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The silver nanoparticles effect on L-sorbose production and the membrane-bound sorbitol dehydrogenase activity

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ABSTRACT

Background: The membrane-bound sorbitol dehydrogenase is a member of the flavoprotein dehydrogenase–cytochrome complex located in the respiratory chain of the genus of *Gluconobacter* oxidizes D-sorbitol to L-sorbose, the vitamin C intermediate production, with high specificity. In this research, the silver nanoparticles effect on L-sorbose production and the sorbitol dehydrogenase activity by *Gluconobacter oxydans* were investigated through response surface methodology.

Methods: The silver nanoparticles effect on L-sorbose production was studied in a 2.5 L laboratory-scale bioreactor. The central composite design was employed for evaluation of the silver nanoparticles effect on sorbitol dehydrogenase activity at different pH and temperatures. The sorbitol dehydrogenase of *Gluconobacter oxydans* activity was evaluated in the membrane fractions by colorimetric method.

Results: The results showed that the addition of 50 mg/L of silver nanoparticles into the culture medium caused a decrease of 2.3 and 1.7 times in L-sorbose production and dry cell weight, respectively. Studying the sorbitol dehydrogenase activity through response surface methodology showed that the highest and lowest activity were observed when 0 and 100 mg/L of silver nanoparticles were added into the culture medium, respectively (35 and 1.5 U/L). The temperature and pH showed a direct effect on the sorbitol dehydrogenase. The effects of the three parameters of temperature, pH, and nanoparticle concentration were linear. The parameters of temperature and silver nanoparticles concentrations showed a positive interaction.

Conclusion: It could be concluded that silver nanoparticles decreased the L-sorbose production by *Gluconobacter oxydans* through inhibiting the membrane-bound sorbitol dehydrogenase activity and cell growth.

KEYWORDS

Gluconobacter, D-Sorbitol, Dehydrogenase, L-sorbose, RSM.

1. Introduction

The strictly aerobic Gram-negative bacteria, *Gluconobacters,* are one genus of the acetic acid bacteria (AAB). *Gluconobacters* are able to oxidize some sugars and sugar-alcohols incompletely. The oxidation products accumulate in the medium at the end of the process. This quality has made these bacteria useful in the production of vitamin C, L-sorbose, dihydroxyacetone, ketogluconic acid 1-deoxygalactonojirimycin, L-tagatose, and the precursor miglitol in the industry (Silva *et al.*, 2022; Adachi *et al.*, 2016). *Gluconobacter* respiratory chains contain coenzyme Q, terminal oxidases and, various membrane-bound dehydrogenases (Soemophol *et al.*, 2008). The membrane-bound dehydrogenases are located on the outer surface of the cytoplasmic membrane. They are linked to the respiratory chain and carry out the oxidation and, consequently generate energy. Among various membrane-bound dehydrogenases in *Gluconobacter* strains, two responsible for the oxidation of D-sorbitol have been reported: glycerol dehydrogenase, which contains pyrroloquinoline quinone (PQQ), and sorbitol dehydrogenase containing FAD as the prosthetic groups, respectively. These enzymes are responsible for producing L-sorbose, the vitamin C production intermediate (Sushigawa *et al.*, 2002). Glycerol dehydrogenase is a polyol dehydrogenase that oxidizes some sugar-alcohols as substrates, such as sorbitol, mannitol, ribitol, erythritol, and arabitol to the ketoses (Wang *et al.*, 2014). While D-sorbitol dehydrogenase, which is a member of the flavoprotein dehydrogenase–cytochrome complex, oxidizes D-sorbitol to produce L-sorbose with high specificity (Toyama *et al.*, 2005).

Nanoparticle substances with dimensions of 1 to 100 nm have a larger surface area per weight (Hajipour *et al.*, 2012). It has been reported that different types of nanoparticles are widely used in medical sciences, antigen and pathogen detection, analytical chemistry, and tissue repair due to their biocompatibility and chemical stability (Cameron *et al.*, 2018). The nanoparticles antibacterial effects on microorganisms have been performed in many studies so far. The results of the studies have revealed that nanoparticles often have shown antifungal, antibacterial, and antiviral activity (Hajipour *et al.*, 2012). There are many studies on the effect of the nanoparticles on microbial metabolites such as microbial cellulose, biosurfactants, vitamins like coenzyme $Q_{10,}$ and polymers (Alamdar *et al.*, 2019; Jalili *et al.*, 2018; Moghadami *et al.*, 2020). It is known that the enzyme function can be modified by nanoparticles. The main properties of nanoparticles, such as structure, size, charge, surface shape, and chemistry, control the interaction between enzymes and nanoparticles. So far, several studies have been performed on enzyme activity influenced by different nanoparticles (Wu *et al.*, 2009; Cha *et al.*, 2015).

This research investigated the silver nanoparticles effect on L-sorbose production and the sorbitol dehydrogenase activity. For this purpose, at first, L-sorbose production was investigated in presence and absence of 50 mg/L of silver nanoparticles and then, the different concentrations of silver nanoparticles effect on sorbitol dehydrogenase of *Gluconobacter oxydans* at different pH and temperatures were studied through the response surface methodology.

2. Materials and methods

2. 1. Chemicals

The L-sorbose was HPLC standard with a CAS number 87-79-6 (Sigma-Aldrich). The silver nanoparticles used in this study were in the dimensions of 20 nm (US Research Nanomaterials Co.). The other chemicals were purchased from Merck (Merck Millipore, Darmstadt, Germany).

2. 2. Microorganism and media

The bacterium used in this research was *Gluconobacter oxydans* ATTC 621H that had been purchased from IROST (Iranian Research Organization for Science and Technology). It was maintained in the GYC Agar consisting of 10 g/L of yeast extract, 50 g/L of D-glucose, 30 g/L of $CaCO_3$, and 25 g/L of Agar. The experiment culture consists of 110 g/L of D-sorbitol, 35 g/L of peptone, 25 g/L of yeast extract, 0.55 g/L of $MgSO_4$ and 0.5 g/L of KH_2PO_4. The temperature, agitation speed, and pH of the cultures were adjusted at 30 °C, 180 rpm and 6.5, respectively. The sorbitol dehydrogenase was measured 40 h after the beginning of the incubation (Moghadami *et al.*, 2021).

2. 3. The silver nanoparticles effect on *L-sorbose* production

To investigate the effect of silver nanoparticles on L-sorbose production and cell growth, the fermentation was performed in the bioreactor (5 L laboratory-scale) containing 2.5 L of the production culture medium with pH 6.5. The temperature was constant and controlled at 30 °C. DO level and agitation speed was controlled at 30% and 500 rpm, respectively. The pH values were adjusted at 6.5 by the addition of 2 M NaOH. The silver nanoparticles concentration was 50 mg/L.

2. 4. Measurement of *L-sorbose* and dry cell weight

The estimation of L-sorbose concentration was carried out by HPLC (Agilent 1100, USA). The used column was an Aminex HPX-87H (300 mm × 7.8 mm). The detector was an RI detector. The eluent and flow rate was 5 mM H2SO4 and 0.6 mL/min at 35 °C, respectively (Hu *et al.*, 2015). The dry cell weight of the bacteria (DCW) was determined by the centrifugation of 1 ml of the bacterial cultures at 10000 ×g for 12 min. The pellets were dried at 60 °C for 8 hours.

2. 5. Enzyme assay

The grown bacteria were centrifuged at 10000 × g for 12 min. Shinagawa's method was used to prepare the membrane fractions (Shinagawa *et al.*, 1982). The cells were suspended in 0.01 M potassium phosphate buffer (pH 6) in the ratio of 1 gram of wet weight in 10 ml of the suspension. The cells were broken by the sonicator for 10 min with six intervals for five times. The intact cells were removed by centrifugation at 8000 × g for 15 min. The resulting precipitate was resuspended with McIlvaine buffer (McB, pH 5.0) and used as a membrane fraction. The measurement of sorbitol dehydrogenase activity was carried out by the reduction ability of an electron acceptor (potassium ferricyanide) (Toyama *et al.*, 2005).

2. 6. The silver nanoparticles effect on sorbitol dehydrogenase activity

The silver nanoparticles effect (AgNPs) on the sorbitol dehydrogenase activity at different pH and temperatures was studied by the Response Surface Methodology (RSM). The three factors were used by the central composite design (CCD). The three factors (silver nanoparticles concentration, pH, and temperature), and sorbitol dehydrogenase activity (SLDH) were selected as independent variables and the response variable, respectively. For designing the experiments and data analysis, version 20.04 of Minitab statistical software was used. The three independent variable levels applied for CCD designing are shown in Table 1. Twenty designated experiments by the Minitab software were used for the experiments. The experiments were carried out at different temperatures and pH in the presence of different concentrations of silver nanoparticles. The proportions and values used can be seen in Table 2. The level of confidence was

95%. In general, 20 experiments were performed with different ratios of all three parameters. In order to adjust different pH values, McIlvan buffer was used with different pH according to table 2.

Table 1. The three independent variables levels applied for CCD designing

	Levels				
Factors	**-1.68**	**-1**	**0**	**1**	**1.68**
AgNPs (mg/L)	0	20	50	80	100.45
pH	3.64	5	7	9	10.36
Temperature (°C)	26.59	30	35	40	43.41

3. Results and discussion

3. 1. The silver nanoparticles effect on *L-sorbose* production

To study of silver nanoparticles effect on the sorbitol dehydrogenase activity, the production of L-sorbose was also measured. The silver nanoparticles effect on L-sorbose production was performed in the batch fermentor. The pH and temperature of the culture were controlled at 6.5 and 30 °C, respectively. The maximum L-sorbose concentration and DCW in the culture without the silver nanoparticles were 36.4 mg/L and 17.3 g/L, respectively. While the highest amount of L-sorbose and DCW in the culture by adding 50 mg/L of the silver nanoparticles were 15.4 mg/L and 10.2 g/L, respectively. The addition of 50 mg/L of the silver nanoparticles caused a decrease of 2.3 times in the L-sorbose level. The comparison of the L-sorbose production and DCW with and without 50 mg/L of the silver nanoparticles in the medium is shown in Fig.1. It revealed the addition of 50 mg/L of the silver nanoparticles into the medium decreased by 2.3 and 1.7 times in L-sorbose production and DCW, respectively. The effect of nanoparticles is different in production processes. It is known that nanoparticles decrease the yield of the production of microbial products as their antibacterial activities. While in some studies, nanoparticles increase the yield of production. For instance, the silver nanoparticles increased the production efficiency of coenzyme Q_{10} in *Gluconobacter* (Moghadami *et al.*, 2020). It is also reported that using SiO_2 nanoparticles for the immobilization of sorbitol dehydrogenase of *Gluconobacter oxydans* increased L-sorbose production from D-sorbitol (Kim *et al.*, 2016).

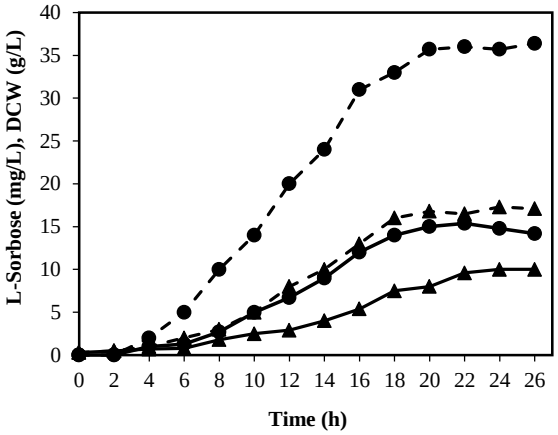

Figure 1. The produced L-Sorbose and DCW by *Gluconobacter oxydans* in a 2.5 L batch fermentor. The culture without silver nanoparticles (dashed line) and the culture with 50 mg/L silver nanoparticles (solid line). The produced L-Sorbose (filled circle) and DCW (filled triangle).

3. 2. The silver nanoparticles effect on the sorbitol dehydrogenase activity

According to the designed experiments of the central composite design, 20 experiments were performed. The results of the experiments are shown in Table 2. Various concentrations of silver nanoparticles decreased sorbitol dehydrogenase activity. It seems that the silver nanoparticles decreased the production of the L-sorbose in *Gluconobacter oxydans* by inhibiting sorbitol dehydrogenase activity and cell growth. FAD-dependent sorbitol dehydrogenase and PQQ-dependent glycerol dehydrogenase are responsible for the D-sorbitol oxidation to L-sorbose in *Gluconobacter* (Bringer *et al.*, 2016). In our previous study, we investigated the effect of the silver

nanoparticles on the glycerol dehydrogenase activity in *Gluconobacter japonicus* FM10 by RSM. It revealed that the silver nanoparticles decrease the activity of glycerol dehydrogenase (Moghadami *et al.*, 2023). The results of this study were the same and showed that the silver nanoparticles decreased the sorbitol dehydrogenase activity and cell growth. This means that silver nanoparticles could decrease the oxidation of D-sorbitol to L-sorbose by decreasing the activity of both enzymes involved in sorbitol oxidation in *Gluconobacter*, sorbitol dehydrogenase, and glycerol dehydrogenase.

The regression models for sorbitol dehydrogenase activity were found as follows:

SLDH = 15.725 – 7.09 AgNPs + 4.12 pH + 5.34 Temp + 3.56 AgNPs*Temp

The adequacy of the regression model was evaluated via analysis of variance (ANOVA). Table 3 and Table 4 show the ANOVA results for sorbitol dehydrogenase and the test of significance for regression coefficients of the equation, respectively. The results showed that the model terms, i.e. the silver nanoparticle concentration, pH, and temperature are significant. The P-value is less than 0.05. The F-value and P-value were 21.12 and <0.05, respectively. These two values indicated that the response surface linear model is significant.

Table 2. Central composite design and the sorbitol dehydrogenase activity

Run number	Coded Levels			AgNPs (mg/L)	pH	Temperature (°C)	SLDH (U/L)
	AgNPs	pH	Temperature				
1	0	0	0	50	7	35	17
2	1	-1	-1	80	5	30	2.5
3	0	0	0	50	7	35	16
4	-1	1	1	20	9	40	30
5	0	-1.68	0	50	3.64	35	2.5
6	0	0	0	50	7	35	16.5
7	0	0	0	50	7	35	17.5
8	0	0	0	50	7	35	16.5
9	0	1.68	0	50	10.36	35	19
10	1	-1	1	80	5	40	15
11	1.68	0	0	100.45	7	35	1.5
12	1	1	1	80	9	40	25
13	0	0	-1.68	50	7	26.59	1.5
14	-1.68	0	0	0	7	35	35
15	-1	-1	1	20	5	40	16
16	-1	1	-1	20	9	30	22
17	-1	-1	-1	20	5	30	19
18	0	0	1.68	50	7	43.41	22
19	1	1	-1	80	9	30	4
20	0	0	0	50	7	35	16

Table 3. Regression analysis by ANOVA for sorbitol dehydrogenase activity

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	4	1409.86	352.464	21.12	0.000
Linear	3	1308.33	436.109	26.13	0.000
AgNPs	1	686.69	686.688	41.14	0.000
pH	1	231.68	231.680	13.88	0.002
Temperature	1	389.96	389.959	23.36	0.000
2-Way Interaction	1	101.53	101.531	6.08	0.026
AgNPs*Temperature	1	101.53	101.531	6.08	0.026
Error	15	250.38	16.692		
Lack-of-Fit	10	248.67	24.867	72.78	0.000
Pure Error	5	1.71	0.342		
Total	19	1660.24			

Table 4. Testing of the significance of the regression coefficients

Term	Coef	SE Coef	T-Value	P-Value
Constant	15.725	0.914	17.21	0.000
AgNPs	-7.09	1.11	-6.41	0.000
pH	4.12	1.11	3.73	0.002
Temperature	5.34	1.11	4.83	0.000
AgNPs*Temperature	3.56	1.44	2.47	0.026

Figure 2 shows the surface plots and contour plots of sorbitol dehydrogenase activity versus the factors of silver nanoparticle concentration, pH, and Temperature. It can be easily seen from the figure that sorbitol dehydrogenase activity tends to increase when pH and Temperature increase. In comparison sorbitol dehydrogenase activity decreases when silver nanoparticles concentration increases. These findings are in completely agree with the previous results obtained from the linear regression model. Many studies have been published on the nanoparticles effect on enzyme activity (Porzani *et al.*, 2021, Mahmoudi *et al.*, 2016). Some studies have shown that using nanoparticles to immobilize of dehydrogenases increases their activities (Alam *et al.*, 2015; Kim *et al.*, 2016; Li *et al.*, 2008). While others have generally revealed that the silver nanoparticles antimicrobial activity is carried out by inhibiting vital enzymes such as dehydrogenases (Cha *et al.*, 2015; Calzolai *et al.*, 2010; Patra *et al.*, 2022). It is reported that silver nanoparticles inhibit the activity of the dehydrogenase located at respiratory chain of *Escherichia coli* (Barbalinardo *et al.*, 2018). The soil enzyme activities, especially dehydrogenases, were affected by citrate-coated silver nanoparticles (Li *et al.*, 2018). It was found that silver nanoparticle inhibits the firefly luciferase enzyme, which can depend on the interaction between silver and the thiol group of this enzyme (Kakinen *et al.*, 2013). Another study showed that silver nanoparticles inhibit glyceraldehyde-3-phosphate dehydrogenase enzyme activity. It is known that the presence of the thiol group in the active site of the enzyme was considered to be the main factor affecting the silver on the enzyme activity (Jiang *et al.*, 2019). It is revealed that enzymes which have a thiol group in their active site can be more sensitive to the silver than other enzymes (Cha *et al.*, 2015; Srivastava *et al.*, 2012). In the case of sorbitol dehydrogenase, it should be studied whether it has the thiol groups in its active site or not. There is no data about the thiol group presence in the active site of FAD-dependent sorbitol dehydrogenase in the Protein Data Bank, which can be used to explain the silver nanoparticles effect on sorbitol dehydrogenase.

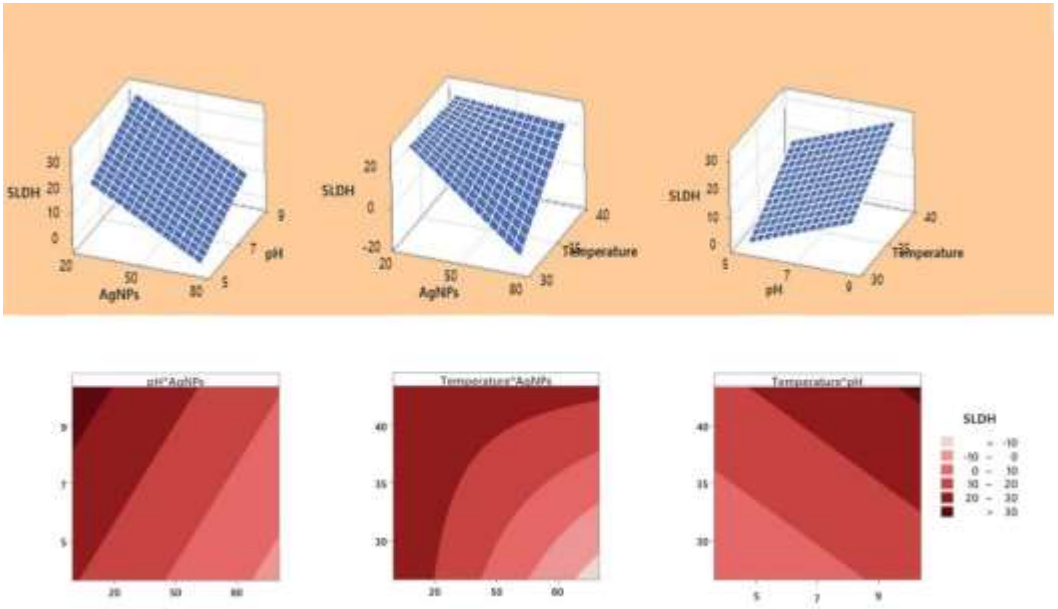

Figure 2. The surface plots and contour plots of sorbitol dehydrogenase activity against silver nanoparticles concentrations, temperature, and pH

3. 3. The Interaction between the silver nanoparticle concentration, pH and temperature

The interactions between the parameters such as silver nanoparticle concentrations, pH values and temperature can be investigated by response surface methodology. The surface plots and contour plots show the interactions between the factors, i.e., temperature and silver nanoparticles, pH and silver nanoparticles, and pH and temperature and their impacts on the sorbitol dehydrogenase activity (Fig.2). According to our previous research, all three parameters of temperature, pH and nanoparticle concentration were independently influenced the activity of PQQ-dependent glycerol dehydrogenase of *Gluconobacter oxydans* (Moghadami *et al.*, 2023). The results of this research showed that the effect of pH on the sorbitol dehydrogenase activity was independent, i.e. temperature and silver nanoparticle concentrations were not affected by the decrease or increase of those two parameters. The two parameters of temperature and silver nanoparticle concentrations showed a positive interaction, which means that the increase of one parameter affects the effect of the other parameter on the enzyme activity. That is, with the increase in temperature, the activity of the enzyme in the presence of higher concentrations of silver nanoparticles not only does not decrease, but also increases. The two parameters of temperature and silver nanoparticle have a positive interaction, which means that the increase of one parameter affects the effect of the other parameter on the enzyme activity. By increasing the temperature, the sorbitol dehydrogenase activity in the presence of higher concentrations of silver nanoparticles increased. Investigating the nanoparticles effect on the activity of the enzymes in different environmental conditions has shown that some parameters can affect the response of enzymes to the effect of nanoparticles by creating interference (Nel *et al.*, 2009; Xia *et al.*, 2011).

4. Conclusion

In this research, the effect of various concentrations of silver nanoparticles on the sorbitol dehydrogenase activity of *Gluconobacter oxydans* was investigated at different pH and temperatures through response surface methodology. The results of the present study showed that all three parameters of temperature, pH, and silver nanoparticle concentration are effective in the activity of sorbitol dehydrogenase. Increasing the concentration of silver nanoparticles decreased the activity of sorbitol dehydrogenase. While temperature and pH showed a direct effect on the sorbitol dehydrogenase activity and increased its activity. All three parameters effects of temperature, pH, and silver nanoparticle concentrations were linear. The results showed that the addition of 50 mg/L of the silver nanoparticles into the culture decreased by 2.3 and 1.7 times in L-sorbose production and DCW, respectively. It could be concluded that silver nanoparticles decreased the L-sorbose production by inhibiting the membrane-bound sorbitol dehydrogenase activity and cell growth.

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Conflict of Interest

There is no conflict of interest.

Author contributions

F Moghadami designed the study and carried out the experiments. M Kalantari carried out the statistical analysis. F Hajmoradi and F Moghadami wrote the manuscript.

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