Bioprocess exploration for thermostable α-amylase production of a deep-sea thermophile \textit{Geobacillus} sp. in high-temperature bioreactor

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\textbf{ABSTRACT}

\textit{Geobacillus} sp. 4j, a deep-sea high-salt thermophile, was found to produce thermostable α-amylase. In this work, culture medium and conditions were first optimized to enhance the production of thermostable α-amylase by statistical methodologies. The resulting extracellular production was increased by five times and reached 6.40 U/ml. Then, a high-temperature batch culture of the thermophile in a 15 l in-house-designed bioreactor was studied. The results showed that a relatively high dissolved oxygen (600 rpm and 15 l/min) and culture temperature of 60°C facilitated both cell growth and α-amylase production. Thus, an efficient fermentation process was established with initial medium of pH 6.0, culture temperature of 60°C, and dissolved oxygen above 20%. It gave an α-amylase production of 79 U/ml and productivity of 19804 U/l·hr, which were 10.8 and 208 times higher than those in shake flask, respectively. This work is useful for deep-sea high-salt thermophile culture, where efforts are lacking presently.

\textbf{Introduction}

Deep-sea microorganisms, with the most extreme environments on earth, usually form their specific metabolic regulation and chemical defense mechanisms. Therefore, these microorganisms easily produced natural products with novel chemical structures, bioactivities, and specific functions.\cite{1} However, as the extreme environment conditions, \textit{i.e.}, physical extremes of temperature and pressure, absence of light and oxygen, receiving less than 1% of organic matter from photosynthetic primary production, and so on., they are commonly difficult to cultivation in laboratory especially the large scale.\cite{3-2}

Thermophiles \textit{Geobacillus} spp. have even been isolated from environments at an altitude as high as 3653 m and at depths as deep as 10,897 m below sea level in the third dimension.\cite{4} Unlike strict anaerobes, barophiles and other extremophiles only surviving under extreme conditions, \textit{Geobacillus} spp. isolated from deep-sea environment can stand but not depend on these extreme factors. Most of them can be cultured in shake flask with an elevated optimal temperature. However, the operation with deep-sea thermophiles \textit{Geobacillus} spp. at bioreactor scale remains a challenging approach considering equipment tolerance and shorter life of sensors and accessories at high temperature and elevated concentration level of salts. Additionally, as a type of thermophiles, one of the traditional challenges faced in the cultivation of these species is the very low biomass yields owing to accumulation of toxic compounds.\cite{4} In spite of all these unfavorable conditions, high-temperature fermentation facilitates to reduce medium viscosity and enhance substrate solubility and diffusion efficiency. Moreover, high-temperature fermentation of \textit{Geobacillus} spp. has been proven to promote high rates of feedstock conversion and reduce cooling costs and risks of contamination in fermentation.\cite{5}

Owing to the extreme environments, \textit{Geobacillus} spp. have the genetic foundation of metabolizing different kinds of plant-derived polysaccharides and their components or even long-chain alkanes.\cite{6,7} These features facilitate them to be attractive sources of thermostable enzymes\cite{8,9} and efficient components of biotransformation\cite{10} and biodegradation.\cite{10,11} Among them, α-Amylase (EC3.2.1.1) is one of the most important industrial enzymes used for starch hydrolysis.\cite{12} The ideal α-amylase should be Ca$^{2+}$ independent, active at nature pH of starch slurry approximately 4.5, and a high temperature of above 105°C. Thus, it can avert adding chemicals, adjusting the pH, and prevent starch granules precipitation in the hydrolysis process, so as to facilitate the downstream refining processes and reduce costs.\cite{13} However, few commercialized α-amylases can fit traditional industrial starch processes perfectly. Therefore, raw starch hydrolysis by α-amylases has been recommended to hydrolyze raw starch. It refers to a type of enzyme acting directly on the starch granules omitting the gelatinization step at a sub-high temperature as a result of reducing the energy requirements significantly and minimizing the formation of undesirable Maillard reaction by-products.\cite{12}

In the previous study, we identified a Ca$^{2+}$-independent raw starch α-amylase with maximal activity at 60–65°C and pH 5.5 from deep-sea high-salt thermophile \textit{Geobacillus} sp. 4j.\cite{15} It exhibited a similar thermostable performance to commercial α-amylase from \textit{Bacillus licheniformis} (BLA) but better than that from \textit{Bacillus amyloquefaciens} (BAA) and \textit{Bacillus subtilis} (BSA). It also showed a universally efficient raw starch hydrolysis performance superior to commercial α-amylases at an acidic pH approaching the nature of starch slurry.\cite{15}
In this work, we aim to establish a high-temperature fermentation process for cell growth and \( z \)-amylase production of the deep-sea high-salt thermophile *Geobacillus* sp. 4j at a 151 bioreactor scale. It may provide useful information for bioreactor culture scale of deep-sea thermophiles and also their productions of valuable products.

**Materials and methods**

**Strains and culture conditions in shake flask**

Yeast extract and tryptone were purchased from Oxoid Ltd, UK. All other organic and inorganic reagents in analytical or biological grade were supplied by Sinopharm Chemical Reagent Company, China. Starch used in this work was corn derived.

*Geobacillus* sp. 4j was first isolated from the subseafloor sediments, which was collected from WP0104 site at the western Pacific “warm pool” (8°53′08″N, 142°59′51″E, 2068 m of water depth) using gravity corer by the Third Institute of Oceanography of the State Oceanic Administration (Xiamen, China). Starch used in this work was corn derived.

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The optimized fermentation medium contained 6 g starch sol and the pH was adjusted to 6.8 with 1 mol/l NaOH solution. 8.92 g MgCl₂·6H₂O, 0.1 g CaCl₂, 0.183 g K₂HPO₄·3H₂O, and 0.02 g H₃BO₃, and 0.02 g NiCl₂·6H₂O in 1 l distilled water.

The composition of original fermentation medium was 10 g yeast extract powder, 2.5 g tryptone, 0.1 g nitrilotriacetic acid, 0.04 g CaSO₄·2H₂O, 0.3 g MgCl₂·6H₂O, 0.167 g MgSO₄, 0.33 g KCl, 0.01 g Fe₂(NH₄)₂SO₄, 0.005 g NiCl₂, and 10 ml metal trace buffer in 1 l distilled water.

The inoculum was prepared by transferring a single colony from the agar plate with a culture age of 10 hr into a 250 ml shake flask containing 80 ml seed medium. The bacterial growth and amylase production were monitored under the assay conditions.

**Bioreactor design and operating conditions**

A deep-sea thermophile culture platform of 151 stirred tank bioreactor was in-house designed and manufactured by high-temperature seawater corrosion-resistant stainless steel (type AISI 904 L). The bioreactor was equipped with two six-blade Rushton turbine impellers and one four-sector blade propeller between them. A heat preservation was designed outside the vessel jacket to enhance thermoking. Dissolved oxygen (DO) was monitored by a polarographic probe. It was set as 100% when a constant value was detected by the DO sensor with an air flow rate of 0.5 vvm, an agitation speed of 600 rpm, and a tank-inside pressure of 0.03 MPa before inoculation. The working volume of the bioreactor was 10 l. The inoculum size was 1% (v/v).

**Analytical methods**

Cell growth was monitored by OD₆₀₀ using a UV-2102PC spectrophotometer (UNICO (Shanghai) Instruments Co., Ltd, China). The offline pH was measured by an FE20 pH meter (Mettler Toledo). The supernatant obtained by centrifugation at 12,000 g for 3 min at 4°C was used for amylase and residual sugar assay. Residual sugar was determined by anthrone-sulfuric acid method.

**Amylase assay**

Amylase assay was performed by adding 10 μl appropriately diluted enzyme solution into 280 μl 1% starch solution prepared in 50 mmol/l sodium phosphate–citric acid buffer (pH 7.5) and incubated at 60°C for 10 min. The reducing sugar was determined by 3,5-dinitrosalicylic acid (DNSA) reagent.

One unit of \( z \)-amylase is defined as the amount of enzyme that liberates 1 µmol of reducing sugars as maltose per minute under the assay conditions.

**Optimizations of medium and culture conditions**

Effects of carbon sources, single organic nitrogen and inorganic nitrogen source, and their combinations on amylase production of *Geobacillus* sp. 4j were investigated. The significant factors were selected by a two-level Plackett–Burman design using Design-Expert Software (version 8.0.6, Stat-Ease Inc., Minneapolis). Thereafter, a central composite design (CCD) with five coded levels was used to optimize the medium to favor the enzyme production. The general polynomial regression model for three independent variables with three various levels was fitted as Eq. (1):

\[
Y = a_0 + \sum_{i=1}^{3} a_i X_i + \sum_{i=1}^{3} a_{ii} X_i^2 + \sum_{i<j}^{3} a_{ij} X_i X_j \tag{1}
\]

where \( Y \) is the response, \( a_0 \) is the value of the fitted response at the center point of the design, \( a_i, a_{ii}, \) and \( a_{ij} \) were the linear, quadratic, and cross-term coefficients, respectively. The designs and coefficients fitting were performed using Statistical Analysis System version 9.2 (SAS Institute Inc.).

**Effect of oxygen supply and culture temperature on cell growth and enzyme production**

Batch cultures with four kinds of combinations of air flow rate and agitation speed were carried on to specify different oxygen supply levels (low, 200 rpm, and 5 l/min; medium, 400 rpm, and 10 l/min; high, 600 rpm, and 15 l/min; high, 800 rpm, and 20 l/min). An initial medium pH was controlled at 6.0,
and it kept natural variation during the whole process. The culture temperature of 60°C and an inoculum volume of 1% (v/v) were used. Initial 600 rpm and 0.5vvm with a tank-inside pressure of 0.03 MPa was adopted to adjust 100% DO saturation, thereafter it was kept more than 20% by adjustment of agitation speed and air flow rate.

Batch cultures were also performed at 50, 60, and 70°C. The DO level was kept not lower than 20% by adjustment of agitation speed and air flow rate. The other conditions were the same as above. Using the mathematics analysis method of interpolation and differentiation by the Origin software (version 8.0), the specific cell growth rate $\mu$ (h$^{-1}$) and specific enzyme production rate $Q_p$ (U/ml) were estimated from experimental data of cell growth ($C_X$, OD$_{600}$) and enzyme production ($C_P$, U/ml) by the following Eqs. (2–3):

$$\mu = \left( \frac{1}{C_X} \right) \frac{\Delta C_X}{\Delta t} \quad (2)$$

$$Q_p = \left( \frac{1}{C_P} \right) \frac{\Delta C_P}{\Delta t} \quad (3)$$

where $t$ is the culture time (h), $C_X$ is the biomass concentration (OD$_{600}$), and $C_P$ is the biomass concentration (U/ml).

**Results and discussion**

**Effects of different carbon and nitrogen sources on cell growth and enzyme production in shake flask**

Nutrition composition is an important for cell growth and enzyme production. Carbon sources, nitrogen sources, and their combinations were firstly screened to favor $\alpha$-amylase production (Fig. 1). Nine carbon sources were studied, and the starch, maltose, and $\alpha$-lactose were more productive than other carbon sources when using 5 g/l yeast extract powder and 5 g/l tryptone (Fig. 1a). Then, the combinations of the three carbon sources were considered, and the results indicated that starch (5 g/l) and maltose (5 g/l) improved $\alpha$-amylase production most (Fig. 1c). Afterward, nitrogen source was screened based on this carbon source combination. As shown in Figure 1b, nine organic nitrogen sources and five inorganic nitrogen sources were evaluated. Yeast extract powder and NaNO$_3$ were the most favorable organic and inorganic nitrogen sources for $\alpha$-amylase production, respectively. A combination of nitrogen sources was also investigated and the results showed that yeast extract powder (4 g/l) and NaNO$_3$ (3 g/l), beef extract (5 g/l) and NaNO$_3$ (3 g/l), sodium glutamate (5 g/l) and NaNO$_3$ (3 g/l), beef extract (5 g/l), and sodium glutamate (5 g/l) significantly improved $\alpha$-amylase production (Fig. 1d). For industrial fermentation, multiple and economic combinations of carbon and nitrogen sources are often used to enhance fermentation efficiency and reduce cost. Thus, combinations of multiple carbon and nitrogen sources were then conducted. As shown in Figure 1e, the combination of starch (10 g/l) and yeast extract powder (4 g/l) and NaNO$_3$ (3 g/l) showed the best effects and produced $\alpha$-amylase of 5.0 U/ml, which was 3.2 folds higher than the original (1.2 U/ml). Thus, the original organic nitrogen source tryptone was then replaced by inorganic NaNO$_3$.

**Statistical optimization of medium in shake flask**

Statistic methods are very useful in medium optimization with the good quality of high performance and factors interaction.$^{[19,20]}$ A L$_{25}(5^6)$ orthogonal design was first used on six important components, i.e., starch, yeast extract powder, NaNO$_3$, NaCl, MgCl$_2$·6H$_2$O, and CaCl$_2$, and the optimal concentrations were determined as 6, 8, 5, 15, 6, and 0.6 g/l, respectively (data not shown). Then, a Plackett–Burman design was performed to select critical factors (Table S1). The analysis of variance (ANOVA) for Plackett–Burman design was shown in Table 1. A model term is considered to be significant when its “p-value” is less than 0.05. Accordingly, NaCl, MgCl$_2$·6H$_2$O, and K$_2$HPO$_4$·3H$_2$O were determined as critical factors. To search the central point for response surface methodology (RSM), the content of three critical factors was optimized by one-factor-at-a-time and determined as NaCl 15 g/l, MgCl$_2$·6H$_2$O 2 g/l, and K$_2$HPO$_4$·3H$_2$O 0.4 g/l, respectively (data not shown). Subsequently, a level of critical components and their interactions on enzyme production were further explored by CCD of RSM. The matrix and ANOVA of experiment data were shown in Tables S2–S4. The following second-order polynomial Eq. (4) was obtained:

$$Y = 6.25 - 0.64X_1 - 0.35X_2 + 0.35X_3 - 0.74X_1^2 + 0.54X_1X_2 - 0.29X_3^2 \quad (4)$$

where $X_1$, $X_2$, and $X_3$ stand for NaCl, MgCl$_2$·6H$_2$O, and K$_2$HPO$_4$·3H$_2$O, respectively. The fit of the model was verified by the coefficient of determination of $R^2 = 0.9677$, implying that 96.77% of the variability could be explained by the model. The low coefficient of variation (CV = 4.69%) also indicated good reliability. The $p < 0.05$ indicated the model terms of significance. Thus, $X_1$, $X_2$, $X_3$, $X_1X_2$, $X_1^2$, and $X_3^2$ were significant. Three-dimensional response surface plots for the effect of interaction between two variables on $\alpha$-amylase production were presented in Figure S1. Surface contour of circular means significant interaction but of parabola or ellipse means not. The interaction between $X_1$ and $X_2$ was significant, which accorded with ANOVA results. By solving the optimization function using SAS software, the maximum enzyme production was predicted as 7 U/ml at optimum of three variables, which was 12.5 g/l for NaCl, 0.32 g/l for MgCl$_2$·6H$_2$O, and 0.484 g/l for K$_2$HPO$_4$·3H$_2$O, respectively.

Additionally, verification for enzyme production by predicted medium composition was carried out in duplicate (Fig. 2). The maximum enzyme production in the optimized medium was obtained as 6.4 U/ml, enhanced by five folds as compared to that in original medium. There was a small gap between real and predicted value, indicating a good accordance between them. The close value between predicted and experimental results corroborated the effectiveness of the model. Besides, the time profiles showed the residual sugar in original medium declined to 4.5 g/l at 30 hr and did not consume thereafter, while it was gradually exhausted during 0–48 hr in optimized medium. The pH in origin medium maintained at a long-term low pH of 4.5, which predicted an abnormal metabolism that may be caused by accumulated organic acids.
However, this phenomenon ameliorated much in optimized medium. These may also be the reasons for the low cell concentration in original medium.

**Effects of dissolved oxygen on cell growth and α-amylase production in bioreactor**

Although *Geobacillus* spp. are thermophilic aerobic or facultatively anaerobic bacilli,[7] the environment for deep-sea thermophile is mostly oxygen deficit. Theoretically, *Geobacillus* sp. 4j should favor oxygen deficiency. However, our previous study showed that cell growth of *Geobacillus* sp. 4j was obviously inhibited in shake flask with rubber plug preventing oxygen transfer but improved by the ventilating gauze or latex plug. Thus, this strain is still aerobic preference. Batch fermentation of *Geobacillus* sp. 4j with high aeration flow in the house made 15 l stirred tank bioreactor (Fig. 3a) with optimized medium under the initial control strategy was performed at first. The microbe grown very well (Fig. 3b).

![Figure 1. Effects of different carbon sources, nitrogen sources, and their combinations on α-amylase production in shake flask at 60°C. (a) Different carbon sources with 5 g/L yeast extract powder and 5 g/L tryptone; (b) different nitrogen sources with 5 g/L starch and 5 g/L maltose; (c) different carbon source combinations with 5 g/L yeast extract powder and 5 g/L tryptone. STA + MAL (5 g/L starch and 5 g/L maltose), STA + MAN + MAL (5 g/L starch, 3 g/L mannitol, and 3 g/L maltose), STA + MAN (5 g/L starch and 5 g/L mannitol), control (10 g/L starch), STA + MAN + LAC (5 g/L starch, 3 g/L mannitol, and 3 g/L α-lactose), STA + LAC (5 g/L starch and 5 g/L α-lactose), STA + MAL + LAC (5 g/L starch, 3 g/L mannitol, and 3 g/L α-lactose); (d) different nitrogen source combinations with 5 g/L of starch and 5 g/L of maltose, TRY (6 g/L tryptone), YEP (4 g/L yeast extract powder), BE (5 g/L beef extract), SG (5 g/L sodium glutamate), NaNO₃ (3 g/L sodium nitrate); (e) carbon and nitrogen source combinations (A—2 or 4 g/L of yeast extract powder, C—3 or 4 g/L of beef extract, and E—3 or 5 g/L of sodium glutamate. The low concentration was for combinations containing three types of nitrogen sources, the high concentration was for combinations containing two types of nitrogen sources. B—3 g/L of tryptone, D—3 g/L of sodium nitrate, S—10 g/L starch, and T—5 g/L of starch and 5 g/L of maltose).
aeration. Four different DO modes, i.e., low (200 rpm and 5 l/min), medium (400 rpm and 10 l/min), high (600 rpm and 15 l/min), and high (800 rpm and 20 l/min), were used, respectively.

As illustrated in Figure 4a, DO dropped dramatically to 0 at 2 hr, which continued until the end in the low DO mode. It dropped to 0 at 4 hr and then rose up at 6 hr in the medium DO mode. However, for the high and high modes, DO rose up quickly and sharply after it decreased to 0 at 3.5 hr. Cell growth and enzyme production were closely related with DO modes. For the low DO mode, the cell growth and enzyme production were severely inhibited (Fig. 4b and c). While, they were obviously increased in the medium DO mode. When the high and high DO modes were adopted, the thermophile grew fast. The cell density in the high mode was a bit higher than that in the high DO mode, while it decreased more quickly, which indicated faster cell autolysis in the high DO mode. Correspondingly, α-amylase production in the high mode (73 U/ml) was also much higher than that in the high mode (58 U/ml), which indicated both cell growth and α-amylase production favored a relatively high DO level (Fig. 4c).

During the fermentation process, DO reflects the dynamic balance between oxygen transfer rate (OTR) and oxygen uptake.

### Table 1: Analysis of variance for Plackett–Burman design.

| Variables               | Sum of squares | DF | Mean square | F value | Adj R² | P > F |
|-------------------------|----------------|----|-------------|---------|--------|-------|
| Model                   | 12.5552        | 9  | 1.3950      | 62.3554 | 0.0159* |       |
| Soluble starch          | 0.1203         | 1  | 0.1203      | 5.3761  | 0.1463 |       |
| Yeast extract           | 0.0637         | 1  | 0.0637      | 2.8456  | 0.2337 |       |
| NaNO₃                  | 0.0221         | 1  | 0.0221      | 0.9864  | 0.4253 |       |
| NaCl                    | 0.9507         | 1  | 0.9507      | 42.4950 | 0.0227*|       |
| MgCl₂·6H₂O             | 9.5830         | 1  | 9.5830      | 428.3442| 0.0023*|       |
| CaCl₂                   | 0.0969         | 1  | 0.0969      | 4.3314  | 0.1729 |       |
| K₂HPO₄·3H₂O            | 1.4723         | 1  | 1.4723      | 65.8115 | 0.0149*|       |
| Trace element buffer    | 0.0015         | 1  | 0.0015      | 0.0676  | 0.8192 |       |
| Initial pH of medium    | 0.2448         | 1  | 0.2448      | 10.9410 | 0.0805 |       |
| Residual               | 0.0447         | 2  | 0.0224      |         |        |       |
| Cor total               | 12.6000        | 11 |             |         |        |       |

R² (determination coefficient) = 0.9964; Adj R² (adjusted determination coefficient) = 0.9805; CV (coefficient of variation) = 3.46%; DF, degree of freedom.

*Model terms are significant (P < 0.05).

Figure 2. Time profiles of growth and α-amylase production of Geobacillus sp. 4j in shake flask at 60°C. (a) Original medium; (b) optimized medium.

Figure 3. The configuration of the 15 L bioreactor in-house designed with core component labeled and the microbe morphology in the bioreactor. (a) Bioreactor configuration, (b) microbe morphology.
rate (OUR). The OTR is a positive correlation with oxygen transfer coefficient ($K_{La}$) and the difference between oxygen saturation concentration and oxygen concentration in the liquid. The $K_{La}$ is proportional to agitation speed and air flow rate in general. Thus, OTR should be a high value with high agitation and aeration rates. However, the DO curves in medium, high, and high DO modes underwent a similar trend of rapid decrease during the first 3.5 hr (Fig. 4a). It should be due to higher OUR in the mode with higher oxygen supply. Moreover, high OUR indicated high metabolic activity of the cells. Thus, residual sugar consumed more quickly in the culture at high oxygen supply level compared to that at low oxygen level (Fig. 4d). Also, pH always kept at a low level and continued a long term in the low DO mode (Fig. 4e), meaning that cells were enduring abnormal metabolism, which then led to bad cell growth and enzyme production.

As for the rapid cell lysis following the high cell growth rate, it exhibited similar variation to *Geobacillus thermoleovorans* T80. It probably ascribed to substrate exhaustion, which showed no substrate specificity. Notably, rapid cell lysis already started before substrate was exhausted, and it could not reverse by feeding substrate despite that it may vary the cell lysis rate. For *Geobacillus* sp. 4j, starch feeding was also tested, and it showed similar effects that improving cell growth but not reverse cell lysis and improve $\alpha$-amylase production (Fig. S2). Therefore, developing a process to maintain cell activity needs a further study, which may be beneficial to the enzyme production.

**Effects of temperature on cell growth and $\alpha$-amylase production in 15 L bioreactor**

For the thermophiles, temperature affected their growth and metabolism importantly. In this case, 50 and 60°C but not 70°C were beneficial to cell growth, and the exponential phase came earlier at 60°C (Fig. 5a). The maximum specific growth rate of *Geobacillus* sp. 4j at 50, 60, and 70°C was 1.3, 2.9, and 2.8 hr$^{-1}$, which was obtained at 4, 1, and 1.5 hr of batch culture, respectively (Fig. 5b). The results proved that 60°C also improved enzyme production and it reached a maximum value of 79 U/ml, which was 1.5 and 6.9 folds higher than that at 50 and 70°C, respectively (Fig. 5c). The enzyme activity seemed

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**Figure 4.** Effects of different combinations of agitation and aeration on growth and $\alpha$-amylase production of *Geobacillus* sp. 4j in 15 L bioreactor at 60°C. (a) DO, (b) OD$_{600}$, (c) enzyme activity, (d) residual sugar. Low—200 rpm and 5 L/min, medium—400 rpm and 10 L/min, high—600 rpm and 15 L/min, high—800 rpm and 20 L/min.
stable once they reached a high value at all three temperatures. The maximum specific enzyme production rate was 1.5, 2.5, and 1.6 hr\(^{-1}\) for 50, 60, and 70°C, respectively, at culture time of 4, 2, and 4 hr (Fig. 5d). Therefore, 60°C was good for both cell growth and enzyme production. For thermophiles, high diffusion coefficient, high substrate solubility, and low liquid viscosity contributed to elevate the temperature that would promote cell metabolism. However, the damage of protein and cell membrane caused by high temperature would severely affect the electron transport chain.[23] It could be the main reason for that 60°C but not 50 or 70°C was the favorable temperature.

Consequently, an efficient batch culture process strategy at a 15 l bioreactor scale for thermostable \(\alpha\)-amylase production by deep-sea high-salt thermophile \textit{Geobacillus} sp. 4j was established. The resulting enzyme production was obtained 79 U/ml, 10.8 folds higher than that in shake flask. The productivity reached 19804 U/(l•hr), which was 208 folds higher than that in shake flask. Moreover, the production phase was greatly shortened in bioreactor. The enzyme production and productivity were superior to most reported cases for \(\alpha\)-amylase production by extreme thermophile strains of \textit{Geobacillus} species at a bioreactor scale.[4]

**Conclusion**

Statistical optimization of medium and culture conditions was conducted to improve thermostable \(\alpha\)-amylase production by deep-sea high-salt thermophile \textit{Geobacillus} sp. 4j. Then, a high-temperature batch culture for the thermophile was investigated. By optimizing the critical parameters, DO and temperature, an efficient batch fermentation strategy at a 15 l bioreactor scale was finally confirmed and the \(\alpha\)-amylase production was highly enhanced. This work provides useful reference for deep-sea thermophile cultivation, especially in bioreactor at very high temperature.

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