Enhanced Biosynthesis of D-arabitol by Metschnikowia Reukaufii Through Optimizing Medium Composition and Fermentation Conditions

Jiaqi Huang
Jiangsu University School of Food and Biological Engineering
Yingfeng An
Shenyang Agricultural University
Hossain M. Zabed
Jiangsu University School of Food and Biological Engineering
Yuvaraj Ravikumar
Jiangsu University School of Food and Biological Engineering
Mei Zhao
Jiangsu University School of Food and Biological Engineering
Junhua Yun
Jiangsu University School of Food and Biological Engineering
Guoyan Zhang
Jiangsu University School of Food and Biological Engineering
Yufei Zhang
Jiangsu University School of Food and Biological Engineering
Xiaolan Li
Jiangsu University School of Food and Biological Engineering
Xianghui Qi (qxh@ujs.edu.cn)
Jiangsu University https://orcid.org/0000-0002-5817-1609

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Abstract

$_D$-arabitol is an important functional sugar alcohol, which can be used in the preparation of foods, chemicals, and medicines. Despite biological production of $_D$-arabitol from low-cost substrates has recently been the focus of research, low yield of this technology has limited its large-scale exploitation. Optimization of this bioprocess could be a promising option to improve the yield of $_D$-arabitol. In this study, one-factor-at-a-time (OFAT) strategy and Box-Behnken design (BBD) were used to increase $_D$-arabitol production by *Metschnikowia reukaufii* CICC 31858 through optimizing the fermentation conditions and medium composition. The OFAT optimization provided the optimal conditions for temperature, agitation speed, and fermentation time of 30℃, 220 rpm, and 144 h, respectively. Likewise, the optimum concentrations of peptone, ammonium sulfate, KH_2PO_4, $MgSO_4{\cdot}7H_2O$, and fumaric acid in the fermentation medium were (g/L) 7.5, 1, 2 , 0.5 and 7.5, respectively. Under these optimum conditions, 80.43 g/L of $_D$-arabitol was produced from 200 g/L of glucose, with a productivity of 0.56 g/L/h. The BBD optimization with three important components of fermentation medium (KH_2PO_4, $MgSO_4{\cdot}7H_2O$ and fumaric acid) showed that the predicted titer of $_D$-arabitol varied from 47.21 g/L to 89.27 g/L, and the actual titer of $_D$-arabitol ranged from 47.36 to 89.83 g/L. The optimum concentrations (g/L) of KH_2PO_4, $MgSO_4{\cdot}7H_2O$, and fumaric acid in the fermentation medium were found to be 1.0, 0.5, and 4.7g/L, respectively. Under the optimum conditions, 92.45 g/L of $_D$-arabitol was finally produced with the yield and productivity of 0.46 g/g and 0.64 g/L/h, respectively.

Introduction

Over the past years, low-caloric sweeteners have become increasingly important to the diabetics and obese patients, such as saccharin, sucralose, aspartame, neotame, and acesulfame potassium, which have higher sweetness than sucrose (Roberts 2016). However, some harmful effects, including the negative effects on intestinal microbial composition, have made them unattractive as the sugar substitutes. In contrast, rare sugars and polyols like $_D$-psicose, $_D$-tagatose, and $_D$-arabitol, have been shown to have health benefits, making them attractive to researchers and food industries (Felipe Hernández-Pérez et al. 2019; Ravikumar et al. 2021). Belonging to a pentitol family, $_D$-arabitol ($C_5H_{12}O_5$, M.wt 152) has a caloric value (0.2 kcal/g) much lower than sucrose (4 kcal/g), which is present naturally in low quantities in yeast, lichens, mushroom, and higher fungi (Kordowska-Wiater 2015). Besides, $_D$-arabitol also can be used as a platform material to synthesize commercially essential compounds such as xylitol, arabinoic and xylonic acids, propylene, and ethylene glycol enantiomeric compounds (Yoshikawa et al. 2014). Owing to such benefits, the United States Department of Energy has included $_D$-arabitol in the list of twelve useful building block chemicals (Kordowska-Wiater 2015).

$_D$-arabitol production by fermentation was mainly done by the osmophilic yeasts, such as species from *Zygosasaccharomyces* (Guo et al. 2019; Qi et al. 2015; Saha et al. 2007), *Candida* (Sánchez-Fresneda et al. 2013; Song et al. 2011; Zheng et al. 2020), *Debaryomyces* (Koganti and Ju 2013), *Metschnikowia*

(Nozaki et al. 2003), and *Candida* (Kumdam et al. 2014; Kordowska-Wiater 2015). The mechanism accumulating polyols by the osmophilic yeasts under osmotic stress is that higher osmolarity levels outside the cells builds hyperosmotic stress, leading to water loss from cells. To prevent water loss under such conditions, yeasts start to synthesize and accumulate various polyols like glycerol, erythritol, xylitol, arabitol, and mannitol in the cytoplasm (Van Eck et al. 1993). The most common pathway for $_D$-arabitol biosynthesis from $_D$-glucose by yeasts is the pentose phosphate pathway (Fig. 1), where glucose is first phosphorylated into glucose-6-phosphate, which is subsequently dephosphorylated into $_D$-ribulose by $_D$-ribulose-5-phosphate. The $_D$-arabitol 2-dehydrogenase (E.C. 1.1.1.250) then converts $_D$-ribulose to $_D$-arabitol. In another alternative pathway, glucose-6-phosphate is converted into $_D$-xylulose-5-phosphate, which is further dephosphorylated to $_D$-xylulose. Finally, the $_D$-arabitol 4-dehydrogenase (E.C. 1.1.1.11) converts $_D$-xylulose into $_D$-arabitol (Saha et al. 2007). Additionally, pathways involved in transforming glycerol, xylose, and sorbitol were reported in *Saccharomyces cervisiae, Aerobacter aerogenes,* and *Zygosaccharomyces rouxii* (Gancedo et al. 1968).

One of the limitations with the reported microorganisms is the low levels of $_D$-arabitol under normal fermentation conditions and that have constrained their industrial applications. Therefore, researchers have attempted various methods to improve the titer and yield of $_D$-arabitol. For example, adaptive laboratory evolution, one of the efficient strategies for strain improvement, was employed on *Pichia pastoris* strain, which resulted 72.7% higher yield than the wild strain (Cheng et al. 2014). By using pyrithioxin dihydrochloride, glucose-6-phosphate dehydrogenase (G6PDH) activity was enhanced, which in turn resulted in greater $_D$-arabitol synthesis (Zheng et al. 2020). These studies, although resulted in enhanced production of $_D$-arabitol, the process involved is laborious and cumbersome. Alternatively, optimization of the fermentation conditions remain an easy way to achieve higher titers and was also found to be highly effective in other biosynthesis approaches(Narisetty et al. 2017; Yang et al. 2019; Zabed et al. 2019). For instance, *Z. rouxii,* a novel strain isolated from raw honey, produced 76.32 g/L $_D$-arabitol with 38.16 % conversion efficiency under the optimum conditions in batch fermentation experiments (Guo et al. 2019). In this studdy, OFAT technique and response surface methodology (RSM) are widely used for optimizing bioprocesses (Chen et al. 2013; Narisetty et al. 2017). At present, there is few research (Nozaki et al. 2003) on the production of $_D$-arabitol by *M. reukaufii*, and the research on optimizing its fermentation conditions and medium composition with OFAT and RSM was not done yet. In this study, the utilization of *M. reukaufii* for $_D$-arabitol synthesis from cheap carbon source, like glucose, was demonstrated. The medium composition and fermentation conditions were optimized by both OFAT and BBD method to achieve a higher conversion of $_D$-glucose with maximum $_D$-arabitol production.

Materials And Methods

Yeast strain and fermentation medium

The yeast strain *M. reukaufii* CICC 31858 was purchased from China Industrial Microbial Culture Collection Management Center (CICC). YPAD medium ($_D$-glucose 20 g/L, peptone 20 g/L, agar 20 g/L, and

yeast extract 10 g/L) was used to streak and separate the strains. Single colony was chosen and inoculated into 50 mL of YPD medium ($_D$-glucose 20 g/L, peptone 20 g/L, and yeast extract 10 g/L) and incubated for 24 h at 30℃ with rotation speed set at 200 rpm. The fermentation medium was then inoculated at a volume ratio of 3% (v/v).

Preparation of fermentation inoculum

To begin the fermentation experiments, the seed culture of *M. reukaufii* CICC 31858 was initially prepared in a 250 mL Erlenmeyer flask consisting of 50 mL YPD medium and grow in a rotary shaker (200 rpm) at 30℃ for 24 h. The biomass concentration of growing cells is periodically monitored by measuring the optical density (OD_{600}). The obtained OD_{600} values are subsequently converted to dry cell weight (DCW) using a pre-defined calibration curve described in Guo et al(Guo et al. 2019).

Determination of the influence of culture conditions on the D-arabitol production

The effects of temperature, agitation speed, and fermentation time on $_D$-arabitol biosynthesis by *M. reukaufii* CICC 31858 were studied by a OFAT approach. The fermentation medium, consisting of $_D$-glucose 200 g/L, peptone 5 g/L, ammonium sulfate 1 g/L, KH_2PO_4 2 g/L, $MgSO_4{\cdot}7H_2O$ 2.5 g/L and fumaric acid 3 g/L, was used for all the experiments unless any changes were specified. During OFAT optimization, the target parameters were studied individually, where the experiments are designed as follows; (i) temperature (28 ℃, 30 ℃, 33 ℃, 35 ℃, 37 ℃, 40 ℃), (ii) rotation speed (100 rpm, 150 rpm, 200 rpm, 220 rpm, 250 rpm) and (iii) fermentation days (1, 2, 3, 4, 5, 6, 7, 8). All experiments were carried out in triplicate, and the results obtained were analyzed and expressed statistically.

Optimization of the medium composition

Five different nitrogen content sources were tested to find the suitable nitrogen source, which were peptone, yeast extract, beef extract, ammonium sulfate, and urea. In the nitrogen source determination experiments, the total content of nitrogen in the medium was maintained 1 g/L. With varying nitrogen substrates, the fermentation medium comprises the following constituents: $_D$-glucose 200 g/L, KH_2PO_4 2 g/L, $MgSO_4{\cdot}7H_2O$ 2.5 g/L, fumaric acid 3 g/L. After obtained the most suitable nitrogen source, all components of the fermentation medium were also optimized by studying varying concentrations using the method of OFAT.

Optimization of culture conditions using Box-Behnken design (BBD)

A BBD experiment was employed for optimizing the three most important components of the fermentation medium, such as KH_2PO_4, $MgSO_4{\cdot}7H_2O$, fumaric acid concentration. The resulting matrix of BBD experiments for the factors tested is summarized in Table 1. The minimum and maximum concentrations for each parameter are determined initially, ensuring optimum concentrations would present within the range obtained. In this design, the titer of $_D$-arabitol was considered as the response

value, the key three variables as the independent variables, and experimental design and data analysis were performed for each single factor.

Table 2 showed the results of tabulating the obtained center value for KH_2PO_4, $MgSO_4 \cdot 7H_2O$, fumaric acid. A total of 17 medium formulations were obtained through BBD design which includes 5 center points. A 200 g/L of $_D$-glucose was used as a substrate and sole carbon source for all the experiments that involve $_D$-arabitol biosynthesis. The fermentation experiments at optimized conditions were done in 20 mL culture volume for 6 days at 220 rpm and 30 ℃.

Analytical Methods

The amount of $_D$-arabitol, glucose, glycerol, and ethanol were measured by using high-performance liquid chromatography (HPLC), where Refractive Index Detector (RID) and Aminex®HPX-87H Column 300×7.8 mm (Bio-Rad Laboratories, Hercules, CA, USA) were equipped. 5 mmol/L sulfuric acid was used as a mobile phase. The column temperature was maintained at 65℃; the flow rate was fixed to 0.6 mL/min and 10 µL sample was injected in HPLC. Before HPLC analysis, samples were centrifuged at 12000 rpm for 10 minutes and supernatant were analyzed.

Statistical analysis

The Box-Behnken design and data analysis were carried out in the Design Expert. In OFAT experiments for optimizing the fermentation conditions, significant variations among the various experimental conditions were determined by one-way ANOVA (analysis of variance) and Tukey Post-hoc test using Minitab Statistical Software. All the statistical significances were considered at a 95% confidence level ($p < 0.05$).

Results And Discussion

Effects of fermentation conditions on the production of $_D$-arabitol

Effects of temperature

The role of temperature in the yeast fermentation process is crucial as the temperature out of optimum range can affect cell growth, biomass and yield. Various studies have proved that during the middle and late stages of fermentation, temperature plays a key role in metabolite synthesis, regulation of enzymes, and biosynthesis of target products (Liszkowska and Berlowska 2021). Considering the significance of temperature, the effects of different temperatures on $_D$-arabitol synthesis were investigated, ranging from 25 to 40 ℃. Biomass , $_D$-glucose conversion, and $_D$-arabitol production were determined at 25, 28, 30, 33, 35, 37, and 40 ℃. As shown in Fig. 2a, the $_D$-arabitol concentration peaked with 21.51 g/L at 30 ℃. A further rise in temperature led to a decrease in $_D$-arabitol production. In particular, a significant decline was observed at 35-40 ℃, and $_D$-arabitol production decreased to 6.75 g/L. These results indicate that a temperature over 30 ℃ is not suitable for producing $_D$-arabitol. The probable reason might be that higher

temperature induces stress on the microbial metabolism, thereby reducing physiological response and curtailment of $_D$-arabitol synthesis.

Furthermore, the synthesis of byproducts such as glycerol and ethanol might also be the reason for the lower production of $_D$-arabitol. As illustrated in Fig 2a, glycerol concentration continuously decreased as the temperature increased. On the other hand, ethanol production increased gradually with an increase of temperature from 25 to 35 ℃. $_D$-glucose utilization, which served as the substrate for $_D$-arabitol biosynthesis, gradually reduced with the rise of temperatures. Maximum $_D$-glucose utilization was observed at 28 ℃. And at the higher temperatures, at 37 ℃ and 40 ℃, lower $_D$-glucose conversion led to producing a lower amount of $_D$-arabitol. Therefore, the temperature had significant roles on substrate utilization, $_D$-arabitol biosynthesis, and byproducts synthesis. Although the production of by-product glycerol was relatively high, considering that the yield of target product $_D$-arabitol reached the highest at 30 ℃, and the yield of by-product ethanol was also low, 30 ℃ was selected as the optimum temperature for further fermentation experiments, which was in accordance with the literature where the optimal temperature for was reported between 25 ℃ and 30 ℃ (Beltran et al. 2008; Toyoda and Ohtaguchi 2011).

Effects of rotation speed and fermentation times

Agitation plays an important role in achieving higher $_D$-arabitol yield during fermentation as it possibly can increase the nutrient permeability across the membrane. It is noteworthy that agitation helps to maintain good oxygen exchange transfer between air and cells present in the medium (Kumdam et al. 2013). Therefore, in order to elucidate the positive effect of agitation speed on $_D$-arabitol biosynthesis, various agitation conditions were explored. The rotation speed (rpm) results on $_D$-arabitol biosynthesis were determined by subjecting the culture flasks containing the host strain at various agitation (150 rpm, 180 rpm, 200 rpm, 220 rpm, 250 rpm), keeping other fermentation conditions unchanged. The results were presented in Fig. 2b. With an increase in rpm, both $_D$-glucose utilization and $_D$-arabitol production increased considerably. Maximum $_D$-arabitol (28.95 g/L) was produced at 220 rpm. A further increase of agitation to 250 rpm showed a reduction in $_D$-arabitol titer and an increase in the accumulation of residual $_D$-glucose. Significant differences in $_D$-arabitol concentrations were found in all the tested rpm conditions, and the optimum agitation speed was selected as 220 rpm for further experiments.

The growth of microorganisms during fermentation is dependent on time. A reasonable growth rate is essential to achieve a good product yield. A shorter fermentation period resulted in lower cell biomass, while a longer duration can exert toxicity to growing cells. This is because some byproducts that were synthesized during fermentation can become toxic to cells (Akinosho et al. 2015). Therefore, it is inevitable to determine the optimal fermentation period for attaining improved product titers. the outcome of time on fermentation was evaluated in accordance with substrate conversion, $_D$-arabitol formation, and byproduct synthesis (Fig. 2c). The results showed that the synthesis of $_D$-arabitol increased rapidly before day 6 and then stabilized. Around 30.82 g/L of $_D$-arabitol was obtained on day 6. Similar trends were

observed in glycerol and ethanol production as byproducts. Furthermore, after the day 6, the substrate D-glucose was no longer consumed. These results indicate that the optimum day for achieving maximum $_D$-arabitol yield was 6. The probable reason for such response could be an accumulation of higher levels of byproducts which could change the culture medium pH. It must also be noted that nutrients in the culture medium might get depleted as the day progresses, and the host cells could have stopped growing after reaching the stationary growth phase. The experimental results obtained here could provide an insight into understanding the relationship between fermentation conditions and $_D$-arabitol production.

Effects of medium composition on $_D$-arabitol fermentation

Effects of nitrogen source

Nitrogen source plays a vital role in microbial growth, metabolism, and product formation. It serves as a precursor material for synthesizing proteins, nucleic acids, enzymes, biological macromolecules, enzymes, and other nitrogen-containing compounds (Fairbairn et al. 2017). Microorganisms like yeast can assimilate organic and inorganic nitrogen sources, although sometimes preferred according to the chemical characteristics. Peptone, yeast extract, and whey act as organic N-sources, while NH_3, NH_4^+, NO_3^- and NO_2^- act as inorganic nitrogen sources. Thus, five different nitrogen sources (peptone, yeast extract, beef extract, ammonium sulfate, and urea) were utilized to demonstrate the effect of nitrogen sources on $_D$-arabitol production, and the most effective nitrogen source was determined through the single factor fermentation experiment. From Fig. 3a, it is clear that organic nitrogen sources (peptone, yeast extract, beef extract) were more effective than inorganic nitrogen sources (ammonium sulfate, urea) in promoting the microbial conversion of $_D$-glucose into $_D$-arabitol. Maximum $_D$-arabitol concentration of 26.24 g/L was obtained using peptone as a nitrogen source, while yeast extract and beef extract did not produce a significant $_D$-arabitol. This yield is lower than that found in section 3.1 (30.82 g/L) because the original nitrogen sources (peptone 5 g/L, ammonium Sulfate 1 g/L) were replaced with a single nitrogen source with a net content of 1 g/L. Therefore, after fixing the nitrogen source concentration to 1 g/L to obtain the optimal nitrogen source, the optimal concentration of the best nitrogen source still needs to be investigated.

On the other hand, no or low $_D$-arabitol synthesis was observed in both inorganic nitrogen sources. The reason might be an insufficient supply of nutrients from nitrogen sources, which was also evident by the low biomass concentration. With these findings, an attempt was made to investigate the combined effect of both organic and inorganic nitrogen sources for achieving a higher $_D$-arabitol concentration. Two nitrogen sources were selected for each time to evaluate the impact on $_D$-arabitol yield. As depicted in Fig.3b, excessive organic nitrogen sources did not influence the growth of *M. reukaufii* and the synthesis of $_D$-arabitol. Among the different combinations tested, peptone and ammonium sulfate (PAS), and beef powder and ammonium sulfate (BPAS) were identified as the best nitrogen sources. Under these two conditions, $_D$-arabitol production was significantly higher than in other conditions. Also, the conversion rate of $_D$-glucose was maximum in PAS than in other nitrogen sources. The highest titer of $_D$-arabitol

reached 38.16 g/L using PAS as nitrogen source, whereas peptone as the nitrogen source alone produced 26.24 g/L $_D$-arabitol. Therefore, combined use of peptone and ammonium sulfate in the fermentation medium would be the best choice for $_D$-arabitol production by *M. reukaufii*.

The effect of different concentrations of peptone on $_D$-arabitol biosynthesis was investigated (Fig. 4a). With the increase of peptone concentration, the consumption of $_D$-glucose increased gradually, which in turn increased the production of $_D$-arabitol, glycerol, and ethanol with the rise in peptone concentration up to 7.5 g/L, and then decreased with the increase in peptone concentration to 10 g/L. The highest yield of $_D$-arabitol was 51.38 g/L, which was significantly higher than that before the optimization of peptone concentration (38.16 g/L). The reduction of product biosynthesis at higher peptone concentration could be due to the excessive growth of the *M. reukaufii* and the increase of $_D$-glucose consumption for the growth and metabolism of the *M. reukaufii*, resulting in the low conversion rate of the product. Next, to determine the optimum ammonium sulfate concentration, five different ammonium sulfate concentrations, ranging from 0 g/L to 2.5 g/L, were studied (Fig. 4b). As seen in Fig. 4b, when the ammonium sulfate concentration was 1 g/L, the yield of $_D$-arabitol was significantly higher than that of other ammonium sulfate concentrations, and the amount of residual $_D$-glucose was much lower at this concentration. The biomass of *M. reukaufii* also reached the highest when ammonium sulfate was 1 g/L. Also, no significant differences in biomass concentration were found in all the tested concentrations. From the above results, it can be determined that the optimal nitrogen sources were peptone 7.5 g/L and ammonium sulfate 1 g/L.

Effects of inorganic salts

Besides sugar and nitrogen concentrations, an adequate supply of inorganic ions is important for enhancing the fermentation process. In particular, metal ions, sometimes called minerals present in culture media, can improve the fermentation efficiency and yield. For example, phosphate-containing salts are required by yeasts for nucleic acid, adenosine triphosphate (ATP), and phospholipid synthesis (Walker and Stewart 2016). Likewise, sulfur-containing amino acids are synthesized from inorganic sulfate compounds. Any deficiency in the supply of these inorganic compounds can lead to improper metabolism and eventually affecting cell growth. Thus, determining the optimum salt concentrations in the fermentation media is essential. By determining the optimal concentration of KH_2PO_4 and $MgSO_4{\cdot}7H_2O$ that affect the metabolism of *M. reukaufii*, a higher production of $_D$-arabitol can be achieved. The fermentation medium was prepared with 0 g/L to 2.5 g/L of KH_2PO_4 and $MgSO_4{\cdot}7H_2O$, respectively, to study their effects on $_D$-arabitol yield. It can be proved that the titer of $_D$-arabitol increased with the increase of KH_2PO_4 concentration, and the maximum of $_D$-arabitol (41.62 g/L) was attained when KH_2PO_4 was 2 g/L (Fig. 4c). Likewise, the highest $_D$-arabitol concentration (53.57 g/L) was found at 0.5 g/L of $MgSO_4{\cdot}7H_2O$ (Fig. 4d). In both the inorganic salts, a similar trend of gradual increase with respect to optimum concentration and decreased at higher concentrations was observed.

Effects of fumaric acid

Fumaric acid, an intermediate in the tricarboxylic acid cycle, can act as an exogenous electron acceptor during the fermentation process (Rhee and Sohn 2003). Since the ratio of NADH/NAD^+ is vital for achieving higher $_D$-arabitol yield, it is worth interrogating the influence of fumaric acid on $_D$-arabitol production, as it helps in balancing the ratio of NADH/NAD^+ within the cells. As shown in Fig. 4e, fermentation with *M. reukaufii* was carried out under varying concentrations of fumaric acid (0–12.5 g/L). It can be noted that the titer of $_D$-arabitol increased significantly during fermentation when the concentration of fumaric acid increased to 7.5 g/L. At this concentration, the residual amount of $_D$-glucose present in the medium was meager. Also, the highest amount of glycerol was observed at 7.5 g/L concentration, while another byproduct, ethanol, was found to be in minimal quantities. Notably, the highest amount of biomass was also measured at this concentration. Overall, the result suggests that 7.5 g/L of fumaric acid could be the optimum concentration for attaining more $_D$-arabitol. The production of $_D$-arabitol obtained under the optimum fumaric acid concentration was 72.67 g/L.

These optimum fermentation conditions together with optimum fermentation medium ($_D$-glucose 200 g/L, peptone 7.5 g/L, ammonium sulfate 1 g/L, KH_2PO_4 2 g/L, $MgSO_4 \cdot 7H_2O$ 0.5 g/L and fumaric acid 7.5 g/L), produced 80.43 g/L $_D$-arabitol.

Optimization of medium composition by Response Surface Methodology (RSM)

Through the BBD experimental design of RSM, the optimal fermentation medium was obtained for achieving maximum $_D$-arabitol production. As glucose was the sole substrate needed for $_D$-arabitol biosynthesis and nitrogen source was essential for microbial growth, these two factors were not considered in this optimization. Therefore, based on the preliminary findings, 200 g/L of $_D$-glucose and nitrogen concentration (peptone 7.5 g/L and ammonium sulfate 1 g/L) was used. Based on the results discussed in previous sections, temperature, rotation rate, and fermentation times were maintained at 30 °C, 220 rpm, 6 d, respectively. The remaining factors, such as KH_2PO_4 (X_1), $MgSO_4 \cdot 7H_2O$ (X_2),and fumaric acid (X_3) were subjected for the optimization. In the OFAT experiment, the yield of $_D$-arabitol was the highest when the concentration of fumaric acid was 7.5 g/L, but there was little difference with the yield of $_D$-arabitol when the concentration was 5 g/L. Consequently, it was chosen as the center point. Similarly, we adjusted the center point of KH_2PO_4 to 1 g/L.

A total of 17 experiments were performed with three independent variables at three levels (1, 0, and +1). The results of 17 experimental runs are given in Table 2. According to these results, the observed values for the concentration of $_D$-arabitol varied from 47.36 to 89.83 g/L under various experimental conditions. The results were analyzed in Design-Expert software. The statistical significance of the quadratic regression equation was checked by analysis of variance (ANOVA) (Table 3). The *p*-value of this regression model was <0.0001, which indicated that the regression equation obtained by the model was highly significant. The *p-value* of the lack of fit was 0.9306, which was not significant. The regression coefficient (R^2) was used to determine the fitting efficiency of the model. The R^2 and corrected quadratic model was 0.9993 and 0.9990, respectively, which were very close to 1. This showed that the model fit

well with the data, revealing that 99% of the variability in the response could be described by this polynomial model. It is also equally important to observe that the p-value and values obtained below 0.05 demonstrated the model terms are statistically significant. Except the term X_1 ($p > 0.05$), all tested model iterms were substantial, having $p < 0.05$. After quadratic regression fitting, the simulation equation between the three factors and the $_D$-arabitol production was obtained. The variables were expressed as their coded values in Eq. (1). One insignificant interaction ($+0.068X_1$) was omitted from this equation due to its higher p-values, $p > 0.05$ ($p = 0.6774$). The significance level of term selection was $p \leq 0.05$.

$$Y=+89.27+2.12X_2-4.21X_3+3.48X_1X_2-2.19X_1X_3-1.34X_2X_3-12.01X_1^2-18.06X_2^2-19.01X_3^2 \quad (1)$$

where, Y is the predicted yield of $_D$-arabitol (g/L); X_1, X_2, X_3 are KH_2PO_4, $MgSO_4 \cdot 7H_2O$ and fumaric acid respectively; $p < 0.01$ indicated that the model is extremely significant.

By evaluating the contour plots and three-dimensional response surface plots, the interaction between three medium additives and the best level of them that significantly impact the response was estimated. Fig. 5 shows the contour plots and response surface plots of the effect of fermentation medium composition on glucose production of $_D$-arabitol. As seen in Fig. 5, the center area has the best clarity. In particular, under various interactions, the concentration of $_D$-arabitol increased with the increase of the amount of three medium additives until the concentration of these components reached the central point value. Then, the titer of $_D$-arabitol decreased at the highest additives value. The Design-Expert software predicted the optimization fermentation medium composition. A maximum of 89.58 g/L of $_D$-arabitol was obtained under the following conditions: KH_2PO_4 concentration of 1.0 g/L, $MgSO_4 \cdot 7H_2O$ concentration of 0.5 g/L, fumaric acid concentration of 4.7 g/L. To validate the RSM findings, in vitro experiments were carried out under the optimum fermentation medium composition as obtained from RSM studies. The $_D$-arabitol concentration reached 92.45 g/L, which was very close to the prediction value (89.58 g/L).

Similar to the results obtained in this study, various studies reported $_D$-arabitol production from glucose were demonstrated by *Z. rouxii*, *M, reukaufii*, *D. hansenii*, *P. miso*, *C. albicans* and *P. ohmerii*. Although several studies with various strains have been reported to date, most of them are not suitable for industrial applications due to lower $_D$-arabitol yield or co-synthesis of other metabolites. Also, few studies have optimized the medium components for the production of $_D$-arabitol, in a previous study, medium optimization was done to improve $_D$-arabitol yield from *Z. rouxii* JM-C46. After using optimized medium composition, a higher $_D$-arabitol of up to 76.32 g/L was produced with 0.8 g/L/h of volumetric productivity. This study reports a higher $_D$-arabitol concentration (92.45g/L) by using *M. reukaufii* CICC 31858 through comprehensive optimization of medium composition and fermentation conditions. Nozaki (Nozaki et al. 2003) studied $_D$-arabitol production by a *Metschnikowia reukaufii* strain, named AJ 14787, and obtained 26.3 g/L $_D$-arabitol (100 g/L $_D$-glucose, fermentation time 24 h) using shaking flask fermentation, while they reported a $_D$-arabitol titer of 206 g/L using resting cells and continuously feeding

high concentration (700 g/L) of $_D$-glucose in bioreactor, the yield of $_D$-arabitol was 0.29 g/g (Nozaki et al. 2003), which was lower than the yield of this study (0.46 g/g). Despite the titer obtained from the later technology was higher than the titer of this study, the present study was confined to only batch fermentation. By continuously monitoring the conditions in the fermentation process, biosynthesis in the bioreactor may lead to higher yield(Loman et al. 2018; Qi et al. 2015).

Conclusions

This work presents the optimization of fermentation medium components for the improved production of $_D$-arabitol by *M. reukaufii*. A total of eight key factors, that are directly associated with glucose consumption, $_D$-arabitol production, and biomass accumulation, were investigated. Based on the $_D$-arabitol production, the optimum conditions for three physical factors, such as temperature, rotation speed, and fermentation time were found to be 30 °C, 220 rpm, and 144 h, respectively. Likewise, the optimum composition of the fermentation media included $_D$-glucose 200 g/L, peptone 7.5 g/L, ammonium sulfate 1 g/L, KH_2PO_4 2 g/L, $MgSO_4 \cdot 7H_2O$ 0.5 g/L and fumaric acid 7.5 g/L. Under the optimum conditions of fermentation medium and conditions, 80.43 g/L of $_D$-arabitol was produced. The $_D$-arabitol titer was further increased to 92.45 g/L under modified optimum conditions of three medium components obtained from the BBD experiments that resulted a change in KH_2PO_4 from 2 to 1 g/L, fumaric acid from 7.5 to 4.7 g/L. The findings of this research work could be an important reference for the improved biosynthesis of $_D$-arabitol, as well as other fermentation-mediated biosynthesis technologies.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors confirm that they have no conflicts of interest with respect to the work described in this manuscript.

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Author Contributions

Jiaqi Huang: Conceptualization, Investigation, Formal analysis, Writing-original draft. **Yingfeng An**: Investigation, Formal analysis, Writing-review & editing. **Hossain M Zabed**: Conceptualization, Writing-review & editing. **Yuvaraj Ravikumar**: Writing-review & editing. **Mei Zhao:** Writing-review & editing. **Junhua Yun**: Formal analysis. **Guoyan Zhang**: Resources, Formal analysis. **Yufei Zhang:** Resources, Formal analysis. **Xiaolan Li**: Investigation, Formal analysis. **Xianghui Qi**: Conceptualization, Writing - review & editing, Supervision.

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Tables

Table 1 BBD for optimizing $_{D}$-arabitol fermentation medium using three components with three levels.

Factors	Unit	Low level (-1)	Medium level (0)	High level (+1)
KH_2PO_4	g/L	0.5	1	1.5
$MgSO_4 \cdot 7H_2O$	g/L	0	0.5	1
Fumaric Acid	g/L	2.5	5	7.5

Table 2 Matrix and results of BBD for medium optimization.

Runs	X_1 (KH_2PO_4, g/L)	X_2 ($MgSO_4 \cdot 7H_2O$, g/L)	X_3 (Fumaric acid, g/L)	$_D$-arabitol concentration (g/L)	
				Observed	Predicted
1	-1(0.5)	0(0.5)	-1(2.5)	60.35	60.20
2	0(1)	0(0.5)	0(5)	88.55	89.27
3	0(1)	0(0.5)	0(5)	88.82	89.27
4	-1(0.5)	1(1)	0(5)	57.76	57.77
5	1(1.5)	0(0.5)	-1(2.5)	64.80	64.71
6	0(1)	1(1)	1(7.5)	48.87	48.77
7	0(1)	0(0.5)	0(5)	89.55	89.27
8	0(1)	0(0.5)	0(5)	89.61	89.27
9	1(1.5)	-1(0)	0(5)	53.68	53.67
10	-1(0.5)	-1(0)	0(5)	60.45	60.50
11	-1(0.5)	0(0.5)	1(7.5)	56.08	56.16
12	0(1)	-1(0)	-1(2.5)	52.86	52.95
13	1(1.5)	0(0.5)	1(7.5)	51.77	51.92
14	0(1)	0(0.5)	0(5)	89.83	89.27
15	0(1)	-1(0)	1(7.5)	47.36	47.21
16	1(1.5)	1(1)	0(5)	64.93	64.87
17	0(1)	1(1)	-1(2.5)	59.72	59.86

Table 3 ANOVA analysis of BBD response variables.

Source	Sum of Squares	Df	Mean Square	F-value	p-Value
Model	4146.63	9	460.74	2380.52	<0.0001
X_1-KH_2PO_4	0.036	1	0.036	0.19	0.6774
X_2- $MgSO_4 \cdot 7H_2O$	35.86	1	35.86	185.29	<0.0001
X_3-Fumaric acid	141.67	1	141.67	732.00	<0.0001
X_1X_2	48.57	1	48.57	250.97	<0.0001
X_1X_3	19.18	1	19.18	99.10	<0.0001
X_2X_3	7.16	1	7.16	36.99	0.0005
X_1^2	607.47	1	607.47	3138.63	<0.0001
X_2^2	1373.07	1	1373.07	7094.35	<0.0001
X_3^2	1521.90	1	1521.90	7863.31	<0.0001
Residual	1.35	7	0.19		
Lack of Fit	0.13	3	0.043	0.14	0.9306
Pure Error	1.23	4	0.31		
Cor Total	4147.98	16			

Figures

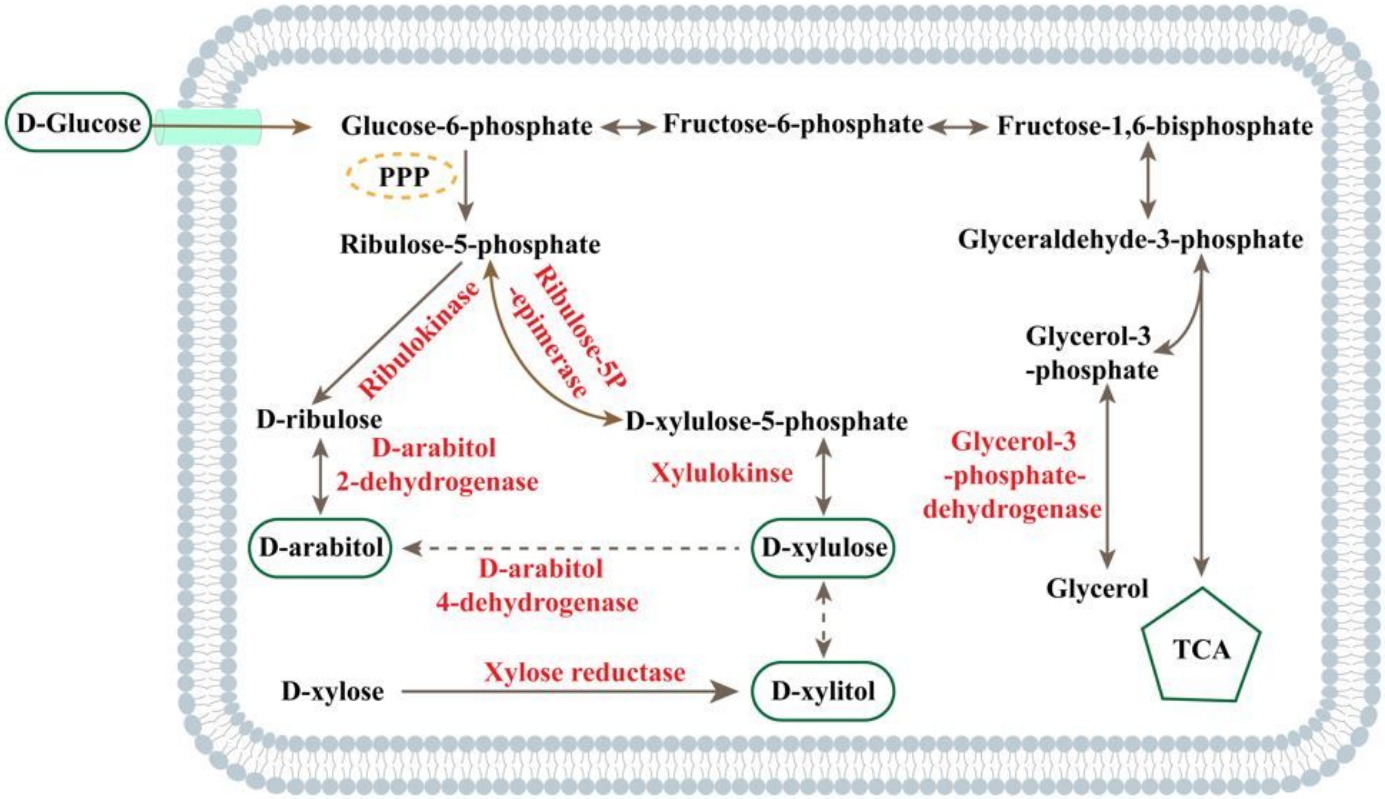

Figure 1

Metabolic pathways for the microbial production of D-arabitol from glucose

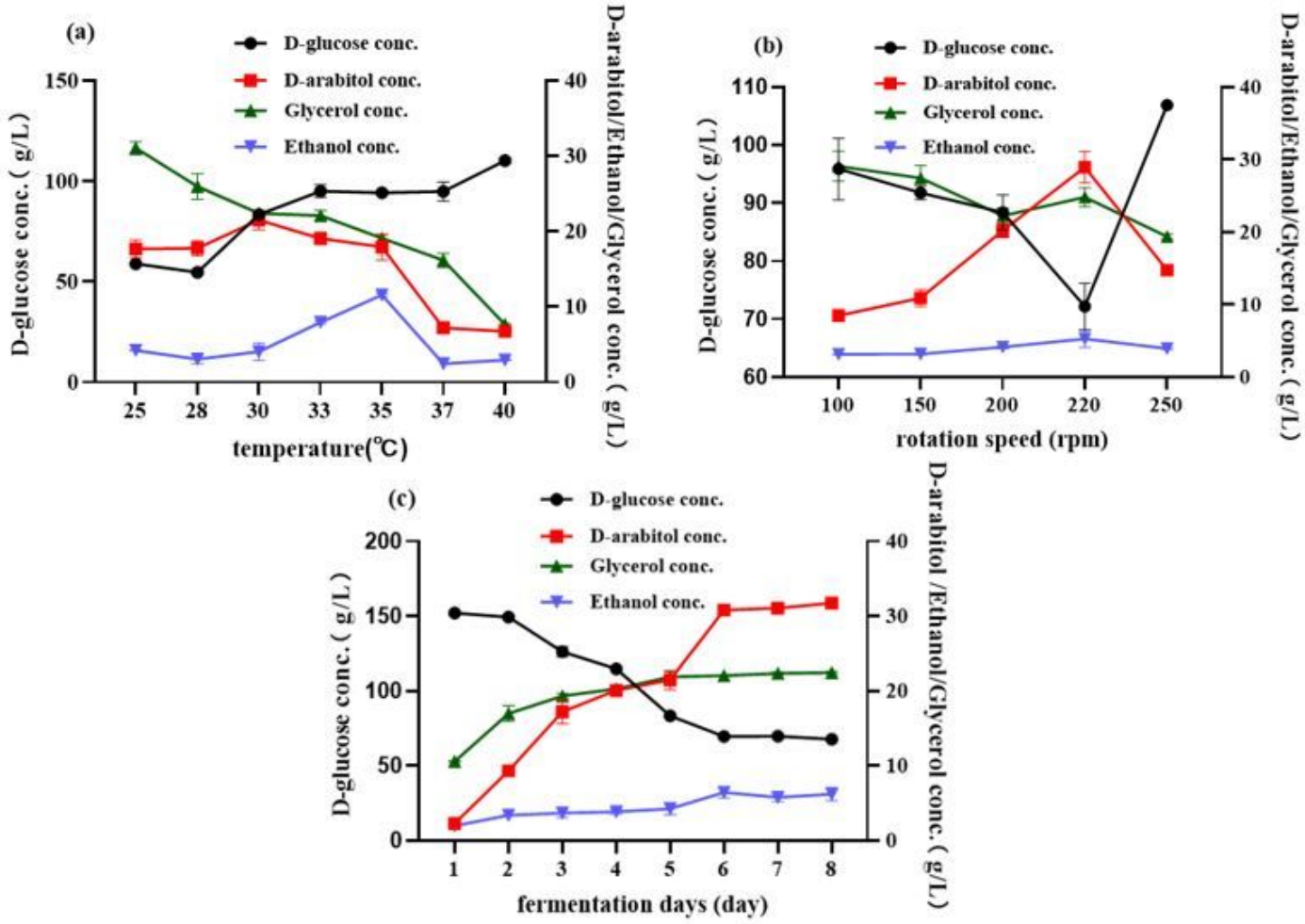

Figure 2

Effects of temperature (a), rotation speed (b), and fermentation time (c) on D-arabitol fermentation

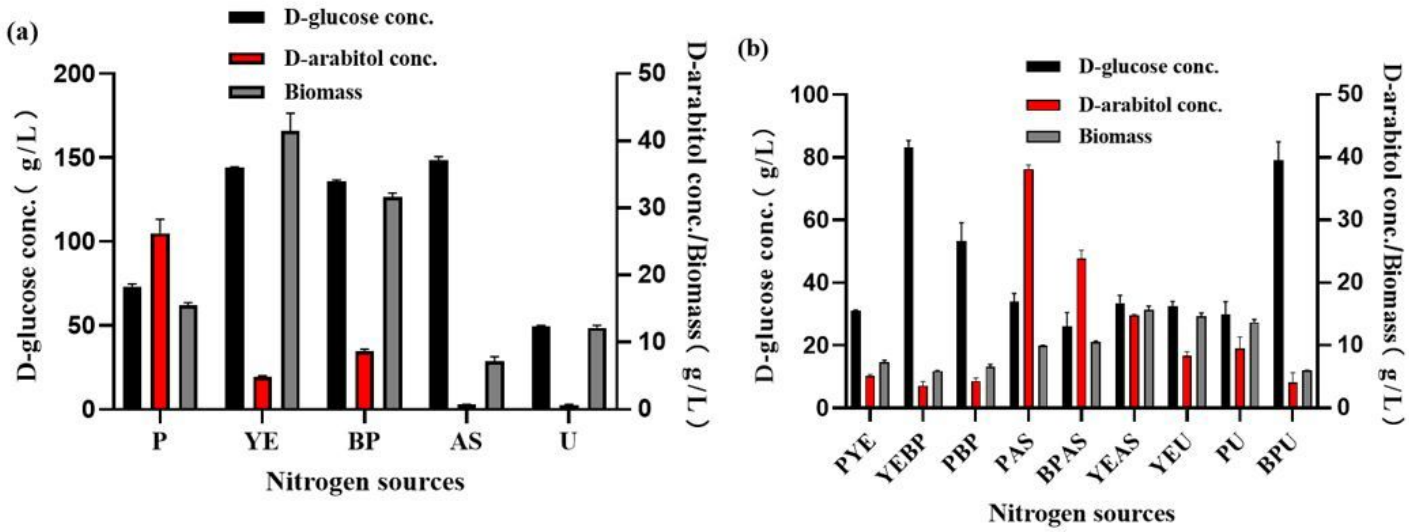

Figure 3

Effects of nitrogen sources on production of D-arabitol

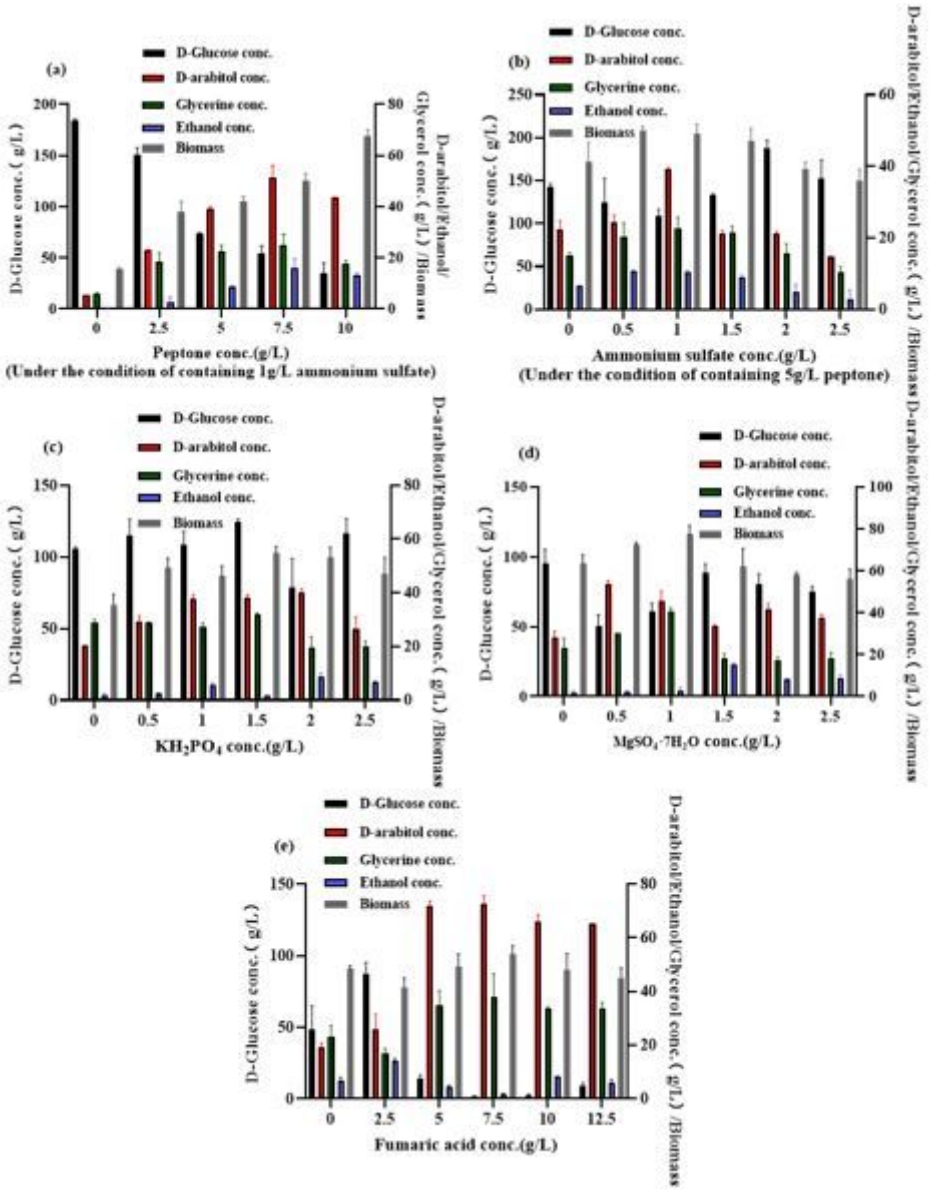

Figure 4

Effects of peptone (a), ammonium sulfate (b), KH2PO4(c), MgSO4·7H2O(d) and fumaric acid (e) on D-arabitol production

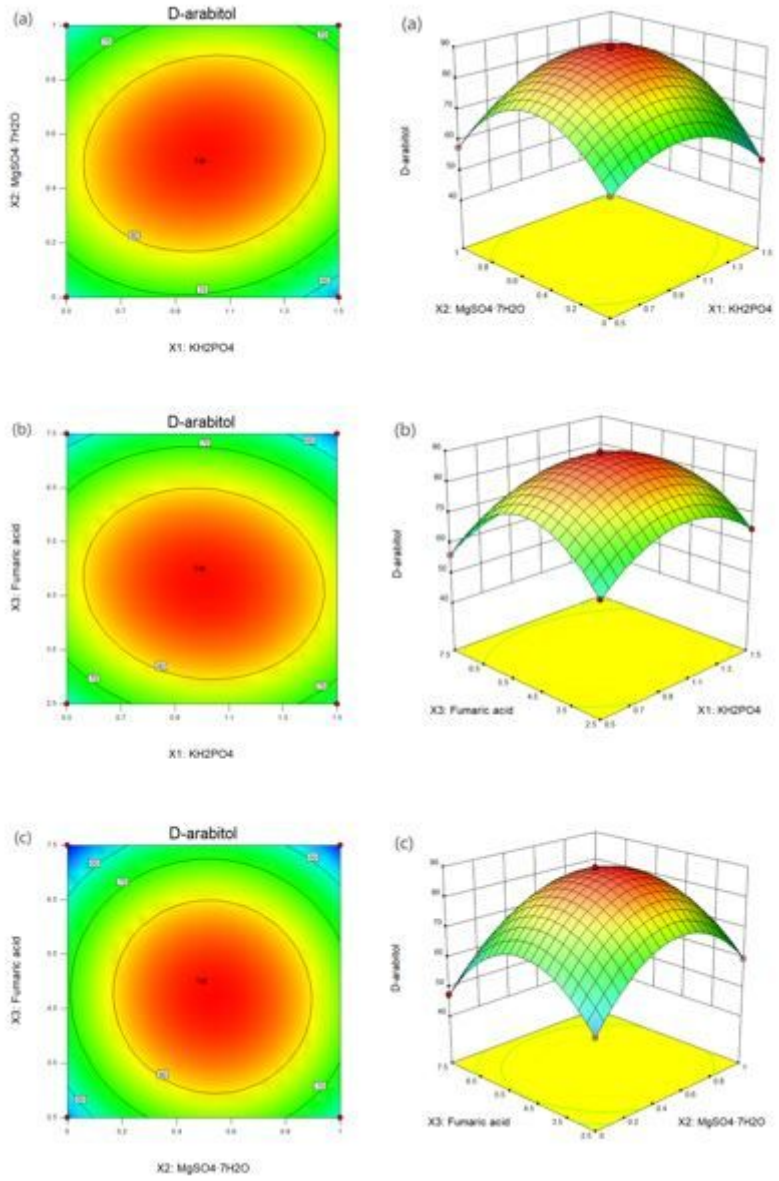

Figure 5

Contour plots and 3D response surface plots for D-arabitol production as a function of KH2PO4 and MgSO4·7H2O (a); KH2PO4 and fumaric acid (b); and MgSO4·7H2O and fumaric acid (c)

Supplementary Files

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- graphicalabstract.png