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Oxygen delivery requirements of *Colletotrichum truncatum* during germination, vegetative growth, and sporulation

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Abstract. Optimization of O_2 delivery was the key to successful conidiation of *Colletotrichum truncatum* in submerged fermentor cultures supplied with 20 g carbon/l and C:N at the optimal 10:1 mass ratio for spore efficacy. Minimal mycelial fragmentation and maximal biomass and spore yields were provided by an O_2 transfer program that called for gradual increases in stirring rate to compensate for rising cell concentration and viscosity. The utility of an event-based O_2 transfer program was further supported by our observation of different O_2 requirements for each phase of the life cycle. Spore germination did not occur in cultures sparged with N_2. However, even low levels of O_2 [10% dissolved O_2 tension (DOT)] allowed 100% germination. The specific growth rate of the mycelia was a Monod-like function of DOT. The maximal growth rate was achieved when ≥15% DOT was provided via O_2 transfer at a specific rate of 5.4×10^{-3} mol/g per hour. Sporulation had a strict O_2 requirement, and its rate and yield were optimized by providing 55% DOT following the cessation of growth. The specific O_2 demand of optimally sporulating mycelia was 4.9×10^{-4} mol/g per hour, an order of magnitude less than that associated with growing mycelia. Behaving as a pseudoplastic fluid, the fermentation broth reached a maximum apparent viscosity of 70 P at the onset of sporulation when the O_2 demand was low. However, the maximum power requirement approx. 7.9 W/l occurred during the last 36 h of growth when the O_2 demand was highest.

Introduction

Interest in using host-specific plant pathogens to control weeds has increased as chemical herbicide registrations have declined and as public interest in farming with fewer chemical pesticides has risen. Many factors, such as host range, weed-killing efficacy, genetic stability, production costs, and market potential, influence the economic feasibility of a biological control product. A primary objective of our research was the development of cost-effective liquid culture technology for producing asexual conidial spores of *Colletotrichum truncatum*, which are able to rapidly infect and kill *Sesbania exaltata,* a weed of cotton, rice, and soybeans.

In our early nutrition studies, the C:N ratio used in the cultivation medium was shown to be a key factor influencing spore morphology and composition (lipid:protein content), as well as spore efficacy against the weed host (Jackson and Bothast 1990; Jackson and Schisler 1992; Schisler et al. 1991a, b). Carbon loading also influenced sporulation, but increasing the loading in a 30:1 C:N medium from 15 g/l to 20 g/l to 25 g C/l, unexpectedly reduced spore accumulations from 1.2×10^7 to 3.3×10^6 to 0 spores/ml. Since dissolved O_2 (DO) is also likely to affect sporulation, these early flask culture observations may reflect changes in O_2 supply and demand brought on by biomass and, consequently, viscosity increases with carbon loading.

The cost of O_2 transfer is a significant part of the total production budget for mycelial cultures. A survey of various processes indicates that fermentation utility costs on average approximately 15–20% of the total production budget (Stanbury and Whitaker 1984). The cost of O_2 transfer can be as much as 20–50% of the utilities budget (Reisman 1988), and in the case of mycelial fermentations, the energy costs tend toward the upper end of this range because of the power needed to maintain O_2 transfer under the viscous conditions of filamentous growth (Van Brunt 1986).

Mycelial cultures typically exhibit time-dependent features, including differentiated physiological states, high O_2 demand, shear sensitivity, high viscosity, and non-Newtonian rheologies, which make DO transfer rates (K_1a) and concentrations difficult to measure, predict, and deliver during the fermentation time course. Scale-up of aerobic cultures is generally based

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on K_1a being maintained at a predetermined optimum for the translation from bench to production fermentors (Hubbard 1987), and in order to make this translation for mycelial cultures, the appropriate fluid rheology data and correlations are needed. Current theory and existing correlations used for predicting K_1a scale translations are predominantly based on time-independent water as the model fluid, and the adaptation of this knowledge base to mycelial culture conditions makes scale-up a complicated and inexact process (Hubbard 1987; Singh et al. 1987). In addition to the O_2 transfer rate, scale-up procedures must also incorporate the concentration of DO needed for optimal culture performance (with respect to production rates and yields). Depending on the microorganism, low levels of O_2 are a possible cause of damage; shear-thinning (pseudoplastic) broths, high O_2 demands, and large-scale operation can accentuate this problem. O_2 toxicity (and/or high agitation rates) have been reported to cause loss of cell performance as well (Nienow 1990).

The first objective of our research was to study the shear sensitivity of *C. truncatum* in bench-scale fermentors and to devise an optimal method of O_2 delivery without cell damage. Once these studies were completed, we determined the O_2 concentrations and delivery rates required for optimal germination, growth, and sporulation (conidiation). Finally, time-dependent fluid rheology and power data were collected from a fermentation given optimal O_2 delivery. This research provides a preliminary view of technical issues and potential costs associated with scale-up.

Materials and methods

Microorganism. C. truncatum (Schw.) Andrus & Moore was originally deposited by D. Boyette, USDA, Stoneville, Miss., USA, in the National Center for Agricultural Utilization Research Culture Collection, where we obtained it as NRRL 13737 (=ARS patent collection culture no. 18434). Stock cultures were stored on 1-mm potato-dextrose-agar (PDA) plugs in 10% glycerol at −80° C, and spore inocula were obtained from conidiated PDA plates as previously described (Jackson and Bothast 1990).

Medium. The liquid culture medium contained (per litre): 2 g KH_2PO_4, 0.247 g $MgCl_2 \cdot 6H_2O$, 0.152 g CaO, 15 mg $MnSO_4 \cdot H_2O$, 36.6 mg $CoCl_2 \cdot 6H_2O$, 14 mg $ZnSO_4 \cdot 7H_2O$, 50 mg $FeSO_4 \cdot 7H_2O$, 0.1 g ethylenediamine tetraacetic acid (EDTA), 0.5 mg each of thiamine, riboflavin, Ca-pantothenate, niacin, pyridoxamine, and thioctic acid, 0.05 mg each of folic acid, biotin, and B_{12}, 25 g Difco vitamin-free casamino acids, and 18.8 g glucose. All ingredients except glucose were combined in 20% of the volume, and the pH was adjusted to 2.0 prior to autoclaving. After sterilization, the glucose and nutrient solutions were combined and adjusted to pH 5.0 with 4 M NaOH. The C:N mass ratio was 10:1 at 20 g C/l loading (glucose + casamino acids C) in the final medium, assuming 40% weight C in glucose and 8 and 50% by weight N and C, respectively, in the casamino acids (Difco, Detroit, Mich., USA). Media with this C:N ratio have been shown to produce spores with optimal efficacy for inciting disease in *S. exaltata* (Schisler et al. 1991b).

Fermentation conditions. Dissolved O_2 tension (DOT) and uptake experiments were carried out in B. Braun Biostat E 2ER fermentors (2 l working volume) equipped with DO control instrumentation. The 0% O_2 saturation condition was achieved by sparging the medium with N_2 instead of air. Experiments focusing on power in-put and fluid rheology were carried out in Biostat ED ES10 fermentors (three Rushton impellers 9.7 cm apart, 12.8 cm from bottom; 10 l working volume). All cultures were inoculated with spores (10^4/l) and controlled at pH 5.0 (4 M HCl/NaOH dosing) and 28° C.

Susceptibility of mycelia to mechanical shearing (see Table 1) was tested by running cultures at various stirring and aeration rates.

Spore germination vs DOT (see Fig. 1a) was studied in fermentors stirred at 200 rpm with air flows automatically regulated between 0–3 l/min. Since germinating spores had a low O_2 demand, a 200 ml N_2/min flow was added to stabilize the control system at each set point.

Growth vs DOT (Fig. 1b) was studied using 24-h-old fermentor cultures (germinated at 1000 ml air/min, 250 rpm) with DOT regulated at the set point by automatic adjustment of both air flow (0–3 l/min) and stirring (150–300 rpm).

Sporulation vs DOT (Fig. 1c) was studied in cultures grown according to an optimal growth program (see Table 2). DO control was initiated when each culture was 88 ± 7 h old, just prior to the onset of sporulation. The timing of control initiation was guided by observation of the following events: glucose ≤0.1 g/l, DOT rising ≥1%, and biomass peaking (Table 2b). Control was achieved by automatic regulation of stirring (350–500 rpm), while the air flow was manually fixed at 250, 500, or 1000 ml/min (higher with DOT setting). To check the susceptibility of sporulating mycelia to shear, the 75% DOT setting was achieved in two ways – 1000 ml/min 50:50 O_2:N_2 at 350–500 rpm and 1000 ml/min air at 350–1000 rpm.

Power and fluid rheology studies (see Figs. 2 and 3) were carried out in 10-l cultures aerated at 2.91 l/min, or 10.5 cm/min superficial velocity (volumetric air flow/cross-sectional area), equivalent to that of the 2-l cultures areated at 1 l/min. The K_1a equivalence between the two culture sizes was observed on the uninoculated medium at 200–500 rpm such that $K_1a = 2.601 \times 10^{-6}$ $(\text{rpm})^{2.0984}$, where K_1a had units of min^{-1}.

Analyses. The DOT was measured in fermentors using Ingold polarographic probes calibrated at 0% and 100% of saturation with N_2 and air, respectively, at atmospheric pressure. The DO concentration in the air-saturated initial medium was 6.28 mg/l at 1 atm and 28° C, as determined by the method of Slininger et al. (1989) using glucose oxidase (grade I, no. 105139, Boehringer Mannheim Biochemicals, Indianapolis, Ind., USA) and peroxidase (P-8375, Sigma, St. Louis, Mo., USA).

Dry cell mass concentrations were determined via filtering, distilled water washing, and drying (1–1.5 h at 105° C) 15–40 ml culture aliquots on prewashed/weighed cellulose nitrate filters.

Glucose concentrations were measured by HPLC (Slininger et al. 1990).

O_2 uptake rates associated with growing and sporulating mycelia were estimated indirectly (due to time-dependent viscosities and K_1a's) via gas chromatography (GC) assay of fermentor exhaust gas CO_2 content (Ramstack et al. 1979). At low air flow rates (air residence times >20 min), GC assays were done to verify that molar O_2 consumption and CO_2 production were equal, a finding consistent with respiration as the primary active pathway. Aeration rates used for most experiments were too rapid (2–8 min air residence times) to allow large enough drops in exhaust gas O_2 content for direct estimation of O_2 consumption, but GC sensitivity remained sufficient for measuring CO_2 production (the molar equivalent of O_2 consumption).

Power consumed by the stirring motor was calculated as VI from the fermentor's recorded output of motor current (I) and rpm, which was a linear function of the armature voltage (V).

Spore concentrations were calculated from hemacytometer counts. The fraction of germinated spores = number of spores with a germ tube present/total spore count. The spores origin of mycelia strands remained obvious at least 8 h after inoculation.

Viscosity was measured using a Brookfield Viscometer Model LVT rotating cylindrical spindle in a fixed container. Shear rates reported were calculated at the spindle wall.

Results and discussion

Mechanical shear sensitivity: need for a stirring program

Increasing the DO transfer by simply increasing the stirring rate for the entire culture time course, led to mycelial fragmentation (observed in microscope specimens) and reduction of the dry cell mass accumulated (Table 1). Although spore accumulation approximately doubled from 2.6 to 6.2×10^5 spores/ml as the stirring rate was increased from 250 to 600 rpm, this concentration was an order of magnitude less than that previously observed in flask cultures provided with 8 g C/l at a 10:1 C:N ratio (Jackson and Bothast 1990). This result suggested that the mycelia were shear sensitive and a "stirring program", which provided for a gradual increase in the stirring rate with time, was tested, the hypothesis being that cell damage could be minimized by increasing stirring rate only as needed to provide adequate O_2 transfer as mycelial mass and viscosity increase. This approach was successful and yielded up to 4.1×10^7 spores/ml, an accumulation nearly two orders of magnitude higher than observed in cultures operated at a fixed stirring rate (Table 1). The decrease in spore yield associated with running the stirring program at 100 ml/min aeration (instead of 1000 ml/min) and the increase associated with using two impeller blades (rather than one) pointed to O_2 as a key factor in regulating conidiation.

Optimal DOT

The DOT required to optimize yield and/or the process rate was different for each of the life cycle stages: germination, growth, and sporulation.

Table 1. Dependence of cell growth and sporulation on aeration and stirring rates: use of a time-based program to avoid mechanical shearing of mycelia

Air flow (ml/min)	Stirring (rpm)	Cell mass (g/l)	Spores (s/ml)
1000	250	7.6 ±0.9	$2.6 \pm 1.2 \times 10^5$
1000	350	5.48±1.1[c]	$5.2 \pm 2.6 \times 10^5$
1000	600	3.85±1.5[c]	$6.2 \pm 3.6 \times 10^5$
1000	Program[a]	6.12±0.31	$1.3 \pm 0.3 \times 10^7$
100	Program[a]	7.38±0.73	$6.0 \pm 1.0 \times 10^6$
1000	Program[a,b]	6.29±0.85	$4.1 \pm 0.8 \times 10^7$

[a] The following time-based stirring program was used in place of a constant rpm setting: 250 rpm (0–22 h); 300 rpm (22–39 h); 350 rpm (39 h onwards)
[b] A second impeller was added to the drive shaft
[c] Mycelial fragmentation was observed in microscope specimens

Germination. Spore inocula in cultures deprived of O_2 by N_2 sparging failed to germinate (Fig. 1a). Cultures that were controlled at 10–20% DOT germinated completely within 6 h despite an initial approx. 1 h lag. Germination proceeded without a lag when DOT was controlled in the 30–80% range but still required 6 h to reach completion. Thus, low levels of O_2 were sufficient to support complete germination, and the duration of this phase was independent of DOT in the 10–80% range. In related studies, the length of the germination phase was controlled by the kind of nitrogen source available; inorganic nitrogen and an incomplete supply of the essential amino acids led to germination periods >6 h (Jackson and Slininger 1992).

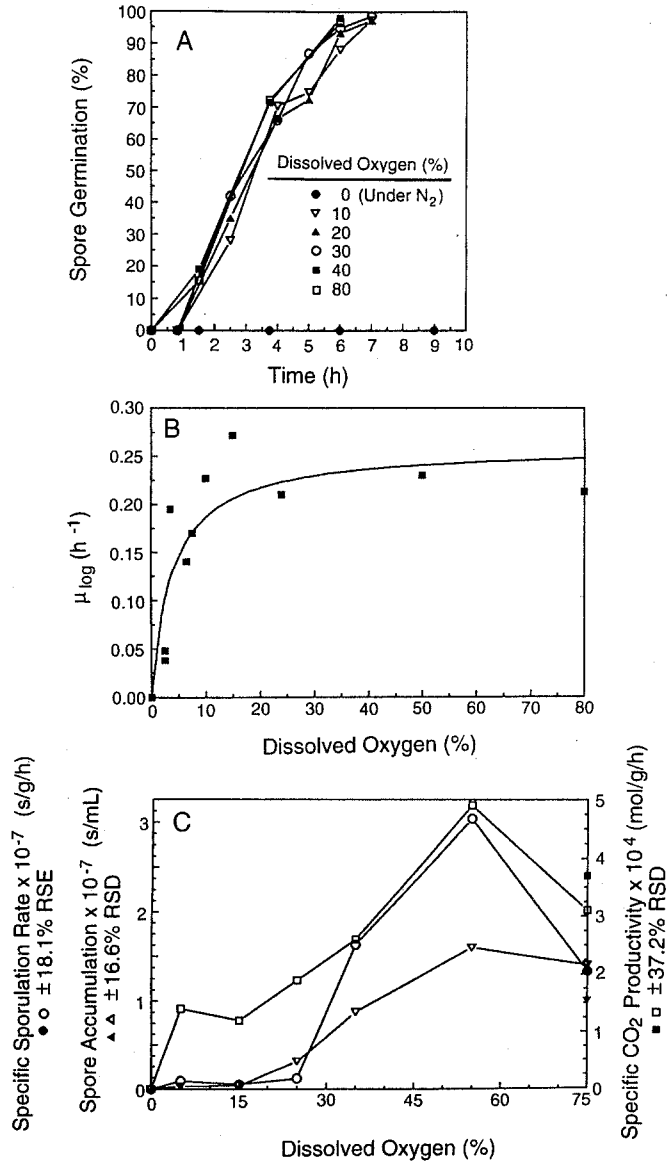

Fig. 1A–C. Effect of dissolved oxygen tension (DOT) on (**A**) spore germination rate, (**B**) specific logarithmic growth rate, where the Monod curve fit parameters were $\mu_{max} = 0.260\ h^{-1}$ and $K_{ox} = 3.93\%$, and (**C**) sporulation and associated respiration: *closed symbols* indicate DOT control with 350–1000 rpm, instead of 350–500 rpm (*open symbols*); RSE, relative standard error; RSD, relative standard deviation

Growth. Calculated from dry cell mass concentration (b) time course (t), the specific growth rate ($d\ln b/dt$) followed a Monod-like dependence on the DO tension (Fig. 1b). However, because the average relative standard error associated with the specific growth rate was ±11%, DOT ≥15% could be interpreted as supporting maximal growth, despite the values of the maximum rate and saturation constant found by the curve-fitting routine. The heterogeneity of mycelial cultures contributed to experimental variation in dry cell concentration and specific growth rate determinations, and the scatter in the data led to a large standard fit error. The curve fit had a correlation coefficient $r^2 = 0.756$, and the standard error in the specific growth rate prediction for any DOT was $\pm 0.0472\ h^{-1}$.

Sporulation. In order to test the effect of DO on sporulation independently of the preceding life cycle stages, mycelia for each trial were grown to stationary phase (89±7 h) according to a stirring and aeration program (Table 2). In the absence of DO, sporulation failed to occur, but spore accumulation increased as the DOT control setting was raised to 55% (Fig. 1c). Both the specific sporulation and CO_2 production (approx. O_2 uptake) rates were maximized by controlling at 55% DOT. Raising the DOT control to 75% did not improve spore accumulation but caused a reduction in the specific sporulation and CO_2 production rates, suggesting that O_2 toxicity may have been a factor. Sporulation at 75% DOT achieved using aeration with 50% O_2 at low shear rates (350–500 rpm) ws not significantly different from that using air at high shear rates (350–1000 rpm). Thus, the use of stirring rates up to 1000 rpm did not appear to cause shear damage to sporulating cultures, and the application of O_2-enriched aeration was not advantageous at this stage. Spore yields were calculated for the experiment controlled at the 55% DOT optimum (Table 3).

Table 2. Description of *Colletotrichum truncatum* growth in sporulation studies

a Stirring and aeration program for culture growth

Air flow (ml/min)	Stirring (rpm)	Start time (h)
1000	250	0
1000	350	42± 6
1000	450	57±10
DOT control initiated (250/500/1000)	(350–500)	88± 7

b. Culture events

Time (h)	Event
0	Inoculation (100% DOT, 18.8 g glucose/l, 10^4 spores/ml)
38± 4	Specific CO_2 productivity (O_2 uptake) peaks
43± 6	DOT falls <1%
73±11	Glucose falls ≤0.1 g/l
74±12	DOT rises >1%
89± 7	Cell mass concentration peaks
101±13	Sporulation begins
166±22	Spore concentration peaks

c. Average kinetic parameters associated with growth

Specific growth rate (log phase)	0.202±0.029 h^{-1}
Specific CO_2 productivity (~O_2 uptake rate)	5.4 ±1.6×10^{-3} mol/g/h
Cell mass accumulation	15.1 ±2.5 g/l
Cell mass yield/O_2 consumed (~/CO_2 produced)	36.8 ±5.6 g/mol
Cell mass yield/glucose consumed	0.82±0.14 g/g
Cell mass yield/C available	0.76±0.13 g/g

DOT, dissolved O_2 tension
~, approximately equivalent to

Table 3. Optimal spore yields at 55% DOT

Yield basis	Value
Glucose consumed (g)	8.7×10^8 spores/g
Carbon available (g)	8.0×10^8 spores/g
CO_2 produced (mol) (~O_2 consumed)	6.2×10^{10} spores/mol

O_2 demand

The O_2 uptake of spores during germination was trivial (≤10^{-6} mol/l per hour) compared to that of mycelia during growth. In cultures grown according to the program described in Table 2, the peak volumetric CO_2 productivity near the end of growth phase averaged 0.012±0.002 mol/l per hour. However, respiration was O_2-transfer limited (DOT <1%) near the end of growth, and the true O_2 demand of the culture was better estimated from the specific CO_2 productivity (5.4×10^{-3} mol/g per hour) measured on log phase cells (Table 2c). The product of this specific rate and the average peak cell accumulation (15.1 g/l) gives the potential volumetric O_2 demand of the culture as 0.082 mol/l per hour, a value seven times the observed respiration rate maximum. Although the DOT level optimizing sporulation was four times that optimizing growth, the specific O_2 demand of sporulating mycelia was only 4.9×10^{-4} mol/g per hour, an order of magnitude less than that of growing mycelia.

It is notable that a relatively high DOT is required to maintain a low O_2 demand during sporulation. Perhaps differentiation causes cell wall changes that lessen permeability and create the need for a high driving force to move O_2 in. Another possibility is that sporulation is stimulated by unfavourably high O_2 tension, as suggested by Churchill (1982) in responce to similar observations of *C. gloeosporioides* f. sp. *aeschynomene.* Generally, spores are produced as a result of conditions unfavorable for vegetative growth (i.e., depletion of nutrients, temperature extremes, unsuitable pH). In the case of our *C. truncatum*, however, DOT >55% supported maximal growth rate (Fig. 1b) but led to reduced sporulation rates (Fig. 1c). Additional research will be needed to identify the mechanism(s) involved in the effect of O_2 on sporulation.

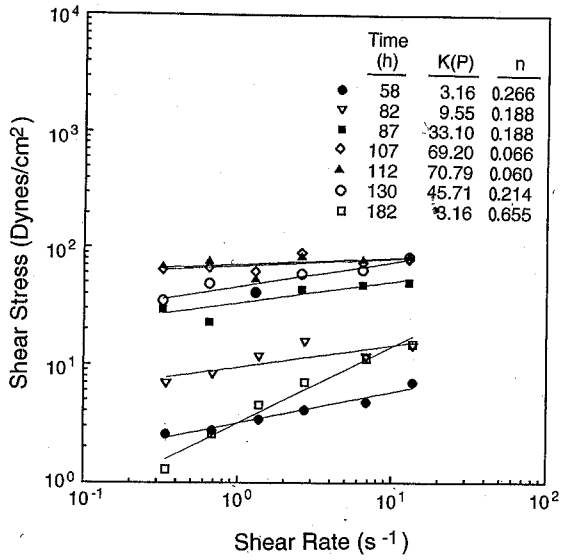

Fig. 2. Time-dependent pseudoplastic behavior of the fermentation broth (K = apparent viscosity; n = flow behavior index)

Optimal O_2 delivery: fluid rheology and power requirements

Rheology and power utilization studies were carried out according to the stirring and aeration program (Table 2) using the optimal 55% DOT control during sporulation. The culture broth exhibited pseudoplastic (shear-thinning) behavior and could be modelled using the power law $F = KS^n$ [F = shear stress, S = shear rate, n = flow behavior index values from 0 (most) to 1 (least pseudoplastic), K = apparent viscosity at S = 1]. The rheological characteristics (K and n) varied during the culture time course (Fig. 2). Both the apparent viscosity and pseudoplastic behavior peaked at oatmeal consistency (K = 69 P, n = 0.06) as sporulation started (107 h), then declined as it finished. Broth thickening continued for approx. 20 h after the cessation of growth – perhaps because of mucin production (Ramadoss et al. 1985) or changes in hyphal morphology. Despite this large change in viscosity, the whole broth density remained constant within 2% (1.0–1.02 g/ml).

The time courses of stirring rate and power required to maintain our observed optimal DOT conditions are shown in Fig. 3. Peak power consumption (and rpm) occurred during the last 36 h of growth and coincided with the O_2-transfer-limited portion of the fermentation (DOT < 15%). Power input fell after the end of growth at 90 h, yet the apparent viscosity at that time was only about half of the maximum, which occurred at 107 h. Thus, the O_2 demand of the culture, rather than viscosity, seemed to be the stronger determinant of the power input. It was fortunate that the highest viscosities occured during a perid of low O_2 demand since the K_1a is expected to decline with rising viscosity. Figures useful for the cost analysis of optimal O_2 delivery include: the power consumption through the end of sporulation (approx. 120 h) calculated as 0.424 kW h per litre culture (via power/volume time plot integration) and the total air flow at 2.1×10^3 l/l.

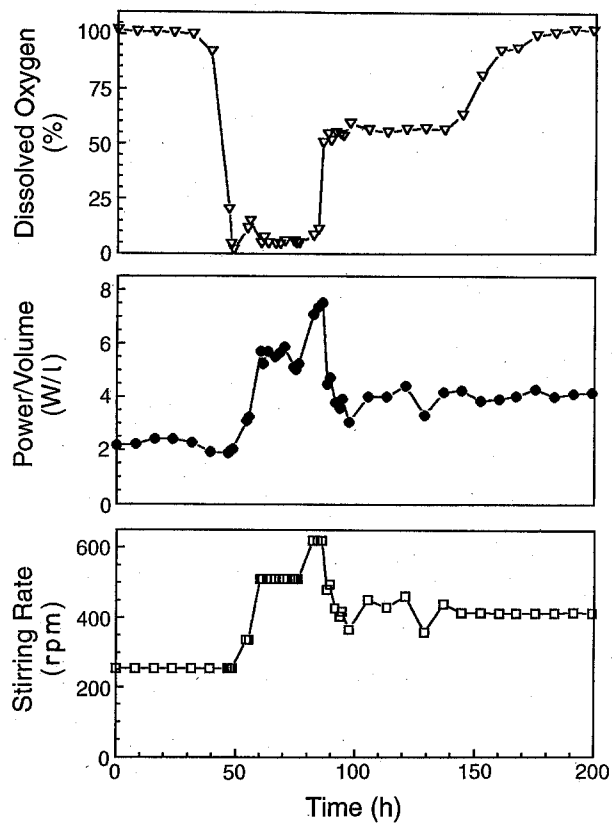

Fig. 3. Optimal O_2 delivery program time courses of DOT, power consumption/volume, and stirring rate (aeration rate = 2.91 l/min)

These results confirm that O_2 transfer effects warrant consideration when designing experiments and interpreting culture behavior, especially sporulation intensity. Conditions (carbon loading, for example) that increase biomass production and viscosity also increase the difficulty (i.e., power requirement) of O_2 transfer by increasing O_2 demand per volume and by decreasing the K_1a per rpm of agitation. Given these circumstances, care must be taken to isolate effects of experimental control variables from those of O_2 transfer, so that variations in observed sporulation can be attributed to the appropriate cause. Use of O_2-controlled fermentors to check flask culture findings is recommended.

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References

Churchill BW (1982) Mass production of microorganisms for biological control. In: Charndattan R, Walker H (eds) Biological control of weeds with plant pathogens. Wiley, New York, pp 139–156

Hubbard DW (1987) Scale-up strategies for bioreactors. In: Ho CS, Oldshue JY (eds) Biotechnology processes scale-up and mixing. American Institute of Chemical Engineers, New York, pp 168–184

Jackson MA, Bothast RJ (1990) Carbon concentration and carbon-to-nitrogen ratio influence submerged-culture conidiation by the potential bioherbicide *Colletotrichum truncatum* NRRL 13737. Appl Environ Microbiol 56:3435–3438

Jackson MA, Schisler DA (1992) The composition and attributes of *Colletotrichum truncatum* spores are altered by the nutritional environment. Appl Environ Microbiol 58:2260–2265

Jackson MA, Slininger PJ (1992) Nitrogen source regulation of *Colletotrichum truncatum* conidial germination and submerged culture conidiation. In: Abstract M858.18, 1992 Joint Meeting of The American Phytopathological Society and The Mycological Society of America, Portland, Ore.

Nienow AW (1990) Gas dispersion performance in fermentor operation. Chem Eng Progr 86:61–71

Ramadoss CS, Uhlig J, Carlson DM, Butler LG, Nicholson RL (1985) Composition of a mucilagenous spore matrix of *Colletotrichum gramini cola*, a pathogen of corn, sorghum, and other grasses. J Agric Food Chem 33:728–732

Ramstack JM, Lancaster EB, Bothast RJ (1979) Gas chromatographic headspace analysis of solid substrate fermentations. Process Biochem 14:2–4

Reisman HB (1988) Economic analysis of fermentation processes. CRC Press, Boca Raton, Fl., p 95

Schisler DA, Howard KM, Bothast RJ (1991a) Enhancement of disease caused by *Colletotrichum truncatum* in *Sesbania exaltata* by coinoculating with epiphytic bacteria. Biol Control 1:261–268

Schisler DA, Jackon MA, Bothast RJ (1991b) Influence of nutrition during conidiation of *Colletotrichum truncatum* on conidial germination and efficacy in inciting disease in *sesbania exaltata.* Phytopathology 81:587–590

Singh V, Fuchs R, Constantinides A (1987) A new method for fermentor scale-up incorporating both mixing and mass transfer effects. I. Theoretical basis. In: Ho CS, Oldshue JY (eds) Biotechnology processes scale-up and mixing. American Institute of Chemical Engineers, New York, pp 200–214

Slininger PJ, Petroski RJ, Ladisch MR, Okos MR, Bothast RJ (1989) Measurement of oxygen solubility in fermentation media: a colorimetric method. Biotechnol Bioeng 33:578–583

Slininger PJ, Bothast RJ, Ladisch MR, Okos MR (1990) Optimum pH and temperature conditions for xylose fermentation by *Pichia stipitis.* Biotechnol Bioeng 35:727–731

Stanbury PF, Whitaker A (1984) Principles of fermentation technology. Pergamon Press, Oxford, pp 231–245

Van Brunt J (1986) Fermentation economics. Bio/Technology 4:395–401