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Effects of Dissolved Oxygen and Agitation on Production of Serratiopeptidase by *Serratia Marcescens* NRRL B-23112 in Stirred Tank Bioreactor and its Kinetic Modeling

Pansuriya, Ruchir C.* and Rekha S. Singhal

Food Engineering and Technology Department, Institute of Chemical Technology, University of Mumbai, Matunga, Mumbai 400 019, India

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The effects of the agitation and aeration rates on the production of serratiopeptidase (SRP) in a 5-L fermentor (working volume 2-l) were systematically investigated using *Serratia marcescens* NRRL B-23112. The dissolved oxygen concentration, pH, biomass, SRP yield, and maltose utilization were all continuously measured during the course of the fermentation runs. The efficiencies of the aeration and agitation were evaluated based on the volumetric mass transfer coefficient (K_La). The maximum SRP production of 11,580 EU/ml with a specific SRP productivity of 78.8 EU/g/h was obtained with an agitation of 400 rpm and aeration of 0.075 vvm, which was 58% higher than the shake-flask level. The K_La for the fermentation system supporting the maximum production (400 rpm, 0.075 vvm) was 11.3 h^{-1}. Under these fermentor optimized conditions, kinetic modeling was performed to understand the detailed course of the fermentation process. The resulting logistic and Luedeking–Piret models provided an effective description of the SRP fermentation, where the correlation coefficients for cell growth, SRP formation, and substrate consumption were 0.99, 0.94, and 0.84, respectively, revealing a good agreement between the model-predicted and experimental results. The kinetic analysis of the batch fermentation process for the production of SRP demonstrated the SRP production to be mixed growth associated.

Keywords: *Serratia marcescens*, serratiopeptidase, fermentor, K_La, kinetic modeling

Serratiopeptidase (SRP) or serrapeptase is a 50 kDa metalloprotease [9] that has gained wide acceptance in Asia and Europe as a potent analgesic and anti-inflammatory drug [13], being used to treat chronic inflammatory diseases, such as atherosclerosis, arthritis, bronchitis, carpel tunnel syndrome, fibrocystic breast disease, and sinusitis [10]. Recent studies have even suggested the use of oral SRP to aid in the treatment or prevention of viral diseases, such as AIDS and hepatitis B and C (Fujisaki *et al.* 1996. JP08040930; Fujisaki *et al.* 1999. JP11199509).

SRP is known to be produced extracellularly in submerged and solid-state fermentation by the bacterium *S. marcescens* (Pansuriya and Singhal, 2010. Indian Patent Application 102/MUM/2010). Research on the production, purification, and characterization of SRP from different strains of *S. marcescens* is already well documented in the literature [6, 15, 22]. In particular, the production of SRP by *S. marcescens* is greatly affected by the constituents of the culture media, especially the organic nitrogen source (Isono *et al.* 1972. US Patent 3,691,014), and various studies have reported high quantities of metalloprotease when using these types of media [3–5, 16]. However, there are relatively few reports on the effects of aeration and agitation on SRP production and its kinetic modeling. Agitation and aeration are both involved to different extents in the overall mass and oxygen transfer during the process flow. McNeil and Harvey [14] reported that agitation controls the nutrient transfer and distribution of air and oxygen, whereas aeration determines the oxygenation of the culture and bulk mixing of the fermentation broth. An inadequate supply of oxygen is one of the major problems in the industrial and lab-scale production of microbial metabolites, as oxygen is sparingly soluble in aqueous media. The fermentative production of several industrially important enzymes, such as streptokinase [26], alkaline protease [7], and cellulase [1] is also well known to be influenced by agitation and aeration.

Fermentation is a very complex process, which makes it very difficult to obtain a complete overview. Thus, the development of kinetic models is important for understanding,

**Corresponding author*
Phone: +91-22-33611111; Fax: +91-22-33611020;
E-mail: ruchir1717@rediffmail.com

Table 1. Specific productivity of SRP with respect to total substrate under different agitation and aeration conditions.

Batch	SRP production (EU/ml)	Time of highest production (h)	K_La (1/h)	Specific productivity (EU/g maltose/h)
300 rpm, 0.05 vvm	2,850	54	4	15
300 rpm, 0.075 vvm	4,450	54	6.6	23.5
300 rpm, 0.1 vvm	5,700	48	6.8	33.9
400 rpm, 0.05 vvm	8,800	48	6.4	52.3
400 rpm, 0.075 vvm	11,580	42	11.3	78.8
400 rpm, 0.1 vvm	10,024	42	13.2	68.1
500 rpm, 0.05 vvm	9,750	48	5.3	58
500 rpm, 0.075 vvm	8,240	42	10.4	56
500 rpm, 0.1 vvm	7,500	42	12.6	51

controlling, and optimizing fermentation processes. Kinetic modeling is regarded as an indispensable step when developing an industrial fermentation process, since it helps to determine the optimal operation conditions for the production of a target metabolite [19, 24]. Although several unstructured kinetic models have already been proposed to describe the fermentative production of industrially important enzymes, such as protease [18], lipase [20], and xylanase [21], there has been no report on the kinetic modeling of the fermentative production of SRP.

In order to optimize the production of SRP for industrial applications, a fundamental understanding of the key fermentation parameters is necessary. In a previous study, the current authors screened different nutritional factors, and concentrations of selected key components were optimized using an evolutionary operation (EVOP) for the production of SRP by *S. marcescens* NRRL B-23112 [18]. Accordingly, the present study systematically investigated the effects of agitation and aeration on the growth, SRP production, protein production, and substrate consumption in batch fermentor cultures of *S. marcescens* NRRL B-23112. K_La studies were also performed as a scale-up parameter. The behavior of *S. marcescens* NRRL B-23112 during fermentation under optimized fermentor conditions was predicted using unstructured kinetic models, and eventually compared with experimental observations. The developed model was able to explain successfully the pattern of cell growth, SRP production, and substrate utilization.

Materials and Methods

Media Components, Microorganism, and Production Medium

The maltose, soybean meal, sodium chloride, potassium dihydrogen phosphate, and trichloroacetic acid (TCA) were all purchased from Himedia Ltd, Mumbai, India. The phenylmethylsulfonylchloride (PMSF) was purchased from S. D. Fine Chemical Ltd., Mumbai, India, and the casein from Merck Chemicals, Mumbai, India.

The *S. marcescens* NRRL B-23112 was a gift sample from the ARS Culture Collection, USA, and was maintained on a soybean casein digest agar medium and subcultured every 15 days.

For the production of SRP, the medium reported by Pansuriya and Singhal [17] was used, which contained (g/l) maltose (45), soybean meal (65), KH_2PO_4 (8.0), and NaCl (5.0). The initial pH of the medium was adjusted to 7.0 using 2 N NaOH and/or 2 N HCl. The medium was sterilized in an autoclave for 15 min at 121°C.

Fermentation

The batch cultivations were performed in a Biostat B5 (B. Braun Biotech-Sartorius) fermentor with a 2-l working volume. The fermentor was inoculated with 1% (v/v) (2×10^8 cells/ml) of a 14-h-old *S. marcescens* NRRL B-23112 seed culture in a sterile production medium, making a 2-l fermentation medium. All the fermentation runs were conducted in batch mode at a controlled temperature of 25°C. The pH was automatically controlled using a sterile pH electrode (Hamilton) at 7.0 ± 0.05 by adding 2 N NaOH. The dissolved oxygen (DO) concentration was measured using a sterilizable polarographic electrode (Mettler-Toledo InPro6000 Series). Pure oxygen gas was used to attain the desired DO concentration in the fermentation medium. The effects of aeration and agitation on the SRP production were evaluated by varying the agitation speed (300, 400, and 500 rpm) and aeration rate (0.05, 0.075, and 0.1 vvm) using pure oxygen. Table 1 shows the detailed conditions of the fermentation batch trails carried out for the optimization study.

SDS–PAGE

The SDS–PAGE of the enzyme samples (20 µl each) obtained at different fermentation times was carried out using a 12% gel, as per the procedure stated in previous literature [8], using an electrophoresis unit (Bio-Rad India Pvt. Ltd., Mumbai, India). The samples were then compared with standard molecular weight markers (Bangalore Genei Pvt. Ltd., Bangalore, India).

Kinetic Modeling for DCW, SRP Production, and Maltose Utilization

The optimum fermentor conditions, 400 rpm/0.075 vvm with oxygen, were used for the kinetic analysis of growth, product formation, and maltose utilization. A modified logistic equation was used for the growth analysis, the Luedeking–Piret equation for the product formation, and a modified Luedeking–Piret equation for the substrate utilization.

Analytical Determinations

The SRP activity was determined based on its caseinolytic property, and the method was adapted from Salamone and Wodzinski [22]. To

the substrate solution [0.75 ml consisting of 1.0% (w/v) casein in 100 mM Tris/HCl, 1 mM $MgCl_2$, and 2 mM PMSF at pH 8.0], 0.1 ml of the assay sample (cell-free centrifuged supernatant appropriately diluted with 50 mM Tris/HCl, pH 8 buffer) was added, and the mixture was incubated at 40°C. After 30 min, the reaction was quenched with 0.5 ml of 10% (w/v) TCA to precipitate the unhydrolyzed casein. After 15 min at 25°C, the reaction mixture was centrifuged at 10,000 ×*g* for 10 min and the absorbance of the supernatant determined at 280 nm. One unit of enzyme activity (EU) was defined as the amount of enzyme that produced an increase in absorbance of 0.1 at 280 nm under the conditions of the assay. For the blank, 0.5 ml of 10% (w/v) TCA was added to 0.1 ml of the assay solution and mixed; a further 0.75 ml of the substrate solution was added and the mixture allowed to stand for 30 min at 40°C, and then the test proceeded as above [17].

The biomass was measured as the log viable count per milliliter of *S. marcescens* NRRL B-23112 based on plating the fermentation broth in a soybean casein digest agar medium at various dilutions. For the kinetic study, a linear relation was established between the viable count and the dry cell weight (DCW) using the soybean casein digest medium, and a conversion factor was used to convert the biomass data into the DCW.

The maltose utilization during fermentation was determined using the dinitrosalicylic acid method, as reported by Pansuriya and Singhal [17].

Determination of K_La

The K_La of the fermentation system was determined using the method reported by Wise [25]. In this method, a static gassing-out technique was used that utilized the broth containing the killed biomass formed at the end of the fermentation. The DO concentration of the broth was lowered by gassing out the liquid with nitrogen gas, followed by aeration and agitation of the liquid to increase the DO concentration. The increase in the DO concentration was monitored using a sterile polarographic electrode (Mettler-Toledo InPro6000 Series). A plot of $\ln(C^*-C_L)$ *vs*. time yielded K_La as the slope of the linear graph obtained, where C* was the saturated DO concentration and C_L was the concentration of DO in the liquid at a particular time.

RESULTS AND DISCUSSION

Optimization of Fermentor Parameters

Effects of aeration and agitation on maltose utilization. Fig. 1 shows the maltose utilization by *S. marcescens* NRRL B-23112 in the batch culture at an agitation of 300, 400, and 500 rpm when varying the aeration at 0.05, 0.075, and 0.1 vvm with oxygen, respectively. As seen, the maltose utilization began after 6 h and continued throughout the fermentation in the case of the aeration settings at 300 rpm/0.05 vvm and 300 rpm/0.075 vvm. However, when using the aeration settings at 400 and 500 rpm, 90% of the maltose was utilized within the first 24 h of fermentation, suggesting more favorable conditions of agitation and aeration than at 300 rpm for the utilization of maltose by *S. marcescens* NRRL B-23112. When the aeration rate was increased from 0.05 to 0.1 vvm at 300 rpm, the amount of unutilized maltose at the end of the fermentation decreased from 13 to 8 g/l, respectively, suggesting that the increase in the oxygen supply played an important role in the maltose uptake by *S. marcescens* NRRL B-23112. This was also confirmed when comparing the maltose utilization profiles, where the amount of unutilized maltose at the end of the fermentation was 6.6 g/l for all the runs at 400 rpm, which was lower than that for the runs at 300 rpm.

Effects of aeration and agitation on biomass production. The biomass was calculated as the log viable count/ml, since the medium employed in this study was turbid because of the presence of soybean meal as the nitrogen source. The biomass was found to increase throughout the fermentation under the conditions of 300 rpm/0.05 vvm and 300 rpm/0.075 vvm; but at 300 rpm/0.1 vvm, the biomass increased for 48 h and then remained almost constant (Fig. 2). Increasing the aeration rate from 0.05 to 0.075 to 0.1 vvm also increased the biomass from 9.8 to 10.7 to 11.0 log viable count/ml, respectively. The explanation is that the increase in the aeration rate not only helped to maintain the DO concentration in the fermentor, but also facilitated the bulk mixing of the nutrients for easy consumption by the microorganism, promoting growth and production.

In the case of the 400 rpm runs, the biomass increased sharply for 30 h of fermentation, after which the growth slowed down. The highest biomass of 11.9, 13.6, and 12.8 log viable count/ml was obtained with 0.05, 0.075, and 0.1 vvm, respectively. The runs at 500 rpm were also found to produce a sharp increase in the biomass for 30 h of fermentation time, after which the growth slowed down. In this case, the highest growths of 12.5, 12.3, and 12.1 log viable count/ml was obtained with 0.05, 0.075, and 0.1 vvm, respectively. A rapid decline in the viability of *S. marcescens* NRRL B-23112 was observed after 42 h (Fig. 2).

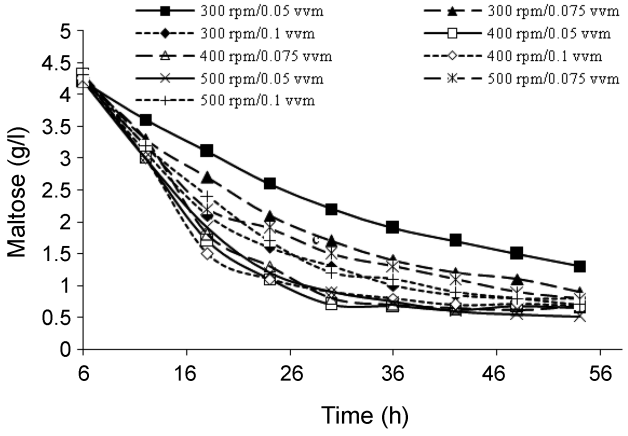

Fig. 1. Effects of agitation at 300, 400, and 500 rpm when varying aeration at 0.05, 0.075, and 0.1 vvm on maltose utilization by *S. marcescens* NRRL B-23112 in batch culture.

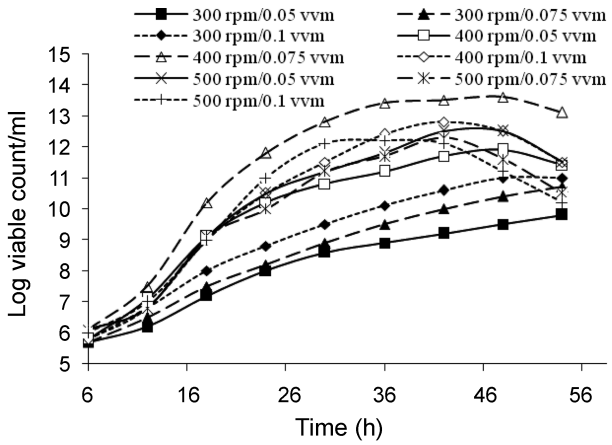

Fig. 2. Effects of agitation at 300, 400, and 500 rpm when varying aeration at 0.05, 0.075, and 0.1 vvm on biomass production by *S. marcescens* NRRL B-23112 in batch culture.

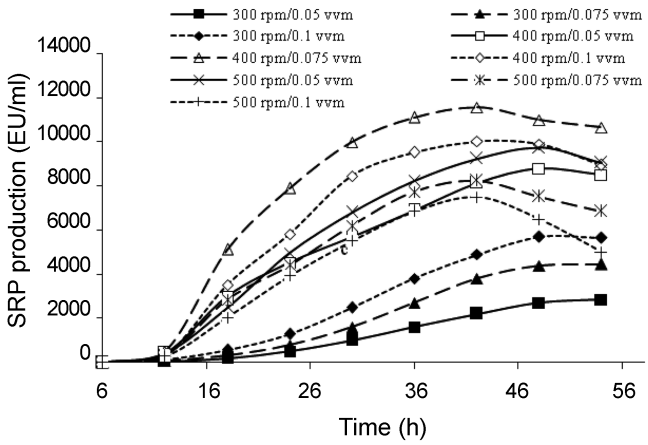

Fig. 3. Effects of agitation at 300, 400, and 500 rpm when varying aeration at 0.05, 0.075, and 0.1 vvm on SRP production by *S. marcescens* NRRL B-23112 in batch culture.

Effects of aeration and agitation on SRP production. The production of SRP began after 12 h and lasted for 48 h of fermentation time (Fig. 3). A higher production of 5,700 EU/ml was observed with 300 rpm/0.1 vvm, followed by 4,450 and 2,850 EU/ml with 300 rpm/0.075 vvm and 300 rpm/0.05 vvm, respectively. It was clearly observed that an increase in the aeration rate also improved the production. Fig. 4A shows the utilization of dissolved oxygen at 300 rpm when varying the aeration at 0.05, 0.075, and 0.1 vvm. Rapid utilization of the DO was observed within the first 8 h of fermentation. In the case of 300 rpm/0.05 vvm and 300 rpm/0.075 vvm, the DO concentration was less than 1% after 8 h of fermentation, suggesting oxygen-depleted fermentation. Although *S. marcescens* NRRL B-23112 is a facultative anaerobe, it was found to be highly dependent on dissolved oxygen for its metabolite production. Hence, it can be concluded that the settings at 300 rpm were not sufficient for the production of SRP by *S. marcescens* NRRL B-23112.

The production of SRP at 400 rpm was observed to be much higher than that at 300 rpm, as the SRP production was 8,800, 11,580, and 10,024 EU/ml at 400 rpm/0.05, 400/0.075, and 400/0.1 vvm, respectively. An enhanced production of alkaline protease has also been observed with higher levels of agitation and aeration [7]. However, in the present study, there seemed to be a limit to the positive effects of agitation and aeration on SRP production, since the SRP production decreased at a high agitation speed and aeration rate. This was confirmed by observing the biomass formation profile at 400 rpm/0.075 vvm and 400 rpm/0.1 vvm, where the log viable count/ml decreased from 13.1 to 11.5, respectively, suggesting a negative effect of high agitation and aeration.

The production of SRP at 500 rpm was lower than that at 400 rpm, as the SRP production was 9,750, 8,240, and 7,500 EU/ml at 500 rpm/0.05 vvm, 500/0.075 vvm, and 500/0.1 vvm, respectively. A high agitation and aeration had an adverse effect on the production of SRP as well as the viability of the microorganism. This was also evident based on the physiological conditions during the fermentation, as the foam formation became so high that it was impossible to control even with an antifoaming agent after 24 h of fermentation. Higher foaming is known to degrade enzymes [11]. Furthermore, since soybean meal is an insoluble medium component, it may have had an abrasive effect on the *S. marcescens* strain at a higher rpm, leading to the lower cell viability.

The DO with the setting at 500 rpm was higher than 40% throughout the fermentation and remained above 80% during most of the fermentation (Fig. 4C). Since *S. marcescens* NRRL B-23112 is a facultative anaerobe, such a high DO during fermentation may not be suitable for metabolite production. This was also verified by SDS–PAGE of the enzyme harvested at different times from fermentation runs at 500 rpm/0.1 vvm (Fig. 5), where the band corresponding to SRP was found to diminish during the progress of the fermentation.

The specific productivity of SRP (EU/g maltose/h), yield (EU/ml), and K_La under different agitation and aeration conditions are shown in Table 1. The maximum specific productivity of 78.8 EU/g maltose/h was obtained at the optimum fermentation conditions of 400 rpm/0.075 vvm in the fermentor. The maximum K_La of 13.2 h^{-1} was obtained at 400 rpm/0.1 vvm, as compared with 11.3 h^{-1} at the optimum fermentation conditions. The K_La often serves to compare the efficiency of bioreactors and mixing devices, plus it is an important scale-up factor used to indicate the oxygen demand of a culture. Thus, the aeration and agitation are often selected to achieve the desired K_La, since this is the controlling parameter in most fermentation systems [2]. The variation in the highest production time of SRP was also observed for the different fermentor

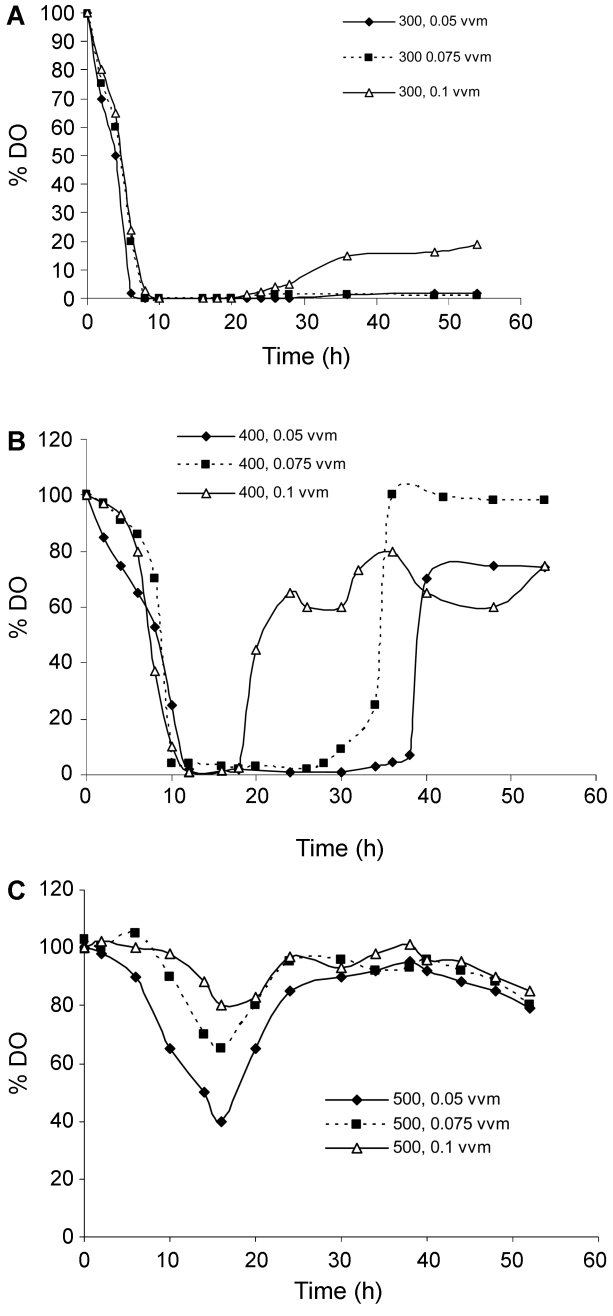

Fig. 4. Percentage of DO during fermentative production of SRP by *S. marcescens* NRRL B-23112 in batch culture at (**A**) 300 rpm (0.05, 0.075, and 0.1 vvm), (**B**) 400 rpm (0.05, 0.075, and 0.1 vvm), and (**C**) 500 rpm (0.05, 0.075, and 0.1 vvm).

batches (Table 1). This phenomenon may have been due to differences in the volumetric mass transfer constraint and availability of oxygen in the different fermentor batches.

Unstructured Kinetic Modeling

Kinetic analysis of microbial growth in batch cultivation. Microbial processes, especially with a modified medium for the prolonged and high production of metabolites, do not necessarily follow the classical kinetic model of substrate-limited biomass growth and product formation proposed by Monod. Therefore, a logistic equation is used as an alternative empirical function [12]. In many fermentation systems, cell growth has already been characterized using a logistic equation [Eq. (1)], which can be described as follows:

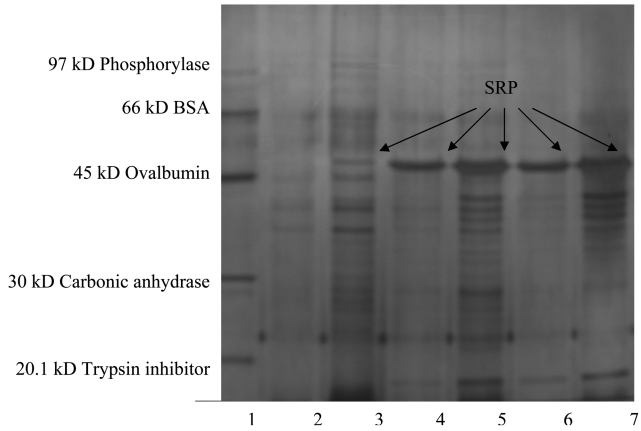

Fig. 5. SDS–PAGE of 500 rpm/0.1 vvm fermentor samples.
Lane 1, Molecular mass marker; Lane 2, 54-h-old broth (diluted); Lane 3, 54-h-old broth (undiluted); Lane 4, 42-h-old broth (diluted); Lane 5, 42-h-old broth (undiluted); Lane 6, 36-h-old broth (diluted); Lane 7, 36-h-old broth (undiluted).

$$\frac{dX}{dt}=\mu_m\left(1-\frac{X}{X_m}\right)X \quad (1)$$

Logistic equations are a set of equations that characterize growth in terms of the carrying capacity. The usual approach is based on a formulation in which the specific growth rate is related to the amount of unused carrying capacity [23].

$$\frac{dX}{dt}=k\left(1-\frac{\overline{X}}{X_m}\right)X \quad (2)$$

The approach used to determine the carrying capacity is to take the log of Eq. (2) to give

$$\log\frac{1}{X}\frac{dX}{dt}=\log k+\log\left(1-\frac{\overline{X}}{X_m}\right)X \quad (3)$$

The intercept of the plot between $\log\left(1-\frac{\overline{X}}{X_m}\right)X$ and $\log\frac{1}{X}\frac{dX}{dt}$ is k.

The integrated form of Eq. (1) using $X=X_0$ and $t=0$ gives a sigmoid variation of X as a function of t, which can represent both the exponential and stationary phases [Eq. (4)].

$$X_{(t)}=\frac{X_o e^{kt}}{1-\frac{X_o}{X_m}(1-e^{kt})} \quad (4)$$

This equation then provides the biomass formation profile during fermentation.

The batch fermentation by *S. marcescens* NRRL B-23112 showed a classical growth trend. After the fermentor (400 rpm/0.075 vvm) was inoculated, the cells entered the exponential growth phase (about 6 h) and then entered the stationary phase (Fig. 2). Cell growth reached a maximum at 42 to 48 h. In contrast, the maximum value for the specific growth rate (μ_m=1.13) was observed at the beginning of the process, and then declined to zero at approximately 42 h. In this case, a logistic equation was used to express the cell growth. The k value was determined using Eq. (3) with a graphical method and found to be 0.34. In this case, X_0 was 0.12 g/l and X_m was 12 g/l. As shown in Fig. 6, the model prediction for cell growth was consistent with the experimental results (the correlation coefficient was 0.99), demonstrating that the model was effective in predicting the experimental growth.

Kinetic analysis of product formation in batch cultivation. The kinetics of product formation are based on the Luedeking–Piret equation [12], which indicates that the product formation rate (r_p) is linearly proportional to the biomass growth rate and instantaneous biomass concentration:

$$r_p=\frac{dp}{dt}=\alpha\frac{dX}{dt}+\beta X \tag{5}$$

where α is the growth-associated parameter and β is the nongrowth-associated parameter. The integration of Eq. (5) (where $P=P_0$ at $t=0$) with Eq. (4) yields

$$P_{(t)}=P_o+\alpha X_o\left(\frac{e^{kt}}{1-(X_o/X_m)(1-e^{kt})}-1\right)+\beta\frac{X_m}{\mu_m}\ln\left(1\frac{X_o}{X_m}(1-e^{kt})\right) \tag{6}$$

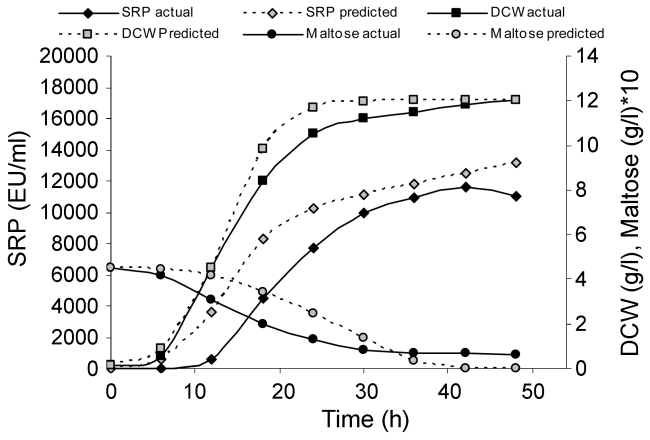

Fig. 6. Experimental and predicted values for optimized fermentor conditions using kinetic modeling.

This equation is used to determine the product concentration formed at different times during the fermentation.

The experimental results were analyzed using the Luedeking–Piret equation [Eq. (6)], and used to express the product formation. The α and β values for SRP production by *S. marcescens* NRRL B-23112 were 792 EU/g cell and 29.94 EU/g cell/h, respectively. Comparisons between the model predictions and the experimental data are given in Fig. 6. Clearly, the model was shown to be effective for describing the SRP yield and specific product formation rate (correlation coefficient was 0.94). The growth-associated constant α was 792 EU/g cell/h, which showed the rate of SRP formation to be high throughout the exponential growth phase. The nongrowth-associated constant β was 29.94 EU/g cell/h, which indicated that SRP formation occurred throughout the stationary growth phase with a comparatively low rate after the cessation of cell growth. This signifies that the SRP production by *S. marcescens* NRRL B-23112 appeared to be mixed growth associated when using the medium employed for the fermentation runs.

Substrate uptake: modified Luedeking–Piret equation. Substrates are used to form cell components and metabolic products, as well as for the maintenance of cells. The substrate consumption equation [Eq. (7)] is a modified Luedeking–Piret equation.

$$-\frac{ds}{dt}=(1/Y_{X/S})\frac{dX}{dt}+(1/Y_{P/S})\frac{dP}{dt}+m_S X \tag{7}$$

Combining Eqs. (5) and (7) gives

$$-\frac{dS}{dt}=(1/Y_{X/S}+\alpha/Y_{P/S})\frac{dX}{dt}+(\beta/Y_{P/S}+m_S)X \tag{8}$$

To simplify Eq. (8), the following model is used to express the substrate consumption:

$$-\frac{dS}{dt}=\gamma\frac{dX}{dt}+\delta X \tag{9}$$

where γ is the sum of $1/Y_{X/S}$ and $\alpha/Y_{P/S}$, and δ is the sum of $\beta/Y_{P/S}$ and m_s.

The integration of Eq. (6) with $S=S_0$ at $t=0$ yields

$$S_{(t)}=S_o-\gamma X_o\left(\frac{e^{kt}}{1-(X_o/X_m)(1-e^{kt})}-1\right)+\delta\frac{X_m}{\mu_m}\ln\left(1-\frac{X_o}{X_m}(1-e^{kt})\right) \tag{10}$$

This equation is then used to determine the unutilized substrate concentration at different times during the fermentation.

During the fermentative production of SRP by *S. marcescens* NRRL B-23112, an increase in the biomass concentration was accompanied by a decrease in the residual substrate concentration. The substrate was consumed

for cell growth, cell maintenance, and product formation and the substrate consumption was represented using Eq. (10). Comparisons between the model prediction data and the experimental results are shown in Fig. 6. Clearly, the model was effective for describing the substrate consumption (correlation coefficient was 0.84). The parameters provided by the model, γ and δ, were 0.02 g maltose/g cell/h and 0.048 g maltose × g cell/h, respectively.

In conclusion, this study demonstrated that the aeration and agitation rate are both key parameters in the fermentative production of SRP by *S. marcescens* NRRL B-23112. Although a high aeration and high agitation favored cell growth and SRP production, it also stimulated excessive foaming. The maximum specific productivity of 78.8 EU/g maltose/h and a K_La value of 11.3 h^{-1} were obtained at the optimum fermentation conditions of 400 rpm agitation and 0.075 vvm aeration. As such, the SRP yield of 11,580 EU/ml under optimum fermentor conditions is the highest in published literature [6, 16, 22]. The production of SRP by *S. marcescens* NRRL B-23112 would appear to be mixed growth associated. The logistic and Luedeking–Piret equations provided an effective description of the cell growth, SRP formation, and maltose consumption versus the batch fermentation time.

Acknowledgment

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Nomenclature and Abbreviations

DO	dissolved oxygen
SRP	serratiopeptidase
TCA	trichloroacetic acid
X	amount of biomass formed
t	time
X_m	maximum biomass formed
μ_m	maximum specific growth rate
$\frac{dX}{dt}$	rate of biomass formation
k	carrying capacity coefficient
$Y_{X/S}$	cell yield coefficient
$Y_{P/S}$	product yield coefficient
m_s	maintenance coefficient (g substrate · g cell/h)
α	growth-associated SRP formation parameter (EU/g DCW)
β	nongrowth-associated SRP formation parameter (EU/g DCW · h)
$\frac{dp}{dt}$	product formation rate (EU/ml · h)
P	amount of product formed (EU/ml)
$-\frac{dS}{dt}$	substrate consumption rate (g/l · h)
S	substrate concentration (g/l)

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