Biomass Conversion and Biorefinery
https://doi.org/10.1007/s13399-021-02096-3

ORIGINAL ARTICLE

Effect of process parameters and microparticle addition on polygalacturonase activity and fungal morphology of *Aspergillus sojae*

Mustafa Germec[1] · Ercan Karahalil[1] · Ercan Yatmaz[2] · Canan Tari[3] · Irfan Turhan[1]

Received: 21 August 2021 / Revised: 24 October 2021 / Accepted: 16 November 2021

Abstract
In this study, the effects of process parameters (initial glucose concentrations, inoculation rates, and nitrogen sources including yeast extract, beef extract, and ammonium nitrate instead of peptone) on polygalacturonase production by *Aspergillus sojae* in the shaking incubator were examined. Subsequently, the effect of talcum microparticles on activity and morphology was investigated. Based on the results, the highest activity and lowest pellet diameter were 41.91 U/mL and 1411.9 μm when initial glucose concentration and inoculation size were 20 g/L and 12% (v/v), respectively. Besides, as inoculation rate increased, maximum specific growth rate and saturation constant decreased from 0.39 to 0.27 day^{-1} and 21.26 to 3.50 g/L, respectively. It was determined that highest activity and lowest pellet diameter were found as 18.53 U/mL and 2953.0 μm when medium was supplemented with 2.5 g/L yeast extract. It was also found that as yeast extract and beef extract concentrations increased, pellet diameter decreased. Additionally, medium was supplemented with talcum as a microparticle (0–25 g/L), and maximum activity was 26.59 U/mL (pellet diameter was 2756.3 μm) when talcum concentration was 5 g/L. In addition, as talcum concentration increases from 0.1 to 5 g/L, the biomass concentration increased relatively, but the enzyme activity increased significantly. However, although talcum concentration in the medium is increased until 20 g/L, while the biomass concentration increased, the activity decreased. Compared to control fermentation (without talcum), the activity increased ninefold. Polygalacturonase was also partially purified via ultrafiltration with the purification fold of 1.84. Consequently, fungal morphology in submerged fermentation can be controlled by microparticle addition fermentation, and thus the enzyme activity can be increased.

Keywords Submerged fermentation · Medium composition · Microparticle addition

1 Introduction

Pectinases are a group of collective enzymes responsible for the breakdown of complex heteropolysaccharides (pectin and pectic substances) commonly found in the middle lamella and primary cell wall of plants [1]. In addition, pectinases are widely found in higher plants, especially some fruits, and are involved in the modification of pectic materials in natural ripening processes. They are capable of breaking, de-branching, or altering glycosidic bonds, which are the most abundant component in fruits [2]. Furthermore, pectinases are commonly used in diverse biotechnological and industrial sectors such as textile, wine, and animal feed industries, modifying pertinacious material, scouring and bleaching of fabric, protoplast fusion technology, fruit juice clarification, industrial wastewater treatment, oil extraction, coffee and tea fermentation, and retting and degumming [3]. On the other hand, pectinases have an important place in the enzyme market, with a 25% share in the global food and beverage market. The market value, which increased 27.6 million dollars ($) in 2013 to 30 million dollars ($) in 2016, is expected to increase to 35.5 million dollars ($) in 2021. This increasing global demand for natural and recombinant pectinases is also closely related to advances

✉ Irfan Turhan
iturhan@akdeniz.edu.tr

[1] Department of Food Engineering, Faculty of Engineering, Akdeniz University, 07058 Antalya, Turkey

[2] Göynük Culinary Arts Vocational School, Akdeniz University, 07980 Antalya, Turkey

[3] Department of Food Engineering, Izmir Institute of Technology, Gulbahce Campus, 35430 Izmir, Turkey

Published online: 23 November 2021

in strain development, genetic engineering, metagenomics studies, site-directed mutagenesis, directed evolution, and media engineering [3]. Pectinases are classified according to their reaction mechanisms as de-esterifying enzymes (pectin esterases), depolymerizing enzymes (hydrolases and lyases), and protopectinases and their effect on the galacturonan structure of pectic materials. Generally, their classification is made in three subcategories: pectate lyases (EC 4.2.2.2), pectin esterases (EC 3.1.1.11), and polygalacturonases (EC 3.2.1.15). The polygalacturonase enzyme is a depolymerizing pectinase that catalyzes the hydrolysis of α-1,4-glycosidic bonds in the homopolygalacturonan backbone [2].

Industrial enzymes are produced by fungi and yeasts (50%), bacteria (35%), and plants or animals (15%) [4]. The efficiency of fungal organisms, which have an important share among microbial resources, in industrial-scale enzyme production has led researchers to focus on fungal species and has led to extensive research for the production of commercial enzymes [3, 5]. Although it is found naturally in the crop maturation process of plants, microbial resources have become more prominent due to the disadvantages of pectinase extraction from plant materials such as seasonal limitations and poor biochemical properties. Apart from this, the production of enzymes that are required for industrial use is possible with microbial resources due to their advantages such as large-scale production, less by-product formation, high activity, and low cost, high stability, and high purity manufacturability. The versatility, broad substrate specificity, inducibility, and ability of microbial pectinases to act on a wide variety of pectic substances have been reported as other reasons for their great biotech potential [6].

Pectinolytic organisms can be isolated from many sources such as rotten fruit walls, soil, decaying agricultural waste, and animals. Major pectinase-producing microorganisms include a variety of bacteria such as *Pseudomonas*, *Xanthomonas*, *Erwinia*, *Actinomycetes*, and *Streptomycetes*. Filamentous fungi, especially *Aspergillus* species, are one of the most important microorganism groups used in commercial pectinase production [7]. The reason why microorganisms of the genus *Aspergillus* are generally preferred in pectinase production is that they have the status of Generally Recognized As Safe (GRAS) [8]. In the production of polygalacturonases, fungi belonging to the genus *Aspergillus* with high activity are generally used. Different species of this genus such as *A. japonicus* [9], *A. awamori* [10], *A. nidulans* [11], *A. oryzae* [12], *A. aculeatus* [13], *A. niger* [14], *A. sojae* [15–18], and *A. fumigatus* [19] have been evaluated in the production of polygalacturonase.

Liquid culture fermentations, which have advantages such as being able to control the fermentation medium more easily against contamination and needless processing after fermentation, have some disadvantages in fungus applications. Excessive cellular growth is one of the most important problems encountered in fermentation studies with fungi. It is known that excessive growth of fungal species in the medium negatively affects the homogeneity of heat-mass-oxygen transfer and substrate transfer through the cell wall [20]. To overcome this problem, changes in fermentation parameters such as agitation speed and impeller type, pH, temperature, and aeration are used together with current techniques [20]. On the other hand, it has been reported that fungal morphology is the most sensitive parameter negatively affecting fermentation efficiency in the growth of filamentous organisms, which have important usage advantages for industrial production, in liquid culture medium [21]. The control of the morphological structures formed by fungal species that have a complex morphological life cycle and show excessive growth causes some difficulties in fermentation studies [22]. It is known that different morphologies are suitable for different metabolites in fermentation with filamentous organisms. While mycelium growth increases the production of enzymes such as amylase, it has been observed that pellet morphology is more suitable in the synthesis of secondary metabolites such as citric acid and penicillin. It has been understood that the frequency of branching in cell morphology is important, as it has been observed in some studies that metabolite secretion occurs mostly at the hyphae ends [23]. Therefore, the desired changes in fungal morphology aim to create smaller and lower-density pellets by preventing the formation of large pellets. Thus, better substrate consumption and product production can be achieved [24]. In recent years, the microparticle-enhance cultivation technique is one of the most remarkable methods among the current methods, and it allows the morphology to be controlled by preventing the spore aggregation of filamentous organisms with a physical effect. It is an easy-to-apply and low-cost method with minimal effect on other parameters of the process [20, 24–26]. Therefore, the microparticle-enhance cultivation technique also appears to be a good alternative to reduce rising energy costs [27]. Another advantage of this method is that microparticles do not interact with the product and cause quality loss [28]. However, the mechanism of action of the microparticle-enhance cultivation technique towards product formation and morphological growth at the molecular level has not yet been explained, and the interest in the subject is increasing day by day.

In this study, the effects of various initial glucose concentrations, inoculation rates, and nitrogen sources (yeast extract, beef extract, and ammonium nitrate instead of peptone) on polygalacturonase production by *A. sojae* (ATCC 20,235) in the shaking incubator were examined. Subsequently, the effect of talcum microparticles added into fermentation media on polygalacturonase activity and fungal morphology was investigated. To our knowledge, there is no study examining the effect of fermentation parameters and medium composition on the fungal morphology of *A.*

sojae in the literature. Besides, magnesium silicate, is also known as talcum, was used as a microparticle agent first to control the fungal morphology in the production of *A. sojae* polygalacturonase. Therefore, the present study is novel in point of the aforementioned topics.

2 Materials and methods

2.1 Materials

Glucose (≥ 99.5%) that used as the sole carbon source, disodium phosphate and monosodium phosphate that uses as mineral sources in the fermentation medium, galacturonic acid that uses to obtain a standard curve for enzyme analysis, and polygalacturonic acid that uses as a substrate for enzyme analysis were purchased from Sigma-Aldrich (Seelze, Germany). Malt extract and agar–agar used to grow the fungus and peptone, yeast extract, beef extract, and ammonium nitrate used as nitrogen sources in the medium were purchased from Merck (Darmstadt, Germany). On the other hand, magnesium silicate ($3MgO.4SiO_2.H_2O$, Talc, Sigma-Aldrich), which prevents the formation of pellets by preventing fungi from sticking to each other and thus increases the surface area [29], was used as a microparticle agent.

2.2 Microorganism and spore suspension

Aspergillus sojae (ATCC 20,235), which was kindly provided by the laboratory of Prof. Dr. Canan Tari (Izmir Institute of Technology, Izmir, Turkey), was used for the production of polygalacturonase. Cultures were cultivated at 30 °C for 7 days on the yeast-malt-extract agar containing 2% (w/v) agar–agar, 1% (w/v) malt extract, 0.4% (w/v) yeast extract, and 0.4% (w/v) glucose. After cultivation, the grown-cultures were stored at 4 °C for a month and renewed periodically each month to have viability and productivity. Besides, for long-term storage, the grown-cultures were also taken into the 20% (v/v) glycerol solution and maintained at − 80 °C [18]. On the other hand, the Tween 80-water solution (100 mL) was prepared at the ratio of 0.02% (v/v) and sterilized for 15 min at 121 °C. A 10 mL of the solution was used to collect the spores grown on the surface of the agar plate. This procedure was performed two times. The number of spores in the obtained solution was counted about 3×10^7 spores/mL using a Thoma counting chamber and was used as inoculum to initiate the fermentation [15, 16, 18].

2.3 Experimental design

The effects of initial glucose concentration (10, 20, and 30 g/L), inoculation rate (8, 10, and 12% (v/v)), and nitrogen sources (yeast extract, beef extract, and ammonium nitrate) on the production of *A. sojae* polygalacturonase were investigated. Besides, as well as the effect of different nitrogen sources instead of peptone used in the basal-medium, the influences of their different concentrations were also examined by the addition of 0.5, 1.5, and 2.5 g/L into the fermentation medium. After determining the best fermentation medium, the impact of microparticle addition (talcum) on the production of polygalacturonase was also studied, and different levels of talcum ranged between 0.05 and 25 g/L in the media were added. Depending on the results of microparticle-enhanced fermentations, the influences of different microparticle concentrations on the fungal morphology and polygalacturonase activity were determined. Nevertheless, it was also examined whether there is a relationship between cell morphology and enzyme activity or not.

2.4 Microparticle preparation

Magnesium silicate (10 μm particle size, $3MgO.4SiO_2.H_2O$, Talc, Sigma-Aldrich, also known as talcum) was utilized to detect the impact of microparticles on fungal morphology and polygalacturonase production in submerged fermentation. For this purpose, talcum solutions prepared in a 50 mM Na-acetate buffer (pH6.5) in a way to 0.05, 0.1, 0.5, 1, 3, 5, 10, 15, 20, and 25 g/L in the medium were then sterilized separately for 20 min at 121 °C to prevent agglomeration. After that, the solutions were aseptically added to the fermentation medium before inoculation [16, 27, 29–31].

2.5 Submerged fermentation

Submerged fermentations were performed with 300 mL medium in 500 mL flasks using a shaking incubator (CERTOMAT® IS, Göttingen, Germany). The temperature and agitation speed were adjusted to 30 °C and 200 rpm. Regarding microparticle-enhanced fermentation, 30 mL of microparticle solution were aseptically mixed with 270 mL of the medium before inoculation. After, the medium was inoculated in varying proportions with the prepared spore solution. All fermentations were carried out two times and according to the experimental design order. The fermentation was terminated after 4 days [16, 17]. Before and after inoculation and during fermentation, the samples were harvested aseptically. The collected samples were stored at 4 °C until analysis in terms of polygalacturonase, residual sugar, biomass, pH, and morphology.

2.6 Analytical methods

2.6.1 Enzyme analysis

The samples taken from the fermentation medium were analyzed with a method prepared by making minor

modifications in the procedure developed by Panda et al. [32], and polygalacturonase activity was determined. Polygalacturonic acid (2.4 g/L, pH 4.8, and 26 °C) was used as a substrate for enzyme analysis. First of all, 0.10 mL of fermentation samples was mixed with 0.4 mL of the substrate in the test tubes, and the mixtures obtained were incubated for 10 min at 50 °C. After the first incubation, copper solution reagent was added to the test tubes and then they were kept at the same temperature for 20 min for the second incubation. In enzyme analysis, unlike the other samples, the control samples prepared separately for each sample were prevented from interacting with the enzyme by adding an iron reagent to the sample before the substrate. After the second incubation, after adding 1 mL of arsenomolybdate solution to the analysis mixtures cooled at room temperature, the absorbance of the samples was measured at 500 nm wavelength against the deionized water used as blank in the spectrophotometer (Thermo Scientific Evolution 201, Waltham, MA, USA). Enzyme activities were calculated using the absorbance values measured at 500 nm wavelength and the galacturonic acid standard curve. One unit of enzyme activity is defined as the amount of enzyme that liberates 1 µmol of galacturonic acid in 1 min under standard assay conditions and activity values are given in U/mL [16–18, 33–38].

2.6.2 Residual sugar analysis

The 3,5-dinitrosalycylic acid method suggested by Miller [39] was used to detect the residual sugar concentration in the fermentation broth. The sugar consumption was calculated by subtracting the residual sugar concentration at the time "d" from the initial glucose concentration.

2.6.3 Biomass analysis and pH

At the end of the fermentation, the biomass amount was detected gravimetrically according to the method suggested by Karahalil et al. [16]. Briefly, samples after fermentation were filtered with pre-weighed filter paper (Whatman No: 1) and solubles were removed. The biomass remaining in the filter was then dried to constant weight at 60 °C [16]. Besides, the medium pH of the collected samples at the end of the fermentation was also measured with a pH meter (Mettler-Toledo AG, 8603 Schwerzenbach, Switzerland). Before pH measurements, the device was calibrated with the special pH calibration fluids (pH 4.01 and pH 7.0) [40, 41].

2.6.4 Morphological analysis

Morphological analysis was performed to determine how the fungal morphology and pellet diameters changed by the studied parameters. For this, a stereo-microscope (Stemi 2000-C, Zeiss, Germany) equipped with an AxioCamERc5 camera (Stemi 2000-C, Zeiss) was used. Before measurements, the biomass was washed with deionized water to remove fermentation broth and microparticles and then taken images to determine their average morphological size. Subsequently, the images were transferred to software (the ImageJ 1.50b, National Institutes of Health, and America) for the image analysis. Initially, the color of the images was converted to black, and then the morphological size of each pellet was determined with the aid of the program [16, 31].

2.6.5 Partial purification of polygalacturonase

The laboratory type Sartocon Slice 200 model ultrafiltration system (Sartorius Stedim Biotech, Göttingen, Germany) was employed to partially purify the resulting bulk enzyme solution from the fermentation with microparticle. In this process, polyether sulfone UF membrane, which has a cut-off value of 10 kDa with a surface area of 200 cm^2 that can withstand up to 4 bar pressure, was utilized. Before the ultrafiltration process, the membrane used was conditioned with the fermented liquid. After that, the ultrafiltration process was begun with 100 mL of the fermented liquid. When the volumes of the retentate and permeate were 10 and 90 mL, respectively, the process was stopped. The enzyme activities in the bulk fermentation medium, retentate, and permeate were determined based on the above method. Besides, the samples collected were analyzed for the protein concentration. By using the obtained results, specific polygalacturonase activity, yield, and purification fold were determined [42, 43].

2.7 Statistical analysis

The research was planned as a factorial arrangement of the randomized plot design. The resulting data were subjected to the analysis of variance and the average of the main variation sources found important to the Duncan multiple comparison test. Besides, if the number of factors did not exceed two, the analysis of variance was used to find out whether there were statistically significant differences between the factor averages. The minimum confidence limit in the analysis of variance and Duncan multiple comparison tests was 95% ($p \leq 0.05$).

3 Results and discussion

3.1 Effect of initial glucose concentration and inoculation rate on enzyme fermentation

In the present study, the impact of initial glucose concentration and inoculation rate on polygalacturonase production was investigated. The fermentation media were

supplemented with 2.5 g/L peptone, 3.2 g/L disodium phosphate, and 3.3 g/L monosodium phosphate. 8, 10, and 12% of the prepared *A. sojae* spore solution on media with initial glucose concentrations of 10, 20, and 30 g/L were inoculated, and the results in Figs. 1 and 2 were obtained from these fermentations carried out separately. According to the results, the highest enzyme activity (41.91 U/mL) was obtained from fermentation with an initial glucose concentration of 20 g/L and an inoculation rate of 12% ($p < 0.05$) (Fig. 1). The lowest activity value (15.82 U/mL) was determined by the fermentation carried out in a medium containing an initial glucose level of 10 g/L where 8% was inoculated (Fig. 1). The maximum and minimum glucose consumption values were determined as 14.45 (30 g/L-8%) and 4.97 g/L (10 g/L-10%), respectively ($p < 0.05$) (Fig. 1). When the microscopic images of the samples taken from the fermentation medium were examined, the largest pellet diameter (4264.4 µm) was determined when there was

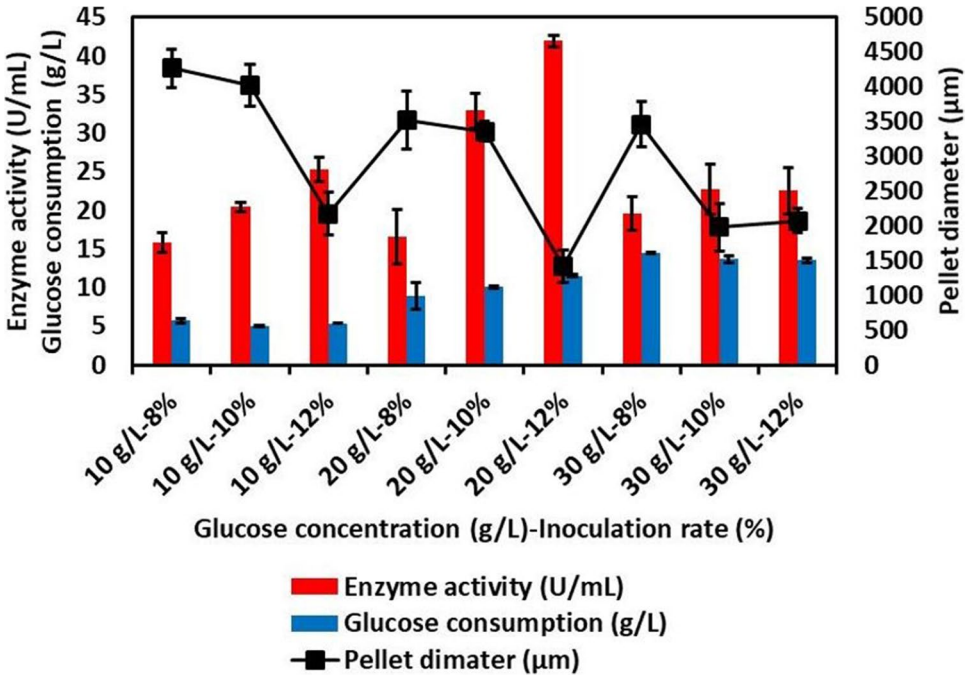

Fig. 1 Effect of initial glucose concentration and inoculation rate on enzyme activity, glucose consumption, and pellet size

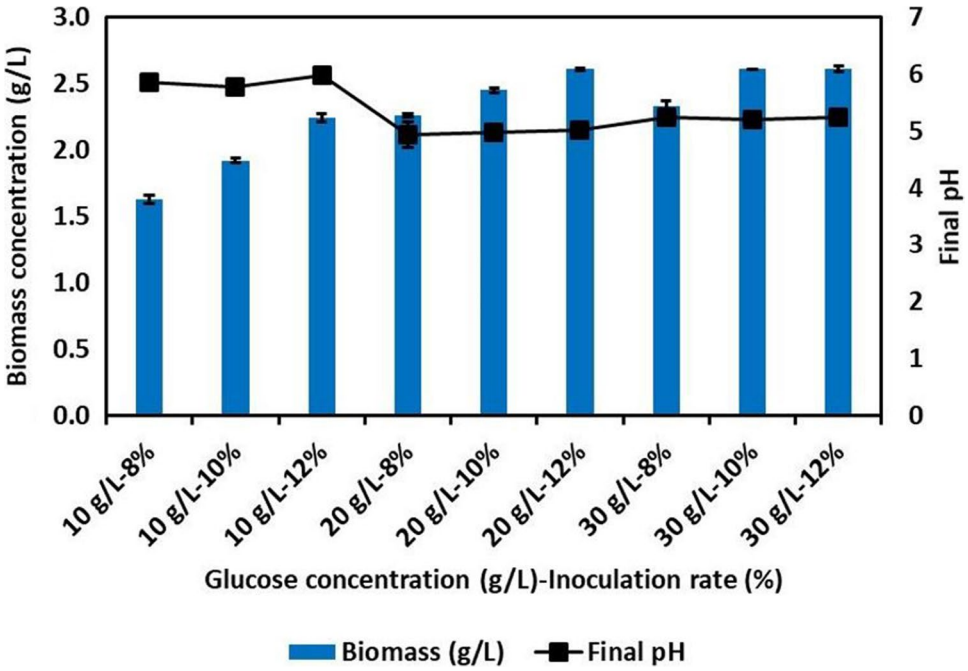

Fig. 2 Effect of initial glucose concentration and inoculation rate on biomass concentration and final pH

10 g/L glucose in the fermentation medium and 8% was inoculated (Fig. 1). On the contrary, the fermentation medium in which 20 g/L initial glucose concentration contained and 12% inoculation, which yielded the highest enzyme activity, gave the minimum pellet diameter (1411.9 µm) (Fig. 1). It was also found a statistically significant difference between the minimum and maximum pellet diameters ($p < 0.05$). Among the fermentations with the same initial sugar content, the largest pellet diameters were determined from the trials in which 8% was inoculated ($p < 0.05$). Besides, when the initial glucose concentration was kept constant, except for that of 30 g/L-12% application, the pellet diameter decreased with an increase in the inoculation rate (Fig. 1). When the inoculation rate was maintained, the pellet diameter decreased with an increase in the initial glucose concentration, apart from that of the 30 g/L-12% trial (Fig. 1). On the other hand, as seen in Fig. 3, it was observed that the spores' morphology formed as round-flat pellets. The highest biomass concentration (2.61 g/L) was reached when fermentation conditions were 20 g/L glucose-12% inoculation rate, 30 g/L glucose-10% inoculation rate, and 30 g/L glucose-12% inoculation rate (Fig. 2). Fermentation with an initial glucose concentration of 10 g/L and an inoculation rate of 8% gave the lowest biomass level (1.63 g/L) (Fig. 2). When the biomass concentrations reached at the end of fermentation were examined, there was no statistically significant difference between the fermentations ($p > 0.05$). On the other hand, the final pH values ranged from 4.93 to 5.86, and it was not found a significant difference between the final pH values ($p > 0.05$).

Additionally, the relationship between the sugar concentration and specific growth rate at different inoculation rates was also examined (Fig. 4). To calculate the maximum specific growth rate (μ_{max}, 1/d) and the limiting substrate concentration when the specific growth rate is equal to half the maximum specific growth rate (K_S, g/L), the Monod equation and thus three velocity equations that include the Lineweaver–Burk, Eadie-Hofstee, and Hanes-Woolf were used. The results are shown in Table 1. Based on the results, depending on the velocity equation, the K_S and μ_{max} values changed between 15.76–21.36 g/L and 0.33–0.39/d, 8.82–9.89 g/L and 0.31–0.33/d, and 2.79–3.50 g/L and 0.265–0.274/d when the inoculation rates were 8, 10, and 12%, respectively. The minimum and maximum K_S and μ_{max} values were completely determined with the Hanes-Woolf and Lineweaver–Burk equations, respectively (Table 1). Besides, with an increase in the inoculation rate, the K_S and μ_{max} values decreased, showing that the increasing inoculation rate increases the substrate affinity of *A. sojae* (Table 1). As a result, based on Figs. 1 and 2, the highest polygalacturonase activity and biomass concentration and the lowest pellet diameter were yielded when the initial glucose concentration and inoculation rate were 20 g/L and 12%. Therefore, for the subsequent fermentation, initial glucose concentration and inoculation rate were kept constant at 20 g/L and 12%, respectively.

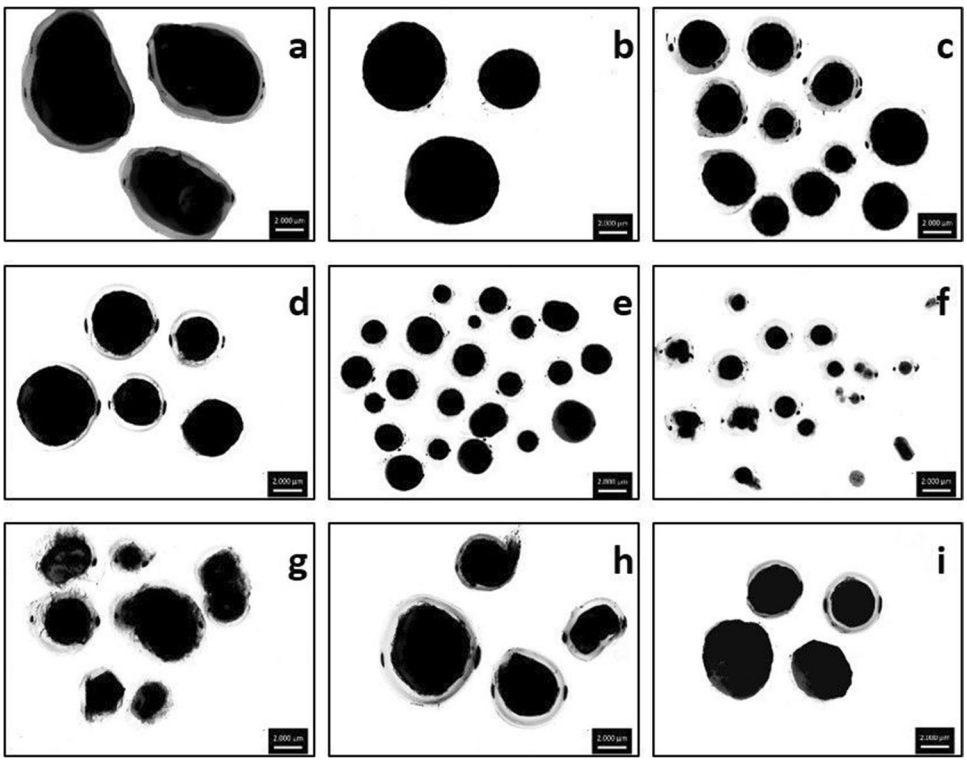

Fig. 3 Effect of initial glucose concentration and inoculation rate on fungal morphology. **a** 10 g/L initial glucose concentration and 8% (v/v) inoculation rate, **b** 10 g/L initial glucose concentration and 10% (v/v) inoculation rate, **c** 10 g/L initial glucose concentration and 12% (v/v) inoculation rate, **d** 20 g/L initial glucose concentration and 8% (v/v) inoculation rate, **e** 20 g/L initial glucose concentration and 10% (v/v) inoculation rate, **f** 20 g/L initial glucose concentration and 12% (v/v) inoculation rate, **g)** 30 g/L initial glucose concentration and 8% (v/v) inoculation rate, **h** 30 g/L initial glucose concentration and 10% (v/v) inoculation rate, and **i** 30 g/L initial glucose concentration and 12% (v/v) inoculation rate. The scale bar is 2000 µm

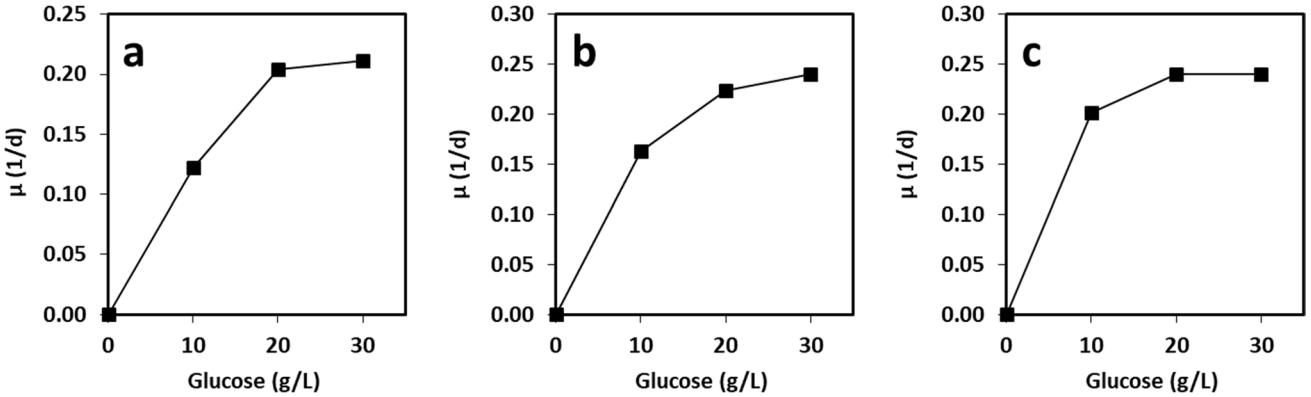

Fig. 4 The relationship between glucose concentration and the specific growth rate of *Aspergillus sojae* in fermentations by inoculating different inoculation rates. **a** 8% inoculation rate, **b** 10% inoculation rate, and **c** 12% inoculation rate

Table 1 The values of K_S and μ_{max} calculating by using velocity equations of the fermentations performed with different inoculation rates

Inoculation rate (%)	Kinetics	Velocity equation		
		Lineweaver–Burk	Eadie-Hofstee	Hanes-Woolf
8	K_S (g/L)	21.36	16.00	15.76
	μ_{max} (1/d)	0.39	0.34	0.33
	R^2	0.9616	0.7078	0.9345
10	K_S (g/L)	9.89	9.48	8.82
	μ_{max} (1/d)	0.33	0.32	0.31
	R^2	0.9894	0.9621	0.995
12	K_S (g/L)	3.50	3.37	2.79
	μ_{max} (1/d)	0.274	0.272	0.26
	R^2	0.9423	0.8961	0.9964

3.2 Effect of nitrogen sources on enzyme fermentation

In this part of the study, yeast extract, beef extract, and ammonium nitrate were used separately and respectively as nitrogen sources instead of peptone. Based on the results, in fermentations enriched with yeast extract, beef extract, and ammonium nitrate, the highest polygalacturonase activities were determined as 18.53, 18.43, and 14.20 U/mL, when 2.5, 2.5, and 1.5 g/L of yeast extract, beef extract, and ammonium nitrate were used in the medium, respectively (Fig. 5) ($p < 0.05$). The lowest polygalacturonase activities were also found as 15.26, 12.09, and 7.50 U/mL when the fermentation media were supplemented with 1.5, 0.5, and 0.5 g/L of yeast extract, beef extract, and ammonium nitrate, respectively (Fig. 5). It was determined that as the beef extract concentration increased in the medium, polygalacturonase activity increased. Besides, polygalacturonase activity decreased first and then increased when the yeast extract concentration was increased in the medium (Fig. 5). Vice versa, the enzyme activity increased first and then decreased when the ammonium nitrate level was raised in the fermentation medium (Fig. 5). It was also found a statistical difference between the lowest and highest polygalacturonase activities belong to each nitrogen source ($p < 0.05$). On the other hand, minimum and maximum glucose consumptions were determined as 8.09 and 12.57 g/L for yeast extract, 6.21 and 11.54 g/L for beef extract, and 2.47 and 4.54 g/L for ammonium nitrate, when the fermentation media were enriched with 0.5 and 2.5 g/L of yeast extract, 0.5 and 1.5 g/L of beef extract, and 1.5 and 0.5 g/L of ammonium nitrate, respectively (Fig. 5). Besides, it was also determined that as the concentration of yeast extract in the medium increased glucose consumption increased (Fig. 5). Also, there was a significant difference between the lowest and highest glucose consumption values ($p < 0.05$). On the other hand, the highest and lowest pellet diameters for yeast extract and beef extract were determined as 7473.8 and 3583.0 µm and 2953.0 and 1876.8 µm on media containing 2.5 and 0.5 g/L yeast extract and beef extract, respectively ($p < 0.05$). However, when the fermentation medium was supplemented with 2.5 and 0.5 g/L of ammonium nitrate, the maximum and minimum pellet diameters were measured as 3786.6 and 3399.2 µm ($p > 0.05$). It was also found that as the yeast extract and beef extract concentrations increased in the fermentation medium, the pellet diameter decreased. However, this was in contrast with ammonium nitrate (Figs. 5 and 6). Additionally, at the end of fermentations, the lowest biomass concentrations (1.55, 2.36, and 1.73 g/L) were detected in the media containing 0.5 g/L yeast extract, beef extract, and ammonium nitrate, while the highest biomass concentrations (4.08, 3.22, and 2.26 g/L) were obtained from the media containing 2.5 g/L yeast extract, beef extract, and ammonium nitrate (Fig. 7). It was also detected that as the concentration of nitrogen sources in the medium used increased, the biomass concentrations also increased ($p < 0.05$) (Fig. 7). Among the effect

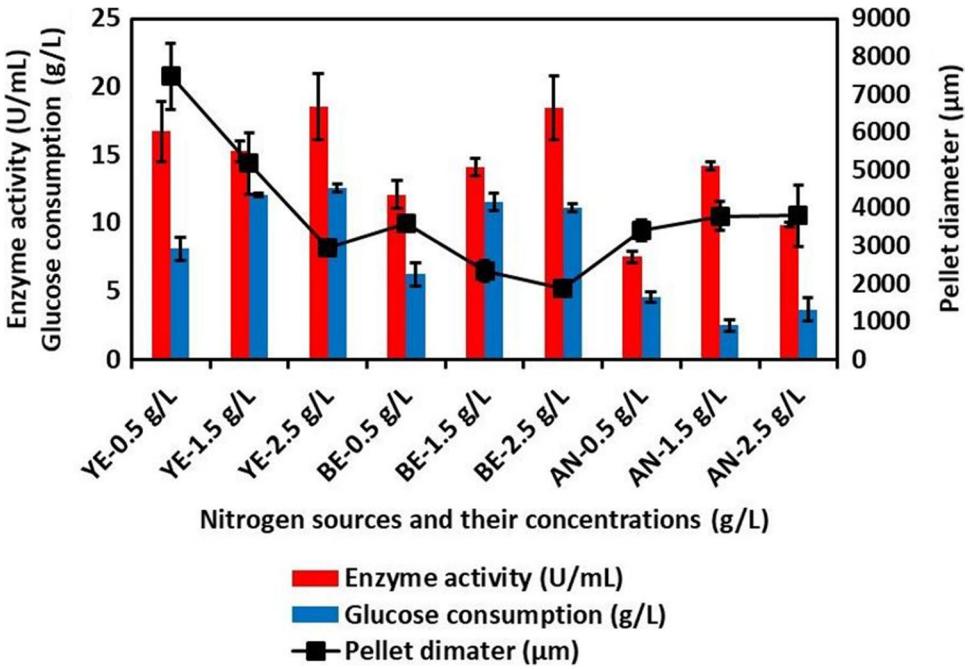

Fig. 5 Effect of nitrogen sources and their concentrations on enzyme activity, glucose consumption, and pellet size. YE is the yeast extract, BE is the beef extract, and AN is the ammonium nitrate

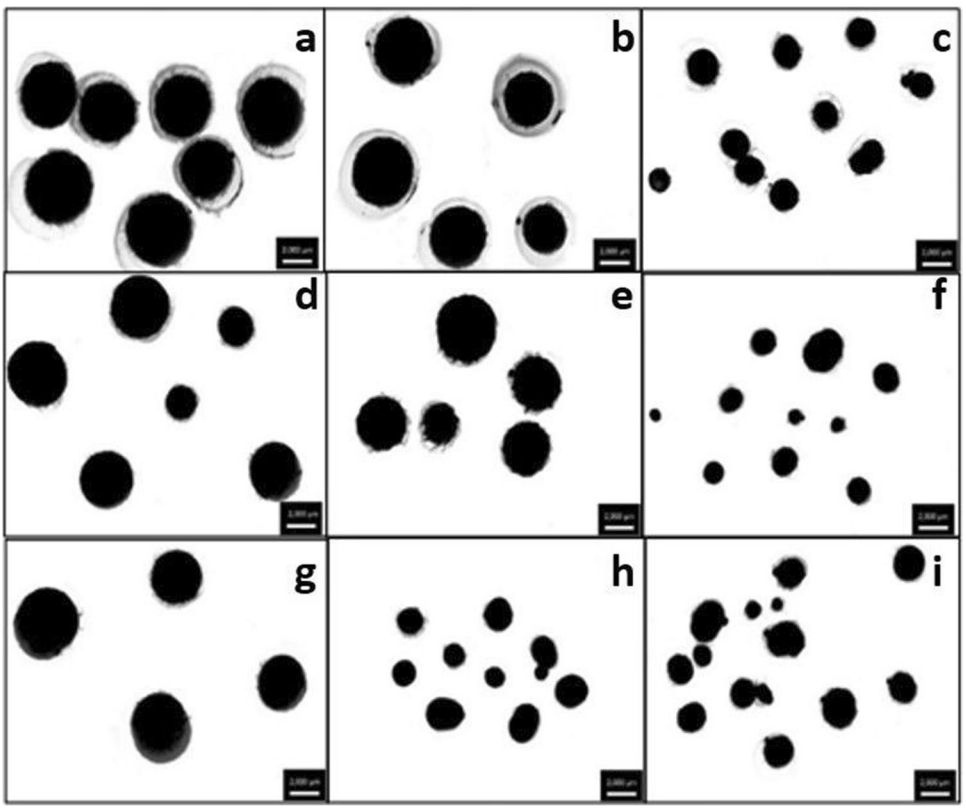

Fig. 6 Effect of nitrogen sources on fungal morphology. **a** 0.5 g/L yeast extract, **b** 1.5 g/L yeast extract, **c** 2.5 g/L yeast extract, **d** 0.5 g/L beef extract, **e** 1.5 g/L beef extract, **f** 2.5 g/L beef extract, **g** 0.5 g/L ammonium nitrate, **h** 1.5 g/L ammonium nitrate, and **i** 2.5 g/L ammonium nitrate. The scale bar is 2000 μm

of nitrogen sources on biomass concentration at the end of the fermentation, the medium supplemented with 2.5 g/L of yeast extract yielded the highest biomass level ($p < 0.05$) (Fig. 7). In addition, at the end of the fermentation, the pH of the fermentation broth varied between 4.41 and 5.24 for yeast extract ($p < 0.05$), 4.56 and 5.15 for beef extract ($p > 0.05$), 5.98 and 6.21 for ammonium nitrate ($p > 0.05$). As the concentration of nitrogen sources in the medium increased, while the pH of fermented broth decreased when ammonium nitrate was used, its level increased when yeast

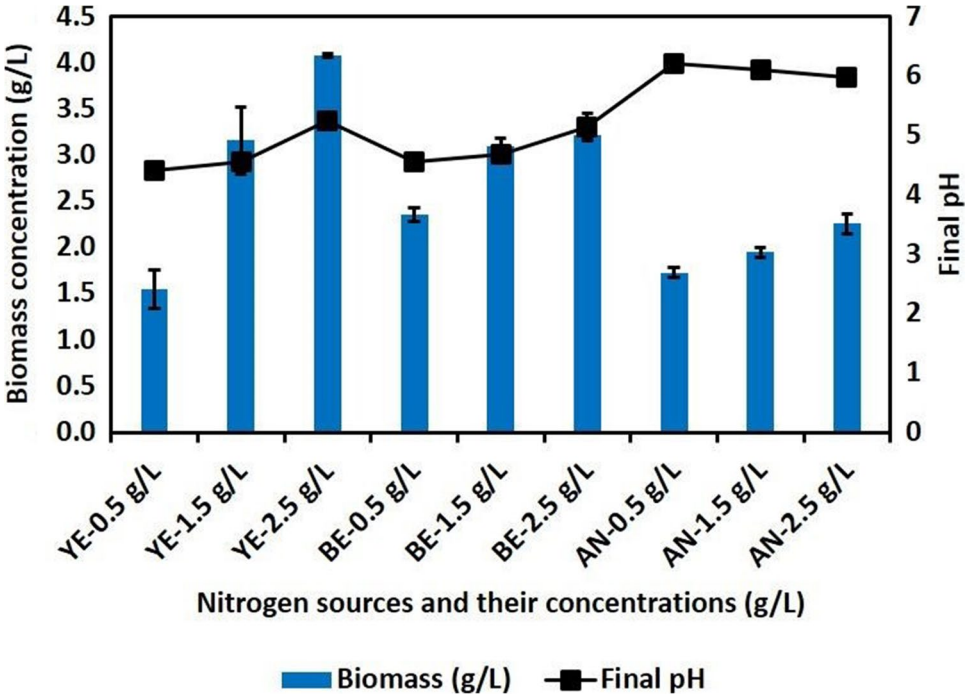

Fig. 7 Effect of nitrogen sources and their concentrations on biomass concentration and final pH. YE is the yeast extract, BE is the beef extract, and AN is the ammonium nitrate

extract and beef extract were utilized (Fig. 7). Overall, it is because the maximum polygalacturonase activity, glucose consumption, and biomass concentration were achieved when the fermentation medium was enriched with 2.5 g/L of yeast extract as a nitrogen source. Therefore, for subsequent microparticle-enhanced fermentation, the fermentation conditions were 20 g/L initial glucose concentration, 12% inoculation rate, and 2.5 g/L yeast extract.

In the literature, the effect of carbon source concentration added into the fermentation on polygalacturonase production was studied. In a study in which wheat bran extract medium including 20 g/L citric pectin and various initial glucose concentrations ranging from 0 to 30 g/L were used as substrate source for the production of polygalacturonase by *A. oryzae* under pH-uncontrolled conditions [12], maximum endo- and exo-polygalacturonase activities were measured as 125.0 and 76.3 U/mL, respectively, when the initial glucose concentration was 10 g/L. When the glucose concentration was increased to 30 g/L, it was determined that both endo and exo-polygalacturonase activities decreased significantly up to 67.3 and 22.5 g/L, respectively [12]. Compared to the enzyme activity yielded by Fontana, Silveira [12], lower polygalacturonase activities were obtained with the present study. On the other hand, it was reported that the maximum biomass concentrations changed between 11.0 and 14.8 g/L and they increased with an increase in the glucose concentration [12]. However, when biomass concentrations are compared with those of the current study, lower biomass concentrations were yielded. Besides, when the sugar consumption values from the study of Fontana, Silveira [12] are examined, almost all of the sugars in the media were consumed by *A. oryzae*. Therefore, it is understood that sugar consumption and polygalacturonase production are directly related to biomass concentration [12]. In the present study, since all sugars were not consumed due to inadequate fungal development, polygalacturonase production was lower than that of Fontana, Silveira [12]. In addition, the final pH of the fermentation broth was also measured, and reported that it ranged from 6.78 to 2.00. It was noted that as the initial glucose concentration increased, the final pH value decreased. The maximal endo- and exo-polygalacturonase activities were yielded when the final pH value was 5.55 [12], which was highly consistent with that of the current study. On the other hand, in another study, Darah et al. [44] studied the effect of inoculation rate (1–4%) and nitrogen sources (yeast extract, ammonium sulfate, peptone, urea, ammonium hydrogen phosphate, ammonium nitrate, and sodium nitrate) on polygalacturonase production from citrus pectin by *Enterobacter aerogenes* under the conditions of pH 6, 30 °C, 150 rpm, and 4 days. It was reported that the optimal inoculation rate was 3% (v/v, 5.4×10^7 cells/mL), which gave 4.77 U/mL polygalacturonase activity. After that inoculation rate, it was noted that the yielded enzyme activity reduced significantly. On the other hand, it was also reported that as the inoculation rate increased, the biomass concentration increased also [44], which validated the result of the present study. Regarding the effect of nitrogen sources, it was reported that the order of best nitrogen sources was yeast extract, ammonium sulfate, peptone, and ammonium nitrate, which yielded 12.90, 12.20, 11.67, and

6.46 U/mL, respectively. It was also noted that urea, ammonium nitrate, and sodium nitrate showed an inhibitory effect on polygalacturonase production. It was also reported that the yeast extract showed the best effect on the cell growth (ranged from 0.12 to 0.23 g/L) in comparison with ammonium sulfate, peptone, and ammonium nitrate [44]. In the present study, when the medium was enriched with yeast extract, the enzyme activity increased, but this was not for ammonium nitrate, which showed an inhibitory effect on polygalacturonase production. Therefore, the yeast extract was the best inducer for polygalacturonase production from *A. sojae* because of the presence of vitamins, minerals, and amino acids [45]. On the other hand, typically, ammonium salts result in acidic circumstances in the medium as the acid is released after the use of ammonium ions, and high acidic circumstances can inhibit the production of polygalacturonase and the development of the fungus [46]. The fact that ammonium nitrate is an inorganic nitrogen source, indicated a negative impact on polygalacturonase production and fungal growth in the current study (Fig. 7). The nutrients in the fermentation medium have a significant impact on the formation, growth, and function of the mycelial pellet. Carbon sources and nitrogen sources are the most essential nutrients for mycelial pellets and product formation [47]. Concerning the use of carbon sources, the carbon sources of the various micellar pellets are different. It has been reported that the most suitable carbon and nitrogen sources for the mycelial pellet of *Aspergillus niger* and metabolite production are sugar beet molasses, yeast extract, and peptone, respectively [40, 41, 48]. Cho et al. [49] investigated the effect of carbon sources on fungal morphology and found that *Paecilomyces sinclairi* had longer mycelium and more biomass when sucrose was used as the sole carbon source. On the other hand, the effect of carbon and nitrogen sources on the production of recombinant *Aspergillus sojae* (DH56) polygalacturonase was also investigated, and it was determined that orange peel, peptone, ammonium nitrate, and ammonium sulfate were the best carbon and nitrogen sources [37]. Therefore, nitrogen sources have an important effect on the formation of mycelial pellets and products [47]. On the other hand, *Streptomyces noursei* failed to grow in media supplemented with sucrose, galactose, maltose, xylose, lactose, and raffinose as carbon sources while using glycerol, glucose, fructose, and soluble starch [50]. However, some strains were able to exploit the difficult-to-decompose carbon source, which makes sense in the application of micellar pellets in the treatment of refractory organic wastewater [51]. As for the effect of inoculation rate, the growth process of the micelle pellet is largely the same as that of normal microorganisms. Under the same feeding conditions, the ratio and number of mycelial pellets are somewhat negatively correlated [47]. Namely, Hosobuchi et al. [52] defined quantitatively the relationship between the size of the micelle pellets and the inoculation ratio ($Dp = -alogN + b$, where Dp is the mean diameter of the micelle pellets (mm) and a and b are constants). As can be understood from this relationship, there is a negative correlation between the size of the micelle pellets and the inoculation ratio of the spores. As the inoculation ratio of spores increases, the mean diameter of the micelle pellets decreases [47], as it is in the current study. As a matter of fact, these information show that it is quite compatible with the results of the present study.

3.3 Effect of talcum concentration on enzyme fermentation

The effects of different amounts of talcum microparticles added to the fermentation medium on the enzyme activity, cell diameter, sugar consumption, biomass concentration, and final pH were examined, and the results are shown in Figs. 8 and 9. When the results are examined, in the control study where no microparticles were added to the medium, the polygalacturonase activity, consumed glucose concentration, pellet diameter, biomass concentration, and final pH were determined as 3.58 U/mL, 10.70 g/L, 3482.1 µm, 2.80 g/L, and 5.60, respectively (Figs. 8 and 9). It is seen that the polygalacturonase activities and biomass concentrations obtained from talcum-enhanced fermentation media are higher than in the control study (Fig. 8). Maximum enzyme activity (26.59 U/ml) was reached when 5 g/L talcum was added to the fermentation medium. Thus, a ninefold increase in polygalacturonase production was achieved by using microparticles in the fermentation medium. Nevertheless, the difference between the polygalacturonase activities of other productions was found to be statistically significant ($p < 0.05$) (Fig. 8). However, lower polygalacturonase activities were detected in fermentations with higher concentrations than 5 g/L talcum addition to the medium (Fig. 8). It was determined that there was no statistically significant difference between the control trial and microparticle-enhanced fermentations in glucose consumption levels ($p > 0.05$) (Fig. 8). Additionally, the lowest average pellet diameter was determined as 2369.3 µm in a medium containing 15 g/L talcum, and a reduction of approximately 32% occurred compared to the control experiment (3482.1 µm) (Fig. 8). When biomass concentrations were examined, the highest value of 11.4 g/L was reached when 20 or 25 g/L talcum was added to the medium. The lowest biomass concentration was measured with control fermentation (without talcum addition) (Fig. 9). It was also determined that the biomass concentration increased parallel to the microparticle concentration (Fig. 9). At the end of the fermentation, the pH of the fermented broth was measured and determined that the pH values ranged from 4.8 to 5.6 (Fig. 9). On the other hand, when Fig. 8 and Fig. 9 are examined, as

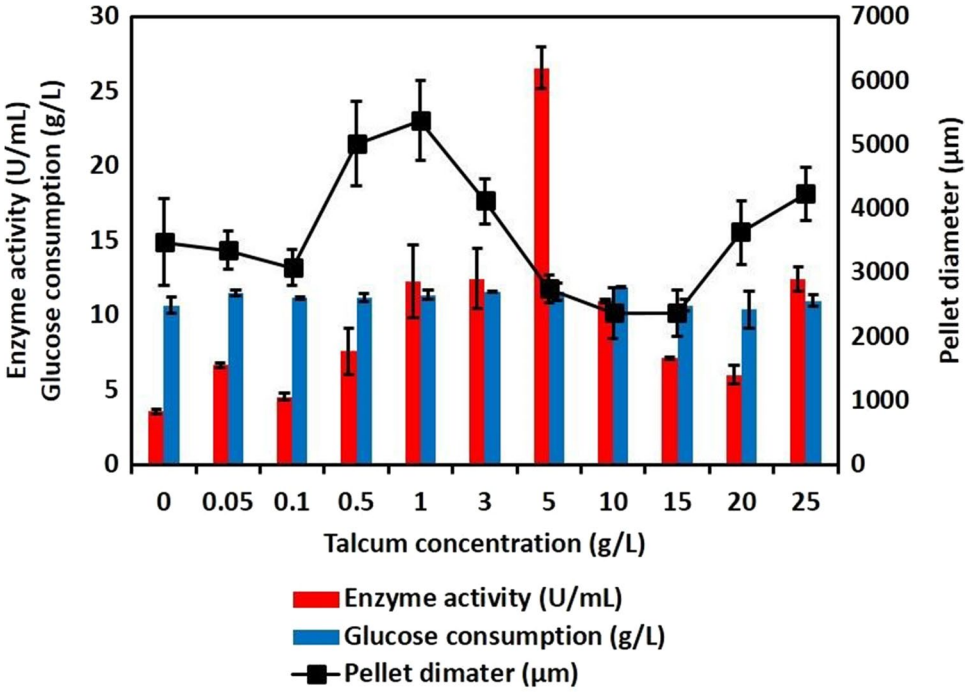

Fig. 8 Effect of talcum concentration on enzyme activity, glucose consumption, and pellet size

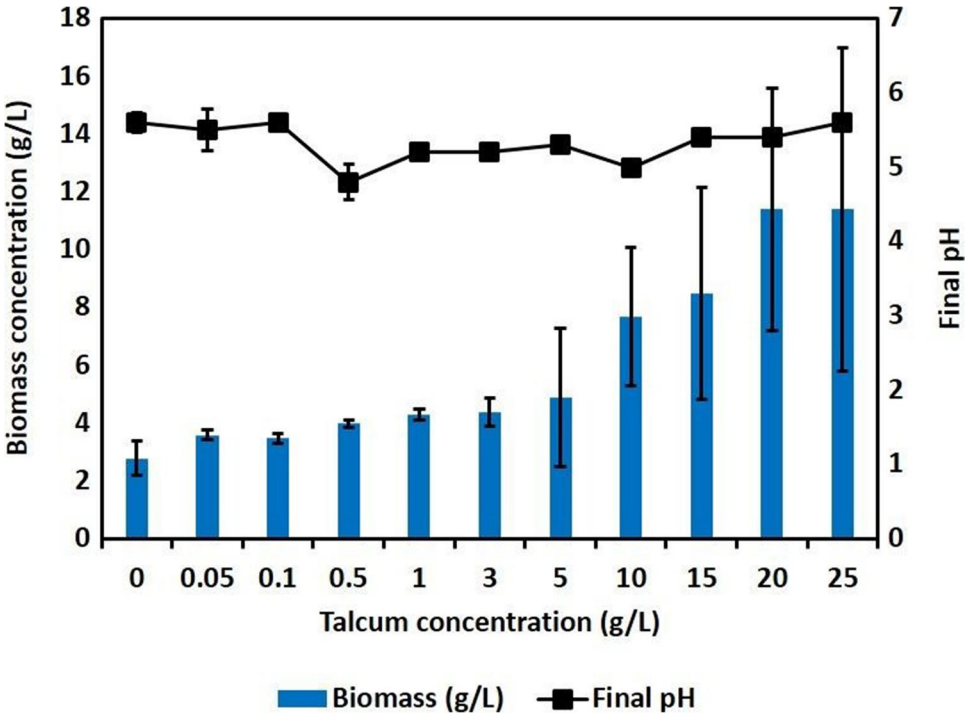

Fig. 9 Effect of talcum concentration on biomass concentration and final pH

talcum concentration increases up to 5 g/L in the medium, although the biomass concentration increases relatively, the enzyme activity generally increases, especially between 0.1 and 5 g/L talcum concentrations added to the medium. However, it is seen that when the microparticle concentration is increased until 20 g/L, although the biomass concentration increased, polygalacturonase activity decreased interestingly. Besides, when the microparticle concentration is 25 g/L, despite the biomass concentration stayed almost the same as that of 20 g/L talcum addition, it was determined that the enzyme activity increased (Fig. 8 and Fig. 9). Overall, when the results of microparticle-enhanced fermentations are examined in general, it can be observed that the addition of more talcum in the selected

concentration range (0–25 g/L) does not always result in higher enzyme activity and smaller pellet formation.

There is only one study in the literature on the production of *Aspergillus sojae* polygalacturonase from glucose by adding microparticles to the medium. In this study, the aluminum oxide was used as a microparticle, and the maximum activity was determined as 34.5 U/mL. Thus, a 2.2-fold increase was obtained compared to the control fermentation [16]. In this study, although the highest activity value (26.59 U/mL) obtained from microparticle-enhanced fermentation could not reach the highest value in the literature, it was observed that it was more efficient as a coefficient of increase and talcum microparticles could be successfully applied in *Aspergillus sojae* fermentations. On the other hand, the data obtained showed that the use of talcum can control fungal growth morphology in the fermentation environment. Recently, the microparticle-enhanced large-scale fermentation was also successfully performed for β-mannanase production from glucose and carob extract [53]. It was reported that the highest β-mannanase activities were yielded as 302.6 and 281.5 U/mL in the carob extract based medium when the medium was supplemented with 3 g/L of talcum and 15 g/L of aluminum oxide, respectively. In the glucose based medium, maximum β-mannanase activity of 117.0 U/mL was obtained when 5 g/L of aluminum oxide was added into the fermentation medium. Consequently, compared to the control fermentation (without microparticles, 102.3 U/mL), β-mannanase activity was increased in the large-scale bioreactor [53]. Therefore, depending on the results of Gürler et al. [53], the microparticle-enhanced large-scale bioreactor is promising for polygalacturonase production. Besides, although there is a limited number of studies regarding microparticle-enhanced polygalacturonase production, many microbial products were produced by adding microparticles into the fermentation medium in the literature. Some of them are summarized in Table 2. According to these data, it is understood that microparticles are successfully used in the medium and the titers of microbial products are significantly increased.

On the other hand, although a certain number of publications have been obtained, the mechanism of action of microparticles on growth has not yet been fully understood; only some researchers have established some hypotheses in their studies [20]. Namely, there are two main mechanisms for this. The first is the collision between microparticles and cells, the shear stress introduced by the microparticles, and the mechanical effects that prevent the recombination of the separated mycelium. Second are physicochemical effects such as surface charge and interaction of microparticles and pH changes in broth [21, 22]. However, more research is needed to fully elucidate the interaction mechanisms between fungi and microparticles [20]. Nevertheless, experimental and computational technologies in systems biotechnology will help take a step towards a better understanding of this complex link between the biological and engineering aspects of filamentous microorganisms [74].

3.4 Partial purification with ultrafiltration

A more concentrated enzyme solution was obtained by the ultrafiltration process of the fermented liquid taken from the fermentation in which 5 g/L talcum was added to the medium. The results of the ultrafiltration process performed with a cut-off value of 10 kDa are given in Table 3. Polygalacturonase activity, which was determined as 11.38 U/mL in the bulk fermented liquid before ultrafiltration, was determined as 22.26 and 4.23 U/mL in retentate and permeate after ultrafiltration with the purification fold of 0.40 and 1.84, respectively. Protein analysis was performed before and after ultrafiltration, and the protein amounts measured in bulk fermented liquid, permeate, and retentate were determined as 0.39, 0.37, and 0.42 mg/mL, respectively. In this case, specific enzyme activities in bulk fermented liquid, permeate, and retentate were determined as 28.74, 11.46, and 52.91 U/mg, respectively (Table 3). In a study, a polygalacturonase produced by *Aspergillus sojae* (ATCC 20,235) was purified by using Q Sepharose and Sp Sepharose chromatographies and a Superdex 75 column. Based on the results, total protein and activity values ranged from 14.73 to 0.41 mg and 12,593 to 5370 U, respectively. Besides, the specific activity values were between 855 and 13,023 U/mg. As the purification process progressed, the specific activity values increased with the purification folds of 3.06 for Q Sepharose, 9.05 for SP Sepharose, and 15.2 for Superdex 75. The results indicated that the purification with chromatographic techniques is superior to the ultrafiltration process. Besides, the ultrafiltration process was successfully used to partially purify the enzymes of inulinase produced by *Aspergillus niger* and mannanase produced by *Aspergillus sojae* [42, 43].

4 Conclusions

In this study, different medium components and talc microparticles were added to the fermentation medium, and the effect of these factors on the production of polygalacturonase from glucose by *Aspergillus sojae* was investigated. According to the results, the highest enzyme activity (41.91 U/mL) and the minimum pellet diameter (1411.9 μm) were obtained from fermentation with an initial glucose concentration of 20 g/L and an inoculation rate of 12%. Among the fermentations with the same initial sugar content, the largest pellet diameters were determined from the trials in which 8% was inoculated. Besides, it was observed that the spores' morphology formed as round-flat pellets. The K_S and μ_{max}

Table 2 Production of microbial metabolites by microparticle-enhanced cultivation technique from various fungal sources and coefficients of increase in yield [37]

Microorganism	Microparticle	Product	Yield increase	Ref
Cerrena unicolor	Aluminum oxide	Laccase	3.5-fold	[24]
Pleurotus sapidus	Aluminum oxide	Laccase	twofold	[24]
Aspergillus ficuum	Aluminum oxide	Fitase	twofold	[54]
Aspergillus ficuum	Magnesium silicate	Fitase	2.9-fold	[54]
Aspergillus ficuum	Magnesium silicate	Fitase	1.9-fold	[54]
Aspergillus ficuum	Magnesium silicate	Fitase	2.8-fold	[55]
Aspergillus ficuum	Magnesium silicate	Fitase	1.3-fold	[55]
Rhizopus oryzae	Aluminum oxide	Lactic acid	2.3-fold	[56]
Rhizopus oryzae	Magnesium silicate	Lactic acid	fourfold	[56]
Rhizopus oryzae	Magnesium silicate	Lactic acid	twofold	[56]
Trichoderma viride	Aluminum oxide	Cellulase	1.2-fold	[57]
Trichoderma viride	Aluminum oxide	Endoglucanase	1.1-fold	[57]
Aspergillus niger	Magnesium silicate	Glucoamylase	3.6-fold	[27]
Aspergillus niger	Magnesium silicate	Fructofuranosidase	twofold	[27]
Aspergillus niger	Titanate	Glucoamylase	9.5-fold	[58]
Aspergillus niger	Titanate	Fructofuranosidase	3.7-fold	[58]
Aspergillus niger	Titanate	Glucoamylase	6.4-fold	[58]
Aspergillus niger	Magnesium silicate	2-phenylethanol	1.3-fold	[59]
Mortierella isabellina	Magnesium silicate	Lipid	2.5-fold	[25]
Aspergillus sojae	Magnesium silicate	β-mannanase	8.6-fold	[60]
Aspergillus terreus	Magnesium silicate	Lovastatin	twofold	[61]
Aspergillus terreus	Magnesium silicate	Lovastatin	3.5-fold	[62]
Aspergillus sojae	Aluminum oxide	Polygalacturonase	2.2-fold	[16]
Caldariomyces fumago	Magnesium silicate	Chloroperoxidase	tenfold	[30]
Caldariomyces fumago	Aluminum oxide	Chloroperoxidase	sixfold	[30]
Aspergillus nidulans	Magnesium silicate	Anidulafungin	1.3-fold	[63]
Grifola frondosa	Magnesium silicate	Phosphoglucose isomerase	1.2-fold	[63]
Grifola frondosa	Magnesium silicate	UDP-glucose pyrophosphorylase	1.2-fold	[64]
Grifola frondosa	Magnesium silicate	UDP-glucose dehydrogenase	1.3-fold	[64]
Grifola frondosa	Magnesium silicate	GDP-mannose pyrophosphorylase	1.3-fold	[64]
Grifola frondosa	Magnesium silicate	UDP-glucose-4-epimerase	3.1-fold	[64]
Aspergillus niger	Magnesium silicate	β-fructofuranosidase	threefold	[65]
Curvularia sp.	Magnesium silicate	Curvulamine	1.9-fold	[21]
Aspergillus sojae	Aluminum oxide	β-mannanase	2.6-fold	[31]
Aspergillus sojae	Magnesium silicate	β-mannanase	1.8-fold	[31]
Aspergillus sojae	Magnesium silicate	β-mannanase	2.96-fold	[53]
Aspergillus sojae	Aluminum oxide	β-mannanase	2.75-fold	[53]
Aspergillus sojae	Aluminum oxide	β-mannanase	14.16-fold	[29]
Aspergillus sojae	Magnesium silicate	β-mannanase	6.80-fold	[29]
Monascus purpureus	Magnesium silicate	Yellow pigments	2.46-fold	[66]
Monascus purpureus	Aluminum oxide	Yellow pigments	1.74-fold	[66]
Monascus ruber	Sodium starch octenyl succinate	Yellow pigments	1.70-fold	[67]
Aspergillus nidulans	Magnesium silicate	Echinocandin B	1.48-fold	[68]
Schizophyllum commune	Aluminum oxide	Schizophyllan	twofold	[69]
Chaetomium globosum	Silica	β-D-glucuronidase	3.24-fold	[70]
Pleurotus ostreatus	Aluminum oxide	Laccase	1.14-fold	[71]
Streptomyces gilvosporeus	Magnesium silicate	Natamycin	1.7-fold	[72]
Streptomyces albus	Magnesium silicate	Pamamycin	threefold	[73]

Table 3 The activities and purification folds of the enzyme solution before and after ultrafiltration process

Ultrafiltration	Volume (mL)	Polygalacturonase activity (U)	Protein (mg)	Specific activity (U/mg)	Yield (%)	Purification fold
Before ultrafiltration	100	1138.0	39.0	28.7	100	1.00
10 kDa permeate	90	380.7	33.3	11.5	33.5	0.40
10 kDa retentate	10	222.6	4.2	52.9	19.6	1.84

values changed between 15.76–21.36 g/L and 0.33–0.39/d, 8.82–9.89 g/L and 0.31–0.33/d, and 2.79–3.50 g/L and 0.265–0.274/d when the inoculation rates were 8%, 10%, and 12%, respectively. Besides, with an increase in the inoculation rate, the K_S and μ_{max} values decreased, showing that the increasing inoculation rate increases the substrate affinity of *A. sojae*. As the concentration of nitrogen sources studied increased in the medium, the biomass concentration also increased. As for talcum-enhanced fermentations, maximum polygalacturonase activity (26.59 U/ml) was achieved when 5 g/L talcum was added to the fermentation medium. Besides, it was detected as talcum concentration in the medium increased, fungal pellet diameter did not increase proportionally. On the other hand, the enzyme activity increased although the biomass concentration stayed relatively stable as talcum concentration increases from 0.1 to 5 g/L. Vice versa, as the microparticle concentration raises from 5 to 20 g/L, despite the biomass concentration increased, the enzyme activity decreased. Besides, it can be said that there is a partial correlation between the enzyme activity and the pellet diameter according to the changing process parameters and microparticle concentration.

Author contribution All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Mustafa Germec, Ercan Karahalil, Ercan Yatmaz, and Irfan Turhan. The first draft of the manuscript was written by Mustafa Germec, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding This work was supported by the Akdeniz University Research Foundation (Grant number # FAY-2016–1547).

Data availability Data available on request from the authors.

Declarations

Conflict of interest The authors declare no competing interests.

References

1. Fratebianchi D, Acosta MA, Cavalitto SF (2018) Harnessing soybean hulls for improved polygalacturonase production by *Aspergillus sojae* through fine-tuning of ambient pH. J Chem Technol Biotechnol 93(3):667–674
2. Nakkeeran E, Gowthaman MK, Umesh-Kumar S, Subramanian R (2012) Techno-economic analysis of processes for *Aspergillus carbonarius* polygalacturonase production. J Biosci Bioeng 113(5):634–640
3. Amin F, Bhatti HN, Bilal M (2019) Recent advances in the production strategies of microbial pectinases-a review. Int J Biol Macromol 122:1017–1026
4. Anisa S, Girish K (2014) Pectinolytic activity of Rhizopus sp. and Trichoderma viride. Int J Res Pure Appl Microbiol 4(2):28–31
5. Garg G, Singh A, Kaur A, Singh R, Kaur J, Mahajan R (2016) Microbial pectinases: an ecofriendly tool of nature for industries. 3 Biotech 6(1):1–13
6. Coskun A (2010) Isolation and characterization of new *Bacillus* sp. strains producing industrial enzymes. Master, Cukurova University, Adana
7. Rebello S, Anju M, Aneesh EM, Sindhu R, Binod P, Pandey A (2017) Recent advancements in the production and application of microbial pectinases: an overview. Rev Environ Sci Bio/Technol 16(3):381–394
8. Naidu G, Panda T (1998) Production of pectolytic enzymes-a review. Bioprocess Eng 19(5):355–361
9. Teixeira MF, Lima Filho JL, Durán N (2000) Carbon sources effect on pectinase production from *Aspergillus japonicus* 586. Braz J Microbiol 31(4):286–290
10. Adedeji OE, Ezekiel OO (2020) Chemical composition and physicochemical properties of mango juice extracted using polygalacturonase produced by *Aspergillus awamori* CICC 2040 on pretreated orange peel. LWT-Food Sci Technol 132:109891
11. Xu H, Zhang P, Zhang Y, Liu Z, Zhang X, Li Z, Li J-J, Du Y (2020) Overexpression and biochemical characterization of an endo-α-1,4-polygalacturonase from *Aspergillus nidulans* in *Pichia pastoris*. Int J Mol Sci 21(6):2100
12. Fontana R, Silveira M (2012) Influence of pectin, glucose, and pH on the production of endo-and exo-polygalacturonase by *Aspergillus oryzae* in liquid medium. Braz J Chem Eng 29(4):683–690
13. de Carvalho SJ, de França PRL, Porto TS (2018) Optimized extraction of polygalacturonase from *Aspergillus aculeatus* URM4953 by aqueous two-phase systems PEG/Citrate. J Mol Liq 263:81–88
14 Anand G, Yadav S, Yadav D (2017) Production, purification and biochemical characterization of an exo-polygalacturonase from *Aspergillus niger* MTCC 478 suitable for clarification of orange juice. 3 Biotech 7(2):1–8
15. Gögus N, Tari C, Oncü S, Unluturk S, Tokatli F (2006) Relationship between morphology, rheology and polygalacturonase production by *Aspergillus sojae* ATCC 20235 in submerged cultures. Biochem Eng J 32(3):171–178
16 Karahalil E, Demirel F, Evcan E, Germeç M, Tari C, Turhan I (2017) Microparticle-enhanced polygalacturonase production by wild type *Aspergillus sojae*. 3 Biotech 7(6):1–9
17. Tari C, Gögus N, Tokatli F (2007) Optimization of biomass, pellet size and polygalacturonase production by *Aspergillus sojae* ATCC

20235 using response surface methodology. Enzyme Microb Technol 40(5):1108–1116
18. Tokatli F, Tari C, Unluturk SM, Gogus Baysal N (2009) Modeling of polygalacturonase enzyme activity and biomass production by *Aspergillus sojae* ATCC 20235. J Ind Microbiol Biotechnol 36(9):1139–1148
19. Wong LY, Saad WZ, Mohamad R, Tahir PM (2017) Optimization of cultural conditions for polygalacturonase production by a newly isolated *Aspergillus fumigatus* R6 capable of retting kenaf. Ind Crops Prod 97:175–183
20. Karahalil E, Coban HB, Turhan I (2019) A current approach to the control of filamentous fungal growth in media: microparticle enhanced cultivation technique. Crit Rev Biotechnol 39(2):192–201
21. Yang J, Jiao R-H, Yao L-Y, Han W-B, Lu Y-H, Tan R-X (2016) Control of fungal morphology for improved production of a novel antimicrobial alkaloid by marine-derived fungus Curvularia sp. IFB-Z10 under submerged fermentation. Process Biochem 51(2):185–194
22. Walisko J, Vernen F, Pommerehne K, Richter G, Terfehr J, Kaden D, Dähne L, Holtmann D, Krull R (2017) Particle-based production of antibiotic rebeccamycin with *Lechevalieria aerocolonigenes*. Process Biochem 53:1–9
23. Walisko R, Moench-Tegeder J, Blotenberg J, Wucherpfennig T, Krull R (2015) The taming of the shrew-controlling the morphology of filamentous eukaryotic and prokaryotic microorganisms. Filaments Bioprocess 1–27
24. Antecka A, Bizukojc M, Ledakowicz S (2016) Modern morphological engineering techniques for improving productivity of filamentous fungi in submerged cultures. World J Microbiol Biotechnol 32(12):1–9
25. Gao D, Zeng J, Yu X, Dong T, Chen S (2014) Improved lipid accumulation by morphology engineering of oleaginous fungus *Mortierella isabellina*. Biotechnol Bioeng 111(9):1758–1766
26. Ren X-D, Xu Y-J, Zeng X, Chen X-S, Tang L, Mao Z-G (2015) Microparticle-enhanced production of ε-poly-l-lysine in fed-batch fermentation. RSC Adv 5(100):82138–82143
27. Driouch H, Sommer B, Wittmann C (2010) Morphology engineering of *Aspergillus niger* for improved enzyme production. Biotechnol Bioeng 105(6):1058–1068
28. Driouch H, Roth A, Dersch P, Wittmann C (2010) Optimized bioprocess for production of fructofuranosidase by recombinant *Aspergillus niger*. Appl Microbiol Biotechnol 87(6):2011–2024
29. Yatmaz E, Germec M, Karahalil E, Turhan I (2020) Enhancing β-mannanase production by controlling fungal morphology in the bioreactor with microparticle addition. Food Bioprod Process 121:123–130
30. Kaup BA, Ehrich K, Pescheck M, Schrader J (2008) Microparticle-enhanced cultivation of filamentous microorganisms: increased chloroperoxidase formation by *Caldariomyces fumago* as an example. Biotechnol Bioeng 99(3):491–498
31. Yatmaz E, Karahalil E, Germec M, Ilgin M, Turhan İ (2016) Controlling filamentous fungi morphology with microparticles to enhanced β-mannanase production. Bioprocess Biosyst Eng 39(9):1391–1399
32. Panda T, Naidu G, Sinha J (1999) Multiresponse analysis of microbiological parameters affecting the production of pectolytic enzymes by *Aspergillus niger*: a statistical view. Process Biochem 35(1–2):187–195
33. Buyukkileci AO, Lahore MF, Tari C (2015) Utilization of orange peel, a food industrial waste, in the production of exo-polygalacturonase by pellet forming *Aspergillus sojae*. Bioprocess Biosyst Eng 38(4):749–760
34. Demir H, Tari C (2016) Bioconversion of wheat bran for polygalacturonase production by *Aspergillus sojae* in tray type solid-state fermentation. Int Biodeterior Biodegradation 106:60–66
35. Demir H, Tari C (2016) Effect of physicochemical parameters on the polygalacturonase of an *Aspergillus sojae* mutant using wheat bran, an agro-industrial waste, via solid-state fermentation. J Sci Food Agric 96(10):3575–3582
36. Fratebianchi D, Crespo JM, Tari C, Cavalitto S (2017) Control of agitation rate and aeration for enhanced polygalacturonase production in submerged fermentation by *Aspergillus sojae* using agro-industrial wastes. J Chem Technol Biotechnol 92(2):305–310
37. Karahalil E (2020) Optimizatıon of microbial polygalacturonase production using different media compositions and fermentation strategies. Akdeniz University, Antalya, Turkey, Doctorate
38. Tari C, Dogan N, Gogus N (2008) Biochemical and thermal characterization of crude exo-polygalacturonase produced by *Aspergillus sojae*. Food Chem 111(4):824–829
39. Miller GL (1959) Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem 31(3):426–428
40. Germec M, Gürler HN, Ozcan A, Erkan SB, Karahalil E, Turhan I (2020) Medium optimization and kinetic modeling for the production of *Aspergillus niger* inulinase. Bioprocess Biosyst Eng 43(2):217–232
41. Germec M, Turhan I (2020) Enhanced production of *Aspergillus niger* inulinase from sugar beet molasses and its kinetic modeling. Biotech Lett 42:1939–1955
42. Germec M, Turhan I (2020) Partial purification and characterization of *Aspergillus niger* inulinase produced from sugar-beet molasses in the shaking incubator and stirred-tank bioreactors. Int J Biol Macromol 164:3789–3799
43. Yatmaz E, Turhan I, Germec M, Karahalil E (2017) Using centrifuge and ultrafiltration as downstream processes for the concentration of microbial β-mannanase fermentation media. Sci Bull Ser F Biotechnol 21:133–136
44. Darah I, Nisha M, Lim S (2013) Enhancement of polygalacturonase production from *Enterobacter aerogenes* NBO2 by submerged fermentation. Adv Stud Biol 5(5):173–189
45. Aguilar G, Trejo BA, García JM, Huitrón C (1991) Influence of pH on endo-and exo-pectinase production by Aspergillus sp. CH-Y-1043. Can J Microbiol 37(12):912–917
46. Ge X-Y, Zhang W-G (2005) Effects of octadecanoylsucrose derivatives on the production of inulinase by *Apergillus niger* SL-09. World J Microbiol Biotechnol 21(8):1633–1638
47. Li L, Liang T, Liu W, Liu Y, Ma F (2020) A comprehensive review of the mycelial pellet: Research status, applications, and future prospects. Ind Eng Chem Res 59(39):16911–16922
48. Germec M, Turhan I (2019) Evaluation of carbon sources for the production of inulinase by *Aspergillus niger* A42 and its characterization. Bioprocess Biosyst Eng 42(12):1993–2005
49. Cho YJ, Hwang HJ, Kim SW, Song CH, Yun JW (2002) Effect of carbon source and aeration rate on broth rheology and fungal morphology during red pigment production by *Paecilomyces sinclairii* in a batch bioreactor. J Biotechnol 95(1):13–23
50. Jonsbu E, McIntyre M, Nielsen J (2002) The influence of carbon sources and morphology on nystatin production by *Streptomyces noursei*. J Biotechnol 95(2):133–144
51. Saraswathy A, Hallberg R (2005) Mycelial pellet formation by *Penicillium ochrochloron* species due to exposure to pyrene. Microbiol Res 160(4):375–383
52. Hosobuchi M, Ogawa K, Yoshikawa H (1993) Morphology study in production of ML-236B, a precursor of pravastatin sodium, by *Penicillium citrinum*. J Ferment Bioeng 76(6):470–475
53. Gürler HN, Erkan SB, Ozcan A, Yılmazer C, Karahalil E, Germec M, Yatmaz E, Ogel ZB, Turhan I (2020) Scale-up processing with different microparticle agent for β-mannanase production in a large-scale stirred tank bioreactor. J Food Process Preserv e14915
54. Coban HB, Demirci A, Turhan I (2015) Microparticle-enhanced *Aspergillus ficuum* phytase production and evaluation of fungal

morphology in submerged fermentation. Bioprocess Biosyst Eng 38(6):1075–1080
55. Coban HB, Demirci A, Turhan I (2015) Enhanced *Aspergillus ficuum* phytase production in fed-batch and continuous fermentations in the presence of talcum microparticles. Bioprocess Biosyst Eng 38(8):1431–1436
56. Coban HB, Demirci A (2016) Enhancement and modeling of microparticle-added *Rhizopus oryzae* lactic acid production. Bioprocess Biosyst Eng 39(2):323–330
57. Dong M, Wang S, Xu F, Li Q, Li W (2018) Addition of aluminum oxide microparticles to *Trichoderma viride* My preculture enhances cellulase production and influences fungal morphology. Eng Life Sci 18(6):353–358
58. Driouch H, Hänsch R, Wucherpfennig T, Krull R, Wittmann C (2012) Improved enzyme production by bio-pellets of *Aspergillus niger*: targeted morphology engineering using titanate microparticles. Biotechnol Bioeng 109(2):462–471
59. Etschmann M, Huth I, Walisko R, Schuster J, Krull R, Holtmann D, Wittmann C, Schrader J (2015) Improving 2-phenylethanol and 6-pentyl-α-pyrone production with fungi by microparticle-enhanced cultivation (MPEC). Yeast 32(1):145–157
60 Germec M, Yatmaz E, Karahalil E, Turhan İ (2017) Effect of different fermentation strategies on β-mannanase production in fed-batch bioreactor system. 3 Biotech 7(1):77
61. Gonciarz J, Bizukojc M (2014) Adding talc microparticles to *Aspergillus terreus* ATCC 20542 preculture decreases fungal pellet size and improves lovastatin production. Eng Life Sci 14(2):190–200
62. Gonciarz J, Kowalska A, Bizukojc M (2016) Application of microparticle-enhanced cultivation to increase the access of oxygen to *Aspergillus terreus* ATCC 20542 mycelium and intensify lovastatin biosynthesis in batch and continuous fed-batch stirred tank bioreactors. Biochem Eng J 109:178–188
63. Niu K, Hu Y, Mao J, Zou S, Zheng Y (2015) Effect of microparticles on echinocandin B production by *Aspergillus nidulans*. Sheng Wu Gong Cheng Xue Bao=Chin J Biotechnol 31 (7):1082–1088
64. Tao T-L, Cui F-J, Chen X-X, Sun W-J, Huang D-M, Zhang J, Yang Y, Wu D, Liu W-M (2018) Improved mycelia and polysaccharide production of *Grifola frondosa* by controlling morphology with microparticle Talc. Microb Cell Fact 17(1):1–10
65. Wucherpfennig T, Lakowitz A, Driouch H, Krull R, Wittmann C (2012) Customization of *Aspergillus niger* morphology through addition of talc micro particles. J Visual Exp 61:e4023
66. Huang J, Guan HW, Huang YY, Lai KS, Chen HY, Xue H, Zhang BB (2021) Evaluating the effects of microparticle addition on mycelial morphology, natural yellow pigments productivity, and key genes regulation in submerged fermentation of *Monascus purpureus*. Biotechnol Bioeng 118(7):2503–2513
67. Huang Z-F, Yang S-Z, Liu H-Q, Tian X-F, Wu Z-Q (2021) Sodium starch octenyl succinate facilitated the production of water-soluble yellow pigments in *Monascus ruber* fermentation. Appl Microbiol Biotechnol 1–16
68. Niu K, Wu X-P, Hu X-L, Zou S-P, Hu Z-C, Liu Z-Q, Zheng Y-G (2020) Effects of methyl oleate and microparticle-enhanced cultivation on echinocandin B fermentation titer. Bioprocess Biosyst Eng 43(11):2009–2015
69. Alizadeh V, Shojaosadati SA, Zamir SM (2021) Enhancement of schizophyllan production in *Schizophyllum commune* using microparticles in medium. Bioprocess Biosyst Eng 44(2):317–328
70. Du L, Gao B, Liang J, Wang Y, Xiao Y, Zhu D (2020) Microparticle-enhanced *Chaetomium globosum* DX-THS3 β-d-glucuronidase production by controlled fungal morphology in submerged fermentation. 3 Biotech 10(3):1–8
71. Prabhu S, Tharayil SAP (2020) Enhanced production of laccase by microparticle induced cultivation of Basidiomycetes and evaluation of its efficiency as deinking agent. J Adv Sci Res 11(4)
72. Yue C, Xu H, Yu Y, Yu X, Yu M, Zhang C, You Q, Xia S, Ding Z, Fu H (2021) Improvement of natamycin production by controlling the morphology of *Streptomyces gilvosporeus* Z8 with microparticle talc in seed preculture. J Chem Technol Biotechnol 96(6):1533–1542
73. Kuhl M, Gläser L, Rebets Y, Rückert C, Sarkar N, Hartsch T, Kalinowski J, Luzhetskyy A, Wittmann C (2020) Microparticles globally reprogram *Streptomyces albus* toward accelerated morphogenesis, streamlined carbon core metabolism, and enhanced production of the antituberculosis polyketide pamamycin. Biotechnol Bioeng 117(12):3858–3875
74. Böl M, Schrinner K, Tesche S, Krull R (2021) Challenges of influencing cellular morphology by morphology engineering techniques and mechanical induced stress on filamentous pellet systems-A critical review. Eng Life Sci 21(3–4):51–67