0273-2289/04/114/0381–0388/$25.00

Gibberellic Acid Production by Free and Immobilized Cells in Different Culture Systems

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Abstract

Gibberellic acid production was studied in different fermentation systems. Free and immobilized cells of *Gibberella fujikuroi* cultures in shake-flask, stirred and fixed-bed reactors were evaluated for the production of gibberellic acid (GA_3). Gibberellic acid production with free cells cultured in a stirred reactor reached 0.206 g/L and a yield of 0.078 g of GA_3/g biomass.

Index Entries: Cell immobilization; gibberellic acid; *Gibberella fujikuroi*; shake flasks; free cells; immobilized cells.

Introduction

Gibberellic acid represents one of the most important plant growth regulators produced from microorganisms owing to its extensive practical uses in agriculture. At present, the production of gibberellic acid by submerged fermentation with the filamentous fungi *Gibberella fujikuroi* can be a practical alternative to increase the production of vegetal biomass for the production of more and better foods, fuels, and chemicals. Gibberellic acid production has been widely studied with different strains of *G. fujikuroi* in different fermentation systems *(1–8)*. In the present work, we studied gibberellic acid production using free and immobilized cells of *G. fujikuroi* NRRL-2278 in order to select the most suitable fermentation system using this particular strain.

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Materials and Methods

Microorganism

G. fujikuroi NRRL-2278 obtained from the US Department of Agriculture, maintained on potato dextrose agar (PDA) slants, and stored at 4°C.

Growth Medium and Inoculum

The culture medium proposed by González et al. *(4)* with a slight modification was used. The composition was glucose (25.0 g/L), KH_2PO_4 (5.0 g/L), NH_4NO_3 (1.33 g/L), and $MgSO_4$ (1.0 g/L). The culture medium was sterilized at 121°C for 20 min. The pH of the culture medium was about 4.0 after sterilization. Inoculum (200 mL) for submerged fermentations was obtained from a 48-h shake-flask culture (120 rpm) at 30°C. The seed medium was inoculated with mycelium grown 5 d in PDA slants.

Fermentation

The microorganism was grown at 38°C within agitation of 200 rpm and a 0.3-vvm aeration in a 5-L laboratory fermentor with a working volume of 3.5-L (Bioflo II; New Brunswick Scientific). For free-cell cultures, 0.7 L of preinoculum was grown in Erlenmeyer flasks for 12 h at 38°C in a rotary shaker and used to inoculate 3.5 L of medium in the fermentor.

Immobilization

The mycelium biomass recovered from 200 mL of the seed culture was mixed with 200 mL of sterile 2% (w/v) κ-carrageenan (E407; Ceca, Paris, France) at 42°C. The mycelium-carrageenan suspension was then pumped through a couple of needles and dropped into sterile KCl solution (0.3 *M*) to form gel beads with an average diameter of 3 mm. Gel beads were soaked for 30 min in the same KCl solution. All immobilization experiments were carried out under aseptic conditions.

Dissolution of Beads

To quantify the immobilized biomass, gel beads were dissolved by the addition of 0.1 *M* sodium citrate *(9)*.

Analysis

Reducing sugars were determined using the dinitrosalicylic acid reagent *(10)*. The biomass was determined by mycelial dry weight. Ammonia was determined by the phenol-hipochlorite reaction *(11)*. Quantification of gibberellic acid was performed by the spectrophotometric method of Holbrook et al. *(12)*, and the purity of gibberellic acid was verified by thin-layer chromatography.

Results and Discussion

The objective of the present study was to compare gibberellic acid production with free and immobilized cells of *G. fujikuroi* in different

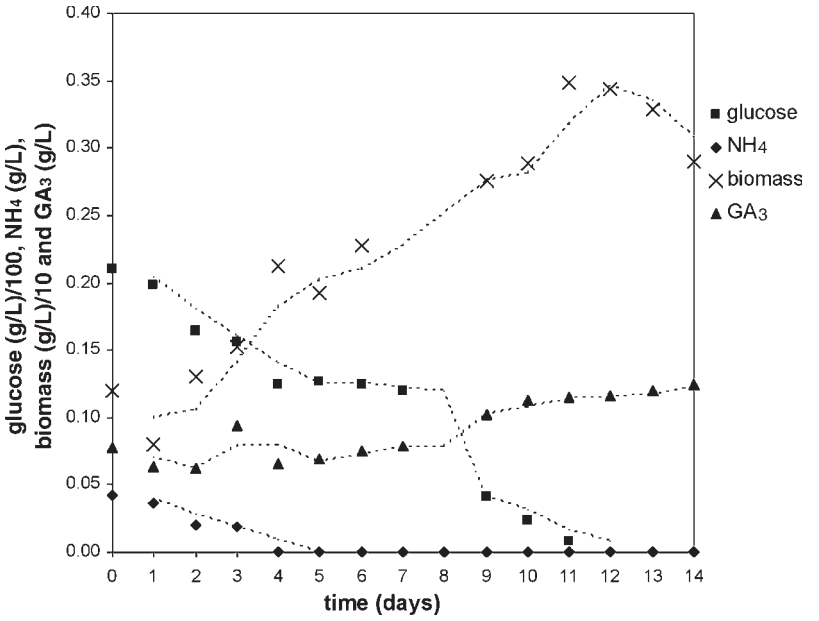

Fig. 1. Gibberellic acid production with free cells of *G. fujikuroi* cultured in Erlenmeyer flasks.

fermentation systems. All experiments were carried out three times, and average results are presented in Figs. 1–5.

Fermentation of Free Cells in Shake Flasks

Figure 1 shows the gibberellic acid production with free cells in shake flasks. Fermentation was carried out at 31° C with an initial pH of 4.5 and agitation of 120 rpm. As can be observed, the nitrogen source was exhausted after 5 d of fermentation. Under nitrogen limitation, gibberellic acid production was effective. Gibberellic acid concentration at the beginning of the fermentation seems to be related to that of the seed inoculum. On d 14, 0.12 g/L of gibberellic acid was produced. Glucose was exhausted after 12 d of fermentation, and at the same time, exponential growth plateaud and then fell. The pH fell because of the production of gibberellic acid. The fermentation of free cells in shake flasks presented a behavior according to that described by Borrow et al. *(13,14)*, in which two phases are presented: producing and nonproducing. The nonproducing phase was characterized by uptake of carbon and nitrogen sources. Under nitrogen limitations, the metabolism of *G. fujikuroi* switches to the production of gibberellic acid and storage compounds such as lipids and polyols, as described by Brückner and Blechschmidt *(15,16)*.

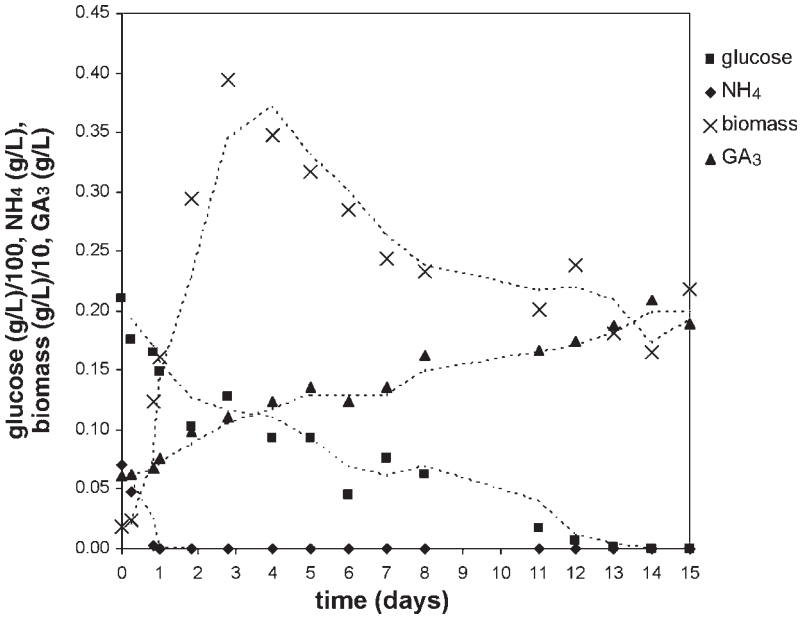

Fig. 2. Gibberellic acid production with free cells of *G. fujikuroi* cultured in stirred reactor.

Fermentation of Free Cells in Stirred Reactor

Figure 2 shows gibberellic acid production by free *G. fujikuroi* cells cultured in a stirred reactor. Some kinetic differences in the stirred reactor regarding shake flasks cultures are observed. The aeration (0.3 vvm) and agitation (200 rpm) in the stirred reactor may be implied in these kinetic differences. As reported by Borrow et al. *(13,14)*, exponential growth ceases when assimilable nitrogenous nutrients are exhausted. However, glucose was exhausted only after 13 d of fermentation. Gibberellic acid production reached a concentration of 0.206 g/L and was almost twice that obtained with free cells in shake flasks.

Fermentation of Immobilized Cells in Erlenmeyer Flasks and in Stirred Reactor

The growth of immobilized cells ceased once the nitrogen source was exhausted, as reported by Nava et al. *(17)* (Fig. 3). After that, immobilized cells were released from the immobilization support and became free cells. Gibberellic acid production (0.160 g/L) was effective once the ammonia was exhausted, and it continued until total glucose consumption. In the case of immobilized cells cultured in a stirred reactor, Fig. 4 shows the fermentation kinetics. One can observe some differences related to the immobilized biomass production, and the uptake in nitrogen and carbon sources. However,

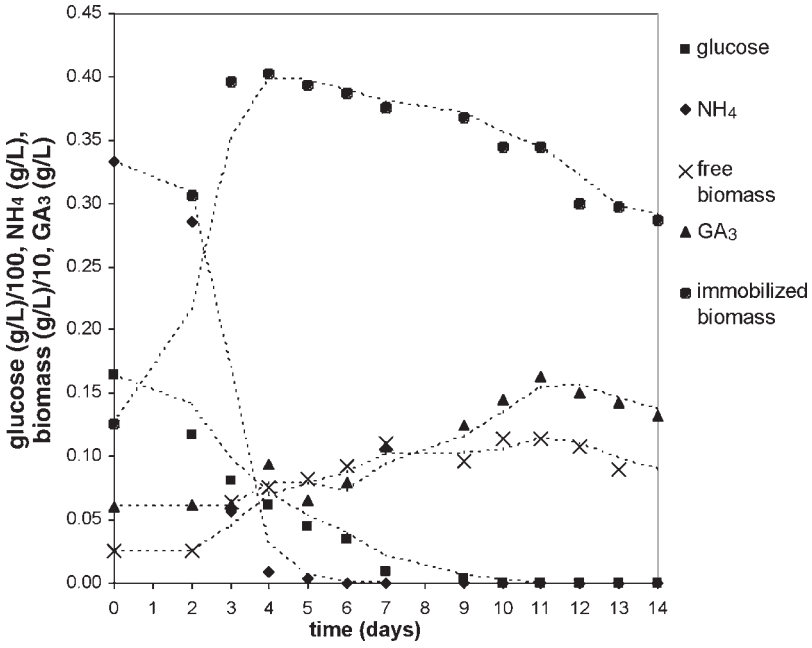

Fig. 3. Gibberellic acid production with immobilized cells of *G. fujikuroi* cultured in Erlenmeyer flasks.

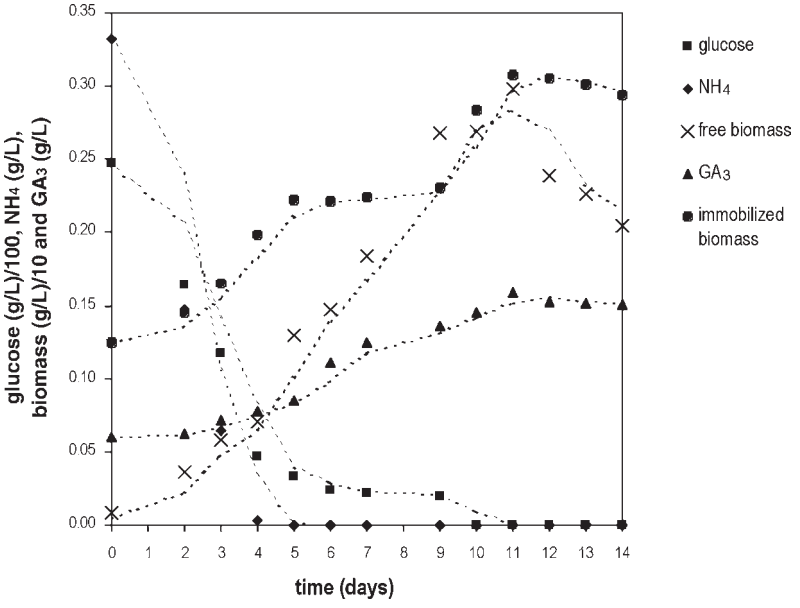

Fig. 4. Gibberellic acid production with immobilized cells of *G. fujikuroi* cultured in stirred reactor.

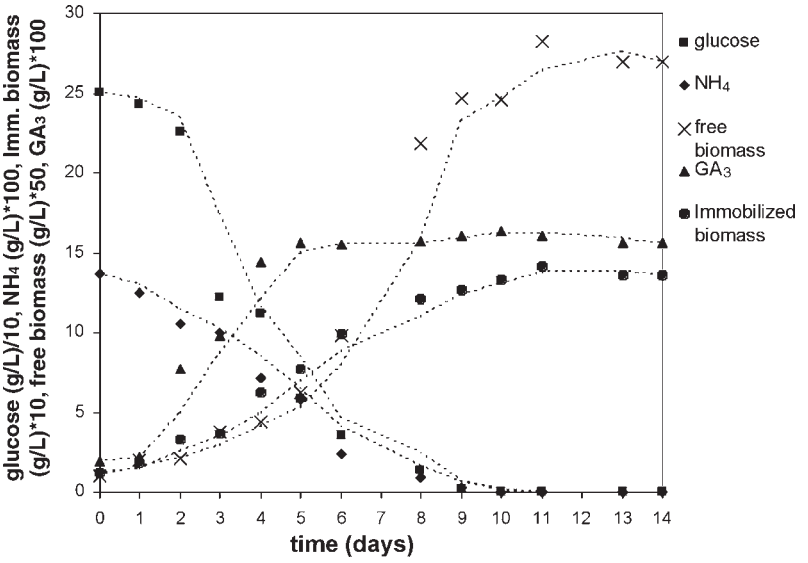

Fig. 5. Gibberellic acid production with immobilized cells of *G. fujikuroi* cultured in fixed-bed reactor.

gibberellic acid concentration (0.163 g/L) had no significant difference regarding the fermentation of immobilized cells in shake flasks. The differences found in the fermentation kinetics seem to be related to the agitation (200 rpm) and aeration (0.3 vvm) in the stirred reactor.

Fermentation of Immobilized Cells in Fixed-Bed Reactor

Figure 5 shows gibberellic acid production by immobilized cells of *G. fujikuroi* in a fixed-bed reactor. Gibberellic acid production after d 4 was effective even under conditions of no nitrogen limitations. Carbon and nitrogen sources were exhausted on d 10, when released and immobilized biomass reached a maximum. The differences observed in that culture may be related to the diffusional limitations imposed by the immobilization support. Cell immobilization allows high biomass concentration and gradient formation inside the immobilization support by diffusional limitations. Diffusional limitations give rise to different microenvironments inside the carrier where fungi cells can develop under nitrogen limitations. Thus, the concentrations dosed into the immobilization support may be different from those dosed in the bulk solution *(9)*.

Gibberellic acid production was almost the same regarding the other immobilized cell fermentation systems (0.164 g/L). There was no significant difference among all immobilized cell systems tested. Further optimization studies should be carried out with immobilized cell systems for the production of gibberellic acid.

Table 1
Production and Yields of Gibberellic Acid by Free and Immobilized Cells of *G. fujikuroi* in Different Culture Systems

	GA_3 (g/L)		$Y_{P/X}$ (g GA_3/g biomass)	
Fermentation system	Free cells	Immobilized cells	Free cells	Immobilized cells
Shake flasks	0.120	0.160	0.052	0.054
Stirred reactor	0.206	0.163	0.078	0.036
Fixed-bed reactor	—	0.164	—	0.027

Conclusion

Table 1 summarizes the results of the fermentations performed in the present study with free and immobilized cells of *G. fujikuroi*. Under the tested culture conditions, no significant differences were found among the immobilized cell systems for gibberellic acid production. However, gibberellic acid production with free cells cultured in the stirred reactor was higher than that obtained in Erlenmeyer flasks or in any other fermentation system assessed. Specific production of gibberellic acid was also the most important in the fermentation of free cells in stirred reactor compared with the other systems evaluated (Table 1). The culture medium and conditions should be further optimized in order to increase gibberellic acid production with immobilized cell culture systems. At present, we trying to meet this goal by using an orthogonal experimental design. In addition, we are working on optimizing the immobilization technique and its use with fixed-bed reactors for the production of gibberellic acid.

Acknowledgments

We wish to thank the Consejo Nacional de Ciencia y Tecnología of Mexico (CONACYT) and the Instituto Politécnico Nacional (CGPI and COFAA), Mexico, for their help and financial support (CONACYT-30206 and CGPI-20010709).

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