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REVIEW

# Recent advances in microbial production of mannitol: utilization of low-cost substrates, strain development and regulation strategies

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## Abstract

Mannitol has been widely used in fine chemicals, pharmaceutical industries, as well as functional foods due to its excellent characteristics, such as antioxidant protecting, regulation of osmotic pressure and non-metabolizable feature. Mannitol can be naturally produced by microorganisms. Compared with chemical manufacturing, microbial production of mannitol provides high yield and convenience in products separation; however the fermentative process has not been widely adopted yet. A major obstacle to microbial production of mannitol under industrial-scale lies in the low economical efficiency, owing to the high cost of fermentation medium, leakage of fructose, low mannitol productivity. In this review, recent advances in improving the economical efficiency of microbial production of mannitol were reviewed, including utilization of low-cost substrates, strain development for high mannitol yield and process regulation strategies for high productivity.

**Keywords** Mannitol · Fermentation · Strain development · Regulation · Metabolic flux

## Introduction

Mannitol is a six-carbon sugar alcohol with several excellent properties that make it widely used in fine chemicals, pharmaceutical industries, as well as functional foods (Soetaert 1991). In addition, mannitol plays an important role in the stress tolerance of microorganisms, lichens, and plants due to its function as a compatible solute (Reed et al. 1985; Stoop and Mooibroek 1998). Mannitol is a food additive permitted by the Food & Drug Administration because it can reduce rise in blood glucose and insulin response due to its slow absorption by the body (Dai et al. 2017). Mannitol is stable and rarely reacts with other ingredients, and its sweet cool taste may cover the unpleasant taste of drugs. Thus, mannitol is used in the manufacture of chewing gum, sober drugs, chewable tables, and granulated powders (Patra et al. 2009). In pharmaceuticals, mannitol is also used as a dehydrating agent and as a diuretic. Hypertonic mannitol can increase the permeability of the blood–brain barrier to allow water-soluble drugs and macromolecules to pass through (Rapoport 2001). Meanwhile, mannitol is a strong radical scavenger that can reduce the harm against neurological functions (André and Villain 2017).

At present, mannitol is mainly produced by chemical methods. In this process, mannitol is synthesized by the hydrogenation of D-glucose/D-fructose mixtures in aqueous solutions at high temperature (120–160 °C) using raney-nickel as a catalyst and hydrogen gas as reducing agent (Dai et al. 2017). In this reaction, β-fructose is concerted to mannitol, while α-fructose and glucose are converted to sorbitol as a byproduct. The hydrogenation of a glucose-fructose mixture (1:1) resulted in mixed products containing only 25% of mannitol and others are in the form of sorbitol, which is caused by the poor selectivity of nickel catalyst (Soetaert 1990). Chemically synthesized mannitol needs to be purified by chromatography to remove metal catalyst, and low-temperature crystallization is required for the remove of sorbitol. The high temperature and pressure conditions, high amount

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of sorbitol, as well as the requirement of pure substrates are the main disadvantages (Korakli et al. 2000). Consequently, this chemical process suffered from higher production costs and low yield (Soetaert et al. 1999). In recent years, research efforts have been extensively directed towards developing biological processes for the production of mannitol due to its economical benefits (Song and Vieille 2009).

Mannitol can be enzymatically synthesized from fructose with the catalysis of mannitol dehydrogenase (MDH) using NAD(P)H as reducing cofactors (Song and Vieille 2009; Dols et al. 1997). The NADH-dependent MDH can be purified from *Lactobacillus brevis, Leuconostoc mesenteriodes, Pseudomonas fluorescens, Rhodobacter spaeroides, Saccharomyces cerevisiae*, and *Torulaspora delbruckii*. NADPH-dependent MDH can be purified from *Aspergillus parasitius, Candida magnoliae, Zymomonas mobilis*, and *Gluconobacter suboxydans* (Martinez et al. 1963; Brunker et al. 1997; Schneider and Giffhom 1989; Schneider et al. 1993; Quain and Boulton 1987; Nidetzky et al. 1996). However, the cost of cofactors and dependence of the pure enzymes are major limitations. The cofactors can be regenerated by enzymatic, electrochemical, chemical, photochemical and biological methods to support the reduction of fructose (Chenault and Whitesides 1987). For example, the NADH consumed in mannitol production by *S. cerevisiae* MDH was regenerated during gluconic acid production by *Bacillus megaterium* glucose dehydrogenase, while the high amount of gluconic acid excited in final products resulted in further purification of mannitol (Kulbe et al. 2010). Another two-enzyme system was devised for cofactor regeneration by simultaneous conversion of two substrates (Wichmann et al. 1981). Formate dehydrogenase (FDH) converts formate to $CO_2$ and reduces $NAD^+$ to NADH. Simultaneously, MDH uses NADH to convert fructose to mannitol and regenerates $NAD^+$. In this system, only $CO_2$ has been generated as by-product, making it easy to be separated from mannitol (Parmentier et al. 2005). Due to high substrate concentration could cause enzyme inhibition; the optimal fructose concentration was 50 mM for the bioconversion system, which would cause low mannitol concentration. Furthermore, poor enzyme stability and complex enzyme system make this process difficult to control, which causes limitation in its application to industrial scale (Parmentier et al. 2003).

Microbial production of mannitol could be conducted at normal pressure and low temperature. High cost of pure hydrogen or cofactors used in chemical or enzymatic processes could be replaced by cheap substrates during microbial fermentation. Therefore, microbial production of mannitol is a promising alternative to chemical or enzymatic processes. Microbes that are able to produce mannitol include several yeasts, fungi, lactic acid bacteria (LAB) with different biosynthetic pathway (Wisselink et al. 2002). To achieve an efficient and competitive biotechnological mannitol production process, low-cost culture media with different substrates and culture conditions optimization are needed (Meng et al. 2017). Simultaneously, to increase the mannitol concentration, yield, and productivity, a number of recombinant microorganisms and regulation strategies have been developed. This review mostly focuses on recent advances in improving the economical efficiency of microbial production of mannitol were reviewed, including utilization of low-cost substrates, strain development for high mannitol yield and process regulation strategies for high productivity.

## Mannitol production by using low-cost substrates

The cost of fructose used in mannitol fermentation takes accounts for almost 50% of the total production cost, thus ultilization of low-cost substrate to produce mannitol is of significant importance. Using low-cost substrate to replace fructose is a way to reduce substrate cost (as shown in Table 1). Glucose is a widely used substrate during microbial fermentation with acceptable price, which is half of fructose. Song et al. (2002) isolated a novel strain of *Candida magnoliae* HH-01 to produce mannitol when grown in presence of 30% fructose and 5% (w/v) glucose as carbon

**Table 1** Mannitol production by using low-cost substrate to replace fructose

| Organism | Process characteristics | Substrate | Yield (g/g) | R [g/(L h)] | Mannitol (g/L) | References |
|---|---|---|---|---|---|---|
| *Candida azyma* NBRC10406 | Batch | 30% w/v raw glycerol | 0.3 | 0.30 | 50.8 | Yoshikawa et al. (2014) |
| *Candida zeylanoides* KY 6166 w | Batch | 10% w/v *n*-alkane | 0.52 | 0.63 | 63 | Hattori and Suzuki (1974) |
| *Candida magnolia* HH-01 | Batch | 5% w/v glucose 30% w/v fructose | 0.50 | 0.74 | 151 | Song et al. (2002) |
| *Candida parapsilosis* SK 26.001 | Shake flask | 20% w/v glucose | 0.34 | 0.95 | 68.5 | Meng et al. (2017) |
| | Batch | 28.4% w/v glucose | 0.40 | 1.12 | 80.3 | |
| | Fed-batch | Glucose /fructose ratio of 1:2 | 0.34 | 0.81 | 97.1 | |

sources, which might partially reduce the substrate cost. *C. magnoliae* HH-01 is not suitable for high substrate fermentation because 200 g/L of glucose could direct the final product toward glycerol. Mannitol was produced at 0.74 g/(L h) with a yield 0.5 g mannitol/g fructose consumed, and reached 151 g/L mannitol after 200 h. *Candida parapsilosis* SK 26.001 was isolated for mannitol production (Meng et al. 2017). When glucose used as sole carbon source, the highest mannitol concentration reached 68.5 g/L in shake flask. During batch fermentation, the strain completely exhausted the fed glucose after 72 h of fermentation, producing 80.3 g/L of mannitol. When fed-batch fermentation was performed, mannitol concentration reached 97.1 g/L. With the complete replacement of fructose, mannitol production by this strain can significantly reduce the substrate cost; however, mannitol production by *C. parapsilosis* SK 26.001 requires high amount of yeast extract (30 g/L), which would highly affect the total cost. Another yeast strain, *Candida azyma* NBRC10406, could produce 50.8 g/L mannitol from crude glycerol, a byproduct of biodiesel production; however, the mannitol productivity was much lower than that by using fructose as the substrate (Yoshikawa et al. 2014). Hattori et al. (1974) studied the large-scale production of mannitol from *n*-alkane by *Candida zeylanoiedes*, and the mannitol concentration reached 63 g/L with a yield 0.52 g/g after 100 h incubation in a 5-L fermenter.

Besides, low-cost substrates rich in fructose also have great potential to replace fructose (as shown in Table 2). Cashew apple juice contains significant amounts glucose and fructose, and when used for mannitol production, production of 18 g/L of mannitol was observed with 67% fructose converted into mannitol. Production of 22 g/L mannitol with a culture medium containing a mixture of cashew apple juice and sucrose increased the mannitol yield to 85% (Fontes et al. 2009). Due to the low content of nitrogen source in cashew apple juice, Fontes et al. 2013 used cashew apple juice supplemented with ammonium sulfate and mannitol of 19 g/L with a high yield of 95% was achieved. The use of ammonium sulfate in replacement of yeast extract for mannitol production showed to be a suitable strategy for mannitol production from cashew apple juice. Molasses and fructose syrup are byproducts of the sugar industry, which could be used as fructose-rich substrate. Production of 40.1 g/L mannitol from molasses for mannitol production by *L. intermedius* NNRL B-3693 was reported, while this process also generated high amount of lactic acid and ethanol. When a mixture of the molasses and fructose syrup (1:1) was used as substrate, enhanced production of 104.8 g/L mannitol was observed in 22 h with the addition of soy peptone and corn steep liquor as the nitrogen source (Saha 2006a, b, c). Inulin is also a suitable substrate for mannitol production. Production of high amount of 207.4 g/L mannitol was achieved with inulin in 72 h by simultaneous saccharification and fermentation. When fructose and inulin mixture (3:5, total 400 g/L) was used as the substrate, the bacterium produced 227 g/L mannitol during 110 h (Saha 2006a, b, c). Furthermore, the corn steep liquor could be used to replace yeast extract, which will be an effective way to reduce production costs. Although the ultilization of low-cost substrate will make mannitol production process more economic, their performance differs greatly. Among all the reported low-cost substrate, fructose syrup and inulin is the most promising substrate for mannitol production due to their remarkable advantages in mannitol concentration, yield and productivity. Although molasses is also suitable for mannitol production, the simultaneous produced glucan would cause difficulties in mannitol separation. Besides, high-cost operation should be avoided, such as addition of enzymes for hydrolysis of inulin. Using genetic approach to express inulinase in the mannitol producers could be a cost-effective way to further reduce production cost (Wang et al. 2016).

**Table 2** Mannitol production by using fructose-rich substrates to replace pure fructose

| Organism | Process characteristics | Substrate | Yield (g/g) | R [g/(L h)] | Mannitol (g/L) | References |
|---|---|---|---|---|---|---|
| *Leuconostoc mesenteroides* B-512F | Batch | CAJ | 0.67 | 1.8 | 18 | Fontes et al. (2009) |
| | | CAJ[a] | 0.95 | 1.6 | 19 | |
| *Lactobacillusintermedius* NNRL B-3693 | Batch | Molasses | 0.50 | 0.74 | 40.1 | Saha (2006a) |
| | | Molasses and fructose syrup (1:1) | 0.87 | 4.76 | 104.8 | |
| *Lactobacillus intermedius* | Batch with SSF | Inulin 300 g/L | 0.69 | 2.88 | 207 | Saha (2006b) |
| | | Fructose 150 g/L and inulin 250 g/L | 0.57 | 2.1 | 227 | |

*CAJ* cashew apple juice; *SSF* simultaneous saccharification and fermentation

[a]Supplement with ammonium sulfate

## Enhanced mannitol production with strain development

Genetic engineering and chemical mutagenesis of yeast, lactic acid bacteria, and even the mannitol non-producer *Escherichia coli* have been demonstrated to convert sugars to mannitol with high efficiency (Costenoble et al. 2003; Saha and Racine 2011). Mannitol production by genetically engineered and chemically mutated mannitol producers were summarized in Table 3. In heterofermentative LAB, a significant part of fructose is reduced to mannitol by mannitol dehydrogenase (MDH), while part of the fructose is simultaneously fermented via the phosphoketolase (PK) pathway to produce a mixture of $CO_2$, ethanol, acetate and lactate (Wisselink et al. 2002). The co-metabolism of fructose results in decreased mannitol yield. To decrease the metabolic flux of fructose into the PK pathway and give improved yield of mannitol from fructose, work of modifying the fructokinase involved in phosphorylation of fructose to fructose-6-phosphate was processed in heterofermentative LAB. A mutant of *L. pseudomensenteroides* ATCC 12291 that unable to grow on fructose was obtained by chemical mutagenesis. The specific fructokinase activity in the mutant was decreased to about 10% of that of the parent strain and as a result, the yield of mannitol was improved from 0.75 g/g to 0.87 g/g (Helantoa et al. 2005). Mutant with complete inactivation of fructokinase in LAB has not been obtained up to now, which might suggest that fructose assimilation through PK pathway is necessary for cell growth of heterofermentative LAB during mannitol production.

In comparison with the heterofermentative LAB, an advantage of mannitol production by the homofermentatives LAB might be the capability to use more sugar substrates to synthesize mannitol with lactate dehydrogenase (LDH) deficient (Gaspar et al. 2004). In these strains, mannitol 1-phosphatase (M1Pase) transforming fructose 1-phosphate to mannitol and M1PDH for the regeneration of $NAD^+$ to fulfill the redox balance are the two key enzymes involved in the mannitol biosynthesis route. Hence, a specific M1Pase gene in *Eimeria tenella* and the *mtlD* gene encoding M1PDH from *Lactobacillus plantarum* were overexpression in *Lactobacillus lactis* to increase mannitol production (Wisselink et al. 2005). High glucose-to-mannitol conversion rate of 50% by *L. lactic* has been achieved, which was close to the theoretical mannitol yield of 67% (Helantoa et al. 2005). *Escherichia coli* is the most common strain to be genetically engineered for D-mannitol. Kaup et al. reported a whole-cell biotransformation system in *E.coli* for the production of mannitol from fructose by constructing a recombinant oxidation–reduction cycle (Kaup et al. 2004). The *mdh* gene encoding mannitol dehydrogenase and the *fdh* gene encoding formate dehydrogenase were functionally over expressed in *E coli* cells. To allow the cells to efficiently take up fructose without simultaneous phosphorylation, the *glf* gene encoding the glucose facilitation protein of *Zymomonas mobilis* was introduced simultaneously. The recombinant strain produced 363 mM mannitol in 8 h under pH control condition. Furthermore, supplementation of this strain with extracellular glucose isomerase resulted in the production of 145.6 g/L mannitol from 180 g/L glucose (Kaup et al. 2005). Although heterofermentative LAB produce excellent quantities of mannitol from fructose, they also produce lactic acid and acetic acid as co-products, and the genetic engineering tools available did not allow the usage of directed inactivation techniques to the strain. In our study, high amount of 23.5 g/L D-lactic acid together with 64.6 g/L mannitol was produced with an optical purity of 99.9% (Zhang et al. 2017). From another point of view, if the high amount of D-lactic acid could be recovered, it would be beneficial to improve the process economy. Furthermore, production of mannitol from glucose by homofermentative LAB is inefficient because of the very low mannitol concentration. Hence a number of recombinant microorganisms have been developed to overproduce mannitol and limit the co-products. To enhance the mannitol production during long-term biotransformation, *Corynebacterium glutamicum* was also used for

**Table 3** Mannitol production by genetically engineered and chemically mutated mannitol producers

| Organism | Process characteristics | Yield (g/g) | R [g/(L h)] | Mannitol (g/L) | References |
|---|---|---|---|---|---|
| *L. pseudomensenteroides* ATCC 12291 | Growing cells | 0.87 | 2.8 | 17.4 | Helantoa et al. (2005) |
| *L. lactis* Δ*ldh*, overexpressing M1PDH and M1Pase | Growing cells | 0.50 | 0.16 | 9 | Wisselink et al. (2005) |
| *E. coli* overexpressing MDH, FDH and GLF | Resting cells | 0.84 | 8.2 | 66 | Kaup et al. (2004) |
| *E. coli* overexpressing MDH, FDH and GLF, plus extracellular XI | Resting cells | 0.80 | 3.6 | 146 | Kaup et al. (2005) |
| *C. glutamicum* overexpressing MDH, FDH and GLF | Fed-batch reactor with resting cells | 0.91 | 2.97 | 285 | Bäumchen et al. (2007) |
| *L. lactis*, Δ*ldh* Δ*mtlF* | Resting cells | 0.33 | 2.7 | 2.4 | Gaspar et al. (2004) |

establishing the recombinant oxidation/reduction cycle for conversion of fructose to mannitol. Expression of the *mdh, fdh* and *glf* genes in *C .glutamicum* led to the production of 87 g/L mannitol from 93.7 g/L D-fructose. In repetitive fed-batch biotransformation, 285 g/L D-mannitol during a time period of 96 h was produced (Bäumchen et al. 2007). Genetically engineered mannitol producers showed great potential to produce mannitol in high concentration over theoretical yield due to the complete escape from fructose leakage compared with the native producers. However, the safety of mannitol produced by genetically engineered producers could be of concerns, especially when used in medicine and health care.

## Process regulation strategy for efficient mannitol production

Mannitol production by microorganism has been extensively studied using batch fermentation; however, batch mode suffers from relatively low mannitol concentration and productivity due to inhibition of substrate and mannitol. Fed-batch fermentation could overcome limitations caused by high substrate concentrations. Tomaszewska et al. (2012) reported that 27.6 g/L mannitol was produced by *Yarrowia lipolytica* A-15 strain, and during fed-batch fermentation, *Y. lipolytica* A-15 produced 41.4 g/L mannitol, which was 50% higher than batch fermentation. Soetaert (1990) have extensively studied the effects of fed batch fermentation on the production of mannitol by *Leuconostoc pseudomesenteroides* ATCC-12291. In fed-batch cultures, an average mannitol productivity of 6.3 g/L/h was observed with a high mannitol yield of 0.95 g/g fructose. It was reported that fed-batch fermentation could prevent the consumption of produced mannitol during the late stages of fermentation, which would facilitate mannitol accumulation. When *C. magnoliae* was used for mannitol production, mannitol concentration reached 209 g/L after 200 h fermentation (Song et al. 2002). Membrane cell-recycle bioreactor (MCRB) could solve these problems satisfactorily. The MCRB has been demonstrated in a number of previous studies of the high-volumetric productivity of lactic acid and ethanol (Vick et al. 1983). Membrane technique could be used to prevent cell loss during continuous fermentation, and consequently increases the cell density gradually. *L. mesenteroides* ATCC-9135 was examined in high cell density membrane cell-recycle cultures. Finally, the volumetric mannitol productivity reached 26.2 g/(L h) with a yield of 0.98 g/g (Weymarn et al. 2002). Racine and Saha (2007) used *L. intermedius* NRRL B-3693 to produce mannitol in continuous cell-recycle fermentation with membrane module. The process was developed using corn steep liquor and glucose as inexpensive industrial nutrient sources, and the productivity of mannitol reached a significant high level of 40 g/(L h), which was mainly contributed by high cell densities in continuous cell-recycle fermentation processes.

The yield of mannitol is mainly affected by aeration, pH, the presence of metal ions. In heterofermentative LAB, 1 mol glucose or fructose is metabolized to 1 mol of lactic acid and 1 mol of acetic acid or ethanol (Wisselink et al. 2002). The produced organic acids decrease the fermentation pH, which significant inhibited the growth of LAB and reduced mannitol production. Consequently, pH control strategy serves as a promising approach to improve mannitol production by heterofermentative LAB. Saha and Racine (2010) optimized the fermentation pH for mannitol production from fructose by *L. intermedius* NRRL B-3693. In the optimal pH condition, the mannitol yield was approximately twice that in uncontrolled pH fermentation. Mannitol production is closely related to cell growth, while the preferable pH for mannitol production may not always be consistent with that for cell growth. Thus, it may not appropriate for maintaining a constant pH throughout the fermentation process for efficient mannitol accumulation. Thus, fermentation with a stage-controlled pH strategy is a valid strategy for improving mannitol production. *Lactobacillus brevis* 3-A5 was observed to produce mannitol efficiently by regulating pH in batch and fed-batch fermentation. The pH value was controlled at 5.5 for the first 12 h to improve cell growth and subsequently shifted to 4.5 to enhance the accumulation of mannitol. The dual-stage pH controlled fed-batch fermentation increased mannitol production to 215 g/L after 98 h of fermentation, which was 109% higher than that without pH control (Yue et al. 2013). The mannitol yield by *L. mesenteroides* varied greatly with aeration strategy used. Under aerated conditions, fructose metabolism involved two distinct pathways: a fraction of fructose was reduced to mannitol associated with the oxidation of NADH, while the remainder fructose was metabolized via the PK pathway and converted to equimolar mixture of lactic acid, acetate and $CO_2$. Under oxygen-limited growth, higher mannitol production was obtained and no ethanol production occurred. In the presence of glucose, coenzyme regeneration would normally be expected to generate ethanol as an end product rather than acetate under anaerobic conditions. When adequate aeration was maintained throughout the culture, no significant production of ethanol occurred. By using a two-stage fermentation strategy, mannitol production has been increased from 60 to 240 g/L with *Candida magnoliae* R9. Besides, the mannitol yield and productivity was at a high level of 0.81 g/g and 4.0 g/L/h. The two-stage fermentation process was comprised of grown phase and production phase. During the growth phase, glucose was supplemented and aerobic conditions were maintained. Thereafter, the production phase was initiated by supplementing fructose and switching to anaerobic conditions by discontinuing aeration and decreasing the speed of agitation (Laxman et al. 2012).

The theoretical yield of mannitol produced by heterofermentative lactic acid bacteria is 66.7 mol% due to 33.3 mol% of sugar is utilized for co-factor regeneration. At optimal conditions, per mol of glucose fermented and 2 mols of fructose are completely reduced to mannitol. The carbohydrate sources for mannitol fermentation of heterofermentative lactic acid bacteria were fructose and glucose in a ratio of 2:1 (Wisselink et al. 2002). For some *Lactobacilli*, the growth rate on fructose is higher than on glucose, and fructose was phosphorylated into fructose-6-phosphate by fructokinase (FK). Then the regeneration of $NAD^+$ must be achieved by using an alternative electron acceptor. Therefore the fructose is simultaneously reduced to mannitol by mannitol dehydrogenase, and one mol of fructose catalyzed the net production of 1 mol of $NAD^+$. Thus, production of a high yield of mannitol requires sufficient NADH to direct the fructose flux toward the mannitol synthesis pathway, so nicotinic acid (NA) was added in fermentation medium to manipulate the intracellular pool of NADH. The specific activity of MDH was consistently increased with the intracellular NADH level and the mannitol overall yield was increased from 0.69 to 0.95 g/g (Zhang et al. 2017). It was demonstrated that manganese ion plays a central role in the production of reducing power NADH and is, therefore, essential for cellular functions and more important for transportation and reduction of fructose. Addition of trace amount of 0.033 g/L manganese sulfate into fermentation medium resulted in the production of 200.6 g mannitol by *Lactobacillus intermedius* NRRL B-3693, during which the mannitol yield has reached its theoretical value of 0.67 (Saha et al. 2006a, b, c). The yield of mannitol during microbial fermentation has been reported to be increased after the addition of certain minerals, such as $Ca^{2+}$ and $Cu^{2+}$. The ion of $Ca^{2+}$ can alter the permeability of cells to mannitol, and the $Cu^{2+}$ can increase the activity of MDH, which was responsible for mannitol biosynthesis. A final mannitol production with *C. magnoliae* HH-01 yeast reached 223 g/L when fructose-glucose medium was supplemented with $Ca^{2+}$ and $Cu^{2+}$ (Meng et al. 2017). Likewise, addition of $Ca^{2+}$ has successfully enhanced the mannitol production from glycerol in *C. azyma* (Yoshikawa et al. 2014).

## Future prospects

Microbial fermentation is a promising approach for mannitol production with high yield, which could not be achieved by chemical or enzyme methods. Research on the utilization of low-cost substrates, strain development and process regulation strategies makes it possible to produce mannitol in an economic way. However, when using low-cost substrates, the mannitol concentration was much lower than that using fructose, which might be caused by the low efficiency in conversion of substrates into the precursor of mannitol. Therefore, further work in improving the utilization of low-cost substrate should be considered. Besides, it seems that LAB is a suitable cell factory for mannitol production, while the technology absence in inactivation genes of LAB has limited its further improvement in mannitol yield. Development in genetic tools for manipulation of LAB metabolic pathway, especially the metabolic flux of reducing cofactors, is also a potential way to obtain high yield of mannitol, while the safety of mannitol produced by genetically engineered producers could be of concerns. Combined strategies in using low-cost substrate, strain development and process regulation should be used to improve process efficiency.

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## Compliance with ethical standards

**Conflict of interest** The authors declares that they have no conflict of interest.

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