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Assessing multiple bioprocess modes for lactic acid production by *Lactiplantibacillus plantarum* ATCC 8014 using lactose as a substrate

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Lactose as a primary carbon source for fermentation is not as thoroughly investigated as glucose, despite certain advantages such as potential sourcing from high volume dairy side-streams. We investigated whether lactose could be a feasible feed source for various lactic acid bacteria (LABs) to produce lactic acid (LA), which is a precursor for the synthesis of the bioplastic polylactic acid. In 1 Litre stirred tank bioreactors under microaerophilic batch growth conditions *Lactiplantibacillus plantarum* ATCC 8014 had the highest LA titre (40 g L^{-1}) and productivity (0.83 g L^{-1} h^{-1}) compared to other LABs tested. When air was supplied to the bioreactor at 10% dissolved oxygen, *L. plantarum* ATCC 8014 fully consumed the lactose supplied to produce 40 g L^{-1} LA and increased the LA volumetric productivity to 1.51 g L^{-1} h^{-1} in 28 hours. Fed-batch fermentations with *L. plantarum* ATCC 8014 achieved the highest LA titre (69.05 g L^{-1}) but productivity was reduced (1.28 g L^{-1} h^{-1}) compared to the best batch cultures. Under continuous culture conditions (D = 0.1 h^{-1}) *L. plantarum* ATCC 8014 had the highest LA yield (0.88 g g^{-1}) from lactose but the titre was low (4 to 6 g L^{-1}) and productivity was not stable.

Sustainability spotlight

This study provides an in-depth investigation of the conversion of lactose to lactic acid. Lactose-containing waste is produced in large volumes worldwide and at present, no scalable process has been developed to deal with this waste. Fermentation of lactose based substrates is a possible valorisation route promoting increased resource efficiency. A first critical step is to identify and test bacterial strains which produce lactic acid, a building block for the synthesis of polylactic acid, a biobased and biodegradable polymer which can replace fossil based non degradable polymers. The research we have undertaken supports UN SDG 12, responsible consumption and production of materials. The use of secondary resources supports the development of a circular bioeconomy.

Introduction

Lactose is a disaccharide sugar formed from one molecule of glucose and one of galactose. It is often produced from waste streams, such as dairy side streams, where lactose can be separated from whey permeate (cheese waste). This makes this sugar an attractive option for feeding bioprocesses as opposed to glucose, which is often produced from food sources such as corn in the form of starch which is then enzymatically hydrolysed to the monosaccharide of glucose.[1] The United Nations' sustainable development goal (SDG) 12 identifies the need to undertake responsible consumption and production, in which significant progress is still needed to meet targets.[2] In the Bioeconomy, the use of secondary resources (side streams, residues, wastes) to manufacture products reduces the burden on primary resources and increases resource efficiency.[3,4] Microorganisms are widely regarded as single cell factories, capable of transforming biobased resources to useful products such as biogas, bioplastics, pharmacologically active agents, and probiotics and thus are a central biotechnological resource in biomanufacturing in a circular bioeconomy.[5–10]

Within the existing literature, there have been multiple assessments of lactic acid bacteria (LABs) for lactic acid production, although these have been mostly tested for production from glucose as opposed to lactose.[11–13] LABs can produce lactic acid (LA) from various carbon sources, along with other co-products of anaerobic metabolism. We investigated well studied LA producers for conversion of lactose to lactic acid with the intention that this could be used to develop a bioprocess for possible lactose-rich wastes such as dairy side streams. Lactic acid is a platform chemical with many useful properties; it can be polymerised into poly-lactic acid (PLA), a bioplastic which is both biobased and biodegradable.[14] Lactic acid is used for food preservation especially in the meat industry and there is a renewed interest in it as a natural antimicrobial.[15] It is also used in cosmetics, flavourings, acidity regulations, drug delivery, and the synthesis of the green solvent ethyl lactate.[16–18]

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The demand for lactic acid in 2022 was approximately 1.5 million metric tonnes, with the demand expected to grow to 2.8 million metric tonnes by 2030.[19] Within this growing area of lactic acid fermentation, batch fermentation is currently the most prevalent. However, various drawbacks such as substrate and product inhibition have led to a focus of research efforts on development of alternate modes of bioprocess such as fed-batch, repeated fermentation, fixed-cell immobilisation, and chemostat continuous culture. We tested multiple bioprocess modes *i.e.* batch, fed-batch and continuous culturing in an attempt to determine the mode that gives rise to the highest LA productivity from lactose.

Methods and materials

Shaken flask fermentations and strain selection

Preliminary experiments into the best LA producer were conducted in flask experiments at small volumes of 100 mL. Glycerol stocks of various LAB strains were collected from the School of Biomolecular and Biomedical Sciences (SBBS) general supply for testing (Table 1). Each strain was screened in biological duplicate in 100 mL duran bottles at 37 °C, 200 rpm for 48 hours. Media used was a simple rich formula dubbed YA media (10 g L^{-1} yeast extract, 0.3 g L^{-1} ammonium phosphate) with 70 g L^{-1} $CaCl_3$ for pH balancing and 60 g L^{-1} lactose as carbon source. Precultures were prepared from a single colony of the selected strain on an MRS-agar plate. Samples for HPLC analysis were harvested at the end of the culture time for analysis of LA productivity by centrifuging ~2 mL of culture at 14 000 rcf for 3 minutes and running the media supernatant on the HPLC (see HPLC method below).

BioLector® I microarray plate mini-fermentations

For testing of toxicity of sodium lactate formation, mini growth experiments were run on a microarray plate in a "BioLector® I" (Beckmann Coulter) with increasing concentrations of sodium lactate. Each well contained cells at 0.05 OD_{600nm}, plus YA media (10 g L^{-1} yeast extract and 0.3 g L^{-1} ammonium phosphate), with added 1 mM magnesium sulphate, 2× Ramsey's trace elements, and 60 g L^{-1} lactose added post autoclaving. Parameters used were set as follows: 10 minutes between measurements, shaking = 400 rpm, temperature = 37 °C, humidity = 80%, used three gains for biomass scatter measurement = 30, 20, and 10. Final gain of 10 was used for all graphical representations. Each condition was conducted in biological duplicate and technical duplicate ($n = 4$), and ANOVA was performed to test statistical significance of the findings.

Table 1 Strains of lactic-acid producing bacteria. Collected for testing from School of Biomolecular and Biomedical Science, University College Dublin

Stock code	Bacterial strain
IMD35	*Lactiplantibacillus plantarum* ATCC 8014
IMD87	*Leuconostoc mesenteroides*
IMD178	*Lactiplantibacillus plantarum*
IMD299	*Streptococcus lactis*
IMD330	*Pediococcus acidilactici* NCIMB 12174
IMD342	*Lactobacillus casei* NCIMB 6375
IMD382	*Lactococcus lactis* ATCC 11454
IMD383	*Lactiplantibacillus plantarum* NCDO1206

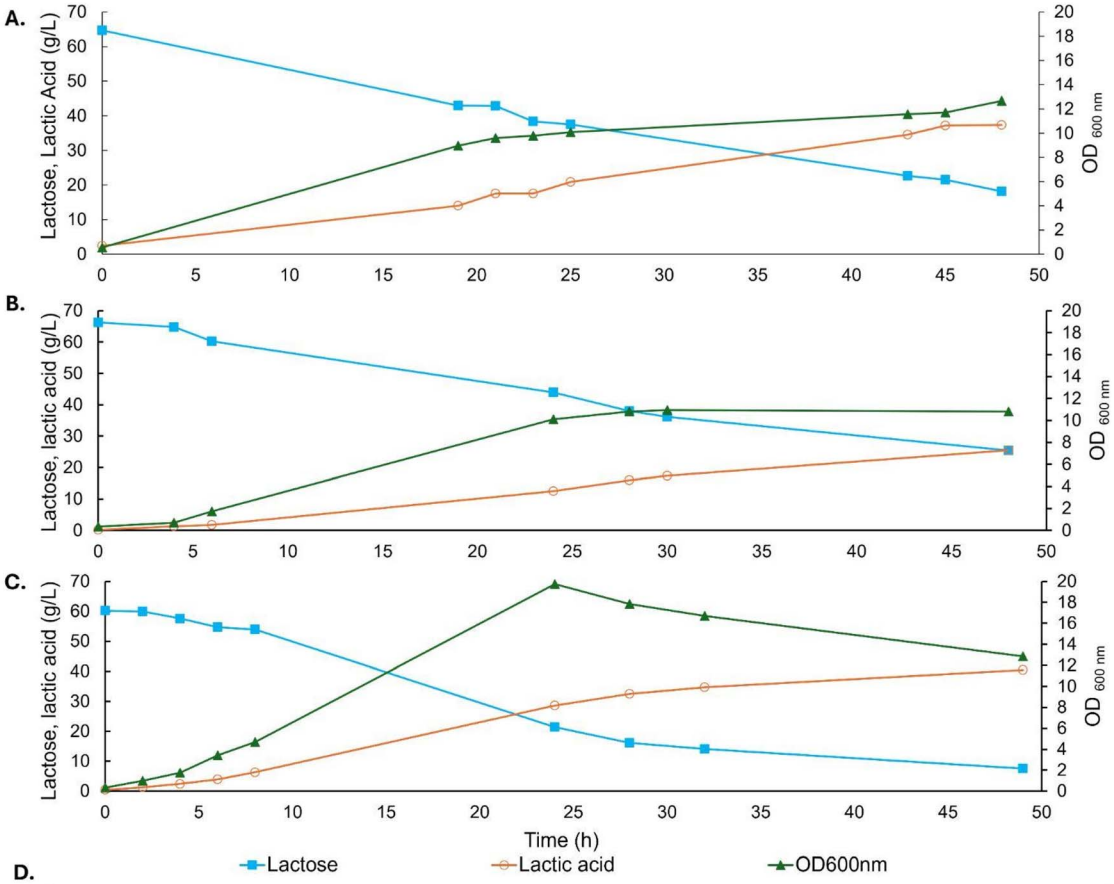
1 L fermentations

Bioreactor precultures were prepared from a single colony of the selected strain on an MRS-agar plate with 1% glucose. Precultures were 200 mL volume, with YA medium (10 g L^{-1} yeast extract, 0.3 g L^{-1} ammonium phosphate, autoclaved at 121 °C for 15 min), 0.5 mM magnesium sulphate (from a 1 M stock solution, autoclaved), and 60 g L^{-1} lactose monohydrate (from a 240 g L^{-1} liquid stock solution, autoclaved). This was then grown in an orbital shaker overnight at 37 °C and 200 rpm. To ensure starting OD_{600nm} was the same across experiments, dilutions were calculated using $C_1V_1 = C_2V_2$ (where C = concentration, V = volume) for a starting OD_{600nm} of 0.5 in the 1 L working volume of the bioreactor, with an inoculum volume of 150 mL (Bionet® Baby-F0 stirred tank reactor). The Baby-F0 with a working volume of 1 L was set up with the following options: airflow = 0.2–10 Lpm (Litre per minute) under dissolved oxygen (DO) control, stirring = 500–1500 rpm under DO control, with DO controlled at 10% (cascade triggered stirring increase first when dropped below 10%, then increased airflow second), pH control = 6.5 (deadband of 0.1) with acid (sulphuric acid 15%) and base (sodium hydroxide 6 M), temperature = 37 °C. Samples were taken periodically for HPLC analysis of lactose and lactic acid concentrations, and OD_{600nm} analysis. In the microaerophilic processes, DO was not controlled, stirring was maintained at a constant of 200 rpm and airflow was shut off. This condition is defined as microaerophilic instead of anaerobic as it does not have nitrogen or carbon dioxide sparging to ensure no oxygen is present.

For batch fermentations, the YA media (10 g L^{-1} yeast extract, 0.3 g L^{-1} ammonium phosphate, except where stated otherwise) was combined with 1 mM magnesium sulphate, 2× concentration of Ramsey's trace elements, and 60 g L^{-1} lactose monohydrate. The incubation was carried out until lactose was exhausted (~48 hours). Fed-batch fermentations contained a modified version of the YA media (15 g L^{-1} yeast extract instead of 10 g L^{-1}, and the same in the precultures for these reactors) due to expected higher cell density after a preliminary test of 10 g L^{-1} yeast extract was found to be not enough (Fig. 3). Fed-batch fermentations were initially supplied with 60 g L^{-1} lactose with a further 60 g L^{-1} lactose added in powder form in a single pulse after 24 hours of culture time. Due to the reduced growth expected in the continuous fermentations, these were carried out in media without Ramsey's trace elements and with half the concentration of magnesium sulphate to prevent nutrient waste in the bleed, and with a yeast extract concentration of 10 g L^{-1}. The continuous cultures also had the following parameters; the bleed rate was set as proportional to the bioreactor weight, with a set point of 1 g, and sensitivity settings of $P = 0.5$, $I = 50$, $D = 0$. The media addition for the continuous feed was applied at a dilution rate of 0.1 h^{-1} based

on the maximal growth rate of the *Lactiplantibacillus plantarum* ATCC 8014 cultures measured at 0.31 (see Results). This was calculated based on a 1 L volume to be the equivalent flow rate of 1.67 mL min^{-1} (0.1 L h^{-1}).

The combination of the clear differences displayed in the results and the reproducibility of bioreactor runs due to the highly controlled environment led to the decision to continue



D.

Lactobacillus casei		
	T25	**T48**
Lactic acid productivity (g/L/h)	0.84	0.78
Lactose consumed (g/L)	24.20	46.51
Yield (g/g lactose)	0.86	0.80
***Lactiplantibacillus plantarum* IMD178**		
	T24	**T48**
Lactic acid productivity (g/L/h)	0.52	0.53
Lactose consumed (g/L)	22.31	40.80
Yield (g/g lactose)	0.56	0.62
***Lactiplantibacillus plantarum* ATCC 8014**		
	T24	**T49**
Lactic acid productivity (g/L/h)	1.19	0.83
Lactose consumed (g/L)	39.56	52.44
Yield (g/g lactose)	0.72	0.77

Fig. 1 Batch fermentations under microaerobic conditions in 1 L bioreactors. OD_{600nm} measurements were taken *via* spectrophotometer while lactose and lactic acid measurements were *via* HPLC. Panel A = *L. casei*, B = *L. plantarum* IMD178, C = *L. plantarum* ATCC 8014, D = table of productivity calculations. Each strain was tested in 1 L bioreactor supplied with 60 g L^{-1} lactose, stirring at 200 rpm and no aeration over 48–49 hours.

with the optimal condition and strain after a single test. Therefore each bioreactor data point is presented once.

DASbox® Quad-reactor fermentations

Fermentations in the DASbox® Quad-reactors from Eppendorf® (maximum working volume 0.25 L) were carried out for the first of the fed-batch experiments. The final volume was 100 mL to account for addition of base during the culture. Preculture and media used was the same as that for the Bionet Baby-F0 reactors, and the parameters were the same, though the airflow was 0.2 Lpm. The pH deadband was also set lower for the smaller volume, at 0.05 due to the smaller tubing for the base addition and therefore slower rate of addition. This fed-batch was then repeated in the larger scale 1 L volume Bionet® Baby-F0 reactor.

High pressure liquid chromatography (HPLC)

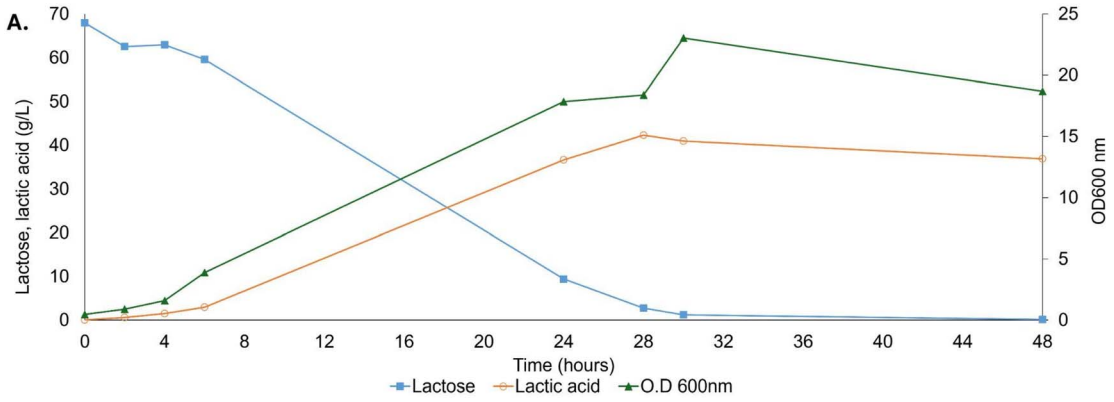
Sugar and organic acid detection was conducted by HPLC on a Shimadzu Prominence unit (SIL-20AC HT autosampler, DGU-20A5 degasser, CTO-20A column oven, RID-10A detector). This unit was fitted with a Bio-Rad Aminex HPX-87H ion exclusion column with an isocratic elution for the mobile phase of 0.014 N sulphuric acid in reverse phase. The method used a flow rate of 0.55 mL min^{-1} for 30 minutes, pressure at 4.41 MPa, and detection *via* refractive index detector (RID). 1 : 10 dilutions of each media supernatant sample were run in Whatman® Mini-UniPrep™ syringeless filter vials. 20 μL of sample was injected onto the column at a time. Standard curves were prepared using a dilution series from 20 g L^{-1} down to 0.3125 g L^{-1} for each metabolite and substrate.

Results

Batch fermentation of top three lactic acid (LA) producers

The three best performing LABs were identified *via* shake flask experiments for the highest lactic acid (LA) titre from 60 g L^{-1} lactose (See supplementary Fig. S1). These were *Lactobacillus casei* NCIMB 6375, and two *Lactiplantibacillus plantarum* species (formally *Lactobacillus plantarum*, and *Lactobacillus arabinoses*), one of which was from an internal University culture collection (*Lactiplantibacillus plantarum* IMD178) and the other the ATCC 8014 substrain (*Lactiplantibacillus plantarum* ATCC 8014) respectively. All three LABs were carried forward into batch bioreactors to investigate their performance at larger scale (Fig. 1). Bioreactor batch fermentations were carried out in a 1 L volume with no airflow *i.e.* micro-aerobically where dissolved oxygen (DO) was below a detectable level, over 48 hours. Of the three strains tested, *L. plantarum* ATCC 8014 demonstrated the highest volumetric productivity at 0.83 g L^{-1} h^{-1} by the end of its culture time (48 h; Fig. 1, panel *D*). The *L. casei* and the *L. plantarum* IMD178 strain were at 0.78 g L^{-1} h^{-1} and 0.53 g L^{-1} h^{-1} respectively. The lactose consumption rate was also fastest in *L. plantarum* ATCC 8014, though the *L. casei* culture demonstrated the highest yield *i.e.* grams of LA per gram of lactose consumed. *L. plantarum* ATCC 8014 achieved the highest biomass (OD_{600nm} of 19.75) in 24 hours (Fig. 1). Coupled with the highest lactose consumption and LA productivity, *L. plantarum* ATCC 8014 was therefore brought forward for further fermentation studies.

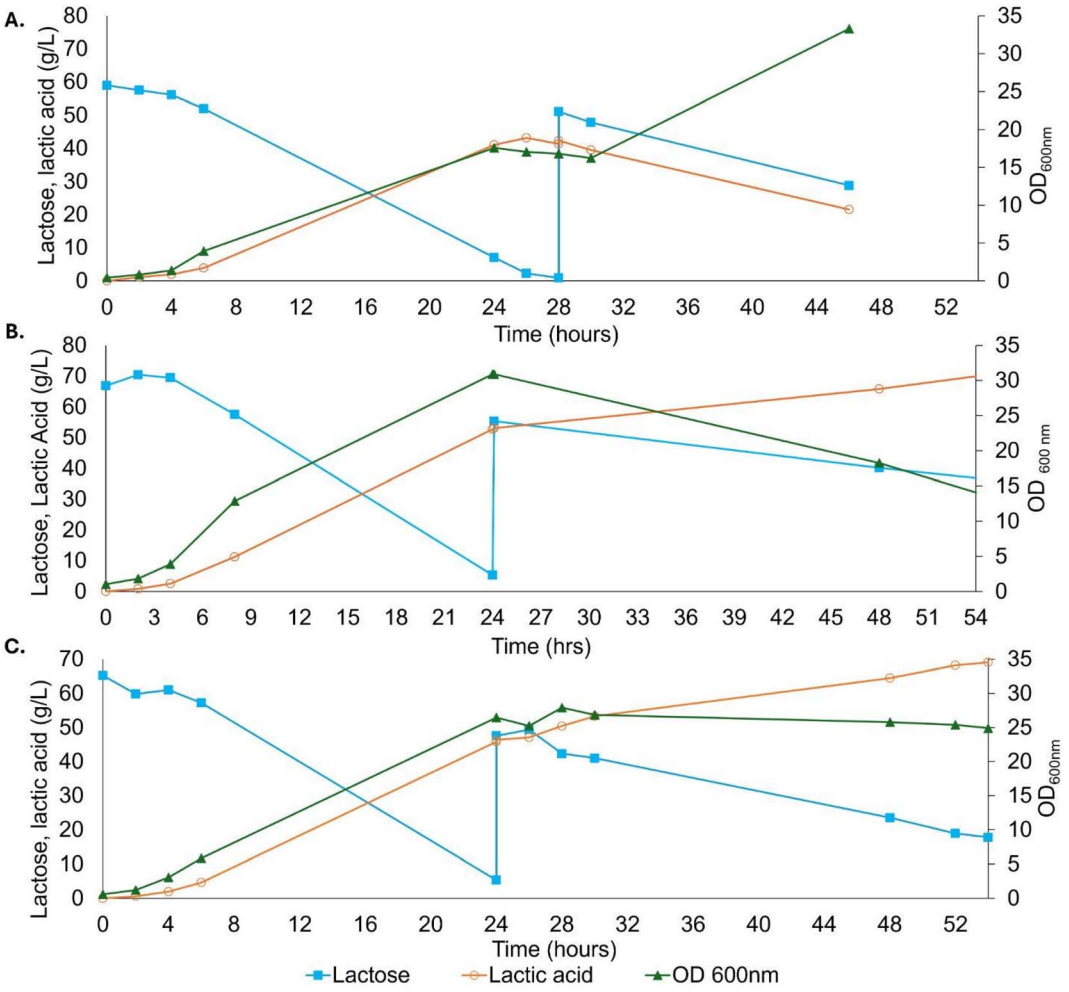
While *L. plantarum* ATCC 8014 is a facultative anaerobe, results of batch fermentation data (Fig. 2) showed that the culture performed better in the presence of oxygen at low levels (10%), with an increased rate of LA production (1.76 g L^{-1} h^{-1})



B.

Lactobacillus plantarum ATCC 8014		
	T24	**T28***
Productivity (g/l/h)	1.76	1.51
Lactose consumed (g/L)	58.52	65.17
Yield (g/g lactose)	0.72	0.65

Fig. 2 *L. plantarum* ATCC 8014 aerobic batch fermentation with lactose (60 g L^{-1}). A. = Growth curve with lactose consumption and lactic acid production. B. Key lactic acid production parameters measured over the growth curve. * = time point at which lactose was approximately exhausted.



D.

Fed-batch A		
	T24	T46
Productivity (g/l/h)	1.71	0.47
Lactose consumed (g/L)	53.02	91.21
Yield (g/g lactose)/ Yield (g/g carbon)	0.78 / 0.54	0.24 / 0.15
Fed-Batch B		
	T24	T55
Productivity (g/L/h)	2.20	1.28
Lactose consumed (g/L)	61.50	85.24
Yield (g/g lactose)/ Yield (g/g carbon)	0.86 / 0.69	0.83 / 0.50
Fed-Batch C		
	T24	T54
Productivity (g/L/h)	1.71	1.28
Lactose consumed (g/L)	56.07	103.25
Yield (g/g lactose)/ Yield (g/g carbon)	0.73 / 0.60	0.67 / 0.49

Fig. 3 Fed-batch fermentations of *L. plantarum* ATCC 8014. Panel A = yeast extract at 10 g L^{-1} in 1 L reactor, B = yeast extract at 15 g L^{-1}, in a 100 mL reactor, C = yeast extract at 15 g L^{-1} in a 1 L reactor, D = table of productivity calculations for all fed-batches. Yield calculations of g g^{-1} lactose were calculated by grams of lactic acid produced divided by the grams of lactose consumed based on HPLC analysis. The yield calculations of g g^{-1} carbon were calculated by grams of carbon contained within lactic acid (40%) divided by the total supplied carbon (carbon contained within lactose (42.10%) and in the yeast extract supplied). Yeast extract carbon composition was taken as 36.94%.[20] As lactic acid concentration reduced after 46 hours in the 10 g L^{-1} test (panel A), the fermentation was halted.

after 24 hours compared to fermentations without air *i.e.* 1.19 g L^{-1} h^{-1} (T_{24}). It also fully consumed lactose after 28 hours in the aerobic cultures compared to 48 hours for the microaerobic cultures. It was therefore decided to test the lactic acid production and growth in aerobic fermentation with a low level of dissolved oxygen (10%) for fed-batch fermentations.

Fed-batch fermentation of *Lactiplantibacillus plantarum* ATCC 8014

The best performing strain, *Lactiplantibacillus plantarum* ATCC 8014, was then tested in an aerobic (10% DO_2) fed-batch mode whereby the culture was initially supplied 60 g L^{-1} lactose and pulse fed another 60 g L^{-1} lactose after 24 hours in a 1 L Bionet Baby-F0 bioreactor. When tested with the same media composition as used in the batch phase (10 g L^{-1} yeast extract, 0.3 g L^{-1} ammonium phosphate, 2× Ramsey's trace elements, 1 mM magnesium sulphate, and 60 g L^{-1} lactose), the fed-batch reduced in LA concentration after 26 hours despite lactose replenishment (Fig. 3, panel A). We suspected this could be due to the depletion of other nutrients and upon repeating the test with 1.5 fold higher yeast extract (15 g L^{-1}) at first 100 mL volume in a DASbox® Quad-reactors from Eppendorf® (Fig. 3, panel B) and then in a 1 L Bionet Baby-F0 bioreactor (Fig. 3, Panel C), ~70 g L^{-1} of LA was successfully produced over 54 hours.

In the subsequent 100 mL (DASbox™) and 1 L (Bionet Baby-F0 bioreactors) fed-batch tests (Fig. 3 B and C), despite the higher yeast extract used, LA production would slow down greatly once over a certain concentration of ~50–60 g L^{-1} (Fig. 3 panel B and C). After the first 24 hours of incubation in a 100 mL reactor, *L. plantarum* ATCC 8014 produced 52.8 g L^{-1} of LA (Fig. 3 B). A pulse feed of 60 g L^{-1} lactose resulted in a further production of only 13.04 g L^{-1} of LA. In the 1 Litre bioreactor, 45.81 g L^{-1} was produced after 24 hours of incubation, and a further 18.63 g L^{-1} LA was produced in the next 24 hours (Fig. 3 C). Despite the reduced LA production rate after 24 hours, these cultures were successful with final LA concentrations of 70.6 g L^{-1} and 69.1 g L^{-1} after 55 and 54 hours respectively. This was equivalent to a productivity of 1.28 g L^{-1} h^{-1} for both fermentations.

Another interesting result was that the yield of LA per gram of lactose was 83% in the 100 mL, yet only 67% in the 1 L fermentation. This difference was due to the higher lactose consumption seen in the 1 L compared to the 100 mL, though the final LA titre was very similar. This higher consumption is reflected in the higher final OD achieved in the 1 L fermentation. Yeast extract is also present in the growth medium (15 g L^{-1}), thus the yield of LA per gram of lactose does not reflect the presence of yeast extract which can act as a carbon source for LA production. Therefore, we also presented the yield of carbon contained in lactic acid per gram of overall carbon present in the medium (from lactose and yeast extract combined). The yield of LA carbon per gram of carbon supplied is 1.23–1.38 fold higher in the first 24 hours (batch) compared to the fed batch phase of the fermentation (Fig. 3).

The reduced rate of LA production seen after 24 hours was attributed to an accumulation of the product and subsequent inhibition of further product formation. This was confirmed by an experiment whereby growth was inhibited relative to the concentration of sodium lactate (the LA product formed in the fermentations) in BioLector® fermentations of 1.5 mL (see SI Fig. S2). The severe growth inhibition by LA concentrations above 30 g L^{-1} suggested this bioprocess may be suitable for continuous fermentation whereby the product would be bled off as it formed and therefore possibly reduce this inhibition.

Continuous fermentation development

A continuous fermentation could allow lower LA concentrations in the medium but still generate high LA productivity (g h^{-1}) compared to batch and fed batch, if the dilution rate could be set at a high enough value. This hypothesis led to the attempt to

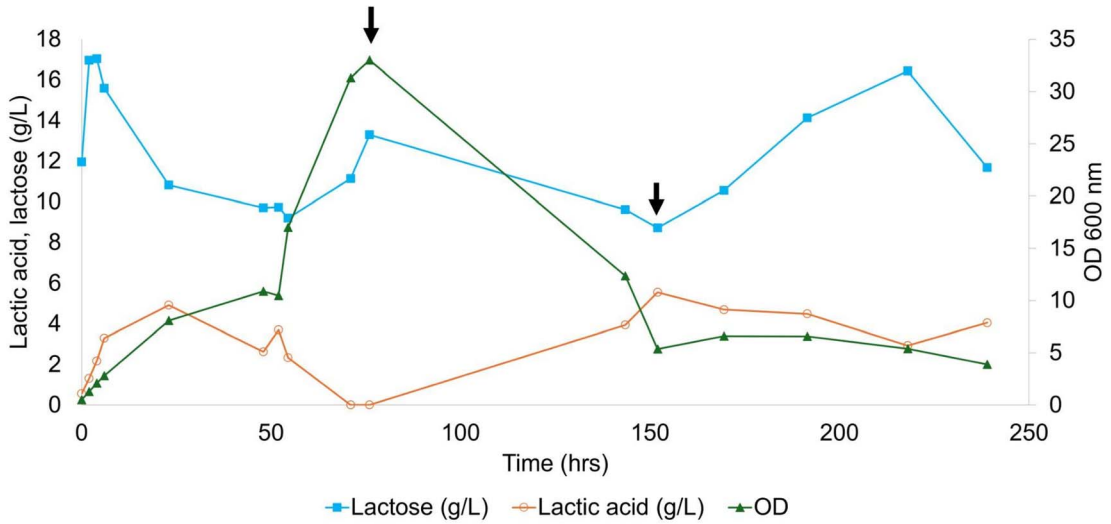


Fig. 4 Continuous fermentation of *L. plantarum* ATCC 8014. Aerobic conditions for growth and lactic acid production were attempted for the first 76 hours after which air supply was turned off (first black arrow, from left to right) due to inhibition of lactic acid production. At 152 hours (second black arrow), lactose was increased from 15 g L^{-1} to 20 g L^{-1}. See Table 2 for productivity values.

develop a continuous process at a dilution rate of 0.1 h^{-1} for *L. plantarum* ATCC 8014 fermentation. The first attempt at this bioprocess used a set point of dissolved oxygen 10%, lactose concentration of 15 g L^{-1}, and yeast extract at 10 g L^{-1}. After approximately 52 hours however, the bacteria appeared to shut off anaerobic fermentation in favour of oxidative respiration leading to a spike in the OD but a corresponding drop in the LA production (Fig. 4). Airflow was shut off completely at 76 hours and this appeared to recover the LA productivity. The LA concentration was maintained at approximately 4–5 g L^{-1}, despite increasing the lactose concentration to 20 g L^{-1}. HPLC analysis revealed that even at 15 g L^{-1}, the lactose was not fully consumed by the bacteria with approximately 10 g L^{-1} still left over at each point.

Further continuous culture experiments were undertaken using a reduced yeast extract concentration to increase resource efficiency *i.e.* supply the yeast extract that is needed and not to supply excess yeast extract. With an approximate OD of 5–6 in the continuous culture experiment described above (the cultures achieved ~20% of the maximum OD obtained in the batch with 10 g L^{-1} yeast extract), then less yeast extract should still support this level of growth. A BioLector® experiment verified that over 3 g L^{-1} of yeast extract appeared sufficient for minimal levels of growth, though 10 g L^{-1} still showed the best growth overall (see SI, Fig. S3).

Further continuous fermentation experiments were undertaken using a reduced yeast extract of 5 g L^{-1}, reduced lactose at 10 g L^{-1}, and with no air from the beginning of the culture, but these attempts resulted in approximately 1 g L^{-1} of LA (Fig. 5). Air was shut off at 95.5 hours (black arrow in Fig. 5A). With the lower resource concentrations used over this continuous culture, there was also a lower level of OD and LA production in general compared to Fig. 4. Fig. 5 panel B then shows a continuous experiment run entirely without air supply. The LA produced never went above 1 g L^{-1} in this continuous mode (except at the beginning when it was still in batch mode prior to being switched to continuous mode). From left to right, the first black arrow indicates where the lactose was increased from 10 g L^{-1} to 20 g L^{-1}, with no visible effect on the LA production which remained at ~0.5 g L^{-1}. The second indicates the lactose being dropped to 5 g L^{-1}, which also had no effect on either OD or LA production. The third arrow indicates the yeast extract being increased from 5 g L^{-1} to 7.5 g L^{-1}, which did cause a minor uptick in the LA production to 1 g L^{-1} demonstrating the close correlation between yeast extract concentration and lactic acid production.

After this lengthy experiment, it was decided that continuous mode for LA production from *L. plantarum* ATCC 8014 was too dependent on oxygen consumption to be suitable for use. Fed-batch mode was determined to be the most productive process. Table 2 shows a productivity comparison of all modes tested in grams per hour, with the highest productivity in the batch (1.51 g L^{-1} h^{-1}) but the overall final titre of lactic acid was best in fed-batch at 69.1 g L^{-1}.

Discussion

Lactic acid (LA) is a valuable molecule, with a range of applications, from food manufacture and skincare, to polymers,

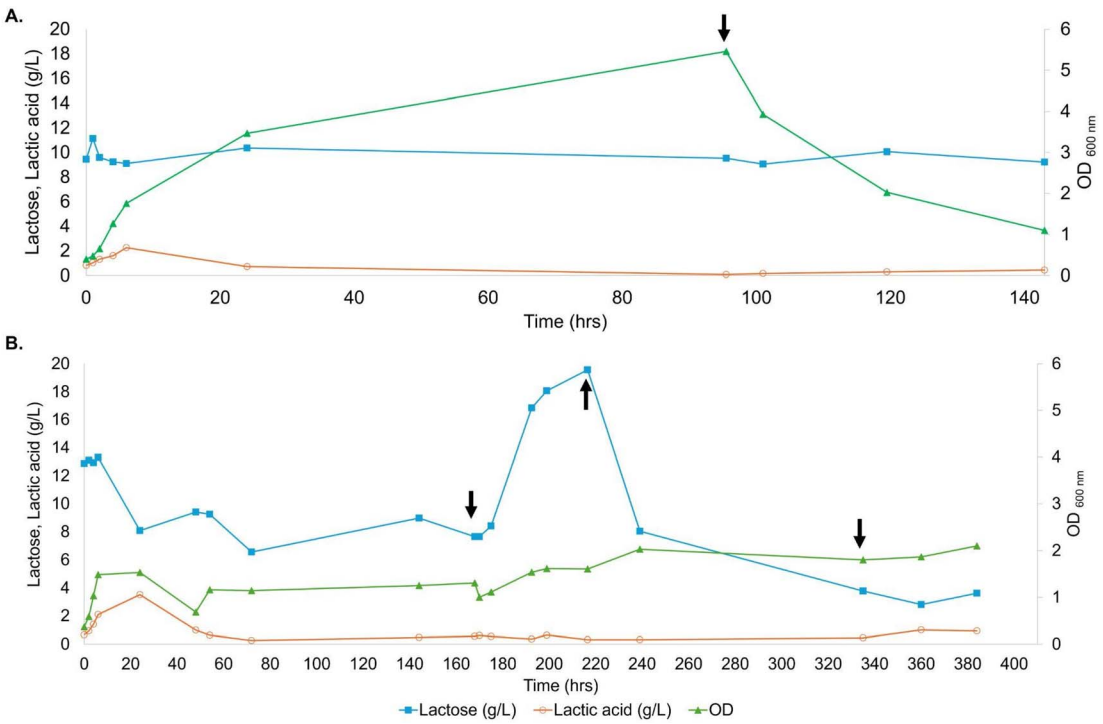


Fig. 5 Continuous fermentation with reduced nutrients. Panel A: aerobic fermentation with airflow set to 0.2 Lpm. Arrow indicates air shut off. Panel B: second attempt at reduced nutrients continuous fermentation with no airflow. From left to right, First arrow = lactose increased to 20 g L^{-1}. Second arrow = lactose decreased to 5 g L^{-1}. Third arrow = yeast extract increased to 7.5 g L^{-1}.

Table 2 Comparison of productivities of the various fermentation modes tested in a 1 L bioreactor. The optimal continuous condition is shown in this comparison, at 15 g L^{-1} lactose, no air, and 10 g L^{-1} yeast extract. Yield calculations of g g^{-1} lactose were calculated by grams of lactic acid produced divided by the grams of lactose consumed based on HPLC analysis. The yield calculations of g g^{-1} carbon were calculated by grams of carbon contained within lactic acid (40%) divided by the total supplied carbon (carbon contained within lactose (42.10%) and in the yeast extract supplied). Yeast extract carbon composition was taken as 36.94% (ref. 20)

Mode	LA titre (g L^{-1})	LA productivity (g L^{-1} h^{-1})	LA yield (g g^{-1} lactose)	LA yield (g g^{-1} carbon)	Monthly LA production[b] (g L^{-1})
Batch	42.32	1.51	0.77	0.55	507.84 g
Fed-batch	69.05	1.28	0.67	0.49	828.60 g
Continuous[a]	5.54	0.55[c]	0.88	0.15	369.60 g

[a] Dilution rate of 0.1 h^{-1}. [b] Assuming three batches and fed-batches per week for four weeks, and 28 days of continuous culture fermentation. [c] Productivity in continuous culture is "g/100 mL h^{-1}" as 100 mL of medium leaves the bioreactor every hour at a dilution rate of 0.1 h^{-1}. At a titre of 5.54 g L^{-1} LA then there are 0.55 g of LA present in the 100 mL of growth medium leaving the bioreactor.

including biobased plastic. The present work focuses on the development of a process to shift the biomanufacturing of this platform molecule from food-based carbohydrates such as corn and starch, to industry side residues-derived lactose such as from whey permeate from dairy side streams, which are produced in massive amounts annually (~200 million tonnes produced in 2023 alone).[21] *Lactiplantibacillus plantarum* ATCC 8014 (also called strain 17–5 and DSM 20205) is a strain of *Lactiplantibacillus plantarum* subsp. *plantarum* which was originally classified as *Lactobacillus arabinosus* (BacDive ID = 6499 (**https://bacdive.dsmz.de/strain/6499**)).[22] This strain is a rod-shaped, homofermentative, exopolysaccharide (EPS), and anti-inflammatory producing member of the lactic acid bacteria (LAB) family.[22,23] At the start of this investigation, the three LAB strains tested performed similarly in batch fermentation in terms of yield, yet the productivity of *L. plantarum* ATCC 8014 after only 24 hours was 1.19 g L^{-1} h^{-1}, 30% higher than the next best performing strain (*L. casei*), and over twofold more efficient than the other strain (*L. plantarum* IMD178) (Fig. 1).

The maximum growth rate achieved by *L. plantarum* ATCC 8014 when grown on lactose was 0.31 h^{-1} (μ_{max}). The μ_{max} of 0.31 h^{-1} is close to the 0.36 value reported by another study utilising lactose as primary carbon source.[24] Other studies using substrates other than glucose show a μ_{max} of anywhere between 0.06–0.64 h^{-1} for various *L. plantarum* strains.[25] The growth rate of 0.31 h^{-1} for *L. plantarum* ATCC 8014 was used as the basis for designing a continuous process in a chemostat with a dilution rate of 0.1 (one third the maximal growth rate) to ensure the culture can maintain its presence in the fermentor when 10% of the culture is being bled off every hour for harvesting of culture media containing lactic acid.

Resource efficiency was highest in fed-batch cultures

Fed-batch fermentation was found to be the most productive bioprocess mode for LA production in terms of final titre with ~70 g L^{-1} and a volumetric productivity of 1.29 g L^{-1} h^{-1} after 54 hours of fermentation. This also surpasses previous results seen in the literature. *i.e. L. plantarum* strains fed with raw corn starch generated 50 g L^{-1} of lactic acid over 72 hours, achieving a productivity of 0.69 g L^{-1} h^{-1}.[26] *L. plantarum* SKL-22 has been shown to utilise rice straw to produce 36.75 g L^{-1} of LA over a 72 hours fermentation, equalling a productivity of 0.51 g L^{-1} h^{-1}.[27] Lactose itself however is not a common substrate with which to investigate LA production, with the majority of studies using glucose or more complex substrates such as corn starch.[11,13] An engineered strain of *Rhizopus oryzae* converted whey to LA (15.6 g L^{-1} over 110 h) with a productivity of 0.142 g L^{-1} h^{-1} in batch fermentation.[28] *Lactobacillus rhamnosus* B103 fed on whey and corn steep liquor, in a fed-batch fermentation achieved a titre of 56.16 g L^{-1} over 48 h and a productivity of 1.17 g L^{-1} h^{-1} (ref. 29) which was lower than the 1.51 g L^{-1} h^{-1} we observed with lactose in a batch fermentation over 28 h (Table 2) and the 1.28 g L^{-1} h^{-1} over 54 h we observed in fed batch (LA titre of 69.05 g L^{-1}).

In the batch fermentation, we observed an LA to lactose carbon yield of 0.55 g g^{-1} while the fed batch fermentation had a carbon yield of 0.49 g g^{-1}, including carbon supplied from both lactose and the yeast extract. Despite the seemingly better productivity and yield in the batch *versus* fed-batch fermentation, the fed-batch in a real world scenario would yield the best resource efficiency. Process mass intensity or PMI, is a measure of the materials efficiency of a product. It equates to the total mass of the input materials (water + raw materials + consumables) divided by the mass of the product.[30] High value products like monoclonal antibodies can have PMI's of around 10 000 kg of materials/kg of product.[31] We calculated a simple PMI for this study's lactose-fed lactic acid production with only the media components from the bioprocesses. The fed-batch in this study was found to have a simplified PMI of 16.218 kg kg^{-1} of LA. By contrast, the batch process had a PMI of 25.592 kg kg^{-1} of LA, most of which is due to the higher titre of the LA in the fed batch process.

High sodium lactate concentration was shown in this study to strongly inhibit cell growth, which also factored into the reduced lactic acid (sodium lactate) production after 24 h in the fed-batch experiments (Fig. 3 and SI Fig. S2), and was the reasoning behind the attempts at developing a continuous process for LA production, whereby the inhibitory product would be bled off as it was produced.

Continuous culture and aerobic metabolism by *L. plantarum*

Continuous culture experiments in this study showed anaerobic conditions must be maintained for lactic acid production to

proceed over more than a few days (Fig. 4). Given that anaerobic growth produces less energy than aerobic growth, the growth rate of the strain was decreased under anaerobic conditions which impacted on the dilution rate at which the cells could be incubated. The dilution rate directly impacts productivity (g h^{-1}) and so faster growing strains incubated under anaerobic growth conditions or microorganisms that can produce larger titres of lactic acid while growing aerobically would be needed for a continuous process to be successful to the same extent as the fed-batch. The maximal productivity we achieved was 0.55 g h^{-1} (Table 2), including a high concentration of carbon source exiting the bioreactor in the spent media which would negatively impact on the PMI also.

Conclusion

L. plantarum ATCC 8014 is a promising strain for the conversion of lactose based substrate to lactic acid with best performance (~70 g L^{-1} LA titre over 54 h and optimal research efficiency) observed under fed batch cultivation. The strain demonstrated critical limitations under continuous culture conditions due to a low growth rate under microaerophilic conditions and lactic acid repression under aerobic growth conditions. Adaptive laboratory evolution or genetic engineering of *Lactiplantibacillus plantarum* ATCC 8014 could be undertaken to overcome these limitations. The resource efficiency was best in the fed-batch bioprocess with a PMI of 16.218 kg kg^{-1} of lactic acid. While lactose is a side stream of the dairy processing industry the next step would be to try a cheaper, less pure, lactose source such as whey. This would promote even greater resource efficiency in the circular bioeconomy compared to using virgin starting materials such as glucose or sucrose for lactic acid production. Valorising waste-produced sugars such as lactose may even reduce costs as well, as fermentation substrates are known to contribute between 40 and 70% of the process costs of lactic acid production.[12]

Author contributions

KOC designed the idea. CL and SL conducted experiments. KOC, CL and SL devised experimental plans. CL, SL, TN and KOC wrote and edited the manuscript.

Conflicts of interest

The authors declare no conflict of interest.

Data availability

The data supporting this work are available in the supplementary information (SI). Supplementary information is available. See DOI: https://doi.org/10.1039/d5su00383k.

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