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Unlocking nature's sweet secret for citric acid production from wood sugars: Evaluation of microbial strains and environmental impacts

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ABSTRACT

This study investigates a novel approach by utilizing wood-derived sugars as a substrate for microbial citric acid (CA) production. Additionally, it examines the environmental implications through an ex-ante life cycle assessment (LCA). Various microbial strains including six yeast strains from the *Yarrowia* genus, three filamentous fungi strains from the *Aspergillus* genus, and eight bacterial strains were evaluated for their potential in CA production. The investigation primarily focused on optimizing citric acid production by considering various factors such as substrate concentrations, nitrogen levels, alcohols, surfactants, and the potential of non-commercial strains. The study yielded promising results, with the highest CA yields recorded as follows: 5.7 g/L from bacterial fermentation, 36.8 g/L from yeast fermentation, and 67.7 g/L from fungi fermentation. Furthermore, by applying an ex-ante LCA, the study assessed the environmental impact of citric acid production. Results indicated a significant reduction in environmental footprint when scaled up, with emissions decreasing from 235 to 585.8 kgCO2eq/kg at the laboratory scale to 4–6 kgCO2eq/kg at the larger scale.

1. Introduction

Citric acid (2-Hydroxypropane-1,2,3-tricarboxylic acid; *abbr.* CA) is a primary metabolic product formed in the tricarboxylic acid (Krebs) cycle. As such, it can be produced in varying amounts by almost all aerobic organisms. CA is widely used in industry as one of the most prominent organic acids, with an approximate production of 2.8 million tonnes worldwide as of 2022 (IMARC, Report ID: SR112023A642). The application of CA encompasses various industries, including food and beverage, pharmaceuticals, cosmetics, and chemistry (Dong et al., 2023). Additionally, CA and its derivatives have been investigated for potential use as components for biodegradable packaging, disinfectants, extracting and preserving agents, as well as for environmental remediation (Seligra et al., 2016) and fire retardants (Dong et al., 2023).

CA production involves microbial fermentation; however, an accumulation of the compound is significantly shaped by the microorganism and the growth media composition. The type and concentration of the carbon source should be considered as crucial factors (Xu et al., 1989). Conventionally, industrial production of CA takes place through the use of filamentous white rot fungus *Aspergillus niger,* with glucose being employed as primary carbon source. To decrease the production cost and alleviate land competition with food production, alternative carbon sources are being explored. These sources include carbohydrates from a range of food industry by-products, such as sugar beet and sugar cane molasses, date syrup, and corn starch (Mores et al., 2021). In addition to *A. niger,* yeast *Yarrowia lipolytica* has been extensively studied as a viable alternative with comparable productivity thanks to its easier cultivation, higher fermentation rates and superior tolerance to culture conditions. Also, *Y. lipolytica* has exposed tolerance to heavy metals, such as copper (Ito et al., 2007) and possesses enzymes that enable successful utilization of hydrophobic substrates like fatty acids (Wang et al., 1999). This organism can utilize both the crude substrates and waste materials for growth. The initial substrates examined for *Y. lipolytica* CA fermentation were *n*-alkanes; however, these substrates were discontinued due to

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increasing petroleum prices and issues with solubility in water (Berovic and Legisa, 2007). Generally, glucose, sunflower oil or pure/raw glycerol are used (Cavallo et al., 2017). Currently, no bacterial strains have been used for large-scale industrial CA bioproduction. There are numerous patents related to bacteria usage for CA production (Anastassiadis et al., 2008). For instance, Sardinas (1973) studied the use of *Bacillus licheniformis* bacterium and cerelose as a carbon source. The bacterium yielded 15.5 g/L of CA during a 38-h fermentation process and 42.0 g/L in five days. Another patent (Tanaka et al., 1972) describes the fermentation process using bacterium *Rhodococcus erythropolis* (*Arthrobacter paraffineus*) and n-dodecane or polyoxyethyleneglycol-monostearate as a carbon source. The reported concentrations of CA in these fermentations range between 9.5 and 28.0 g/L in a span of 72–96 h. *Brevibacterium flavum* (with glucose), *Rhodococcus fascians* (*Corynebacterium fascians*) (with n-paraffin) and *R. erythropolis* (with n-hexadecane) were likewise reported by Suzuki et al. (1974). Fermentation lengths ranged from 3 to 6 days, while final CA concentrations ranged from 28.0 to 61.0 g/L. Nonetheless, limited information exists regarding the crucial composition of the fermentation media, particularly with regard to the use of waste material-derived carbohydrates. In recent years, various research has highlighted the potential of using lignocellulosic waste from agriculture as a substrate for the production of CA. Although fruit syrups and molasses are commonly used in microbial CA fermentation, they still have demand in food and animal feed industries. Therefore, using inedible parts of plants present a more favourable approach as it does not directly compete with food production. Potential use of pre-treated straw (Liu et al., 2015) or corn waste as carbon sources (Zhou et al., 2017) have been reported in recent studies that have employed either solid-state or submerged fermentation methods. The capacity of CA-producing microorganisms to utilize xylan, xylan hydrolysate, and sugars present in hemicellulose hydrolysate has been explored (Kirimura et al., 1999; Maddox et al., 1985). Maddox et al. (1985) discovered that in addition to glucose, *Y. lipolytica* can metabolise mannose and generate 9.0 g/L of CA within eight days of shake flask cultivation. They also discovered that *A. niger* can ferment mannose, xylose and arabinose, and yield 17.0, 22.0 and 6.0 g/L of CA respectively after fourteen days of shake flask cultivation. Kirimura et al. (1999) reported maximum CA yields of 72.4 g/L from xylose and 52.6 g/L from arabinose in a five-day culture. Additionally, yields of 51.6 g/L from xylan hydrolysate and 39.6 g/L from xylan were achieved in a three-day semi-solid culture. The potential of woody biomass hydrolysate as a substrate for CA production is highlighted by these findings.

Lignocellulosic biomass from forestry industry residues is an emerging feedstock that has primarily been used for biofuel production, specifically biogas and bioethanol. Several studies have proposed the use of wood-based feedstock for manufacturing bio-based materials, including polyhydroxyalkanoates (Koller et al., 2015), yeast proteins (Sharma et al., 2018) and sophorolipids (Ingham et al., 2023). However, these bio-based materials are emerging technologies with low technology readiness levels (TRL), challenging a robust environmental assessment and benchmark against their fossil counterparts. Ex-ante life cycle assessment (LCA) can be applied to assess the environmental impacts of chemicals and materials from wood-based feedstock. This is an approach that overcomes the limitations of conventional LCA studies by scaling-up low TRL technologies to industrial scale, applying process simulations, process-based calculations, scenarios, learning curves, and scaling factors (Cucurachi et al., 2018). This study marks the first examination of the feasibility and environmental impact of using wood-derived sugars as a novel substrate for microbial CA production. In addition to conventional fungal and yeast strains, the study also explores the use of bacterial strains. The environmental impacts are evaluated using an ex-ante LCA, and a methodology to scale-up the emerging bio-based technologies is applied.

2. Materials and methods

2.1. Materials

Lignocellulosic sugar substrate from wood hydrolysate was kindly gifted by the company Fibenol (Estonia). It is produced via an enzymatic hydrolysis of hardwood and the resulting C6 wood sugar syrup composition provided by the manufacturer consists of glucose 550 g/L, xylose 60 g/L, other sugars 8 g/L, formic acid 3 g/L, acetic acid 10 g/L, HMF (hydroxymethylfurfural)1 g/L, Cl^- 0.18 g/L, SO_4^- 3.9 g/L, Na^+ 7.8 g/L, K^+ 0.2 g/L, Mg^{2+} 0.1 g/L, Ca^{2+} 0.3 g/L. Luria-Bertani Broth, Miller (Fisher Scientific), bacteriological grade agar (Acros Organics) and nutrient broth (Oxoid), Czapek dox agar were used to culture microorganisms. Bromocresol purple (Sigma-Aldrich) was used as an indicator. The media were supplemented with GMO-free soya peptone (Oxoid), casamino acids (Amresco) and KH_2PO_4, potassium phosphate monobasic (Merck) accordingly. Ammonium chloride, iron (II) sulphate heptahydrate (Mallinckrodt) and extra pure magnesium sulphate-7-hydrate (Riedel-de Haën) were also added. Yeast extract, granulated (Merck), potassium hydroxide (Chempol); trypticase soy broth (BD); ammonium sulphate (Acros Organics), zinc sulphate heptahydrate (Sigma-Aldrich), manganese sulphate pentahydrate (Sigma-Aldrich), $FeCl_2{\cdot}4H_2O$ known as iron (II) chloride tetrahydrate, with a purity of 98% from Alfa Aesar were also employed. The granules of $CaCl_2$ with purity of 96% from Acros Organics, sodium phosphate dibasic (Merck), Triton X-100 (Sigma-Aldrich), copper (II) sulphate pentahydrate (Merck), methanol, ≥99.9% (Merck) and absolute ethanol of ≥99.8% (Riedel-de Haën) were also used as reagents. Polyoxyethylene sorbitan monooleate, commonly known as Tween 80 (Sigma-Aldrich), was used alongside Lucrafoam® as antifoam agent. The glucose was used anhydrous D (+) of analytical grade (SERVA), while glucose monohydrate D (+) was in the form of crystalline powder (Fisher Scientific). The neocuproine hydrochloride monohydrate used was of 99% purity (Acros Organics). CaO, calcium oxide from Sigma-Aldrich; sulfuric acid, 96%, extra pure, solution in water from Acros Organics, toluene (from Sigma Aldrich), phosphorus pentoxide, 99+%, for analysis from Acros Organics, copper sulphate from Merck, and glycine, which was in the form of white crystals or crystalline powder from Fisher Scientific.

2.2. Microorganisms

Bacterial strains *Pseudomonas fluorescens* DSMZ 50090 and *Bacillus licheniformis* DSMZ 8785, along with yeast strains *Yarrowia lipolytica* DSM 1345 and *Y. lipolytica* DSM 3286 as well as filamentous fungi strains *Aspergillus niger* DSM 821 and *A. niger* DSM 823 were purchased from DSMZ – German Collection of Microorganisms and Cell Cultures GmbH (Braunschweig, Germany). All purchased strains were obtained for scientific purposes. Wild-type bacterial strains including *Bacillus cereus*, *Bacillus pumilus*, *Streptomyces* sp., *Streptomyces thermocarboxydus*, *Microbacterium oxydans*, *Acinetobacter calcoaceticus* and yeast strains *Y. lipolytica* 71/80, *Y. lipolytica* 72/63, *Y. lipolytica* 73/64, *Y. lipolytica* 74/73 were acquired from the collection of Sector of Applied Biocatalysis at Vilnius University (Lithuania).

2.3. Agar plate cultivation

Organisms were cultured on agar plates according to the following protocol.

- *P. fluorescens* DSMZ 50090 and B. *licheniformis* DSMZ 8785 on LB agar at 30 °C;
- *B. cereus*, *B. pumilus*, *S. thermocarboxydus* on nutrient agar plates at 37 °C;
- *Streptomyces* sp., *M. oxydans*, *A. calcoaceticus* on nutrient agar plates at 37 °C;
- All *Y. lipolytica* strains on YEPD agar plates at 30 °C;

- All *A. niger* strains on Czapek Dox agar plates at 30 °C.

Organic acid production in bacteria was achieved by cultivating microorganisms at the appropriate temperatures on agar plates supplied with media containing 0.02 g/L of bromocresol purple to indicate pH levels. The microorganisms were cultured for 48 h, followed by observation of yellow zones around the colonies, indicating secretion of organic acid.

2.4. Shake flask cultivation

For the bacterial fermentation, 250 mL volume flasks were filled with 50 mL of the media. The media comprised of sugar substrate (100 g/L), casamino acids (0–5 g/L), KH_2PO_4 (4 g/L), NH_4Cl (0.5–8 g/L), $FeSO_4{\cdot}4H_2O$ (0.4 g/L), $MgSO_4{\cdot}7H_2O$ (1 g/L), and yeast extract (2 g/L). The pH was adjusted to 7.0 initially with 1 M KOH. Overnight cultures were grown in NB, LB, or tryptic soy broth, harvested through centrifugation, washed, and inoculated. The fermentation was carried out for 144 h with shake flasks, at 30 °C and 220 rpm. Media samples were collected every 24 h to test the change in pH, reducing sugar and CA concentrations.

For yeast *Y. lipolytica* fermentation, 100 mL of medium was prepared in 500 mL flasks. The medium consisted of the following components per litre (L): sugar substrate (100 g), $(NH_4)_2SO_4$ (0–1 g), KH_2PO_4 (7 g), $ZnSO_4{\cdot}7H_2O$ (0.04 g), $MnSO_4{\cdot}5H_2O$ (0.1 g), $FeCl_2{\cdot}4H_2O$ (0.24 g), $MgSO_4{\cdot}7H_2O$ (3.1 g), $CaCl_2$ (0.15 g), yeast extract (0.5 g), Na_2HPO_4 (2.5 g); and Triton X-100 (0–1.0% w/v). The initial pH was adjusted to 6.0 using 1 M NaOH. Overnight cultures were grown in YEPD broth at 30 °C and 220 rpm. The cultures were then harvested and washed by centrifugation, before being inoculated. Shake flask fermentation was conducted for a duration of 144 h under the same conditions with media samples collected every 24 h to analyse changes in pH, reducing sugar and CA concentrations.

For the fermentation of fungi *A. niger*, 250 mL flasks containing 50 mL of medium were prepared. The medium consisted of the following components per litre (L): sugar substrate (100 g), $(NH_4)_2SO_4$ (2 g), $MgSO_4{\cdot}7H_2O$ (0.5 g), KH_2PO_4 (2 g), $FeCl_2{\cdot}4H_2O$ (0.48 mg), $ZnSO_4{\cdot}7H_2O$ (0.48 mg), $CuSO_4{\cdot}5H_2O$ (0.24 mg); and methanol/ethanol (0–3%). The spores were collected from Czapek Dox agar plates, thereafter washed with a sterile saline solution containing 0.05% Tween 80 and then inoculated into the shake flasks ($\sim 6{\cdot}10^5$ spores per millilitre in the fermentation medium). The shake flask fermentation conducted over 120 h at 30 °C and 200 rpm. Samples of the media were obtained every 24 h to assess the alterations in pH, reducing sugars, and CA concentrations.

2.5. Cultivation in the bioreactor

Y. lipolytica DSM 3286 strain was selected for the seed culture. The colonies from YEPD agar plates were transferred to a 500 mL flask that contained 100 mL of media. The culture was incubated at 30 °C and 220 rpm for 24 h. The seed culture (10% (v/v)) was then inoculated into a 2.5 L bioreactor with the working volume of 1 L that was equipped with a Rushton impeller. Batch fermentation took place for 96 h at 30 °C with an aeration rate of 0.2 vvm (50 ± 2% pO_2) and agitation between 200 and 1400 rpm. A Lucrafoam® antifoam agent was used and NaOH was added to maintain a pH value of 6.0 ± 0.5. The seed culture and fermentation media were identical to those used in shake flask fermentation, with a concentration of $(NH_4)_2SO_4$ at 1 mg/L and 1% (w/v) Triton X-100.

2.6. Assay techniques

The concentration of reducing sugars in the medium was determined using the Neocuproine method, as described by Dygert et al. (1965). To determine the reducing sugar concentration in the sample, a calibration curve was created using a set of glucose standards with a concentration range of 0.1–0.6 mM.

The determination of CA and ICA was carried out spectrophotometrically using enzymatic test kits from Megazyme, following the manufacturer's protocol. CA was determined by measuring the NADH consumed in the conversion of CA to oxaloacetate and its decarboxylation product pyruvate. The reactions were catalysed by citrate lyase, L-malate dehydrogenase and D-lactate dehydrogenase. ICA determination, on the other hand, relied on the measurement of NADPH produced during the conversion of ICA to 2-oxoglutarate, which was catalysed by isocitrate dehydrogenase.

2.7. Isolation of citric acid

The method for isolating citric acid through precipitation was adapted from Behera et al. (2021) and Sawant et al. (2018). Firstly, small particles were filtered from the fermentation broth using a 0.22 μm pore size PVDF membrane (Merck Millipore Ltd.). Then, a portion of the soluble proteins was denatured and removed by incubating the filtrate at 70 °C for 15 min, followed by centrifugation (10000×*g*, 10 min, 20 °C) to remove them. The solution was reduced from 640 mL to 60 mL via evaporation under reduced pressure for 3 h at 45 °C temperature and pressure of ~0.15 atm. A $Ca(OH)_2$ suspension, otherwise known as milk of lime, was formed by adding 0.644 g of CaO to 0.5 l of dH_2O. Calcium citrate tetrahydrate ($Ca_3(C_6H_5O_7)_2{\cdot}4H_2O$) was then precipitated from the concentrated broth at a temperature of 90 °C by gradually introducing the milk of lime until the pH hit 7.0. The solid material was collected by filtration through a 100 μm fritted glass filter, washed with 100 ml of heated deionized water to eliminate soluble impurities, and left to dry in the open air for 48 h. The dried solid (2.884 g) was suspended in 10 mL of dH_2O and treated with 70% H_2SO_4 dropwise until the suspension reached a pH of 2.0. Calcium sulphate ($CaSO_4$) was separated from the solution by filtration using a 100 μm fritted glass filter and subsequently washed with 2 mL of dH_2O. Residual impurities were eliminated by passing 36 mL of the citric acid solution through a column of activated carbon (20 g). Subsequently, citric acid was precipitated by reducing the solution volume from 35 mL to 5 mL at a reduced pressure of 0.15 atm and a temperature of 40 °C via 4 h of evaporation. In order to obtain citric acid monohydrate crystals (0.35 g), a 40 μm fritted glass filter was used for filtration, followed by washing with 5 mL of toluene and drying in a P_2O_5 exicator for 24 h. The process produced a yield of 2.8%. ATR-FTIR confirmed the following peaks: 3330, 3203, 2969, 2325, 1646, 1231, 1073, 992, 952, 860, 667, 567, 485 cm^{-1}.

2.8. Ex-ante life cycle assessment (LCA)

The environmental impacts of the technology are assessed by implementing the so-called ex-ante LCA (Cucurachi et al., 2018). We scale-up process data from lab to industrial scale to identify potential improvements prior to large-scale implementation, this happens by means of process-based calculations, estimation of energy consumption, and screening of processes synergies such as material, energy, and waste recovery. The ex-ante LCA of CA produced from wood sugars was performed applying the Phase I and Phase II of the approach developed by Souza et al. (2023) (Fig. 1a). We have identified an acrylic acid and formaldehyde, as a well-established fossil-based building blocks, which could potentially be replaced by CA in, e.g., super absorbent polymers or adhesives (Li et al., 2023). Further, the environmental performances of CA are benchmarked against conventional CA production from food crops (namely, corn and sugar beet) which involved fermentation using *Aspergillus niger*. The functional unit was defined as 1 kg of CA. The system boundaries encompassed a biorefinery that produces CA from wood sugars in a cradle-to-gate approach (Fig. 1b). The wood sugars are mainly hexoses produced by Fibenol, and they carry impacts from the collection, handling, storage, and transportation of wood residues to the

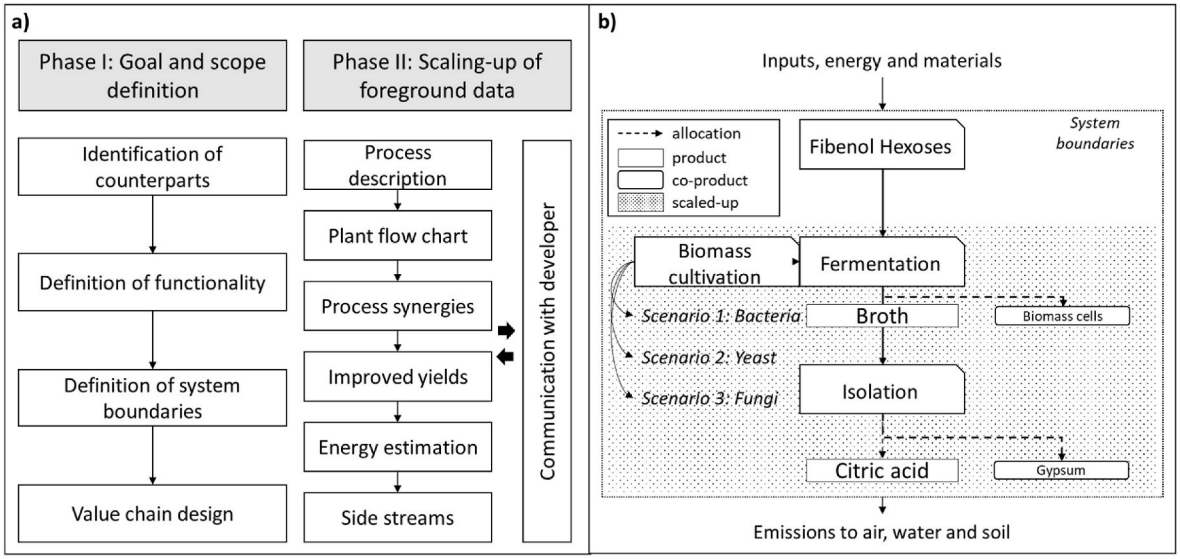

Fig. 1. Schematic diagrams are presented for the ex-ante LCA (a) and the biorefinery system that produces CA from wood residues (b).

biorefinery, pretreatment of wood residues, in addition to cellulose conversion to hexoses. The sugar fermentation to critic acid and CA isolation are based on lab-scale data provided in the previous sections. The assessed environmental impacts are marine eutrophication, freshwater eutrophication, ozone layer depletion, and photochemical oxidant formation, using Recipe 2016 Midpoint (H) (Huijbregts et al., 2017). The impacts of climate change consider a 100-year global warming potential (IPCC, 2013).

We examined three scenarios that varied the microorganism used to produce CA, based on the best performance at the lab experiments: Scenario 1 utilized bacteria, Scenario 2 yeast, and Scenario 3 fungi. *Y. lipolytica* DSM 3286 (Scenario 2) and *A. niger* DSM 823 (Scenario 3) were chosen as the most productive strains, because they produced the highest CA concentrations in shake flasks.

The foreground inventory was compiled by including required inputs within the system boundaries. Fibenol produced the wood sugars, which carry environmental impacts from hexose production (refer to Table S1). The wood residues were treated with dilute acid and further fractionated into hexoses, pentoses, and lignin, applying a mass allocation, as detailed in Fibenol's Environmental Production Data (2023). For the production of CA from wood sugars, the scaling-up of foreground data starts with a process description and a plant flow chart (Fig. S1), identifying all necessary equipment and machinery for industrial scale operations. To attain viable cell yield, heated reactors under stirring and centrifugation are required for microorganism inoculation and yeast, bacteria, and fungal fermentation. The isolation of CA involves centrifugation, filtration, heated reactors, and evaporators. The identified process synergies include the reuse of sulfuric acid and activated carbon during the isolation of CA, that can be reused with a 97% and 50% recovery yield, respectively. The inputs for bacteria, yeast and fungi growth and fermentation are entirely consumed as nutrients, and no material recovery is possible. Despite low overall yield rates in CA production, improvements are being sought. For scenarios 1 and 2, it is assumed that they are double the lab yields, with a range of 8%–16% and 25%–50%, respectively. Lab-scale yields for scenario 3 are already at a high level of 92% (i.e., 67.7 g/L) and no assumptions were made. The overall yield of CA isolation reached only 2.8%. However, at higher levels of maturity, up to 60% overall yield can be achieved (Wang et al., 2020), which is considered as an improvement. Energy requirements for the recovery of chemicals, as well as for heating, stirring, filtration, centrifugation and process integration through pumping, were calculated using equations S1 and S2, and Tables S2–S4 (see Supplementary material), according to Piccinno et al. (2016). All three scenarios resulted in leftover of microorganisms' cells, residue of the fermentation process, and their nutritional value as animal feed has been considered (Amara and El-Baky, 2023). Consequently, they can be commercialized and treated as co-products of CA production. Gypsum is a co-product in the isolation of CA, owing to its utility in the construction industry (Mores et al., 2021). Allocation of environmental impacts was based on mass values among the main product and the co-products, as outlined in Table S5. No fugitive emissions or wastes were detected in any of the three scenarios considered. Table S6 presents a summary of all the scaling-up methods.

Electricity was assumed to be supplied by the European average grid and the necessary heat was generated through the combustion of wood residues. The background data were obtained from the Ecoinvent 3.8 database cut-off version (Moreno-Ruiz et al., 2021). The complete inventory for the three scenarios is provided in Table S7.

3. Results and discussion

Since the discovery of microbial production of CA, numerous attempts have been made to improve fermentation efficiency. It has been established that both the concentration and the type of substrate are pivotal factors. High substrate concentrations promote the accumulation of CA in both filamentous fungi (Xu et al., 1989) and yeasts (Papanikolaou et al., 2002), as they perturb the tricarboxylic acid cycle (TCA). Contrary, nitrogen should be maintained at low and limiting concentration to ensure optimal CA synthesis in both *Y. lipolytica* and *A. niger* fermentations. Several publications highlight the significance of a high C/N ratio for this purpose (Gupta et al., 1976; Papanikolaou et al., 2002). The type of nitrogen used also plays a crucial role, with ammonium salts proving to be the most suitable and cost-effective option (Mattey, 1992). Optimizing CA production requires consideration of several parameters, including pH, media additives, and accumulation of by-products. Due to variations in cell morphology, metabolism and growth conditions among different microorganisms, optimization steps are not universal. Therefore, this study screened multiple microorganisms for their ability to convert wood sugar substrates to CA as there are no data on fermentation of such sugars to CA. Classical strains of microorganisms known to produce CA, including *A. niger* and *Y. lipolytica,* were investigated alongside several novel bacterial strains. All microorganisms were cultured in liquid shake flasks, with monitoring of pH, sugar, and CA concentration. Cultivation conditions were optimized for the best selected strains within each microorganism group (bacteria, yeasts, filamentous fungi) to achieve sufficient CA yields. One of the

yeast strains was fermented in a 2.5 L bioreactor (with the working volume of 1 L) in order to obtain ample amount of CA for isolation experiments.

3.1. CA production by bacteria

Thirty-four bacterial strains were cultured on agar plates containing bromocresol purple, serving as a pH indicator. After 48 h of incubation, eight strains exhibited yellow zones around their cells, indicating a pH below 5.2, suggesting organic acid accumulation, potentially citric acid (CA) (Fig. 2). These strains included *M. oxydans*, *B. cereus*, *B. pumillus*, *A. calcoaceticus*, two strains of *Streptomyces* sp., *Pseudomonas fluorescens* DSM 50090, and *Bacillus licheniformis* DSM 8785. These eight strains, showing organic acid production, were selected for shake flask cultivation.

During the shake flask experiments conducted over six days, *B. licheniformis* showed the highest CA production at 3.0 g/L, consuming 69.2% of the available reducing sugars (Table 1). Conversely, *Streptomyces* sp., *S. thermocarboxydus*, and *M. oxydans* exhibited minimal CA production (<1.0 g/L) and low wood sugar consumption. Although *B. cereus* exhibited low wood sugar utilization (21.8%), it produced 2.7 g/L of CA, potentially due to its preference for proteins and amino acids over polysaccharides. (Ivanova et al., 2003). There was no consistent correlation between CA accumulation and media acidification. While *B. licheniformis* fermentation led to only a 0.5 pH decrease, *A. calcoaceticus* fermentation resulted in a pH drop from 7.0 to 4.0 despite producing 2.1 g/L of CA. Even *P. fluorescens*, with 1.3 g/L CA, showed a slight increase in pH.

Bacillus licheniformis DSMZ 8785 was identified as a promising CA producer from wood sugars and used for further optimization which involved exploring nitrogen sources, namely ammonium chloride and casamino acids. Using casamino acids alone led to a lower CA concentration (0.4 g/L) compared to ammonium chloride (Table 2). In fact, 8 g/L of ammonium chloride resulted in the highest CA concentration (5.7 g/L), indicating a direct correlation between ammonium salt concentration and CA accumulation. However, casamino acids inhibited CA production, rendering them unsuitable as a nitrogen source. This study sheds light on *B. licheniformis* potential for CA production, typically known for polyglutamic acid production (Xavier et al., 2020). The CA concentration obtained from *B. licheniformis* fermentation, following optimization, was 5.7 g/L and accumulated wet cell biomass was 2.0 g. Despite its yield not matching conventional CA-producing organisms like *A. niger* or *Y. lipolytica*, the results indicated the potential of bacterial strains for CA production.

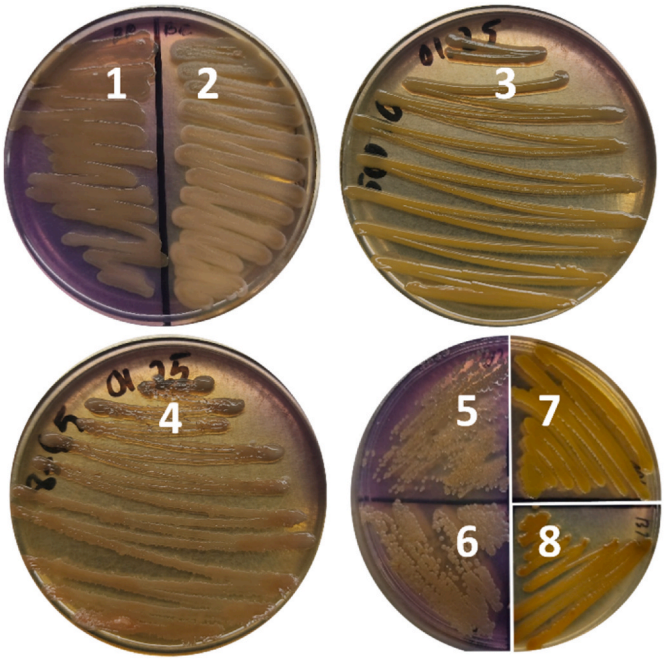

Fig. 2. Citric acid production by bacteria. Qualitative screening of bacterial strains for organic acid production on media plates supplied with bromocresol purple as a pH indicator. The bacterial strains have been numbered as follows: 1 - *Bacillus pumilus,* 2 - *Bacillus cereus,* 3 - *Pseudomonas fluorescens,* 4 - *Bacillus licheniformis,* 5 - *Streptomyces* sp., 6 - *Streptomyces thermocarboxydus,* 7 - *Microbacterium oxydans,* and 8 - *Acinetobacter calcoaceticus*. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 1
The comparison of CA production, wood sugar consumption and media pH change of various bacterial strains after six days of cultivation.

Strain	CA conc. G/L	Sugar consumption, %	Final pH
Bacillus cereus	2.7	21.8	5.6
Bacillus pumilus	2.2	41.3	6.2
Streptomyces sp.	0.2	5.0	5.7
Streptomyces thermocarboxydus	0.9	25.8	5.1
Microbacterium oxydans	0.2	9.9	5.8
Acinetobacter calcoaceticus	2.1	47.7	4.0
Pseudomonas fluorescens DSMZ 50090	1.3	47.7	7.1
Bacillus licheniformis DSMZ 8785	3.0	69.2	6.5

Table 2
The effect of nitrogen source type and its concentration for CA production by *B. licheniformis*.

Casamino acid conc., g/L	NH_4Cl conc., g/L	Produced CA conc., g/L
5	8.0	3.0
5	0	0.4
0	8.0	5.7
0	4.0	3.3
0	1.0	3.3
0	0.5	2.8

3.2. CA production by yeasts

Two commercial strains of *Y. lipolytica*, specifically DSM 1345 and DSM 3286, were selected to establish optimal growing conditions in shake flasks. A shake flask cultivation was carried out for 144 h at 30 °C, with monitoring pH of media, sugar, CA, and ICA concentrations. Both strains consumed about 50% of the wood sugars in the cultivation media. The pH decrease in the media was consistent in this experiment (see Fig. 3a). Generally, pH plays a crucial role in CA production. Thus, the pH was initially adjusted to 6.0 using NaOH with monopotassium phosphate and disodium hydrogen phosphate serving as buffering agents in the medium. The final pH values following fermentation were 4.5 (DSM 1345) and 4.8 (DSM 3286), which aligns with the optimal pH range identified in the literature. CA yield of DSM 1345 was greater than that of DSM 3286 (3.0 and 1.8 g/L respectively) as illustrated in Fig. 3b. Nonetheless, both strains produced isocitric acid (ICA) as a by-product. Thus, efforts have been made to optimize the cultivation conditions for *Y. lipolytica* CA fermentation.

One approach to enhance the overall CA yield involves elevating its secretion into culture media, which can be accomplished by treating yeast cell culture with surfactant chemicals. We investigated the effect of 1% (w/v) Triton X-100 on CA production conducted by two commercial *Y. lipolytica* strains. The CA yield was markedly increased in the culture medium supplemented with Triton X-100 during 144-h shake flask cultivation, while maintaining the pre-set parameters. CA concentration rose by 1.5 times in DSM 1345 and by 2.5 times in DSM 3286 cultivation (see Fig. 3c). Notably, the ICA secretion was even more significantly enhanced, with a 1.9-fold and 2.8-fold increase in DSM 1345 and DSM

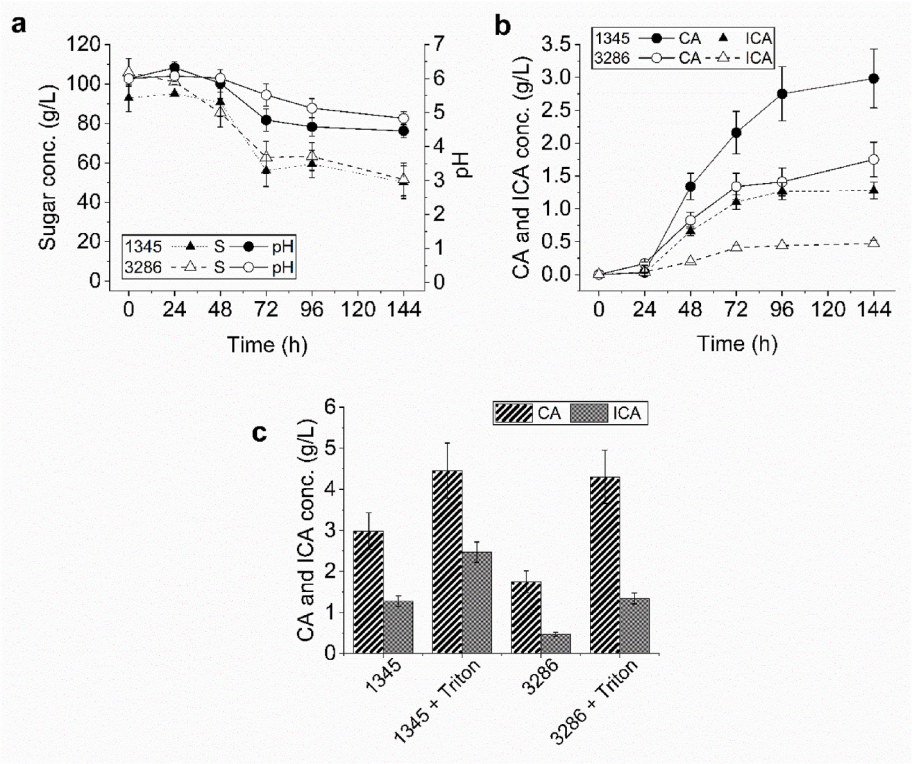

Fig. 3. Citric acid production by yeast. **(a)** Media acidification (pH) and reducing sugar consumption (S) during the 144-h shake flask cultivation of *Y. lipolytica* strains DSM 1345 and DSM 3286. **(b)** The production of citric acid (CA) and isocitric acid (ICA) by two *Y. lipolytica* strains during a 144-h shake flask cultivation is investigated. **(c)** The influence of 1% (w/v) Triton X-100 on the production of CA and ICA by *Y. lipolytica* strains DSM 1345 and DSM 3286 is analyzed during a 144-h shake flask cultivation.

3286, respectively.

The accumulation of the by-product ICA, along with other growth characteristics is probably reliant on the particular strain. DSM 1345 and DSM 3286 are commercial strains that have been widely used in CA fermentation processes. However, wild type *Y. lipolytica* strains have not been researched extensively and could serve as potential alternatives to conventional industrial strains. This study screened four wild type *Y. lipolytica* strains (71/80, 72/63, 73/64, 74/73) from Vilnius University collection along with DSM 1345 and DSM 3286 (refer to Table 3). Cultivations were conducted using previously optimized conditions in shake flasks. All of the strains utilized more than 50% of reducing sugars from wood residues hydrolysate and generated varying amounts of CA. The variation in pH of the media correlated with the production of CA, the greatest reduction was observed in the DSM 1345 (from pH 6.0 to 3.7) and DSM 3286 (from pH 6.0 to 3.4) strains, which produced the highest CA yields – 10.6 and 17.9 g/L, respectively. Wild type *Y. lipolytica* strains did not generate a comparable quantity of CA. Interestingly, ICA production in wild type *Y. lipolytica* was low or non-existent. The wild type strain *Y. lipolytica* 72/63 did not secrete any ICA, but had the highest yield of CA (3.1 g/L) out of all the screened wild type strains. This observation provides insight into the peculiarities of yeast metabolism and variability between strains of the same organism. We observed, that all strains accumulated around 11 g of wet cell biomass after the fermentation, although commercial strains differ from wild-type strains producing higher yields of CA. DSM 3286 yielded higher amounts of CA and a more favourable CA/ICA ratio (5.1:1 compared to 2.6:1) than DSM 1345. Consequently, DSM 3286 was selected for subsequent optimization experiments. Ammonium sulphate was chosen as a nitrogen source since it is a relatively cheap compound. The eight various concentrations (ranging from 0.0 to 1.0 g/L) of ammonium sulphate underwent testing (refer to Table 4) during the 144-h shake flask cultivation with the initially established parameters. The lowest concentration of $(NH_4)_2SO_4$ ($1{\cdot}10^{-3}$ g/L) resulted in the

Table 3
The comparison of CA and ICA production, wood sugar consumption and media pH change of various *Y. lipolytica* strains after six days of cultivation.

Strain	CA conc., g/L	ICA conc., g/L	CA/ICA ratio	Sugar consumption, %	Final pH
Y. lipolytica DSM 1345	10.6	4.1	2.6:1	52.5	3.7
Y. lipolytica DSM 3286	17.9	3.5	5.1:1	71.7	3.4
Y. lipolytica 71/80	1.7	0.03	56.7:1	55.8	5.8
Y. lipolytica 72/63	3.1	0	–	50.4	6.1
Y. lipolytica 73/64	0.9	0.02	45:1	58.3	5.6
Y. lipolytica 74/73	3.0	0.05	60:1	50.5	5.6

Table 4
The effect of ammonium sulphate concentration on *Y lipolytica* DSM 3286 CA and ICA production, wood sugar consumption and media acidification.

Ammonium sulphate conc., g/L	CA conc., g/L	ICA conc., g/L	CA/ICA ratio	Sugar consumption, %	Final pH
0	20.8	2.9	7.1:1	44.1	3.7
0.001	36.8	2.3	16:1	53.6	3.3
0.010	30.6	3.2	9.6:1	53.9	3.5
0.050	18.7	2.7	6.9:1	60.5	3.4
0.100	24.1	2.5	9.6:1	53.3	3.5
0.250	12.8	2.9	4.4:1	56.5	3.4
0.500	11.8	3.1	3.8:1	59.8	3.4
1.000	9.1	1.8	5:1	52.7	3.6

highest CA concentration (36.8 g/L). Conversely, complete deprivation of ammonium sulphate led to reduced acid concentration (20.8 g/L). Furthermore, nitrogen deprivation conditions notably improved the CA/ICA ratio. The optimal ratio (16:1) was attained using the minimum concentration of nitrogen source, indicating that $(NH_4)_2SO_4$ is essential in the cultivation medium, but lower concentrations boost CA production. The acidification of cultivation medium remained stable throughout all the trials, with pH values ranging between 3.3 and 3.7, which had no significant effect on CA accumulation. Sugar consumption ranged from 44.1 to 60.5%. Interestingly, there was little to no correlation observed between sugar consumption and CA accumulation. Therefore, it could be argued that the main factor in CA production by *Y. lipolytica* DSM 3286 is N source deprivation. Nitrogen deprivation appears to be one of the effective strategies to increase CA production and improve the CA/ICA ratio. Studies have shown that nitrogen deprivation can lead to increased CA yield, with a clear correlation between ammonium sulphate concentration and CA accumulation. This approach highlights the importance of nitrogen availability in microbial metabolism and its impact on CA production. The results of Egermeier et al. (2017) and Darvishi et al. (2009) highlighted the versatility of certain microbial strains in citric acid (CA) production utilizing different carbon sources: *Y. lipolytica* DSM 1345 and DSM 3286 stains using glycerol and for *Y. lipolytica* DSM using plant oil as carbon sources, respectively. It is also clear that pH regulation is crucial for CA production, with optimal ranges varying depending on the organism used. Unlike fungal cultivation, which typically requires acidification of the media, *Yarrowia lipolytica* has been shown to achieve peak CA production within a pH range of 4.5–6.0 (Moeller et al., 2007). Understanding the pH requirements of different microbial strains is essential for fine-tuning fermentation conditions to maximise CA yield. A major challenge in CA fermentation is the formation of unwanted by-products, in particular the structural isomer of CA - isocitric acid (ICA). Yeasts, in particular, tend to produce ICA, which not only reduces the overall CA yield but also interferes with the crystallisation process. While neither filamentous fungi nor bacteria have been reported to produce noteworthy quantities of CA. Efforts to optimize cultivation conditions, particularly for *Y. lipolytica*, have typically focused on minimising ICA production to increase CA purity and yield (Aurich et al., 2012). In addition, enhancing CA secretion into the culture media is a promising approach to improve overall CA yield. The use of surfactant chemicals in culture media, such as Triton X-100, has been shown to significantly increase CA production. This strategy increases cell wall permeability, facilitating the release of CA into the surrounding medium (Mirbagheri et al., 2011). Overall, the obtained data demonstrates the potential of *Y. lipolytica* in CA fermentation process utilizing sugars derived from wood biomass as a carbon source. In traditional practice, glycerol or plant oils serve as the substrates for *Y. lipolytica.* The use of carbohydrates from wood biomass is therefore a novel approach. Under nitrogen limitation conditions, the commercial strain DSM 3286 produced a maximum of 36.8 g/L of citric acid utilizing wood sugars, the result that is consistent with previously reported concentration of 31.5 g/L when aspen hydrolysate was used as a carbon source (Morgunov et al., 2018). The obtained results open up the possibility for further exploration and application of *Y. lipolytica* DSM 3286 in larger scale fermentations incorporating wood hydrolysate as a C source.

3.3. CA production by fungi

The strains *A. niger* DSM 821 (ATCC 9142) and DSM 823 (ATCC 10577) have a previous history of being used for CA production. DSM 821 was previously applied in CA production with orange peel hydrolysate as a substrate, as reported by Rivas et al., in 2008, and DSM 823 was applied with distillers grains as a substrate, according to West et al., 2004 report. The pH of the growth medium is a critical factor in CA production, particularly during fungal fermentation. Mattey (1992) has already shown that nitrogen metabolism during fermentation leads to a decrease in pH, and that low pH (<3) enhances CA production whilst preventing contamination and the formation of by-products, such as glucuronic acid. Two commercial strains of filamentous fungi *A. niger* were chosen to test whether they could produce CA using wood sugars as a carbon source. The selected strains were cultured for 120 h in a shake flask at a temperature of 30 °C. During this time, the level of CA accumulation was monitored (as shown in Fig. 4a), along with media acidification and sugar consumption (as illustrated in Fig. 4b).

In our *A. niger* cultivation experiments, we did not make any pH adjustments or regulations, thus enabling media acidification. The DSM 821 strain yielded 0.2 g/L of CA, whereas the DSM 823 produced 3.3 g/L of CA over a 5-day cultivation period. The pH decrease was concurrent with the CA accumulation in both cultures – in DSM 821 the pH reduced from 5.0 to 3.9, and in DSM 823, the pH lowered from 5.0 to 2.1. In the case of DSM 823 cultivation, CA production commenced only after 72 h, coinciding with the drop of pH below the 3.0 mark. Our results indicated that DSM 821 and DSM 823 have only consumed a small amount of wood sugars – 14,7% and 26.7% respectively. Despite this, the production ratio of grams of CA per grams of sugar is high. *A. niger* DSM 823 was chosen for further optimization experiments to enhance CA yield and the impact of methanol and ethanol on CA production was examined at pre-established cultivation parameters. At the initiation of fermentation, 1%, 2% or 3% of either ethanol or methanol was introduced into the cultivation medium (refer to Table 5).

The CA yield was enhanced with the addition of alcohols, regardless of their type and concentration. For instance, the addition of 1% ethanol caused an approximately three-fold increase in CA yield whereas higher concentrations yielded only a two-fold increase. Methanol was even more effective, enhancing CA production to 67.7 g/L with a 2% addition. This quantity was nearly eight times higher than that of the control. The addition of 1% and 3% MeOH in the media yielded beneficial results, with CA yield increasing 4.6-fold and 2-fold, correspondingly. In general, media acidification occurred in all tested conditions with no consistent trends noticed. The concentration of added alcohol directly affected sugar consumption - higher alcohol concentration led to lower utilization of sugars. The addition of 3% of methanol resulted in over threefold reduction of wet cell weight (from 13.6 g in the control to 4.2 g) and addition of 3% of ethanol resulted in over fourfold reduction (to 3.1 g). Therefore, it could be noted that the addition of alcohol could disrupt not only the TCA cycle but also other processes of cell metabolism.

Overall, we have demonstrated the effective implementation of *A. niger* DSM 823 in the CA fermentation process using sugars from waste wood biomass as a carbon source. The CA concentration we obtained after optimizing cultivation was 67.7 g/L, comparable to yields reported by Kirimura et al. (1999), who used carbohydrates found in plant biomass. Furthermore, our obtained data support the finding that alcohols, such as methanol or ethanol, can significantly enhance the CA production when *A. niger* is used for fermentations (Haq et al., 2003). They reported CA yields of 39.6–72.4 g/L in a semi-solid process. Our findings suggest that *A. niger* DSM 823 could be applied in large-scale production processes using wood-derived sugars as a substrate.

3.4. Isolation step of citric acid

To conduct ex-ante LCA experiments, it was necessary to determine the required parameters for the CA extraction step. To ensure comparability of results, a larger scale cultivation was carried out, and CA was isolated using published precipitation methods. The yeast strain *Y. lipolytica* DSM 3286, which produced the highest amount of CA under optimal conditions in flasks, was selected for scale-up in a stirred 1 L working volume bioreactor to obtain a sufficient quantity of CA. The optimized production media was used to carry out fermentation for 96 h, resulting in a CA concentration of 17.5 g/L. When scaling up the process, changes in cultivation conditions, such as increased aeration, agitation, and cell biomass accumulation, should be considered. *Y. lipolytica* was

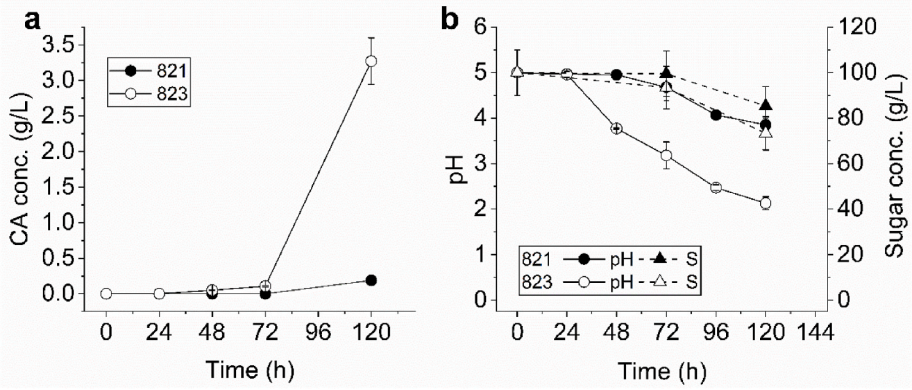

Fig. 4. Citric acid production by filamentous fungi. (a) Citric acid production of *A. niger* DSM 821 and DSM 823 strains in a 120-h shake flask cultivation. (b) Media acidification (pH) and reducing sugar consumption (S) of *A. niger* DSM 821 and DSM 823 strains in a 120-h shake flask cultivation.

Table 5
The effect of alcohols on *A. niger* DSM 823 CA production, sugar consumption and media acidification.

Ethanol conc. %	Methanol conc., %	CA conc. G/L	Sugar consumption, %	Final pH
0	0	8.8	33.9	2.5
1	0	25.1	49.9	2.0
2	0	16.4	41.5	1.9
3	0	17.1	28.1	3.1
0	1	40.5	71.8	1.8
0	2	67.7	53.1	1.9
0	3	17.5	18.5	3.6

cultured in a bioreactor and the resulting fermentation broth was filtered using a 0.22 μm pore size PVDF membrane. To reduce interference from soluble proteins, the broth was incubated at 70 °C for 15 min to denature and coagulate them. The resulting protein suspension was then centrifuged, and the liquid fraction was collected by decantation. Sawant et al. (2018) state that a minimum CA concentration of 15% (w/v) is required for the isolation process. However, in our case, the concentration was only 1.41% (w/v), so excess water was removed through evaporation under reduced pressure (rotary evaporation), resulting in an increase of the CA concentration by more than ten-fold, up to 15%. The CA was precipitated in calcium citrate form by adding a slurry of $Ca(OH)_2$ to the CA solution until the pH reached 7.0. To achieve maximum yield, the CA solution temperature was maintained at 90 °C throughout the process. This is because calcium citrate follows an inverse solubility dependency, whereby salt solubility decreases with increasing temperature (Vavrusova and Skibsted 2016). The soluble impurities, such as carbohydrates, proteins, and salts, were removed from the calcium citrate through filtration and hot water washing. The calcium citrate was suspended in water and converted back to a CA solution by adding 70% H_2SO_4 until the pH reached 2.0. The Ca^{2+} ions were then liberated and combined with SO_4^{2-} to precipitate as insoluble $CaSO_4$ (gypsum). The precipitate weighing 5.357 g was obtained as a side product of the CA isolation process after being filtered and washed. To obtain purified CA in a sulfuric acid solution, the crude CA solution was passed through an activated carbon column to absorb impurities. The solution volume was then reduced using rotary evaporation to obtain CA crystals. The crystals were subsequently filtered, washed with toluene to remove any residual sulfuric acid, and dried in a P_2O_5 exicator. The isolation process yielded 2.8%. The purity of CA and its mono hydration were confirmed using attenuated total reflectance Fourier-transform infrared spectroscopy (ATR-FTIR). The obtained yield was low, but the process parameters required for the ex-ante LCA study were defined. It is important to note that during industrial CA production, activated carbon and ion exchange resins are used for isolation experiments (Grewal and Kalra 1995).

3.5. Environmental impacts of citric acid from wood sugars

The LCA applied to input and emission data from lab-scale protocols resulted in 235–585.8 $kgCO_2$-eq per kg of CA (Fig. 5a). The stepwise application of ex-ante LCA reduced climate change impacts of CA production to 4–6 $kgCO_2$eq-kg CA, comparable to those identified for CA produced from corn (which range from 3 to 5 $kgCO_2$eq per kg CA) (Wang et al., 2020). The recovery of solvents (Process synergies) was responsible for up to 46% of total emission reduction compared to the results obtained at the lab-scale as the case of Scenario 3. Improving yields produced the greatest effect on reducing GHG emissions, achieving a decrease up to 80% of the total reductions in Scenario 1 and 53% in Scenario 3 (i.e., from isolation step). The consideration of an industrial scale energy balance increased impacts up to 28% compared to the results obtained from enhancing the yield (Improved yields), but this represented only 1% increase in the total emission reductions. This occurs because most reactions happen at room temperature and heat integrations are possible (e.g., CA isolation requires heating up to 70 °C but the fermented broth is already at 30 °C). The allocation of a share of the environmental burdens to the identified co-products (Side stream), contributed to 2% of total emissions reductions, and up to 63% reduction compared to the results of scenario 1 prior to allocation (Energy estimation). After the application of ex-ante LCA, Scenario 3, which involved CA production through fungi, shows the best environmental performance among the three evaluated scenarios due to its higher conversion yields (Fig. 5b). Ozone depletion and marine eutrophication in this scenario resulted in 2.3E-07 kg CFC-11 eq./kg CA and 1.7E-03 kg N eq/kg CA, respectively.

The climate change impacts from CA from wood sugars are still currently between 2 and 7 times higher than those from some possible fossil counterparts, i.e., acrylic acid and formaldehyde, but only 0.8 and 1.5 times higher than those of conventional CA production in Europe (Moreno-Ruiz et al., 2021). However, the ozone depletion and marine eutrophication of this study are significantly lower compared to a previous study based on CA from corn (Wang et al., 2020), which found ranges from 5.80E-07 to 1.32E-06 kg CFC-11 eq./kg CA and 1.3–5.4 kg N eq/kg CA. This might be primarily connected to the relatively higher impacts of the agricultural phase when CA is produced from annual food crops, as they require fertilizers and various on-farm operations. Marine eutrophication impacts from Scenario 3 are 72% lower than the European average CA production, while ozone depletion levels are 8% higher (Moreno-Ruiz et al., 2021). Overall, CA production from forest residues offers the advantage of reduced agriculture-related impacts, such as avoiding the use of fertilizers and agrochemicals, when compared to CA from food crops like corn and beet.

Additional process improvements are necessary beyond those

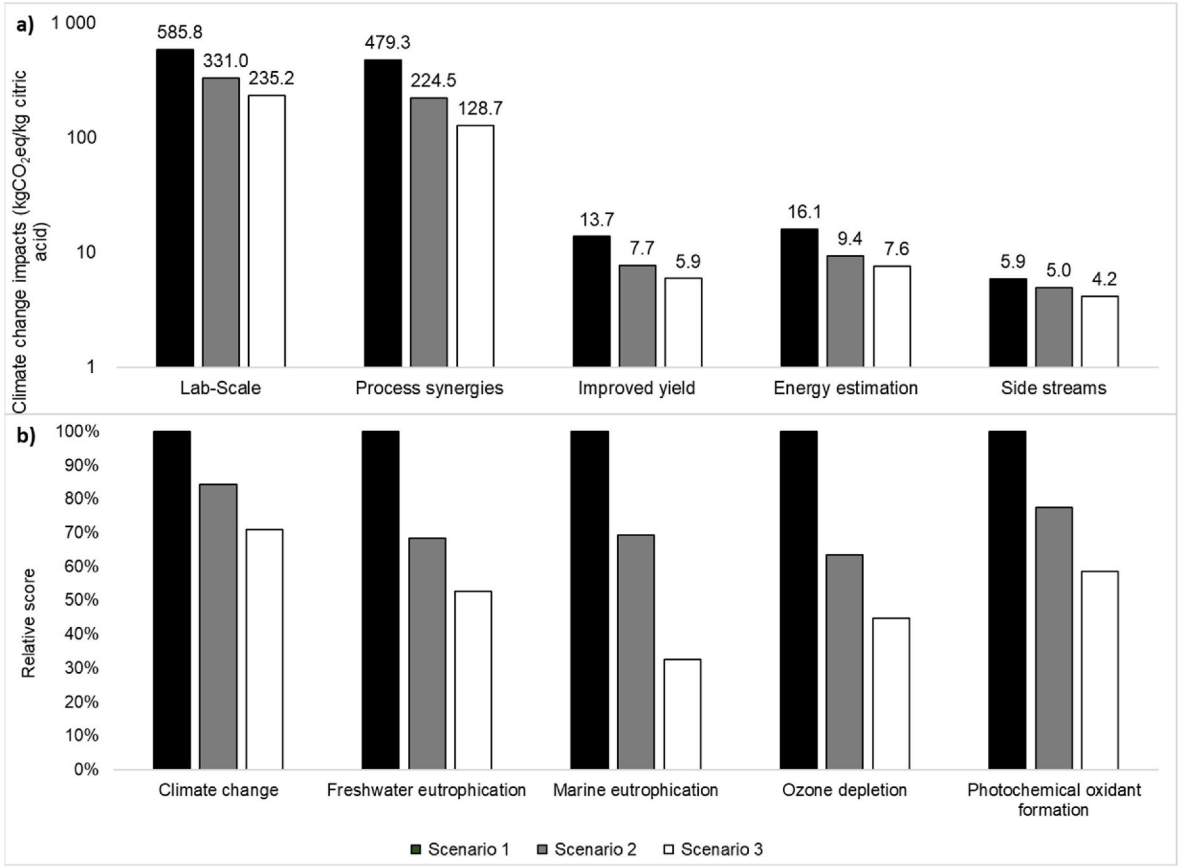

Fig. 5. Life cycle impacts of citric acid production from wood sugars. Panel (a) shows how the gradual application of ex-ante LCA affects the climate change impacts. The axis y in panel (a) is in a log scale for better visualization. Panel (b) shows the relative environmental impacts of the scale-up results (e.g., all steps up to Side streams) normalized to the worst impact, whereby the highest the relative score, the highest the impact. Absolute values are provided in Table S8.

considered here in the ex-ante LCA. For both yeast and filamentous fungi, possible improvements in cultivation conditions including increased aeration, agitation, and cell biomass accumulation have to be considered when scaling-up the process to achieve high concentrations of CA. Reducing or substituting activated charcoal in the process of CA isolation and purification is also necessary to improve the environmental performance of citric acid from wood sugars. These two factors are identified as environmental hotspots for each of the five assessed environmental impacts. Furthermore, the utilization of gypsum as a co-product is typically deemed a resourceful measure for environmental assessments, although varying allocation methods can produce substantial disparities in the outcomes.

4. Conclusions

The classical strains of microorganisms known to produce CA, including *A. niger* and *Y. lipolytica*, were verified alongside several bacterial strains, the latter of which were assessed for the first time. It was found that substrate and nitrogen concentrations were critical determinants in enhancing CA yields. Furthermore, alcohols, such as methanol and ethanol, were confirmed as influential enhancers of CA production in filamentous fungi. The incorporation of Triton X-100 surfactant proved effective in enhancing CA production in yeast strains. Furthermore, the potential of non-commercial strains of *Y. lipolytica* for CA production was explored. The study has produced promising outcome, showing the highest CA yields achieved by different fermentation processes: 5.7 g/L from bacterial fermentation, 36.8 g/L from yeast fermentation and 67.7 g/L from fungal fermentation. Moreover, the application of *ex-ante* LCA reduced the impact of citric acid on the environment from 235 to 585.8 kgCO2-eq per kg of CA when produced at the laboratory scale to 4 to 6 kgCO2eq/kg CA when produced at a larger scale. These results highlight the potential for further exploration and optimization of these processes for CA production.

CRediT authorship contribution statement

Dovilė Daunoraitė: Writing – original draft, Investigation, Formal analysis, Data curation. **Narie Rinke Dias de Souza:** Writing – original draft, Visualization, Software, Formal analysis. **Justinas Babinskas:** Writing – original draft, Investigation, Formal analysis, Data curation. **Francesco Cherubini:** Writing – review & editing, Supervision. **Lauri Vares:** Writing – review & editing, Project administration, Conceptualization. **Inga Matijošytė:** Writing – review & editing, Writing – original draft, Supervision, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jclepro.2024.142914.

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