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Repeated-batch Culture of Immobilized *Gibberella fujikuroi* B9 for Gibberellic Acid Production: An Optimization Study

Chang-Joon Kim[1]*, Sang Jong Lee[2], Yong Keun Chang[3]*, Gie-Taek Chun[4], Yeon-Ho Jeong[5], and Sung Bae Kim[1]

[1] Department of Chemical and Biological Engineering and ERI, Gyeongsang National University, JinJu 660-701, Korea
[2] STR Biotech. Co., Ltd., Chunchon 200-161, Korea
[3] Department of Chemical and Biomolecular Engineering, Korea Advanced Institute of Science and Technology, Daejeon 305-701, Korea
[4] Department of Molecular Bioscience, Kangwon National University, Chunchon 200-701, Korea
[5] Department of Molecular and Medical Biotechnology, Kangwon National University, Chunchon 200-701, Korea

Abstract The performance of immobilized fungal cells on celite beads for the production of gibberrelic acid was investigated in flasks and 7-L stirred-tank reactor. Repeated incubations of immobilized fungal cells increased cell concentrations and volumetric productivity. The maximum volumetric productivity obtained in the immobilized-cell culture was 3-fold greater than that in suspended-cell culture. The concentration of cotton seed flour (CSF), among the various nutrients supplied, most significantly influenced productivity and operational stability. Notably, insoluble components in CSF were found to be essential for production. CSF at 6 g/L with 60 g/L glucose was found to be optimal for gibberellic acid production and stable operation by preventing excessive cell growth.

Keywords: immobilized fungal cells, celite beads, repeated-batch operations, gibberellic acid, cotton seed flour

Gibberellic acid (GA_3), a plant growth promoter, is one of the more important secondary metabolites in modern agricultural industries. The worldwide production of GA_3 exceeded 25 tons with a market value of US $100 million as recently as 1999 [1]. The GA_3 is commercially produced by the fermentation of *Gibberella fujikuroi* [1]. Efforts for process optimization and strain improvement to enhance commercial GA_3 production by using suspended-cell cultures have approached a saturation-point [2]. Thus, there is at present heightened interest in the production of secondary metabolites through use of immobilized-cell technologies. The immobilized-cell culture systems are known to achieve high cell density while maintaining high mass transfer rates and thereby offer the advantage of high productivity [3,4,18-20]. With immobilized-cell cultures, cells can be used repeatedly thereby minimizing substrate consumption for cell growth, and the substrate feeding process can be optimally controlled further enhancing substrate utilization efficiencies [5-7]. In spite of the considerable potentials of immobilized-cell systems, however, the industry continues to be reluctant towards their application. The critical issue to the resistance by industry underlying the development and utilization of immobilized-cell processes are relate to the stability of the cells in the long-term and their repeated operation [7,8].

Kumar *et al.* [2] performed repeated-batch cultures of *G. fujikuroi* P-3 immobilized in sodium alginate or κ-carrageenan gel beads in an inverted conical fluidized bioreactor. The level of GA_3 production was shown to decrease as the culture was used repeatedly and the maximum productivity of GA_3 represented 20% less than that from a suspended-cell culture. Furthermore, the outgrowth of mycelial cells ultimately resulted in cell detachment and bead rupture [2]. GA_3 production by *G. fujikuroi* immobilized on polymeric fibrous carriers was also reported to be maintained at a constant value of about 210 mg/L during 12 consecutive batch fermentation cycles over an 84 day period in flask cultures. No comparable results, however, were reported for bioreactor cultures [9]. Escamilla *et al*. optimized the pH, C:N ratio, rice flour concentration, and temperature for GA_3 production by immobilized *G. fujikuroi* in Ca-polygalacturonate in a batch fluidized bioreactor, and obtained a product concentration of 3.9 g/L, more than 3-fold greater than those previously reported for either suspended and solid cultures [10]. However, a recent report regarding batch cultures of *G. fujikuroi* cells immobilized in κ-carrageenan in bioreactors demonstrated that the presumed advantages of immobilized-cell cultures over

***Corresponding authors**
Tel: +82-42-869-3927 Fax: +82-42-869-3910
e-mail: ychang@kaist.ac.kr
Tel: +82-55-751-5391 Fax: +82-55-753-1806
e-mail: cj_kim@gnu.ac.kr

suspended-cell cultures for achieving high productivity could not be achieved [11]. To date, there is an absence of research results which demonstrate the full potential of immobilized-cell culture for GA_3 production, at a reactor level, in terms of microbial and operational stability and high productivity.

In the present study, flask cultures of *G. fujikuroi* B9 cells immobilized on celite beads were carried out to enhance productivity. Repeated-batch cultures of immobilized cells were performed in a stirred-tank bioreactor. During the course of repeated-batch culture, nitrogen source concentrations in the medium were optimized to minimize the out-growth of cells thereby improving operational stability while maintaining a high level of the productivity.

The microorganism used, *G. fujikuroi* B9, was a mutant of *G. fujikuroi* (ATCC # 12616). The original producer microorganism was purchased from the American Type Culture Collection (Rockville, MD, USA) and improved to a stable high-yielding mutant through a rational screening program using UV and/or NTG mutagenesis in our laboratory. Glycerol stock cultures were prepared from 2-day old cultures in the synthetic production medium (see below) and were stored in a −80°C freezer. These stock cultures were used for preservation of seed cultures for both immobilized- and suspended-cell cultures. Fresh stock cultures were prepared every three months.

Suspended-cell cultures in the flasks were performed using a seed culture medium containing (g/L) 80 soybean oil, 2.5 $(NH_4)_2SO_4$, 35 corn steep liquor, and 10 KH_2PO_4. A synthetic production medium was developed and contained (g/L) 60 D-mannitol, 0.8 NH_4NO_3, 0.5 KH_2PO_4, 1.5 $MgSO_4$, 0.05 $FeSO_4$, 0.05 $ZnSO_4$, and 0.05 g $MnSO_4$ [12].

In the immobilized-cell cultures, Celite R 633 beads (Celite Korea, Seoul, Korea) were used as the biosupports. Bead sizes ranging from 100 to 500 μm in diameter were obtained by sieve fractionation. The preparation of fungal spores to be immobilized on celite and the celite bead pretreatment were performed according to the procedures described by Lee *et al.* [13]. For flask cultures, the same synthetic production medium as in the suspended-cell cultures was used while for the bioreactor cultures eight different complex media were used and evaluated and involved different nitrogen source concentrations. The basal composition of the complex production media was (g/L) 60∼120 glucose, 1∼12 cotton seed flour (CSF), 1.5 $MgSO_4$, 0.05 $FeSO_4$, 0.05 $ZnSO_4$, and 0.05 $MnSO_4$.

Suspended and immobilized cells were cultivated in 500-mL Erlenmeyer flasks placed on a rotary shaking incubator (Vision Co. Ltd., Korea) at 28°C and 230 rpm for 8 days. For the suspended-cell cultures, flasks that contained 50 mL of the synthetic medium were inoculated with 5 mL of 2-day-old seed cultures. For cell immobilization, 50 mL of the spore solution (10^8 spores/mL distilled water) that had been prepared in advance was added to 8 g of pretreated celite beads in a 500-mL flask. After 1 h of mixing, the spore-immobilized beads were washed three times with sterile distilled water to remove free spores. A concentrated synthetic medium was added to the flask to adjust the liquid volume and the medium concentration to be identical to those for the suspended-cell cultures. The immobilized-cell cultures were carried out at 28°C and shaken at 230 rpm. After each batch cycle, the spent medium was drained and the immobilized cells were washed three times with sterile water, and a fresh volume of medium was filled for the next batch cycle. All experiments described were conducted in triplicate.

Repeated-batch cultures of immobilized cells were performed in a bioreactor. A 7-L top-driven jar fermentor (Kobiotech Ltd., Korea) with a working volume of 4.0 L including 700 g of celite beads (50%, v/v) was used. The incubation temperature was maintained at 28°C and the pH controlled at 4.5∼5.0 with 2-N H_2SO_4 or 2-N NaOH solutions. The dissolved oxygen concentration was maintained at over 20% of air saturation by manipulating the agitation speed. When glucose was depleted at the end of each batch cycle, the reactor was drained, washed with sterile water, and refilled with fresh medium for the next batch. All chemicals used in these studies were reagent grade purchased from Sigma-Aldrich (St. Louis, USA), except for the glucose which was purchased from Daesang Co., Ltd. (Seoul, Korea).

The glucose concentration was measured by using a glucose analyzer (YSI Life Science, USA). Cell concentrations were determined in the same manner as previously described by Kim *et al.* [8]. The GA_3 concentration in the supernatant of the culture broth was measured by isocratic high-performance liquid chromatography. A mighty sil C18 column (Kanto Co., Ltd, Japan) was used. The mobile phase consisted of methanol (40%, v/v) and 10-mM H_3PO_4 solution (60%, v/v) and was used at a flow rate of 1.5 mL/min; column temperature was maintained at 40°C. The GA_3 was detected at 204 nm using a UV detector (LC-10ADvp, Shimadzu, Japan).

As a preliminary experiment, the production of GA_3 by both suspended and immobilized cells were compared through a series of flask cultures. As shown in Table 1, the final cell and GA_3 concentrations for the suspended-cell culture were 13 g/L and 35 mg/L, respectively. In the repeated-batch cultures of immobilized cells, GA_3 concentration increased from 23 mg/L at the end of the first batch to 98 mg/L at the end of the fourth batch. Thereafter, GA_3 concentrations appeared to remain fairly constant and reached 103 mg/L by the end of the seventh batch. Cell concentrations also increased from 10 g/L at the end of the first to 36 g/L at the end of the seventh batch.

The amount of GA_3 produced by immobilized cells in the first batch was less than that by suspended-cell culture, however, repeated incubations of immobilized cells resulted in a significant productivity enhancement likely due to the increasing cell number and thus concentration. A 3-fold higher volumetric productivity (Q_p) was obtained compared to the suspended cell culture. No notable differences in specific productivity (q_p) between suspended and immobilized cells were observed. These re-

Table 1. A comparison of methods: productivity of suspended-cell culture vs. repeated-batch culture of immobilized cells

	Batch	GA_3 conc. (mg/L)	Cell conc. (g/L)	Vol. prod. (Q_p) (mg-GA_3/L/day)	Specific prod. (q_p) (mg-GA_3/g-cells)
Suspended cells[a]	–	35 (±1)	13.0 (±0.7)	4.4	2.7
Immobilized cells[b] (Repeated batch)	1st	23 (±1)	10.0 (±0.4)	2.9	2.1
	2nd	57 (±2)	–	7.1	–
	3rd	54 (±5)	–	6.8	–
	4th	98 (±21)	–	12.3	–
	5th	78 (±0)	–	9.8	–
	6th	97 (±11)	–	12.1	–
	7th	103 (±5)	36.0 (±1.5)	12.9	2.9

[a]8-day culture.
[b]Synthetic production medium was replaced with fresh medium every 8 days.

Table 2. Repeated-batch cultures of immobilized cells in the bioreactor

Batch	Medium	Composition (g/L)			Culture time (days)	Residual glucose (g/L)	Final GA_3 (mg/L)	Glucose uptake rate (g/L/day)	Vol. prod. (Q_p) (mg-GA_3/L/day)	Prod. yield ($Y_{p/s}$) (mg-GA_3/g-glucose)
		Glucose	CSF	KH_2PO_4						
1st	M1	60	1	1	7.5	35	–	2.9	–	–
2nd	M2	60	4	3	8.5	20	27	5.2	3.2	0.62
3rd	M2	60	4	3	7.0	7	49	8.1	6.1	0.75
4th	M2	60	4	3	7.0	6	77	7.7	9.0	1.17
5th	M3	60	extract	3	7.0	29	41	4.8	3.9	0.82
6th	M2	60	4	3	7.0	6	78	7.4	10.1	1.37
7th	M2	60	4	3	8.0	0	117	9.5	14.0	1.48
8th	M5	60	8	3	4.0	0	112	18.0	20.3	1.13
9th	M4	60	6	3	4.0	0	113	17.0	23.5	1.38
10th	M6	60	12	3	2.0	0	39	28.5	11.4	0.40
11th	M7	90	9	3	5.0	0	147	32.8	28.3	0.86

[*]All complex production media included 1.5 g/L of $MgSO_4 \cdot 7H_2O$.

sults clearly indicate that the enhancement of GA_3 production was mainly due to the increased cell concentration in the immobilized-cell culture.

In the repeated-batch cultures of immobilized cells in the bioreactor, the sole nitrogen source of NH_4NO_3 was replaced by cotton seed flour (CSF). The latter was subsequently found by our study team to be an improved source for GA_3 production than NH_4NO_3 (data not shown). This finding was supported by reports that plant extracts in general, such as corn steep extracts, soybean, peanuts meals, and rice flour, positively affect GA_3 biosynthesis [1,14].

The performance of immobilized-cell culture was examined for seven different media (M1 – M7, mainly different in CSF concentrations), by changing the medium from batch to batch (Table 2). The time profile of each batch is given in Fig. 1. For the first batch, M1 medium containing rather low CSF and KH_2PO_4 concentrations was chosen based on the general knowledge that high concentrations of nitrogen and/or phosphate suppress the production of secondary metabolites. No production of GA_3 was observed even after 7 days at which time a considerable amount of glucose remained in the first batch using M1. It was reported that GA_3 production was not induced in spite of phosphate depletion in the presence of excess nitrogen in a suspended-cell culture of *G. fujikuroi* [12,14]. No significant adverse effect of high concentrations of phosphate (KH_2PO_4) on GA_3 had been reported for immobilized-cell cultures [10]. Therefore we primarily investigated the effects of the nitrogen source (CSF) concentration, while maintaining KH_2PO_4 concentration at 3 g/L [10] in subsequent batches. GA_3 production was observed in the next three batches using M2 which contained 4-g/L CSF; GA_3 concentration was 27 mg/L at the end of the second, 49 mg/L at the end of the third, and 77 mg/L at the end of the fourth batch (Table 2).

We hypothesized that dissolved solids from the CSF source might block the pores of cell-immobilized beads and interfere with nutrient uptake by the cells. To test our hypothesis, a solid-free CSF extract was prepared by fil-

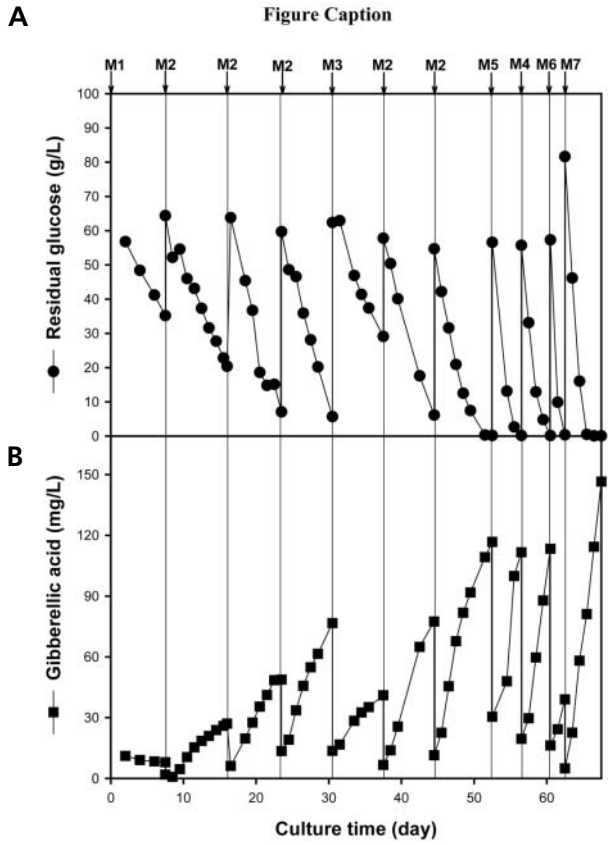

Fig. 1. Time profiles of gibberellic acid production in repeated-batch cultures of immobilized cells in the bioreactor: (A) residual glucose concentration (●); (B) gibberellic acid concentration (■).

tration and used as the nitrogen source for the fifth batch. Unexpectedly, a significant drop of GA_3 production was observed with a greatly reduced glucose uptake rate. The residual glucose concentration at the end of the culture was as high as 29 g/L. When M2 was used again for the next batch, that is, the sixth batch, the production of GA_3 and glucose uptake rate recovered to the previous levels noted in the fourth batch. This result clearly indicated that the insoluble components of CSF were essential for the production of GA_3 and that no harmful effects could be attributed to solid components (pore-clogging). A large quantity of GA_3 (117 mg/L) was produced by cells which completely consumed glucose in the 8-day culture of the seventh batch using the M2 medium.

As the incubation cycles were repeated using M2, the Q_p increased 4.4-fold progressively from a 3.2 mg-GA_3/day level at the second batch to 14.0 mg-GA_3/day at the seventh batch. The corresponding glucose uptake rate also increased from 5.2 g-glucose/L/day to 9.5 g-glucose/L/day. As demonstrated in the flask cultures of immobilized cells, the enhancement of glucose uptake rate and the volumetric productivity of cells were due to the increased cell concentration without loss of specific productivity as the number of batches increased. The yield coefficient, $Y_{p/s}$ increased from 0.62 mg-GA_3/g-glucose to 1.48 mg-GA_3/g-glucose for the corresponding batch cultures.

The effect of initial CSF concentration on GA_3 production was investigated in the seventh to the tenth incubation cycles. When a medium with 8-g/L CSF (M5) or 6-g/L CSF (M4) was used for the eighth or ninth batch, approximately 112 mg/L of GA_3 was produced while completely consuming the glucose. However, only a small amount of GA_3 (36 mg/L) was produced and glucose completely depleted when a medium with 12 g/L-CSF (M6) was used for the tenth batch. The importance of initial concentration of CSF on the productivity and yield at 60-g/L glucose (the seventh to the tenth batches) is clearly shown by Table 2. The Q_p increased as the CSF concentration increased going from 4 to 6 g/L, however, the Q_p progressively declined beginning at 8-g/L CSF and reached the lowest value at 12-g/L CSF. One the other hand, the glucose uptake rate continued to increase with increasing in CSF concentrations and reached the highest value at 12-g/L CSF. The $Y_{p/s}$ remained constant up to 6-g/L CSF and then decreased with the increase in the CSF concentration, reaching the lowest value at 12-g/L CSF. This result indicates that the initial CSF concentration as a nitrogen source is critical for achieving maximal GA_3 productivity with efficient utilization of the carbon source. A 6-g/L CSF concentration was found to be optimal for a 60-g/L glucose considering Q_p and $Y_{p/s}$. Such a dominant effect of nitrogen concentration and the presence of an optimal concentration of nitrogen source for GA_3 production conditions are well known for suspended-cell cultures [12,14,15]. The GA_3 production was also examined when the concentrations of glucose and CSF were increased to 90-g/L and 9-g/L, respectively (M7) in the eleventh batch. The glucose to CSF ratio of 10 was considered to be optimal as seen in the ninth batch. GA_3 production started at 1 day and was seen to continue even after glucose was depleted at day 3 (Fig. 1); GA_3 concentrations reached a maximum of 147 mg/L at day 5. Compared with the culture using M4 (the ninth batch), a higher Q_p but much lower $Y_{p/s}$ were observed.

It is notable that GA_3 production recovered from the suppression by the high CSF concentration in the tenth batch when the medium was changed to M7 which contained less CSF. Such reversibility of GA_3 production has been also reported in suspended-cell cultures [12]. When ammonium-repressed mycelia were transferred from a high-nitrogen culture to a low-nitrogen medium, cells produced GA_3 with the original activity but after a lag period, possibly the time needed for enzyme synthesis.

Another important observation was the characteristics of GA_3 production by the immobilized cells. It has generally been reported for suspended-cell cultures that GA_3 production begins at, or soon after, nitrogen exhaustion [1,12,14,15]. We also observed that GA_3 production began only in the later stage of cultivation in suspended-cell cultures (data not shown). Alternatively, the immobilized cells started to produce GA_3 day 1. The same physiological characteristics of GA_3 biosynthesis have been observed in immobilized-cell cultures [11,16]. One of the

possibilities to consider is the existence of a nutrient concentration gradient inside the biosupports that are limited by diffusion. Diffusion limitation can give rise to different microenvironments within the biosupport where the fungal cells could be under nitrogen limitation [11].

In repeated-batch cultures, when batch cycle has finished, the stirring and aeration actions cease allowing immobilized cells to settle. The supernatant is then withdrawn; the immobilized cells rinsed and new freshly prepared medium is filled for the next batch cycle. Although the highest volumetric productivity was achieved by using M7 in the eleventh batch, the viscosity of the culture broth was remarkably high. We were unable to run the next batch because the beads failed to sediment after the batch had been finished and thus a medium change was not possible. Such a high viscosity was caused by the presence of a large amount of free cells in the culture medium, due to an overgrowth of cells on the biosupports [6,8,17]. The use of M4 resulted in the best performance considering volumetric productivity and substrate utilization ($Y_{p/s}$). Therefore, it was essential to consider the degree of free cell formation as well as the productivity when evaluating the medium. We observed that CSF was mostly digested so that very little insoluble material remained in the culture broth after cultivation. Therefore, we considered that free cell formation might be estimated from measuring dry cell weight which led to a set of experiments to evaluate M4 for free cell formation.

A series of repeated-batch cultures using immobilized cells were initiated using M2 for stable operations and minimal formation of free cells. The GA_3 concentration and the corresponding Q_p were 106 mg/L and 12.9 mg-GA_3/L/day, respectively, at the end of the third batch. The concentrations of immobilized cells and free cells after this batch were 100 mg-cells/g-celite and 1.9 g/L, respectively. When M4 was used in the fourth batch, the GA_3 concentration and corresponding Q_p were observed to be 145 mg/L and 24.8 mg-GA_3/L/day, respectively; the immobilized and released free cells were 152 mg-cells/g-celite and 1.0 g/L, respectively. These results indicated that a minor amount of released free cells were produced in M4 while immobilized-cell concentration continued at a much higher level.

In summary, medium composition, especially the concentration of CSF as a nitrogen source had potent effects upon GA_3 production as well as on free cell formation. The formation of a high amount of free cells caused problems detrimental to repeated-batch culture. The optimal concentration of CSF was determined as 6 g/L in support of improved GA_3 productivity and free cell formation.

REFERENCES

[1] Tudzynski, B. (1999) Biosynthesis of gibberellins in *Gibberella fujikuroi:* biomolecular aspects. *Appl. Microbiol. Biotechnol.* 52: 298-310.

[2] Kumar, P. K. R. and B. K. Lonsane (1988) Immobilized growing cells of *Gibberella fujikuroi* P-3 for production of gibberellic acid and pigment in batch and semi-continuous cultures. *Appl. Microbiol. Biotechnol.* 28: 537-542.

[3] Gbewonyo, K. and D. I. C. Wang (1983) Confining mycelial growth to porous microbeads: A novel technique to alter the morphology of non-newtonian mycelial cultures. *Biotechnol. Bioeng.* 25: 967-983.

[4] Gbewonyo, K. and D. I. C Wang (1983) Enhancing gas-liquid mass transfer rates in non-newtonian fermentations by confining mycelial growth to microbeads in a bubble column. *Biotechnol. Bioeng.* 25: 2873-2887.

[5] Constantinides, A. and N. Mehta (1991) Periodic operation of immobilized live cell bioreactor for the production of candicidin. *Biotechnol. Bioeng.* 37: 1010-1020.

[6] Sarra, M., C. Casas, and F. Godia (1997) Continuous production of a hybrid antibiotic by *Streptomyces lividans* TK21 pellets in a three-phase fluidized-bed bioreactor. *Biotechnol. Bioeng*. 53: 601-610.

[7] Deo, Y. M. and G. M. Gaucher (1985) Effect of nitrogen supplementation on the longevity of antibiotic production by immobilized cells of *Penicillium urticae*. *Appl. Microbiol. Biotechnol.* 21: 220-227.

[8] Kim, C. J., Y. K. Chang, G. T. Chun, Y. H. Jeong, and S. J. Lee (2001) Continuous culture of immobilized *Streptomyces* cells for kasugamycin production. *Biotechnol. Prog.* 17: 453-461.

[9] Lu, Z.-X., Z.-C. Xie, and M. Kumakura (1995) Production of gibberellic acid in *Gibberella fujikuroi* adhered onto polymeric fibrous carriers. *Process Biochem.* 30: 661-665.

[10] Escamilla, E. M., L. Dendooven, I. P. Magana, S. R. Parra, and M. De la Torre (2000) Optimization of gibberellic acid production by immobilized *Gibberella fujikuroi* mycelium in fluidized bioreactors. *J. Biotechnol.* 76: 147-155.

[11] Duran-Paramo, E., H. Molina-Jimenez, M. A. Brito-Arias, and F. Robles-Martinez (2004) Gibberellic acid production by free and immobilized cells in different culture systems. *Appl. Biochem. Biotechnol.* 113-116: 381-388.

[12] Candau, R., J. Avalos, and E. Cerda-Olmedo (1992) Regulation of gibberellin biosynthesis in *Gibberella fujikuroi*. *Plant Physiol.* 100: 1184-1188.

[13] Lee, T. H., G. T. Chun, and Y. K. Chang (1997) Development of sporulation/immobilization method and its application for the continuous production of cyclosporin A by *Tolypocladium inflatum*. *Biotechnol. Prog.* 13: 546-550.

[14] Bruckner, B. (1992) Regulation of gibberellin formation by the fungus *Gibberella fujikuroi.* pp. 129-143. In: D. J. Chadwick and J. Whelan (eds.). *Ciba Foundation Symposium* 171. *Secondary Metabolites: Their Function and Evolution*. John Wiley & Sons Publishers, Chichester, UK.

[15] Borrow, A., S. Brown, E. G. Jefferys, R. H. J. Kessell, E. C. Lloyd, P. B. Lloyd, A. Rothwell, B. Rothwell, and J. C. Swait (1964) The kinetics of metabolism of *Gibberella fujikuroi* in stirred culture. *Can. J. Microbiol.* 10: 407-444.

[16] Nava Saucedo, J. E., J.-N. Barbotin, and D. Thomas (1989) Continuous production of gibberellic acid in a fixed-bed reactor by immobilized mycelia of *Gibberella fujikuroi* in calcium alginate beads. *Appl. Microbiol. Biotechnol.* 30: 226-233.

[17] Park, Y. H., E. Y. Kim, W. T. Seo, K. H. Jung, and Y. J. Yoo (1989) Production of cephalosporin C in a fluidized-bed bioreactor. *J. Ferment. Bioeng.* 67: 409-414.

[18] Na, J.-G., H. H. Kim, and Y. K. Chang (2005) On-line

estimation of cell growth from agitation speed in DO-stat culture of a filamentous microorganism, *Agaricus blazei*. *Biotechnol. Bioprocess Eng.* 10: 571-575.

[19] Lim, J. S., J. H. Lee, J. M. Kim, S. W. Park, and S. W. Kim (2006) Effects of morphology and rheology on neo-fructosyltransferase production by *Penicillium citrinum*. *Biotechnol. Bioprocess Eng.* 11: 100-104.

[20] Ibrahim, S., S. Vikineswary, S. Al-Azad, and L. L. Chong (2006) The effects of light intensity, inoculum size, and cell immobilization on the treatment of sago effluent with *Rhodopseudomonas palustris* strain B1. *Biotechnol. Bioprocess Eng.* 11: 377-381.

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