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Biotechnological production of calcium malate from sugarcane molasses using *Acetobacter persici* BX1

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Abstract The production of calcium malate (calcium salt of malic acid) by *Acetobacter persici* BX1 using sugarcane molasses as low-cost carbon source was explored. Further investigation on untreated and pretreated molasses revealed that the highest concentration of calcium malate (22.90 ± 0.25 g/L) was obtained from the acid pretreatment using sulfuric acid. Under optimized conditions, *A. persici* BX1 produced a maximum of 36.07 ± 0.32 g/L calcium malate from sulfuric acid-pretreated molasses with initial sugar concentration of 250 g/L as the only carbon source without additional nitrogen supplementation within 96 h. The strain consumed 24.31% of the initial sugars with maximum production rate of 0.88 ± 0.02 g/L/h in first 24 h. These results indicated the potential of sugarcane molasses as an efficient and cheap.

Keywords: *Acetobacter persici* BX1, Acid pretreatment, Bioproduction, Calcium malate, Sugarcane molasses

Introduction

Malic acid, also known as 2-hydroxybutanedioic acid, is a C4 dicarboxylic acid that exists in two stereoisomeric forms: L-form and D-form. It is a white crystalline compound that is highly soluble in water (Goldberg *et al*., 2006). In 2024, the global malic acid market was valued at USD 233.9 million and is projected to grow at a compound annual growth rate (CAGR) of 4.8%, reaching USD 309.8 million by 2030 (Grand View Research, 2025). Malic acid can be produced from biomass and has a wide range of applications across various industries, including food, chemicals, pharmaceuticals, and agriculture. It is also used in metal cleaning, wastewater treatment, textile dyeing, and as a precursor for the synthesis of poly β-L-malic acid (PMA), a biodegradable plastic. Additionally, malic acid serves as a starting material for synthesizing several C4-based chemicals such as 3-hydroxybutyrolactone, 1,2,4-trihydroxybutane, and 3,4-dihydroxybutyric acid-1-methyl ester, which are regarded as promising

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alternatives to petrochemical-derived substances (Cheng *et al*., 2017; Kövilein *et al*., 2020; Wei *et al*., 2017; Xi *et al*., 2023; Yadav *et al*., 2024). Owing to its versatility and potential as a renewable resource, malic acid has been identified by the U.S. Department of Energy as one of the top 12 value-added platform chemicals with significant potential to replace petroleum-based counterparts (Werpy and Petersen, 2004).

Malic acid production can be achieved through two primary methods: chemical synthesis and biological synthesis. The chemical route of manufacture is the hydration of maleic acid or fumaric acid at high temperature and pressure, yielding a racemate of L- and D-forms of malic acid, and no optical purity. Although this technique is possible, it is rarely used in commercial applications because of the high cost of starting materials and energy-intensive methods needed, which results in environmental damage and global warming (Goldberg *et al*., 2006). Moreover, chemical synthesis may lead to the formation of hazardous by-products that can be health-damaging in high numbers (Damalas and Eleftherohorinos, 2011).

Biological synthesis, however, offers a more sustainable option. One method involves using the enzyme fumarase. This enzyme catalyses the conversion of fumaric acid to malic acid. Microorganisms with a high fumarase activity such as *Brevibacterium ammoniagenes* have been utilized in immobilized cell systems for this conversion (Battat *et al*., 1991). However, the use of this approach is limited by the expensive cost of fumaric acid. Therefore, microbial fermentation has become a potential approach for malic acid synthesis. This technique produces the stereochemically pure L-isomer of malic acid, which may be generated economically from inexpensive agricultural and industrial waste products as a carbon source, making it environmentally friendly.

Different micro-organisms have been reported to produce malic acid via fermentation with glucose as only carbon source. These are *Aspergillus niger*, *A. flavus* (West, 2011), *A. oryzae* (Knuf *et al*., 2013), *Saccharomyces cerevisiae* (Nakayama *et al*., 2012), *Monascus araneosus* ST91 (Lumyong and Tomita, 1993) and *Schizophyllum commune* (Kawagoe *et al*., 1997). Interestingly, the bacterial strain *Acetobacter persici* BX1 isolated from longan by Tepgun and Yodsanga (2014) was also found to generate malic acid utilizing glucose or xylose as carbon sources.

Sugarcane is an important agricultural product in Thailand, the third largest sugarcane producer in the world after Brazil and India. In 2024, the total area of sugarcane cultivation in Thailand was about 11.1 million rai and produced approximately 98.8 million tons of cane, with an average extraction rate of 0.109 tons of sugar per ton of cane. Currently, 58 sugar factories are in operation across the country, collectively producing up to 10 million tons of sugar annually

(Office of the Cane and Sugar Board, 2025). A major by-product of sugar production is molasses, which contains approximately 50% total sugars (sucrose, glucose and fructose) by weight, together with suspended solids, minerals, vitamins, nitrogen compounds and other substances, including heavy metals. (Jamir *et al*., 2021; Palmonari *et al*., 2020; Zohri *et al*., 2022). Due to its high sugar content and low cost, molasses has become a widely used carbon source in the production of various biochemicals, including ethanol, lactic acid, citric acid, and polysaccharides (Xia *et al*., 2014; Xu *et al*., 2025).

The study aimed to investigate the production of calcium malate (a calcium salt of malic acid) via microbial fermentation using *A. persici* BX1 and molasses, a low-cost by-product of the sugar industry.

Materials and methods

Bacterial activation and seed culture preparation

A. persici BX1 preserved in glycerol at -20 °C was activated in 20 mL selective medium (Tabuchi *et al*., 1981) containing 0.5% (w/v) $CaCO_3$ in 125 mL flasks and incubated at 30 °C, 200 rpm for 20 h. Growth was confirmed by reduction of $CaCO_3$ precipitate. For seed culture, 2% (v/v) of the activated culture was transferred into 60 mL fresh medium with 0.5% $CaCO_3$ in 250 mL flasks and incubated under the same conditions for 20 h. The seed culture was adjusted to OD_{600} about 2.5 prior to inoculation.

Molasses pretreatment

Commercial molasses was diluted 1:1 (v/v) with distilled water and subjected to different pretreatments: (i) acid pretreatment with 3 M H_2SO_4 (pH 2.0, 100 °C, 30 min), neutralized with NaOH and centrifuged; (ii) alkaline pretreatment with 15% (w/v) $Ca(OH)_2$ (65 °C, 30 min), neutralized with H_2SO_4; (iii) potassium ferrocyanide pretreatment with 0.3% (w/v) solution (98 °C, 60 min), followed by neutralization; (iv) activated charcoal pretreatment with 5% (w/v) charcoal stirred for 60 min; and (v) combined acid-alkaline pretreatment involving pH adjustment with H_2SO_4, boiling, cooling, incubation at 4 °C, and neutralization with $Ca(OH)_2$ (Cheng *et al*., 2017; Xu *et al*., 2015). Supernatants were centrifuged (8,000 rpm, 15 min) and stored at 4 °C.

Effect of individual sugars on calcium malate production

Sugars in molasses were identified by HPLC (BP-800 Ca^{2+} carbohydrate column) as glucose, fructose, and sucrose (data not shown). Each sugar (100 g/L)

was tested individually as sole carbon source in calcium malate production medium (Khan *et al*., 2014). Cultures (30 mL in 125 mL flasks) were inoculated with 5% (v/v) seed culture and incubated at 30 °C, 200 rpm for 2 days.

Effect of pretreated molasses

Untreated and pretreated molasses samples were adjusted to 150 g/L sugar and used as carbon sources in the same medium. Cultivation followed the conditions above, with incubation for 2 days.

Effect of initial sugar concentration

Sulfuric acid-pretreated molasses was adjusted to 100-300 g/L sugar and used as carbon source. Cultures were incubated under the same conditions for 2 days.

Effect of nitrogen source concentration

Using sulfuric acid-pretreated molasses (250 g/L sugar) as carbon source, corn steep liquor was supplied at 0.0-0.9 g/L. Cultures were incubated for 2 days.

Calcium malate production without nitrogen supplementation

To confirm nitrogen effects, cultures were grown in sulfuric acid-pretreated molasses (250 g/L sugar) without nitrogen supplementation for 4 days, following previous protocols. Experiments were done in triplicate with daily analyses.

Calcium malate precipitation

Cell-free supernatant was treated with methyl alcohol to remove exopolysaccharides, followed by additional methyl alcohol (3 volumes) and incubation at 4 °C for 24 h. Precipitated calcium malate was collected by centrifugation, dried at 70 °C, and weighed (Wang *et al*., 2013).

Quantification of calcium malate

A 0.2000 g sample of dried calcium malate was dissolved in distilled water, acidified with HCl, adjusted to 100 mL, and titrated with 0.05 M EDTA after adding NaOH and hydroxy naphthol blue indicator. Calcium malate

concentration was calculated using FAO (2008), where 1 mL EDTA equals 8.067 mg calcium malate.

The calcium malate concentration (g/L) was calculated using the following formula:

$$\frac{\left[\frac{(\text{Total precipitate weight (g)})\times(\text{EDTA volume (mL)})\times(0.008607)}{\text{Sample weight used for titration (g)}}\right]}{\text{Volume of fermentation broth used for precipitation (mL)}}$$

Analytical methods

Reducing sugars were analyzed by DNS method (Miller, 1959), total sugars by phenol-sulfuric acid assay (DuBois *et al*., 1956), and malate quantified as described above.

Statistical analysis

Data were analyzed using Minitab version 21. One-way ANOVA was applied, and means were compared by Tukey's test at $p < 0.01$ (Tukey, 1949).

Results

The effect of sugars in molasses as carbon sources on calcium malate production by A. persici BX1

The sugar content of molasses from Wang Kanai sugar factory was evaluated by HPLC with a BP-800 Ca^{2+} carbohydrate column. Analysis indicated the major sugar components to be glucose, fructose and sucrose (data not shown). Cultures were incubated for 2 days at 30 °C, with shaking at 200 rpm. Results indicated that *A. persici* BX1 produced calcium malate differently depending on the sugar source. The strain produced 32.42 ± 0.29 g/L calcium malate with glucose, whereas only 1.14 ± 0.02 and 1.55 ± 0.02 g/L were obtained with fructose and sucrose, respectively (Table 1).

Table 1. Effect of molasses-derived sugars as a carbon source on calcium malate production by *A. persici* BX1

Carbon source	Calcium malate (g/L)	Residual sugar (g/L)
Glucose	$32.42^{a} \pm 0.29$	7.29 ± 0.01
Fructose	$1.14^{b} \pm 0.02$	79.28 ± 0.34
Sucrose	$1.55^{b} \pm 0.02$	84.62 ± 0.41

Effect of pretreated molasses as a carbon source on calcium malate production by A. persici BX1

Commercial molasses was subjected to various pretreatment methods to evaluate their effects on total and reducing sugar contents (Table 2). Among all treatments, sulfuric acid pretreatment yielded the highest concentrations of total sugar and reducing sugar, at 1001.30 ± 3.67 g/L and 845.44 ± 2.81 g/L, respectively. In comparison, untreated molasses and molasses pretreated with calcium hydroxide, potassium ferrocyanide, activated carbon, and a combination of sulfuric acid and calcium hydroxide showed lower sugar contents. The total sugar concentrations obtained from these treatments were 563.33 ± 8.67, 444.67 ± 4.67, 801.33 ± 2.33, 563.33 ± 6.01, and 703.33 ± 3.33 g/L, respectively. Corresponding reducing sugar concentrations were 419.33 ± 1.56, 150.18 ± 1.82, 644.88 ± 3.45, 420.44 ± 2.18, and 512.11 ± 4.11 g/L, respectively.

Table 2. Total and reducing sugar contents after molasses pretreatment by different methods

Pretreatment method	Total sugar (g/L)	Reducing sugar (g/L)
Untreated molasses	$563.33^{d} \pm 8.67$	$419.33^{d} \pm 1.56$
Sulfuric acid pretreatment	$1001.30^{a} \pm 3.67$	$845.44^{a} \pm 2.81$
Calcium hydroxide pretreatment	$444.67^{e} \pm 4.67$	$150.18^{e} \pm 1.82$
Potassium ferrocyanide pretreatment	$801.33^{b} \pm 2.33$	$644.88^{b} \pm 3.45$
Activated carbon pretreatment	$563.33^{d} \pm 6.01$	$420.44^{d} \pm 2.18$
Combination of sulfuric acid and calcium hydroxide pretreatment	$703.33^{c} \pm 3.33$	$512.11^{c} \pm 4.11$

The strain produced calcium malate at concentrations of 17.64 ± 0.15, 22.90 ± 0.25, 19.34 ± 0.14, 19.97 ± 0.17, 20.21 ± 0.14 and 20.48 ± 0.17 g/L, with corresponding production rates of 0.37 ± 0.004, 0.48 ± 0.007, 0.40 ± 0.004, 0.42 ± 0.005, 0.42 ± 0.004, and 0.43 ± 0.005 g/L/h when using untreated molasses, sulfuric acid-pretreated molasses, calcium hydroxide-pretreated molasses, potassium ferrocyanide-pretreated molasses, activated charcoal-pretreated molasses, and combined sulfuric acid-calcium hydroxide-pretreated molasses, respectively (Table 3). The highest calcium malate production was achieved using sulfuric acid-treated molasses as the carbon source.

Effect of initial sugar concentration in sulfuric acid-pretreated molasses on calcium malate production by A. persici BX1

The resulting calcium malate titers were 16.06 ± 0.15, 21.89 ± 0.19, 26.44 ± 0.23, 31.01 ± 0.37, and 28.73 ± 0.34 g/L, respectively, with corresponding

productivities of 0.33 ± 0.004, 0.46 ± 0.006, 0.55 ± 0.007, 0.65 ± 0.011, and 0.60 ± 0.010 g/L/h (Table 4). Comparative analysis revealed that increasing the initial sugar concentration enhanced both calcium malate titers and productivities up to 250 g/L, which yielded the maximum production (31.01 ± 0.37 g/L, 0.65 ± 0.011 g/L/h). However, a slight decline at 300 g/L (28.73 ± 0.34 g/L, 0.60 ± 0.010 g/L/h) suggested that excessively high substrate concentrations may exert inhibitory effects, thereby limiting further improvement in calcium malate production.

Table 3. Effect of different molasses pretreatments as carbon sources on calcium malate production by *A. persici* BX1

Pretreatment method	Calcium malate (g/L)	Productivity (g/L/h)
Untreated molasses	$17.64^{e} \pm 0.15$	$0.37^{e} \pm 0.004$
Sulfuric acid pretreatment	$22.90^{a} \pm 0.25$	$0.48^{a} \pm 0.007$
Calcium hydroxide pretreatment	$19.34^{d} \pm 0.14$	$0.40^{d} \pm 0.004$
Potassium ferrocyanide pretreatment	$19.97^{c} \pm 0.17$	$0.42^{c} \pm 0.005$
Activated carbon pretreatment	$20.21^{bc} \pm 0.14$	$0.42^{bc} \pm 0.004$
Combination of sulfuric acid and calcium hydroxide pretreatment	$20.48^{b} \pm 0.17$	$0.43^{b} \pm 0.005$

Table 4. Calcium malate production by *A. persici* BX1 using sulfuric acid-pretreated molasses with different initial sugar concentrations

Initial sugar concentration (g/L)	Calcium malate (g/L)	Productivity (g/L/h)
100	$16.06^{e} \pm 0.15$	$0.33^{e} \pm 0.004$
150	$21.89^{d} \pm 0.19$	$0.46^{d} \pm 0.006$
200	$26.44^{c} \pm 0.23$	$0.55^{c} \pm 0.007$
250	$31.01^{a} \pm 0.37$	$0.65^{a} \pm 0.011$
300	$28.73^{b} \pm 0.34$	$0.60^{b} \pm 0.010$

Effect of nitrogen source concentration on calcium malate production by A. persici BX1

Cultures were incubated at 30 °C with shaking at 200 rpm for 2 days. The bacterium produced calcium malate at 30.05 ± 0.25, 29.57 ± 0.30, 28.60 ± 0.30, 28.08 ± 0.30, 27.05 ± 0.29 and 26.34 ± 0.29 g/L, with corresponding production rates of 0.63 ± 0.007, 0.62 ± 0.009, 0.60 ± 0.009, 0.59 ± 0.009, 0.56 ± 0.009 and 0.55 ± 0.009 g/L/h (Table 5). Maximum calcium malate production was observed in the absence of additional nitrogen, indicating that the supplementation of corn steep liquor did not enhance malic acid synthesis under the tested conditions.

Table 5. Effect of nitrogen source concentration on calcium malate production by *A. persici* BX1

Nitrogen source concentration (g/L)	Calcium malate (g/L)	Productivity (g/L/h)
0.00	$30.05^{a} \pm 0.25$	$0.63^{a} \pm 0.007$
0.10	$29.57^{b} \pm 0.30$	$0.62^{a} \pm 0.009$
0.30	$28.60^{c} \pm 0.30$	$0.60^{b} \pm 0.009$
0.50	$28.08^{c} \pm 0.30$	$0.59^{b} \pm 0.009$
0.70	$27.05^{d} \pm 0.29$	$0.56^{c} \pm 0.009$
0.90	$26.34^{e} \pm 0.29$	$0.55^{c} \pm 0.009$

Calcium malate production and productivity of A. persici BX1 in sulfuric acid-treated molasses medium (250 g/L sugar) without additional nitrogen supplementation

Based on the previous findings, the highest calcium malate production by *A. persici* BX1 was obtained when sulfuric acid-treated molasses containing an initial sugar concentration of 250 g/L was used as the carbon source without additional nitrogen supplementation. The results are summarized in Table 6.

Table 6. Calcium malate production by *A. persici* BX1 in sulfuric acid-treated molasses (250 g/L, no nitrogen supplementation)

Day	Residual sugar (g/L)	Calcium malate (g/L)	Productivity (g/L/h)
0	250.19 ± 0.51	$0.00^{e} \pm 0.00$	$0.00^{e} \pm 0.000$
1	217.86 ± 0.34	$21.12^{d} \pm 0.37$	$0.88^{a} \pm 0.015$
2	197.97 ± 0.88	$30.06^{c} \pm 0.43$	$0.63^{b} \pm 0.009$
3	192.63 ± 0.33	$33.86^{b} \pm 0.31$	$0.47^{c} \pm 0.004$
4	189.37 ± 0.51	$36.07^{a} \pm 0.32$	$0.38^{d} \pm 0.003$

According to Table 6, after 4 days of cultivation, *A. persici* BX1 consumed 60.82 g/L of sugar and produced 36.07 ± 0.32 g/L calcium malate, with an overall productivity of 0.38 ± 0.003 g/L/h. The sugar concentration declined sharply during the first two days of cultivation, decreasing from 250.19 ± 0.51 to 197.97 ± 0.88 g/L, corresponding to a consumption of 52.22 g/L. Between day 2 and day 3, the sugar level declined more slowly, from 197.97 ± 0.88 to 192.63 ± 0.33 g/L (5.34 g/L consumed), and by day 4 it further decreased to 189.37 ± 0.51 g/L. In total, 60.82 g/L of sugars were consumed over 4 days. The highest calcium malate productivity was observed on day 1, reaching 0.88 ± 0.015 g/L/h.

Discussion

After 2 days of cultivation, *A. persici* BX1 exhibited varying levels of residual sugar and calcium malate production depending on the carbon source

used. When glucose was used, the residual sugar concentration was only 7.29 ± 0.01 g/L, indicating efficient sugar utilization, and resulted in the highest calcium malate production at 32.42 ± 0.29 g/L. In contrast, much higher residual sugar concentrations were observed with fructose (79.28 ± 0.34 g/L) and sucrose (84.62 ± 0.41 g/L), corresponding to significantly lower calcium malate yields. These findings indicate that *A. persici* BX1 preferentially metabolizes glucose, likely due to its direct entry into glycolysis and subsequent supply of precursors for malic acid biosynthesis, whereas fructose and sucrose require additional metabolic steps that may constrain efficiency under the tested conditions (Cheng *et al.,* 2017; Kövilein *et al*., 2020; Xi *et al*., 2023).

The production of calcium malate by *A. persici* BX1 using molasses pretreated by various substances as the carbon source showed the highest calcium malate production by using sulfuric acid pretreated molasses compared with untreated molasses or molasses pretreatment with calcium hydroxide, potassium ferrocyanide, activated carbon or a combination of sulfuric acid and calcium hydroxide. This finding can be linked to the fact that pretreatment affects the chemical composition of molasses that usually contain high levels of sugars, suspended solids, minerals, vitamins, organic compounds and sometimes heavy metals (Jamir *et al*., 2021; Palmonari *et al*., 2020; Zohri *et al*., 2022). Untreated molasses may include an excess level of metals such as calcium, potassium, sodium, magnesium and copper and colloids that may be toxic to cells, disrupting growth and metabolism and lowering calcium malate production. Pretreatment methods such as flocculation, sedimentation and precipitation minimize the inhibiting components. For example, activated carbon adsorption removes metals and toxic organics; potassium ferrocyanide removes metal ions by chelation-like precipitation; sulfuric acid removes metals as insoluble sulfates and hydrolyzes disaccharides (e.g., sucrose) to fermentable monosaccharides (glucose and fructose); and calcium hydroxide removes metals as hydroxides, but this method is limited by the formation of low-density sludges and amphoteric hydroxides. The combined treatment of sulfuric acid and calcium hydroxide can increase sugar hydrolysis, but it still has the disadvantages of hydroxide precipitation, which is responsible for its lower productivity than sulfuric acid alone. These results are consistent with those of Xu *et al*. (2015) who demonstrated that the pretreatment with sulfuric acid was the best technique for the removal of heavy metals compared to other strategies. Thus, the sulfuric acid treated molasses was selected as the optimal carbon source to produce calcium malate for further study.

The results indicated that initial sugar concentrations of sulfuric acid-pretreated molasses affected the calcium malate titer and productivity by *A. persici* BX1 significantly. The initial sugar content is of great importance for malic acid biosynthesis, since it directly affects biomass formation and organic

acid generation. The best synthesis of calcium malate was produced at starting sugar concentration of 250 g/L, indicating that this concentration is the most beneficial in terms of the availability of the substrate and the cellular metabolism. The main precursor for the production of malic acid is glucose degradation by glycolysis and then either the conventional TCA cycle, the reverse TCA cycle or the glyoxylate route depending on the kind of microbe (Cheng *et al*., 2017; Kövilein *et al*., 2020; Xi *et al*., 2023). When glucose is present, malic acid production occurs, but the large amount of the substrate involves inefficient substrate use and substrate inhibition (Zou *et al*., 2013). Higher sugar concentrations raise the osmotic pressure causing loss of water and intracellular solutes thereby compromising the cell activity and fermentation efficiency. Other key parameters for fermentation efficiency are temperature, pH, oxygen transfer rate and cell density that all need to be set for improving microbial growth and metabolic flux towards malic acid production (Fang *et al*., 2011).

The effect of varying nitrogen source concentrations on calcium malate production by *A. persici* BX1 was investigated. The bacterium was cultivated in a calcium malate production medium containing sulfuric acid-treated molasses as the carbon source, with corn steep liquor added at 0.0, 0.1, 0.3, 0.5, 0.7 and 0.9 g/L. Results showed that calcium malate production differed across the nitrogen levels. The highest calcium malate concentration (30.05 ± 0.25 g/L) and production rate (0.63 ± 0.007 g/L/h) were obtained in the absence of added nitrogen. Increasing concentrations of corn steep liquor progressively reduced both calcium malate yield and production rate. This pattern can be attributed to the inherent nitrogen content in molasses, which varies by source (Jamir *et al*., 2021; Palmonari *et al*., 2020; Zohri *et al*., 2022). Bacteria utilize sugars as a carbon source for biomass formation and organic acid synthesis; under nitrogen-limited conditions, however, cell growth slows, redirecting carbon flux toward organic acid production through related metabolic pathways (Mondala, 2015; Ochsenreither *et al*., 2014). Thus, the native nitrogen content in the treated molasses was sufficient to support bacterial growth while maximizing calcium malate production, eliminating the need for additional nitrogen supplementation and reducing production costs.

In this study, *A. persici* BX1 cultivated in a calcium malate production medium (Khan *et al*., 2014) containing sulfuric acid-treated molasses with an initial sugar concentration of 250 g/L, without additional nitrogen supplementation, produced a maximum of 36.07 ± 0.32 g/L calcium malate after 4 days. The highest malic acid productivity (0.88 ± 0.015 g/L/h) was observed on day 1, which corresponded with a rapid decrease in sugar concentration from 250.19 ± 0.51 to 217.86 ± 0.33 g/L between day 0 and day 1. From day 2 to day 4, sugar comsumption decreased. This tendency is associated with the sugar

composition of molasses, which is mostly glucose, fructose and sucrose. Glucose as a monosaccharide has direct access to the glycolytic cascade and the related pathways of organic acid formation (Cheng *et al*., 2017; Kövilein *et al*., 2020; Xi *et al*., 2023) as opposed to fructose and sucrose, which require further metabolic conversion prior to entry into the pathways. Thus, *A. persici* BX1 probably preferred to consume glucose in the early period of cultivation, and this led to a rapid depletion of sugar and large formation of calcium malate, whereas fructose and sucrose were used later. Additionally, the lower production after day 2 could be due to the cells entering the stationary phase, in which the growth rates are slower but metabolic activity and generation of metabolites can still take place (Navarro Llorens *et al*., 2010). Molasses is a by-product of the sugar industry. It is utilized effectively to reduce downward manufacturing costs and to create value for the industrial waste streams.

In conclusion, *A. persici* BX1 was capable of efficiently manufacture calcium malate utilizing molasses as a carbon source under different growing circumstances. Glucose was identified as the most favorable sugar, supporting a maximum yield of 33.39 ± 0.20 g/L. The highest titer of calcium malate among the pretreatment procedures was produced from molasses treated with sulfuric acid. Optimization of initial sugar concentration reaching to 31.01 ± 0.37 g/L at 250 g/L sugar further improved output. Interestingly, the absence of additional nitrogen supplementation also did not alter the peak production. The maximal concentration of calcium malate produced by *A. persici* BX1 under optimum conditions was 36.07 ± 0.32 g/L after 4 days and the maximum productivity (0.88 ± 0.015 g/L/h) was reached on the first day that was in accordance with the rapid sugar consumption. These results suggest the possibility of economical and long-term synthesis of malic acid from low-cost industrial by-products by *A. persici* BX1.

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Conflicts of interest

The authors declare no conflict of interest.

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