35 ± 0.10	0	0
5.50 ± 0.10	35.5	28.2
4.00 ± 0.10	71.8	50.2
3.00 ± 0.10	80.0	78.5
2.50 ± 0.10	87.3	91.1

[a] Reaction conditions: $T = 130$ °C, $pH_2 = 3.5$ MPa, $m_{cat} = 0.5$ g, V = 20 mL, $C_{C_6H_{10}CaO_6} = 0.1$ M.

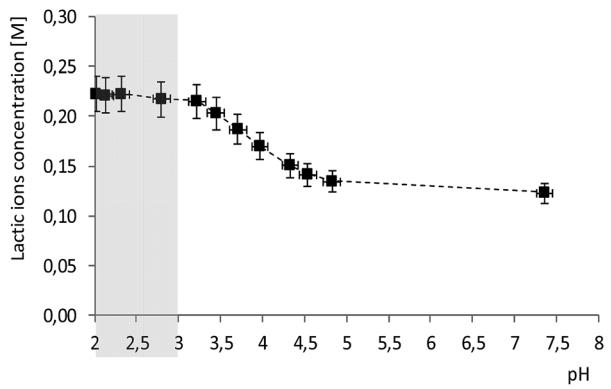

Fig. 2 Changes in the concentration of lactate ions in the solution ($C_6H_{10}CaO_6$ 0.1 M) relative to pH (the amount of concentrated H_2SO_4 added).

Table 6 Influence of the addition of HCl to 0.1 M calcium lactate solution on the conversion of lactate ions and selectivity to propylene glycol

pH of solution	X [%]	S [%]
7.35 ± 0.10	0	0
5.50 ± 0.10	8.2	27.9
4.00 ± 0.10	19.6	64.4
3.00 ± 0.10	35.8	74.9
2.50 ± 0.10	65.2	59.8
2.00 ± 0.10	79.4	55.7

3.3. Propylene glycol synthesis

3.3.1. Effect of substrate. Initially, we investigated the suitability of commercial calcium lactate ($C_6H_{10}CaO_6$, POCh S.A, p.p.a.) as a substratum in the production of PG. It was observed that the reduction of 0.1 M solution of $C_6H_{10}CaO_6$ did not produce PG. The lactate ions were too strongly bound in the salt molecules to be selectively reduced to the desired product (Table 5). However, the addition of strong acid, H_2SO_4 (Table 5, Fig. 2) or HCl (Table 6), led to the formation of free lactate ions, which could be effectively reduced into PG over 5% Ru/C catalyst.

On the basis of the results presented in Tables 5 and 6, it can be concluded that sulphuric acid(VI) is more effective as an acidifying agent than hydrochloric acid. Moreover, the acidification of crude calcium lactate solution with sulphuric acid(VI) is a process already used by industry (Fig. 2). Adding H_2SO_4 until the pH of the calcium lactate solution was around 2.5 ÷ 3.0 resulted in almost 90% substrate conversion, while maintaining more than 90% selectivity to PG.

We therefore decided to acidify the post-fermentation broth using H_2SO_4 to pH 2–3 prior to purification on activated carbon. The diluted water solution of lactate ions obtained was reduced over commercial Ru-based supported catalyst.

3.3.2. Hydrogenation of lactic ions obtained from fermented sugar beet pulp hydrolyzates. Once the biological samples had been acidified with H_2SO_4 and purified on activated carbon, catalytic hydrogenation of lactic ions was performed. The reaction was conducted in a high-pressure batch reactor. Table 7 shows a comparison of lactic ion conversions over ruthenium-supported catalyst after 4 h. The activity of the 5% Ru/C catalyst was determined as the percentage of lactate ions converted, according to the equation:

$$X = [1 - (C/C_0)] \times 100\%$$

where: X is the percentage of the lactate ions converted [%]; C_0 is the initial concentration of lactate ions [M]; C is the concentration of lactate ions at time t [M].

Selectivity to PG was determined using the equation:

$$S = [C_{PG}/(C_0 - C)] \times 100\%$$

where C_{PG} is the concentration of PG [M].

The reaction conditions were optimized in terms of time and weight of catalyst to achieve a concentration of lactic ions of

Table 7 Activity and selectivity of 5% Ru/C catalyst for reduction of lactate ions to propylene glycol

Biological substratum	pH	Initial concentration of lactate ions [M]	Final concentration of lactate ions [M]	Conversion of lactate ions [%]	Concentration of propylene glycol [M]	Selectivity to propylene glycol [%]
Lactobacillus delbrueckii PCM 490	2.5 ± 0.2	0.488 ± 0.024	0.049 ± 0.002	**89.96**	0.428 ± 0.034	**97.49**
Lactococcus lactis PCM 2379	2.5 ± 0.2	0.464 ± 0.23	0.211 ± 0.011	**54.53**	0.173 ± 0.014	**68.38**
Lactobacillus plantarum HII	2.5 ± 0.2	0.556 ± 0.028	0.307 ± 0.015	**44.78**	0.238 ± 0.019	**95.58**
Lactobacillus acidophilus PCM 2510	2.5 ± 0.2	0.631 ± 0.032	0.370 ± 0.019	**41.36**	0.256 ± 0.020	**98.08**
	2.5 ± 0.2	0.509 ± 0.025	0.252 ± 0.013	**50.49**	0.245 ± 0.020	**95.33**
Lactobacillus acidphilus PCM 2510 *and Lactobacillus plantarum* AXG	2.5 ± 0.2	0.838 ± 0.042	0.588 ± 0.029	**29.83**	0.089 ± 0.007	**35.60**
	2.5 ± 0.2	0.523 ± 0.026	0.323 ± 0.016	**38.24**	0.134 ± 0.011	**67.00**
Lactococcus lactis PCM 2379 *and Lactobacillus plantarum* AXD	2.5 ± 0.2	0.809 ± 0.040	0.483 ± 0.024	**40.30**	0.143 ± 0.011	**43.87**
	2.5 ± 0.2	0.492 ± 0.025	0.243 ± 0.012	**50.61**	0.210 ± 0.017	**84.34**

around 0.5 M. Biological samples with higher concentrations of lactic ions were diluted to 0.5 M. The 0.5 g ruthenium catalyst showed similar activity and selectivity with diluted biological samples to that observed for 0.5 M solution of commercial LA after 4 h of hydrogenation.[26] This result proves the effectiveness of the method of purification used on the biological samples prior to the catalytic process.

4. Conclusions

This study has proposed a new method for the treatment of post-fermentation broth containing calcium lactate, obtained from SBP. The post-fermentation broth was acidified to pH 2–3 and purified using activated carbon. Catalytic reduction of a diluted water solution of LA was performed over Ru-based supported catalyst. The yield of PG was satisfactory. Propylene glycol is an example of a product derived from LA with a rapidly growing market. The water solutions of PG obtained using our method could be used in concentrated form as a component in antifreeze.

Acknowledgements

The authors would like to acknowledge the contribution of the National Centre for Research and Development funded Applied Research Programme – Project "Sugar beet pulp biomass as a raw material for new fermentation media" PBS1/B8/3/2012.

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