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Impact of Environmental Factors on the Production of Silver Nanoparticles by *Saccharomyces Ellipsoideus BSU-XR1*

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In our presented work we have studied the effect of various environmental factors on the synthesis of silver nanoparticles by Saccharomyces ellipsoideus BSU-XR1. Silver nanoparticles were formed by growing the yeast in broth medium at 30oC. Filters were used to seperate biomass from broth. Biomass was washed 3 times with 0.1L of distilled water (DW). 10 grams of wet biomass was poured to beaker containing 90 ml of sterile DW. 1 ml of 10-3 molar solution of Silver nitrate was poured to it, and this mixture with silver nitrate salt was incubated in a dark environment. It was determined that, the optimal condition for the production of silver nanoparticles was being on the 21st day of incubation, in 10 grams of wet biomass. For the cultivation of the studied yeast strain, the synthesis of silver nanoparticles took place in the range of 25-30°C. Tthe initial pH of the medium was 7.0. and the concentration of silver nitrate was used as salt at conntration of 0.5 mM and 1.0 mM. The optimal conditions of incubation were in dark environment in all variotions of experiments.

Keywords: Biomass, Medium Acidity (pH); Concentration of AgNO3 salt; Incubation Time; Nanoparticles; temperature; Yeast.

Currently, particular consideration is given to the study of interaction of microorganisms with silver ions. One of the latest achievements of nanotechnology has been the biological production of silver nanoparticles from yeast cells for use in medicine, biotechnology, and the food industry[1–2]. Different types of yeast have variable ability to produce silver nanoparticles i.e. *Candida albicans* release silver ions that infiltrate into the cell leading to the formation of nanoparticles through reduction by organic compounds present in the cell wall and cytoplasm[3]. In addition of this, it was found that the synthesis of silver nanoparticles changes depending on physico-chemical parameters (for example, cell age and mass, temperature, pH, incubation time, concentration of $AgNO_3$ salt, and light)[4-6].

As a result of large-scale scientific research works, it was determined that various environmental factors play a significant role in obtaining metal nanoparticles from Saccharomyces yeast strains[7–9].

Therefore, major aim of this research was to study the effect of various environmental factors on the creation of silver nanoparticles by yeast i.e. *Saccharomyces ellipsoideus* BSU - XR1.

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MATERIALS AND METHODS

Saccharomyces ellipsoideus BSU-XR1 yeast strain stored in the "Microbiology" laboratory of Baku State University was used as the research object and the effect of various environmental factors was studied on the creation of silver nanoparticles by this strain. *Yeast* was firstly grown in a broth having composition as follows.

The yeast was grown in a thermostat at 30°C for 48 hours.Filters were used to seperate biomass from broth. Biomass was washed 3 times with 0.1L of distilled water (DW). 10 grams of wet biomass was poured to beaker containing 90 ml of sterile DW. 1 ml of 10^{-3} molar solution of Silver nitrate was poured to it, and this mixture with silver nitrate salt was incubated in a dark environment, and samples were analyzed periodically for every 7, 11, 21, 30, 42 days.

The formation of silver nanoparticles was initially determined by the change in the colour of mixture from yellow to brown. Then, it was determined based on the absorption peak at 370-450 nm in a UV spectrophotometer ("UV-VIS specord 250")

The shape and size of silver nanoparticles were studied in a field emission scanning electron microscope (SEM) (JEOL SEM 7600F) after making preparation from the colloidal solution.

In Rentgen's spectroscope (X-ray diffraction), the elemental properties of the samples were determined and it has been stated that, that the nanoparticles belonged to silver or those nanoparticles are silver nanoparticles.

After determining the optimum incubation time, the formation of silver nanoparticles was studied depending on the biomass. For this purpose, after cultivation in the above-mentioned nutrient medium 5, 10, 15, 20 grams of wet biomass of the studied strain was detached from the culture liquid by using centrifuge machine and filtration at 5000 period/min, then it was washed 3 times in 100 ml of distilled water and used. Biomass obtained from these samples added into a flask containing 99ml of distilled water, then 1 ml 10^{-3}molar silver nitrate salt ($AgNO_3$) solution was added onto it and incubated in a dark environment under the same conditions as the control sample (no $AgNO_3$ solution added).

Optimum wet biomass was found for the formation property of silver nanoparticle by studied yeast strain.

The impact of different temperatures for the synthesis of nanoparticles by the yeast was observed at the optimal incubation period and the optimal amount of biomass.

At this time, after preparation of the reaction mixture of wet biomass and silver nitrate salt in flasks, the optimal temperature limit for the creation of Ag nanoparticles was determined by incubating it in a thermostat at different temperatures.

Then, the creation of Ag nanoparticles depending on environmental acidity (pH 4.0; 5.0; 6.0; 7.0; 8.0) of this strain was studied at the optimal incubation time, the optimal amount of biomass and the optimal temperature.

For making the initial environmental acidity (pH 4.0; 5.0; 6.0; 7.0; 8.0) in the reaction mixture of wet biomass and silver nitrate salt were used 0.1n NaOH and 0.1n HCl solutions.Flasks with colloidal solutions with different levels of initial medium acidity were cultured under the same conditions in the dark, and the optimal pH limit for the production of silver nanoparticles by the yeast strain was found.

The ability of yeast to synthesize Ag nanoparticles, depending on the concentration of silver nitrate salt (0.5; 1.0; 3.0; 5.0; 10.0 mM of $AgNO_3$) was studied on the optimal incubation period time, optimal amount of biomass , at optimum temperature and in optimum acidity

At this time, 10 g wet biomass of the cultured strain was collected and after washing 3 times in 100 ml of distilled water, different amounts of silver nitrate salt were added into it and placed in a dark environment for incubation.

For comparison, samples without added silver nitrate salt also were incubated under the same conditions as a control. The optimal concentration of silver nitrate salt was found for this strain to form silver nanoparticles during the incubation period

The incubation process of the wet biomass of the studied yeast strain and the reaction mixture of silver nitrate salt in dark and light environments was carried out at the optimal incubation time, the optimal amount of biomass, the optimal

temperature, in the optimal acidity, and optimal amount of the salt.

As a result of incubation, optimal conditions for the creation of Ag nanoparticles were studied for this strain. All experiments were performed in 4-5 repetitions.

RESULTS AND DISCUSSIONS

The dependency of the creation of Ag nanoparticles by yeast strain from incubation period was studied. There was a change in colour on the 7th, 11th, 21st, 30th and 42nd days of incubation the in mixture of wet biomass of yeast strain and silver nitrate salt darkened as a primary indicator of silver nanoparticles[10].

On the 7th and 11th days of incubation, a sample taken from the dark colored reaction mixture was analyzed in a spectrophotometer and absorption was observed at 415 nm wave length. For Ag nanoparticles absorption at this wavelength is a characteristic feature. However, no silver nanoparticles were found when these samples were observed under a Scanning Electron Microscope

The sample taken on the 30th and 21st days of the incubation period was analyzed in a spectrophotometer and the absorption was observed at wave length of 420 nm. After making a preparation from the samples and examining them under a scanning electron microscope, it was determined that there were 22.4 and 17.2 nm in sizes spherical silver nanoparticles. During X-ray spectrum analysis, AgLa1 peak was noted in the samples which is characteristic for silver nanoparticles

On the 42nd day of incubation, absorption became increasingly weak when the samples were analyzed in a UV-VIS spectrophotometer. The power of the studied strain to synthesize Ag nanoparticles was optimal on the 21st day of incubation.As the incubation time increased, the speed of silver nanoparticles creation weakened.

Depending on the species and strains each fungal strain gives a color change as an early indicator for the formation of Ag nanoparticles at various times of incubation. For example, reaction mixture of the commercial strain of yeast *Saccharomyces cerevisiae* started to darken from light yellow to brown after 4 days of incubation, and in UV spectrophotometer for silver nanoparticles had absorption at 450 nm wavelength. The reaction mixture of another strain of *Saccharomyces cerevisiae* yeast began to darken on the 6th day of incubation. The silver nanoparticles formed at this time showed absorption at a wavelength of 430 nm[7, 9].

The formation of nanoparticles with the biomass of another strain of the Saccharomyces cerevisiae yeast used in baking bread has been studied.In this case, the formation of nanoparticles has started after 24 hours of incubation. The processed nanoparticles were in cuboidal shape, and their size was very small, 67.2 A (angitherm). The formation of nanoparticles with the biomass of another strain of the Saccharomyces cerevisiae yeast used in baking bread has been studied. In this case, the formation of nanoparticles started after 24 hours of incubation. The processed nanoparticles were cuboidal in shape, and their size

Table 1. Composition of broth medium for cultivation of yeast

Ingredient	Quantity
yeast extract	10 g
sucrose	20 g
peptone	10 g
distilled water	1 liter

Table 2. Incubation day and Wavelength for absorption of silver nanoparticles

Incubation day	Wavelength for absorption
7th	415 nm
11th	415 nm
21st	420 nm
30th	420 nm
42nd	No absorption

Table 3. Effect of amount of biomass on size of nanoparticles

Amount of biomass	Size of nanoparticles
5g	8.6-16.9 nm
10g	17.2 nm
15g	22.4 nm

was very small, 67.2 A (angitherm). As a result of the study of the creation of Ag nanoparticles with the culture liquid of another strain of fungus, it was determined that after 48 hours, nanoparticles with a spherical shape and a size of 25 nm were formed. The absorption of nanoparticles in the UV system was at a wave length of 413 nm[9]. The production of silver nanoparticles by *Candida* yeasts was slightly different from *Saccharomyce* yeasts. Silver nanoparticles produced by *Candida albicans* NCIM-3100 strain gave absorption at 420 nm wavelength in the UV spectrum During 48 hours of incubation time. The size of silver nanoparticles varied between 20-60 nm and formed aggregates. Nanoparticles were in spherical shape. Another strain of the yeast *Candida albicans* MIC 50 produced silver nanoparticles after 96 hours of incubation.Nanoparticles had a size of 80 nm and had an absorption at 370 nm wavelength in the UV spectrum. They were in spherical appearance[11].

Synthesis of silver nanoparticles with wet biomass of *Candida macedoniensis* BDU-MI44 strain took place on the 3rd-7th days of incubation. Intensive formation of silver nanoparticles by *Candida guilliermondii* BDU-217 yeast biomass occurred on days 3-10 of incubation, and then the process weakened[4, 6,12]

From the above literature data and from our experimental results, it has cleared that, the creation time of Ag nanoparticles significantly varies by depending on yeast and its type. And of course, this process initially depends on the incubation conditions and the metabolic characteristics of the fungal organism.

During the research work, it was found that at the incubation period time of the reaction mixture of 5, 10, 15, 20 g of wet biomass of used yeast strain with silver nitrate salt, it darkened from yellowish to brown. No colour change was observed in the flask without silver nitrate solution.

During analysis in a UV spectrophotometer, the reaction mixture of 5 grams of biomass and silver nitrate salt had absorption at a wavetlength of 400 nm which is suitable for silver nanoparticles, at a wavelength of 420 nm at 10 g, and at a wavelength of 415 nm at 15 g.However, absorption was not observed during the analysis of the reaction mixture with 20 g of wet biomass. As have we seen, absorption was not observed in studied yeast strain when the amount of wet biomass increased in reaction mixture[13].

The SEM depicted that the Ag nanoparticles have a roundish shape and different sizes.The size of the nanoparticles varied depending on the amount of biomass. So, the size of nanoparticles synthesized with 5g of biomass was 8.6-16.9 nm, with 10 g of biomass - 17.2 nm, and with 15 g of biomass - 22.4 nm.So, depending on the amount of wet biomass, size of formed Ag nanoparticles was also different.

X-ray spectra of samples, the AgLa1 peak was noted which is characteristic for silver nanoparticles. The optimal biomass of yeast strain was 10 g for the creation of Ag nanoparticles.

The formation of Ag nanoparticles by *Candida utilis NCIM3469* yeast strain has shown itself in 8 grams of wet biomass and by another strain of *Candida utilis* species has shown itself in 8-9 grams of wet biomass[14,15].

Creation of Ag nanoparticles by the yeast strain was observed on different temperatures (25, 30, 35, 40 and 45 °C)[16]. The creation of Ag nanoparticles was noted in mixtures incubated at 25°C and 30°C due to the color change. There was not any color change in a control flask under similar conditions (no silver nitrate added).

Spectrophotometric analysis of the taken samples showed that, absorption (peak) at 410-413 nm wavelength was observed in samples which had incubated at 25°C and 30°C. And this is suitable adsorption that characteristic for silver nanoparticles.The reaction mixture incubated at 35°C had a very weak absorption (peak) at the wavelength of 404 nm considered suitable for silver nanoparticles during UV spectrophotometric analysis, and very little absorption was observed at 40 and 45°C.So, as it can be seen from the experiment, as the temperature increases, the creation of Ag nanoparticles is weakened during incubation of the reaction mixture of silver nitrate salt with the wet biomass of the studied fungal strain.

To elaborate temperature dependency of production of Ag nanoparticles by this strain, a preparation was made from the samples and examined under SEM. It was noted that there were spherical shaped silver nanoparticles with sizes of 22.4 and 17.2 nm. With the optical absorption

peak (AgLa 1) obtained during the analysis with an X-ray phase spectroscope determined that the nanoparticles were pure.

Thus, it was found that the optimal temperature limit for the studied strain to synthesize silver nanoparticles is in the range of 25-30°C.In this interval, absorption (peak) at the wavelength of 410 to 413 nm was observed in the UV spectrophotometer during cultivation.Very weak creation of Ag nanoparticles was seen at 35°C, and at 40 to 45°C no activiy was observed.

It is known from the literature that the creation of Ag nanoparticles in *Candida albicans NCIM-3100* strain occurred at 25°C and in *Candida glabrata* at 37°C[11].

Optimal temperature for formation of silver nanoparticles in *Candida guillermondi BDU-217* strain was 25°C, in *Candida macedoniensis BDU-MI44* strain it was 30°C[5, 12].

Depending on the species and strain, the formation of nanoparticles in Saccharomyces yeast fungi took place under different temperature conditions. For example, the yeast strain *Saccharomyces cerevisiae* used in baking uses silver nanoparticles at a temperature of 30°C[7], but another *S. cerevisiae* fungus applied in baking optimally carried out this process at a temperature of 35°C, some strains of the *Saccharomyces cerevisiae* species took silver nanoparticles at 25°C and They formed at temperatures of 27°C. The optimum temperature for the creation of Ag nanoparticles for the extromophilic *Saccharomyces cerevisiae* was 22°C[9]. Optimal synthesis of silver nanoparticles by the yeast *Saccharomyces bourlardii* occurred at a temperature of 35°C[8]. Thus, it is clear from the above literature data that depending on the type and strain of the fungus, the optimal temperature for the formation of silver nanoparticles can change.

Depending on the initial medium acidity (pH4.0 to 8.0), the production of silver nanoparticles by *Saccharomyces ellipsoideus* BSU-XR1 strain was studied[17], and it was determined that the colour of the mixture was light when silver nanoparticles were collected in the medium and then it changes from yellowish to brown. From the 7th day of the incubation period, the color change was noticeable in the reaction mixtures in the thermostat

During the spectrophotometric analysis, it was determined that the absorption peak between the wavelength of 408-412 nm was observed in the reaction mixtures with pH of 6.0, 7.0, 8.0.

Very little absorption was found in samples obtained from mixtures with medium acidity (pH 4.0, 5.0), where a weak color change was noted.The silver nanoparticles produced by the yeast strain at pH 7.0 were characterized by their spherical appearance in the scanning electron microscope.The size of nanoparticles was equal to 22.4 nm.

Therefore, it is confirmed that the nanoparticles are Ag nanoparticles during the X-ray spectrum analysis.

The effect of pH on the synthesis of Ag nanoparticles by the yeast strain was determined and it was found that if the creation of Ag nanoparticles occurs between pH 6.0-8.0, the optimal pH for biomass is pH 7.0 was considered.

It is known from the literature that silver nanoparticles can be actively synthesized during the incubation period of *Saccharomyces cerevisiae* yeast cells with silver nitrate salt when the pH of the solution is above 8. Metabolism products collected by the yeast played the role of regulator for the formed silver nanoparticles. It is believed that silver nanoparticles are formed as a result of the reduction of silver ions by secretion, and this is one of the possible causes of formation[9]. As a result of the research, it was found that microbial cultures in alkaline environment have the ability to synthesize more silver nanoparticles than in acidic environment. When the acidity of the environment exceeds pH 10, it causes the death of cells. More nanoparticles are synthesized by microorganisms under neutral alkaline conditions than under acidic conditions.

The yeast *Saccharomyces cerevisiae* using in baking had the ability to synthesize silver nanoparticles in a wide pH range (pH 4-10)[7, 9]. Optimum biosynthesis of silver nanoparticles for extromophilic yeast was pH 2.5[18]. Although the yeast *Saccharomyces boulardii* can synthesize silver nanoparticles in a wide pH range, the optimal pH was 7[8]. *Candida guillermondi BDU-217* and *Candida macedoniensis BDU-MI44* yeast fungi were also able to synthesize silver nanoparticles in the pH range of 7[4, 6]. In general, it can be noted that the optimal biosynthesis of silver nanoparticles in *Saccharomyces* and *Candida* yeasts occurs mainly in a neutral environment (pH 7). Our results using

the strain *Saccharomyces ellipsoideus BDU-XR1* confirm this again.When studying the creation of Ag nanoparticles by the used strain relying on the concentration of $AgNO_3$ salt (0.5, 1.0, 3.0, 5.0 and 10.0 mM). It was observed that when Ag nanoparticles are collected in medium, the reaction the color of the mixture starts to darken [[19]. The synthesis of Ag nanoparticles was seen on the 7th day of incubation in the mixtures containing silver nitrate at concentrations of 0.5 and 1.0 mM due to the color change. During the spectrophotometric analysis, absorption peaks at 405 and 408 nm wavelength were observed in the samples of silver nitrate salt incubated at concentrations of 0.5 and 1.0 mM. This is typical for silver nanoparticles. The SEM depicted that the Ag nanoparticles are roundish in shape and have different sizes. The size of nanoparticles was equal to 22.4 nm in 0.5 mM mixture of silver salt and 35.5 nm in 1.0 mM concentration.

In the result, as the amount of Ag nitrate salt increased, size of the formed Ag nanoparticles also increased slightly. Our results are similar to those obtained in the yeast *Saccharomyces cerevisiae*. Thus, the size of the Ag nanoparticles produced by fungus at concentrations of 0.5 and 1.0 mM Ag nitrate salt was 34.2 nm and 37.5 nm, respectively[4].

Thus, it was clear from the obtained results that the ability of used strain to form Ag nanoparticles was optimal at concentrations of 0.5 and 1 mM of silver nitrate salt. The synthesis process did not proceed at concentration of 3.0, 5.0 and 10.0 mM of Ag nitrate. In the UV spectrophotometer, an absorption peak at a wavelength of 405-408 nm was observed in two of the reaction mixture samples (0.5 and 1.0 mM) 7th day post incubation. The size of Ag nanoparticles formed at quantity of 0.5 and 1.0 mM of Ag salt was observed SEM as 22.4 nm and 35.5 nm, respectively. The Ag nanoparticles found in two samples had a roundish shape.

Different strains of fungus of *Saccharomyces cerevisiae* used in baking[7, 9] and *Saccharomyces boulardii*[8] produced silver nanoparticles in 1.0 mM concentration of silver nitrate salt. Extremophilic yeast was tolerant to a greater quantity of Ag nitrate salt and was able to form Ag nanoparticles at a quantity of 0.3 mM of $AgNO_3$ salt[18]. It is known that silver nitrate salt has a toxic effect on microorganisms. Therefore, yeast fungi studied by various researchers were able to produce silver nanoparticles at a very low concentration (0.1-0.3 mM) of silver nitrate salt.

However, the studied strain of *Saccharomyces ellipsoideus* BDU-XR1 was able to synthesize silver nanoparticles at concentrations of 0.5 and even 1.0 mM of silver nitrate salt. Therefore, *Sacch. ellipsoideus BDU-XR1* strain, unlike other strains, is resistant to relatively high concentration of silver nitrate salt and can be considered as a practically very prospective producer. In order to study the effect of dark and light factor on the creation of Ag nanoparticles, incubation of reaction mixture of silver nitrate salt with wet biomass of *Saccharomyces ellipsoideus* BDU-XR1 strain was carried out in two environments (dark and light).

During incubation, the reaction mixture underwent a sharper color change in the dark medium and a weak color change in the light medium. This depicts that the synthesis process of Ag nanoparticles is relatively weak in light environment. Colour change of the biomass in the mixtures from light yellow to dark brown was observed in the dark environment. This shows that the process of synthesizing silver nanoparticles is faster.

As a result of UV-spectrophotometer analysis of the reaction mixture of the researched yeast strain and silver nitrate, it was determined that the absorption peak was observed at the wavelength of 414 nm in the sample incubated in the dark environment, and at the wavelength of 409 nm in the sample incubated in the light environment. However, when viewed under a scanning electron microscope, roundish Ag nanoparticles with a diameter of 17.2 nm were identified in a sample incubated in a dark environment. However, silver nanoparticles were not observed in the sample incubated in the light environment.

It should be noted that microbiological creation of Ag nanoparticles was mainly performed in dark conditions (thermostat) [4, 6, 20, 21 and others].

Thus, by studying the effect of various environmental factors on the creation of Ag nanoparticles by the yeast strain. It was found that optimal conditions were on the 21st day of incubation, at 10 grams of wet biomass. The creation of Ag nanoparticles took place at

temperature range of 25-30°C, the initial medium pH was 7.0, and the concentration of Ag salt was 0.5 and 1 mM for the cultivation of the studied yeast strain. In all variants of experiments, the optimal conditions of incubation were dark environment.

CONCLUSION

For *Saccharomyces ellipsoideus* BDU-XR1, the synthesis of silver nanoparticles took place in the range of 25-30°C. Tthe initial pH of the medium was 7.0. and the silver nitrate was used as salt at concentration of 0.5 mM and 1.0 mM. The optimal conditions of incubation were in dark environment in all variations of experiments.

Conflict of Interest

The authors declare that there is no conflict of interest regarding this paper.

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