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Impact of dilution rate on CGTase activity and productivity from an alkalophilic *Bacillus* sp. G1 in continuous culture

Khosiya Sali*, Aidil Abdul Hamid and Wan Mohtar Wan Yusoff

University Kebangsaan Malaysia
*Corresponding Author: E-mail address: khosiya@yahoo.com

Abstract

Cyclodextrin glucanotransferase (CGTase) production by a locally isolated bacteria *Bacillus* sp. G1 was investigated in continuous culture with different dilution rates (D) (0.03, 0.05, 0.07, 0.1, 0.3 and 0.5h^{-1}). The CGTase activity profile was successfully maintained at steady state using dilution rates of 0.03 to 0.3h^{-1}. Maximum CGTase activity was obtained at low dilution rate range (0.03-0.07h^{-1}). CGTase activity decreased from 26.4 to 12.4 U/ml when the dilution rate was increased to 0.3h^{-1}. The critical dilution rate (D_c) was established to be at dilution rate greater than 0.3h^{-1} and that the maximum CGTase productivity (R_{max}) (3.72 U/ml/h) was achieved at 0.3h^{-1} dilution rate. This represented an increase of CGTase productivity by 4.8 times than that obtained at the dilution rate of 0.03h^{-1}. We conclude that the CGTase productivity in steady state continuous culture can be readily achieved and that the impact of dilution rate is significant on activity and productivity.

Key words: CGTase, Bacillus sp. G1, dilution rate, continuous culture

1. INTRODUCTION

Alkalophilic bacilli have received major attention for industrial applications because of their high CGTase activity over a range of pH and temperature. Most productions of the CGTase were carried out in batch processes (Gawande *et al.*, 1998; Rosso *et al.*, 2002; Ibrahim *et al.*, 2005). However, many reports employing continuous fermentation as more economical than the batch. Report by Vassileva et al. (2005) indicated that the CGTase activity and CGTase productivity in the continuous culture was higher than in batch culture. This may be due to biomass growth and product formation during the batch culture are limited by the accumulation of metabolic inhibitory products, whereas a continuous culture may overcome this limitation by providing a continuous replacement of growth medium. The dilution rate is the most important factor effecting microbial growth, CGTase activity and CGTase productivity at steady state in a continuous culture as it directly controls the specific growth rate. Most reports indicated that at low dilution rate, microbial growth and CGTase production increased and that CGTase productivity increased with increasing dilution rate (Jamuna *et al.*, 1993). This may be obvious from the formula for CGTase productivity ($R = D\bar{E}\ (1-t_{iii}/T)$. Therefore, when using high dilution rate, the total output (R) for enzyme activity at steady state ($\bar{E}$) increased until a maximum value at D close to the critical dilution rate (D_c). In addition, the success in the industrial continuous culture must employ

microorganisms that have high specific growth rate so that the system can be operated at high dilution rate (Diers 1976), necessitating the estimation of D_c.

Therefore, this study reports a suitable dilution rate for steady state continuous CGTase production by a local isolate *Bacillus* sp. G1 for high CGTase activity and productivity and the limiting operational value of D.

2. MATERIALS AND METHOD

2.1 Microorganism, inoculum and culture condition

Bacillus sp. G1 was isolated from an alkaline environment in Malaysia (Rosli *et al.*, 2000) and obtained from the Industrial Biotechnology Group, KBTI at the School of Biosciences and Biotechnology, Faculty of Science and Technology, UKM. Bacteria from the stock culture were grown in 100ml of Horikhoshi medium (Horikhoshi, 1979) in 250ml conical flask. The culture was incubated at 35^{o}C in an incubator (Incubator shaker, INFORS) with agitation of 150rpm and 18h and used as inoculum.

The continuous culture fermentation was operated using 2L fermenter (LH Fermentor LTD., Stoke Poges, UK). The medium for CGTase production was optimized by Ibrahim et al. (2005), which composed of 40g/L tapioca starch, 20g/L peptone, 0.4g/L $MgSO_4.7H_2O$, and 10g/L Na_2CO_3. A 10 % (v/v) inoculum was transferred into the fermenter with the final culture volume of 1000ml. Fermentation was carried out at 35^{o}C, 150rpm, and the aeration was fixed at 1v/v/m. The continuous fermentation was initialized by a batch operation for 12 hours when the fresh medium was fed at different dilution rates (0.03, 0.05, 0.07, 0.1, 0.3 and 0.5h^{-1}) using a peristaltic pump (Watson-Marlow LTD., Falmouth, Cornwall, UK). For each dilution rate, an experiment was carried out. Samples were collected at 12h interval and the CGTase activity and biomass concentration determined. The schematic representation of the continuous culture is illustrated in Figure.1.

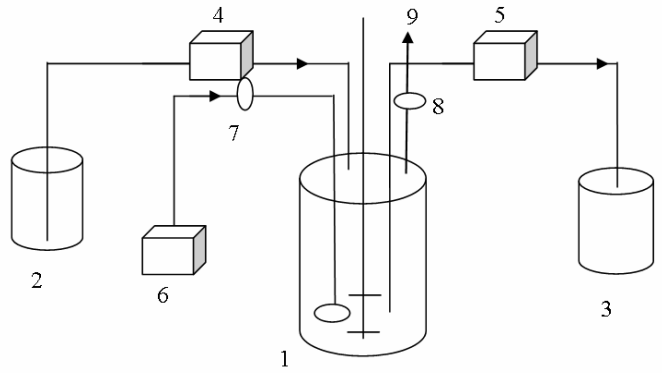

Figure 1 Schematic diagram of the continuous culture system. (1) Bioreactor. (2) Medium reservoir. (3) Product receiver. (4) Peristaltic pump for medium inlet. (5) Peristaltic pump for medium outlet. (6) Air pump. (7) Inlet air filter. (8) Outlet air filter. (9) Air outlet. The temperature was controlled using a constant temperature water bath.

2.2 Analytical methods

2.2.1 Estimation of cell growth

Each sample was appropriately diluted with 0.85% (v/v) NaCl solution and absorbance was measured at 600nm. The OD values were then converted to cell dry weight (g/l) form the standard curve.

2.2.2 Activity of CGTase

Enzyme assay was carried out according to the method of Kaneko et al. (1987). After incubation, the culture sample was centrifuged at 10,000g for 10 min. 0.5ml of crude enzyme solution was added to 1.0ml of 0.04g soluble starch in 1.0ml of phosphate buffer pH7 and incubated at 70^{o}C for 10 min in water bath, 3.5ml of NaOH (30mM) was immediately added to the solution to stop the reaction. Then 0.5ml of 0.02% phenolphthalein in 5mM Na_2CO_3 was added. The color intensity of the reaction mixture was measured at 550nm using Hitachi spectrophotometer. One unit of enzyme activity was defined as 1μmol of β-CD formed per minute under standard assay condition.

2.3 statistic analysis

All data were analyzed using one-way analysis of variance (ANOVA). A Duncan Multiple Comparison Test was used to test the significant difference between variable at $p < 0.05$ (SPSS software package version 11.5, USA).

3. RESULTS AND DISCUSSION

3.1 Effect of dilution rate on cell growth and CGTase production

igure 2 shows the effect of dilution rate in the range of 0.03 to 0.5h^{-1} on biomass concentration in continuous culture. The results indicated that the dilution rate of 0.03 to 0.07h^{-1} did not give significant change in biomass concentration (2.2 to 2.1g/l) at the steady state operation. The biomass concentration (1.9g/L) decreased at dilution rate 0.1 and 0.3h^{-1} (1.2g/l) until washout happens at dilution rate of 0.5h^{-1}. The corresponding CGTase activity profile (Figure 3) showed that the CGTase activity increased after 12h of the batch culture for dilution rate of 0.03-0.1h^{-1} but decreased when the dilution rate was fixed at 0.3h^{-1} and with CGTase activity maintained at steady state after 36h of continuous culture. The dilution rate from 0.03-0.1h^{-1} gave CGTase activity in the range of 23.6-26.4 U/ml at steady state whilst at dilution rate of 0.3h^{-1} CGTase activity immediately decreased at the beginning of medium flow but remained constant at 12.4 U/ml after 36 hours of cultivation. However, CGTase activity profile did not achieve steady state at the dilution rate of 0.5h^{-1}. The results showed that CGTase activity decreases with increasing dilution. This may be due to a decrease in contact time between the medium and cell with the increase in dilution rate. The inverse relationship between CGTase activity and dilution rate was in agreement with Vassileva et al (2005). Abdel-Naby et al. (2000) also reported a reduction of 57% in enzyme activity when residence time decreases from 10 to 1.42h. Our results indicated that the steady state production of CGTase are closely associated with cell growth and that low dilution rate is preferred.

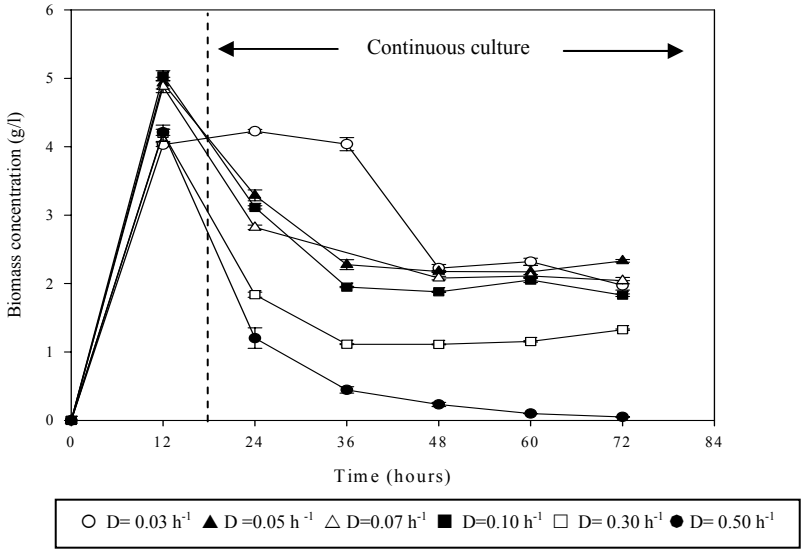

Figure 2 Time courses of the biomass concentration of *Bacillus* sp. G1 in continuous culture at various dilution rates. After batch culture for 12h, continuous culture was operated at 35°C with aeration of 1v/v/m and agitation at 150rpm for 72h.

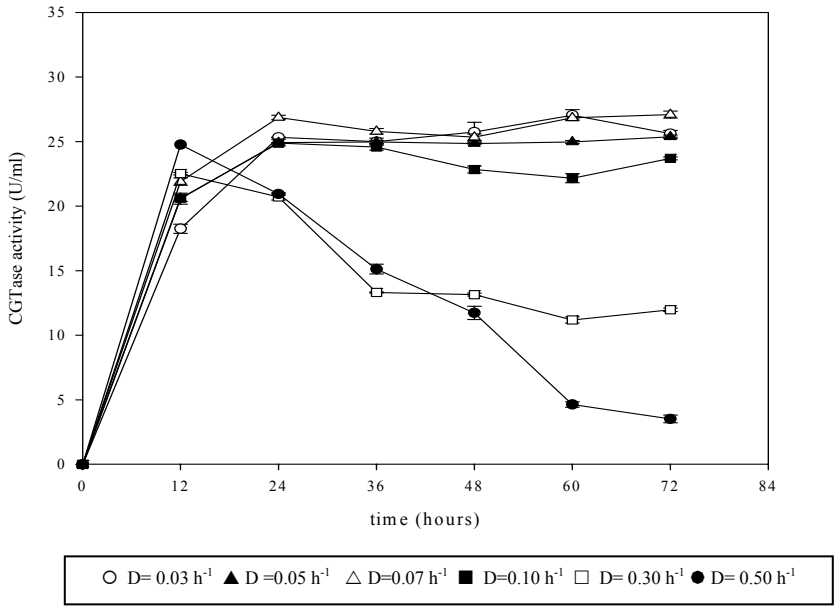

Figure 3 Time courses of the CGTase activity of the effluent of the continuous culture at various dilution rates operating condition are an stated in Figure 1.

3.2 Effect of dilution rate on productivity CGTase at steady state

Figure 4 shows the relationship between CGTase activity and CGTase productivity plotted against dilution rate. The highest CGTase productivity was obtained at the dilution rate of $0.3h^{-1}$ and that the productivity increases with on increase in dilution rate although the CGTase activity decreased significantly at dilution rate $0.3h^{-1}$. Therefore, in agreement with the theory of continuous culture, the productivity increased with increasing dilution rate and that the total amount of enzyme output from the reactor increases are due to high flow rate more than that due to enzyme activity. In this study, increasing the dilution rate greater than $0.3h^{-1}$, resulted in decreased productivity as steady state was not achieved at dilution rate at $0.5h^{-1}$. This is due to the wash-out phenomenon in the continuous culture when D is operated at value greater than D_c. This result is in agreement with the study of Jamuna & Ramakrishna (1992) for α-amylase production in continuous culture by *Bacillus cereus*. The α-amylase productivity increased with increasing dilution rate up to $2.85h^{-1}$ and decreased thereafter. Similar result was reported by Vassileva et al. (2005).

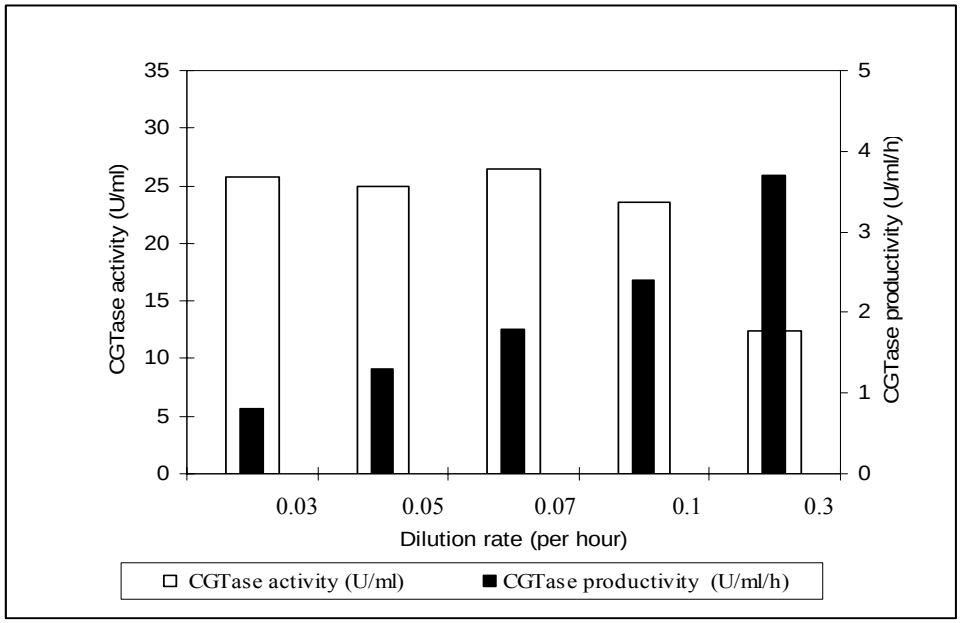

Figure 4 Effect of dilution rates on CGTase activity and productivity at steadystate. When D equals $0.5h^{-1}$, steady state was not achieved.

4. CONCLUSION

The CGTase productivity in steady state continuous culture can be improved by increasing the dilution rate and that a high dilution rate ($0.3h^{-1}$) close to the critical dilution rate (D_c) was important to achieve maximum CGTase productivity (3.72 U/ml/h).

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