Galactose oxidase production by immobilized cells of
**Dactylium dendroides**

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**SUMMARY**

Dactylium dendroides cells were immobilized with calcium alginate, calcium pectate and k-carrageenan. Alginate immobilized cells produced relatively small amounts of (D-galactose:O₂ oxidoreductase, EC 1.1.3.9, GOase). Pectate immobilized cells gave the best yield of GOase, which was comparable with that obtained with free cells, and productivity could be extended up to 28 days (7 cycles). Controlled dosage of phosphate to the medium markedly improved GOase production with higher yields per cycle than with free cells.

**INTRODUCTION**

Galactose oxidase (D-galactose:O₂ oxidoreductase, EC 1.1.3.9, GOase) catalyses the oxidation of D-galactose to galacto-hexodiallde with the concomitant reduction of O₂ to H₂O₂. GOase is an extracellular enzyme produced by several fungi (Gancedo et al., 1967). The production of GOase by free cells of D. dendroides and GOase purification have been already described (Avigad et al., 1962). The production of GOase can be improved by using immobilized cells. We report a method for immobilization of D. dendroides cells with calcium alginate, k-carrageenan, calcium pectate as carriers, for the semicontinuous production of GOase.

**MATERIALS AND METHODS**

Sodium alginate Protanal LF 60/10 was kindly donated by Protan A/S (Norway), pectate Genu LM 111 and k-carrageenan Genugel X-0828 were donated by Copenhagen Pectinfabrik (Denmark).

Dactylium dendroides was maintained on Sabouraud agar at 4°C. It was grown on medium containing sorbose (55 mM) as a carbon source, (NH₄)₂SO₄ (13 mM), NH₄NO₃ (13 mM), KH₂PO₄ (68 mM), Na₂HPO₄.12H₂O (60 mM), thiamin.HCl (6 mM) and trace elements (Tressel et al., 1980) was inoculated with a stock culture and cultivated on a rotary shaker at 22°C for 72 h. The biomass was filtered, washed with distilled water and briefly homogenized by Ultra Turrax mixer (Janke-Kunkel, Germany) before use. For control experiments with free cells the nutrient medium of the same composition which was described before, was inoculated
with 5% (v/v) of inoculum and cultivated on a rotary shaker at 22°C for 96 h.

**Immobilization:** Mycelium suspension of 13 ml in distilled water (dry wt was determined for each immobilization separately) was blended with 6% (v/v) solution of appropriate carrier to make 20 ml of suspension. (The solutions of the carriers were sterilized by autoclaving, all procedures were performed under sterile conditions.) Suspension in alginate or pectate was dropped into 2.5% (w/v) CaCl₂ or into 3% (w/v) KCl (carrageenan) to form beads of average diameter 2 to 3 mm. After 1 h the precipitating solution was removed and the beads were washed twice with sterile distilled water.

Immobilized cells were incubated in 250 ml conical flasks containing 40 ml of medium of the same nutrient composition as before except the concentration of phosphate. Different experiments were carried out in medium containing no phosphate, 20 mM KH₂PO₄ or 40 mM KH₂PO₄ (see below) and no Na₂HPO₄·12H₂O. The flasks were shaken on a rotary shaker at 22°C. Chloramphenicol (Kfen et al., 1985) to give 40 mg/l was added to prevent bacterial contamination. The medium was changed in 4 to 7 day-intervals.

**Assay of galactose oxidase activity:** Enzyme activity was monitored by the method of Kosman et al. (1974).

### RESULTS AND DISCUSSION

The cells entrapped in three different polymers showed marked differences in GOase production and in their kinetics. The cells entrapped in alginate produced only traces of GOase at the beginning of the cultivation independently of phosphate concentration in the medium. However *D. dendroides*, immobilized in pectate or in carrageenan, produced considerable amounts of GOase (see Fig. 1 and 2).

In both latter cases, phosphate (40 mM KH₂PO₄) in the medium stimulated the onset of GOase production. However, after two cycles extracellular GOase activity sharply dropped. In the cultivation medium, which contained no phosphate, the production increased more gradually, but was prolonged up to fifth cycle (32 days) and therefore resulted in a substantially higher overall yield of the enzyme. The production profile during each incubation reached its maximum on day 4 and then sharply decreased probably due to depletion of nutrients. Thus, in the later experiments the medium was always changed on the forth day of incubation of the cycle.

Pectate was the best carrier for the immobilization of *D. dendroides*. Influence of pectate concentration on GOase production was also investigated. Although 1% pectate concentration on GOase production was also investigated. Although 1% pectate concentration on GOase production was also investigated. Although 1% pectate beads were mechanically unstable and started to decay at the beginning of the cultivation, cells entrapped in 4% (w/v) pectate exhibited rather high activity in the initial phases of the process, but later GOase formation decreased markedly probably due to decreased O₂ transfer into the beads. The best results were obtained with 2% (w/v) pectate beads, giving good overall production during the whole period.

Phosphate concentration in the medium is one of the main controlling elements of the GOase synthesis by immobilized