Growth and Anthraquinone Production of *Morinda elliptica* Cell Suspension Cultures in a Stirred-Tank Bioreactor

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The effects of medium strategy, number of impellers, aeration mode, and mode of operation on *Morinda elliptica* cell suspension cultures in a stirred-tank bioreactor are described. A lower number of impellers and continuous aeration contributed toward high cell growth rate, whereas a higher number of impellers reduced cell growth rate, although not anthraquinone yield. The semicontinuous mode could indirectly imitate the larger scale version of production medium strategy and improved anthraquinone production even with 0.012% (v/v) antifoam addition. Production medium promoted both growth (maximum dry cell weight of 24.6 g/L) and anthraquinone formation (maximum content of 19.5 mg/g of dry cell weight), without any necessity for antifoam addition. Cultures in production medium or with higher growth rate and anthraquinone production were less acidic than cultures in growth medium or with lower growth rate and anthraquinone production. Using the best operating variables, growth of *M. elliptica* cells (24.6 g/L) and anthraquinone yield (0.25 g/L) were 45% and 140%, respectively, lower than those using a shake flask culture after 12 days of cultivation.

Keywords: *Plant cell culture; Morinda elliptica; anthraquinone; colorant; bioreactor; medium strategy*

INTRODUCTION

There is much concern nowadays about the safety of synthetic food colorants because many of them have been banned in food products (Timberlake and Henry, 1986). This has spurred research on food colorants from natural pigments as their production is seen as a potential alternative to synthetic colorant. Several pigments such as shikonins from *Lithospermum erythrorhizon*, anthocyanins from *Daucus carota*, β-carotenes from *Ruta graveolens*, flavonoids from *Citrus limon* (Nickell, 1982), and anthraquinones from Rubiaceous species (Schulte et al., 1984), have been produced in plant cell and tissue cultures.

Anthraquinones from *Morinda* species are used traditionally in the Malay Peninsula as dyes (Burkill, 1935). In the pharmaceutical industry, anthraquinone glycosides are used to treat gingivitis, stomatitis, mouth ulcers, inflammatory oral mucosa, constipation, and periodontal conditions (*DIMS*, 1992; British National Formulary, 1994). Anthraquinones extracted from the root of *M. elliptica* have shown antibacterial, antifungal, and antileukemic properties (Ismail et al., 1997). We have reported an anthraquinone yield of 4.5 g/L from a 21-day cell suspension culture of *Morinda elliptica* using a shake flask system (Abdullah et al., 1998). This is among the highest yields ever reported for plant cell suspension culture.

Nonetheless, the exploitation of plant cell cultures commercially has been hampered mainly by the scaling-up problem. Cultivation of plant cells at large scale involves volumes above those normally used in a shake flask, generally in a bioreactor of 2 L or above (Scragg and Fowler, 1985). The idea among others is to test the applicability of optimized medium formulation and environmental conditions established at shake flask level in a bioreactor. This step is precarious as a shake flask and a bioreactor are entirely two different systems in geometry, mixing and gas regime (ten Hoopen et al., 1994). The response of cells to growth in bioreactors cannot be predicted as some cultural parameters change with the cube of the volume, whereas others increase only with the square of the volume (Scragg, 1991).

In this paper, the determination and maintenance of optimal conditions for cell growth and anthraquinone formation in a stirred-tank bioreactor and the determination of factors that are important upon scale-up are addressed. The profile of activities of each operating strategy, which include the dry cell weight and anthraquinone production, the dissolved oxygen tension (DOT) level, air flow rate, the cells' oxygen requirement, and the pH, is analyzed. Comparison of cells' activities in shake flask and bioreactor systems and factors that may contribute to any differences in the performances between the two systems are also highlighted.

MATERIALS AND METHODS

Cell Cultures and Medium Preparation. Callus of *M. elliptica* was induced from young leaves (Aziz et al., 1997). Cell suspension cultures were initiated after callus was 1.5 years old. At the time of experiment, the freely suspended stock cultures were 2 years old. Cultures were maintained on maintenance medium (M), which was MS medium (Murashige and Skoog, 1962) to which had been added 3% (w/v) sucrose, 2 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D), and 1 mg/L

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Table 1. Effect of Different Strategies Applied in the Cultivation of *M. elliptica* Cell Cultures in a 2 L Stirred-Tank Bioreactor (1.8 L Working Volume) for a 12 Day Experimental Period, at 30 °C and an Agitation Speed of 75 rpm (Impeller Tip Speed = 21.6 cm/s) on Growth and AQ Production[a]

expt	inoculation medium	antifoam[b] (% v/v)	mode of air supply	stirrer configuration[c]	mode of operation	DCW (g/L)	AQ content (mg/g of DCW)	AQ yield (g/L)
B1	G		6 s interval	3/45°downward	batch	5.6 ± 0.7[a]	3.1 ± 0.4[a]	0.017
B2	G	0.002	continuous	3/45°downward	batch	14.4 ± 0.4[b]	3.9 ± 0.5[a]	0.056
B3	G	0.012	continuous	3/45°downward	semicontinuous	28.8 ± 1.5[e]	10.7 ± 0.8[b]	0.300
B4	G	0.025	continuous	3/45°downward	semicontinuous	12.4 ± 0.9[b]	4.2 ± 0.8[a]	0.052
C1	P	0.012	continuous	3/45°downward	batch	18.2 ± 0.9[c]	19.5 ± 1.1[c]	0.270
C2	P		continuous	3/45°downward	batch	23.8 ± 0.8[d]	17.7 ± 0.9[c]	0.420
C3	P		6 s interval	2/vertical	batch	23.4 ± 1.2[d]	9.1 ± 1.0[b]	0.210
C4	P		continuous	2/45°downward	batch	24.6 ± 1.5[d]	10.3 ± 1.2[b]	0.252

[a] Values are means of three replicates ± standard deviation. Mean values in the same column with different superscripts are significantly different ($P < 0.05$). [b] Amount of antifoam (% v/v) added into the medium before autoclaving. [c] Number of impeller/position of impeller.

6-furfurylaminopurine (kinetin). Chemicals for culture media were supplied by Sigma Chemical Co. The flasks containing media were sealed with steristoppers (H. Herenz) and autoclaved at 121 °C for 15 min. Subculture of freely suspended stock cultures in M medium was made at a regular period of 6−8 days. Subculturing involved the transfer of 5−10 mL suspension cultures into 90 mL of medium in 300 mL Erlenmeyer flasks. Stock cultures were placed on an orbital shaker (130 rpm) at 24 ± 2 °C under a 16 h photoperiod with illumination of a white fluorescent light of 500 lx intensity.

Growth and production medium strategies, termed G and P medium, were MS medium to which had been added 80 g/L sucrose, 0.5 mg/L α-naphthaleneacetic acid (NAA), and 0.5 mg/L kinetin. The only difference between G and P medium strategies is that the inoculum for G medium was grown in M, whereas the inoculum for P medium was grown in G. Details of optimization strategies have been described elsewhere (Abdullah et al., 1998).

Bioreactor Experiments. Bioreactor experiments were carried out in a commercially available 2 L stirred-tank bioreactor (Biostat M, B Braun Biotech International). The cultures were aerated through a sintered steel sparger. The air flow rate was adjusted manually to the required level using an air flow control system. Each set of impeller constitutes two paddles and can be positioned on the axis at three different positions such that for clockwise rotation the paddles can be orientated to exert three different pumping modes: vertically orientated for liquid flows away from the motor axis, inclined at 45° for downward liquid pumping mode, and inclined at 45° for upward liquid pumping mode.

Inoculum for bioreactor experiments was prepared in a 500 mL bioreactor inoculum flask (BIF). For experiments using G medium, 18 mL of 5−6-day-old stock culture was inoculated into 162 mL of M medium in a BIF. Cell suspension culture in a BIF was grown for 4−5 days before inoculation into the bioreactor containing G medium. For experiments using P medium, 18 mL of 5−6-day-old stock culture was inoculated into 162 mL of G medium in a BIF. Cell suspension culture in a BIF was grown for 6−8 days before inoculation into the bioreactor containing P medium. Medium in the bioreactor vessel was prepared at 1620 mL volume and autoclaved at 121 °C for 20 min. Upon inoculation of cultures into the bioreactor, the initial working volume was 1.8 L.

The strategies and conditions applied for each culture cultivated in the bioreactor are shown in Table 1. Cultures in experiment B were grown using G medium strategy, whereas cultures in experiment C were grown using P medium strategy. Chemical antifoam used in experiments B2, B3, B4, and C1 was silicon antifoam (Fluka Chemica). All cell cultivations were carried out in batch mode except for experiments B3 and B4, which were in semicontinuous mode. For semicontinuous mode, a portion of culture was harvested on day 9 such that 900 mL of culture remained as a seed culture. A volume of 900 mL of fresh sterile G medium with 0.012% (v/v) antifoam was subsequently added to give a final volume of 1.8 L.

Analytical Procedures. For biomass determination upon sample removal, cells were harvested by suction filtration via a Büchner funnel with a filter paper (Whatman No. 4). Cells were dried in an oven at 70 °C for 24 h to obtain dry cell weight (DCW). For sugar analysis, ∼6 mL of medium filtrate was filtered through a Sep-Pak cartridge C18 (Waters Co.) attached to a syringe and injected into a vial. The vials were frozen at −30 °C for analysis at a convenient time later.

For anthraquinone content analysis, 0.020 ± 0.001 g of dried cells was deposited in a 10 mL vial. Anthraquinones were extracted using 2 mL of dichloromethane several times until the extractant in the final extraction became colorless. To speed up the release of anthraquinones, dried cells were initially slightly wetted with deionized water (without excess water) and left for a few minutes before dichloromethane was added, and the samples were gently shaken on a shaker at 90 rpm. The anthraquinones extracted were measured spectrophotometrically at 420 nm using alizarin as a reference substance (Zenk et al., 1975).

Medium samples for sugar analysis were defrosted and prepared for analysis using a high-performance liquid chromatograph (LC-10AS, Shimadzu), equipped with a refractive index detector RID-6A. The column used was NH_2 (amino) of 25 cm length and 0.4 cm internal diameter (LiChrosorb, E. Merck, Darmstadt, Germany) maintained at 28 °C in an oven. The mobile phase was acetonitrile and deionized water at an 80:20 ratio and run at 1.5 mL/min flow rate. A medium sample of 10 μL was introduced to the manual injection port of the chromatograph. Three standard solutions of known fructose, glucose, and sucrose concentrations were injected to produce standard peaks.

Cells' oxygen requirements were determined according to the method normally used in microbial fermentation (Taguchi and Humphrey, 1966). In this method, air supply during cultivation was stopped and the drop in DOT level with time (every 10 s interval) was recorded to give oxygen uptake rate, before aeration is resumed.

Statistical Analysis. The results were compared by one-way analysis of variance (one-way ANOVA) and tested by Duncan's multiple range test to find the differences between treatment means at the 5% (0.05) significance level. Data were analyzed using the General Linear Model Procedure of the SAS Institute Inc. (1988).

RESULTS AND DISCUSSION

Cell Growth and Anthraquinone Formation. Growth of *M. elliptica* cell cultures in a stirred-tank bioreactor and anthraquinone (AQ) formation were greatly affected by the medium strategy, mode of aeration, the effect of the number of impellers, the effect of antifoam, and mode of operation (Table 1). For experiment B, the growth rate up to day 9 was exceptionally high in experiment B3, followed by experiments B2, B4, and B1 in decreasing order with maximum DCW values on day 12 of 28.8, 14.4, 12.4, and 5.6 g/L, respectively. For experiment C, experiment C4 showed

the highest growth rate, followed by experiments C3, C2, and C1 with maximum DCW values of 24.6, 23.4, 23.8, and 18.2 g/L, respectively.

Although addition of 0.025% (v/v) antifoam retarded growth in experiment B4, addition of 0.012% (v/v) antifoam in experiment B3 conversely produced higher DCW than 0.002% (v/v) antifoam in experiment B2. Low biomass yield as a result of antifoam addition has been attributed to a decline in mass transfer or lower oxygen diffusivity (Su, 1995; Smart and Fowler, 1981). In this case, more serious foaming in experiment B2 was the major factor in causing greater losses of cells into the foam or wall growth, rendering lower numbers of cells in suspended form. In experiment B1, however, despite not having antifoam present, the DCW was the lowest. The intermittent air supply employed in this experiment may have yielded more inefficient hydrodynamic condition for optimum cell growth than that attained under continuous aeration. In experiment C3, the same explanation caused by intermittent air supply was applicable for only the first 6 days, during which the growth rate was lower than in experiment C2. From day 6 onward, cell growth started to pick up, surpassing even experiment C2 before finally reaching almost the same DCW as that in a better-performing experiment C4. We attributed this to the lower number of impellers in experiment C3 with consequent lower shearing effects than in experiment C2. The higher growth rate in experiment C4 than in experiment C3, nevertheless, could confirm our earlier postulation that continuous aeration actually assists mixing by providing greater turbulence than could be achieved via intermittent air supply.

The profile of AQ content and yield in experiment B was similar to its DCW. Although other experiments obtained AQ contents and yields no higher than 5 mg/g of DCW and 0.06 g/L, respectively, experiment B3 showed higher AQ content than the rest starting from day 9 onward. On day 12, its AQ content increased 2-fold to 10.7 mg/g of DCW and the yield increased by almost 6-fold to 0.3 g/L. This sudden increase starting from day 9 coincides with the removal of a portion of culture and replenishment with fresh medium. The semicontinuous mode employed in experiment B3 indirectly imitates the larger scale version of P medium strategy, in which inoculum is initially grown in G medium before inoculation into P medium (Abdullah et al., 1998). The only difference is that in this case, a portion of culture is harvested and the remaining culture is used as a seed culture. The elevation of AQ content shows that P medium strategy is applicable at larger scale and the addition of 0.012% (v/v) antifoam does not interrupt the anthraquinone formation.

For experiment C, although experiments C3 and C4 showed higher growth rates, experiments C1 and C2 produced higher AQ contents of 19.5 and 17.7 mg/g of DCW, respectively. These values were twice those obtained in experiments C3 and C4. Experiment C2 also showed the highest AQ yield of 0.42 g/L. Comparison of experiments C2 and C3 shows that the higher number of impellers with consequent higher shearing effect in the former appears to reduce only cell growth rate but not AQ content and yield. This may support the common understanding that low cell growth will normally be associated with higher secondary metabolite production and vice versa (Rokem and Goldberg, 1985; Kurtz and Constabel, 1985). However, the effect of higher shear, which potentially elevates secondary metabolites as part of plant stress signal (Berglund and Ohlsson, 1995), could not also be discounted. As in shake flask culture (Abdullah et al., 1998, 1999), P medium strategy (experiment C) in a bioreactor system again showed better cell growth and anthraquinone formation than G medium strategy (experiment B).

Dissolved Oxygen Tension (DOT) Level, Air Flow Rate, and Cells' Oxygen Requirements. The profile of DOT level, air flow rate, and oxygen uptake rate (xQO_2) of *M. elliptica* cell cultures in a stirred-tank bioreactor is shown in Figure 1. The DOT level of fast-growing cells as in experiment B3 was rapidly decreasing, and the air flow rate had to be continuously adjusted starting from day 6 onward to maintain the DOT level above the critical level at 20% of saturation. The moderately fast growing cells in experiment C3, in which air was supplied intermittently, also called for continuous adjustment of the air flow rate from day 4 onward. However, due to the intermittent air supply, the DOT level in experiment C3 remained between 20 and 30% of saturation despite the use of a maximum air flow rate of 1.6 L/min. The maximum oxygen uptake rate was observed near the stationary phase in all experiments. This was similarly observed in strawberry cell cultures (Hong et al., 1989) and *Dioscorea deltoidea* cell cultures (Drapeau et al., 1986). Experiments B3 and C4 showed the highest oxygen uptake rates of 1.97 and 2.78 mmol/L·h, with corresponding DCW values of 28.8 and 24.6 g/L and AQ contents of 10.71 and 10.25 mg/g of DCW, respectively. These values are within the limits of maximum oxygen uptake rate suggested for plant cells, which are in the range of 1−3.5 mmol/L·h (Taticek et al., 1991).

The DOT level in experiment B1 was able to be maintained above 20% of saturation utilizing an intermittent air flow rate <0.3 L/min up to day 9. Thenceforth, the air flow rate was progressively increased up to 0.8 L/min on day 12. In experiment C1, the DOT level remained high at 70% for 7 days before dropping progressively, although the level did not go lower than 30%. This was achieved with an air flow rate consistently low at 0.16 L/min in a continuous mode. Although it was difficult to draw any conclusion from this contradiction between experiments B1 and C1 as the mode of aeration was different, the interesting observation was that the aeration strategies in both cases were applied with the DCW in experiment B1 below 5 g/L, whereas in experiment C1 the DCW reached 18.2 g/L. Indeed, a similar trend was observed in experiments B4 and C2 under continuous aeration. In experiment B4, the DOT level was high, above 25%, with air flow rate no higher than 0.3 L/min, but the DCW and AQ content stayed lower than 10 g/L and 5 mg/g of DCW, respectively. In experiment C2, the DOT level remained between 60 and 70% with air flow rate consistently low at 0.2 L/min, with comparatively high DCW and AQ content of 23.8 g/L and 17.7 mg/g of DCW, respectively. In experiment C4, the air flow rate was adjusted from 0.2 to 0.3 L/min on day 9 before being progressively increased to 1.0 L/min on day 12.

All of the observations above indicated that the DOT levels were generally higher in continuously aerated cultures in P medium (experiment C) and in the antifoam-added experiments B4 and C1 than in G medium (experiment B). This phenomenon can be explained by correlating it with the oxygen uptake rate.

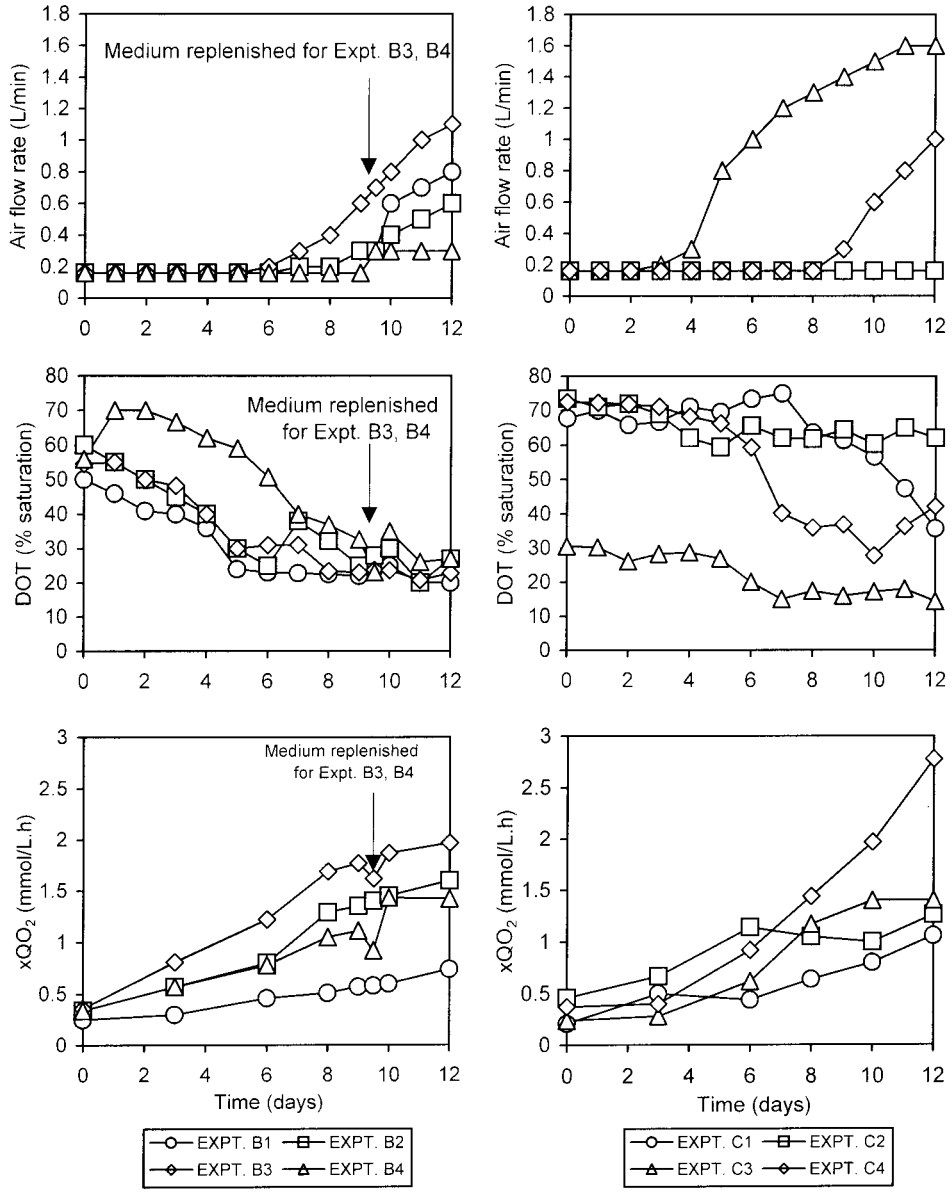

Figure 1. Profile of DOT level, air flow rate, and oxygen uptake rate during the cultivation of *M. elliptica* cell suspension cultures in the stirred-tank bioreactor (see Table 1 for a detailed description of each experiment).

The oxygen uptake rate profiles for both experiments B and C reflect the profile of DCW (data not shown). In experiments B3 and C4, the high cell growth rate compares well with the high oxygen uptake rates, resulting in a sharp drop in DOT level. Hence, the lower cell growth rate and consequently lower oxygen uptake rates in experiments C1 and C2 and antifoam-added experiment B4 explain the higher DOT levels of between 50 and 70% over substantial duration in those experiments. The early entrance of cells in experiment C4 into the deceleration phase (data not shown) suggests the suppression of growth at high air flow rates, as a result of increased turbulence and shear within the reactor. The same deceleration of growth rate was conversely not observed in experiments B3 and C3 despite the high air flow rates used. The increased air flow rate starting from day 9 in experiment C4 pushed the DOT level from 28 to 45% of saturation, whereas in experiments B3 and C3, the DOT levels were only ~20% of saturation. Hence, the possible oxygen toxicity at high levels of DOT on the final days of cultivation in experiment C4 could not be ruled out, leading to inhibiton of metabolic activity (Taticek et al., 1991).

In both experiments B1 and C3, lower AQ content and yield under intermittent air supply may signify the role of air in elevating AQ production. This, however, is not reflected in experiments C1 and C2, for which the AQ contents were highest at 19.5 and 17.7 mg/g of DCW, respectively, with corresponding low oxygen uptake rates. This may prove instead that oxygen uptakes by the cells are more important in promoting biomass buildup than for anthraquinone formation. This hypothesis is further supported by the considerably higher cell growth rates with corresponding high oxygen uptake rates but low AQ contents in experiments B2, C3, and C4. In experiment B3, a high AQ content with a high oxygen uptake rate observed still could not disqualify the preceding hypothesis as the high oxygen uptake rate in this experiment also compares well with the high cell growth rate. The high AQ content in experiment B3 could be attributed to the semicontinuous mode applied instead of the high oxygen uptake rate. A similar obser-

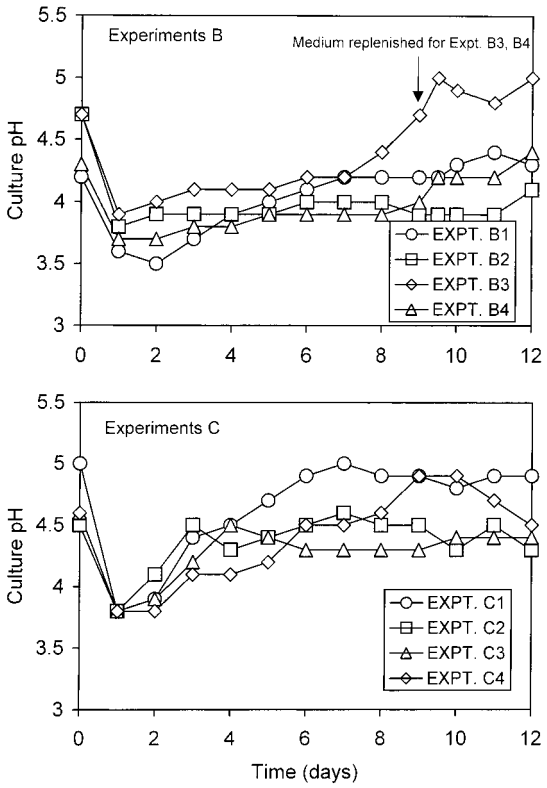

Figure 2. Culture pH profile of *M. elliptica* cell suspension cultures in the stirred-tank bioreactor (see Table 1 for a detailed description of each experiment).

vation has been made in *Morinda citrifolia* cell cultures, in which 2,4-D cells, whose number of cells are higher, indicate higher metabolic activities for cell division and maintenance and AQ production is completely absent, register higher respiration rates than NAA cells, whose number of cells are lower and AQ production is maximal (Hagendoorn et al., 1994; van der Plas et al., 1995).

pH Profile. The pH profile for experiments B and C is shown in Figure 2. The pH dropped soon after cell inoculation, to values of 3.5−3.7, before rising slowly to 4 or above as cell growth ensued. In experiment B3, the lowest pH level attained was 3.9 before the pH rose slowly and stayed in the range of 4−4.5 up to day 8. From day 9 onward, the culture pH remained >4.5 and was close to 5 on the last 2 days. In other experiments, pH values were mostly below pH 4.5; cultures in experiments B2 and B4 spent considerable time below pH 4. The profile in experiment B3 was also observed in experiment C, in which pH levels were below 4 for only 1 or 2 days but remained above pH 4 the rest of the time. During experiment C1 there was considerable time during which the pH level was >4.5 and close to 5.0, whereas in experiment C3 the pH level was constantly below 4.5.

The duration of time spent under a more acidic pH of <4 may have great impacts on cell growth and AQ production. With longer time spent above pH 4, high DCW and AQ production were achieved as observed in experiments B3, C1, and C4. In general, cultures in P medium or with higher growth rate and AQ production were less acidic than cultures in G medium or with lower growth rate and AQ production. As emphasis in P medium is laid on production, we had proposed that the expansion of organic acid anions such as mevalonic, acetic, *o*-succinoylbenzoic, shikimic, oxoglutarate, chorismate, and α-ketoglutarate to take part in AQ biosynthesis may have triggered high efflux of OH^- to maintain the intracellular pH (Abdullah et al., 1999), hence the more basic pH observed for cultures grown in P medium.

Comparison of Performance between Shake Flask and Bioreactor Systems. Comparison of growth, AQ formation, and sugar consumption of 36-month-old *M. elliptica* cell cultures in P medium between shake flask and stirred-tank bioreactor system is shown in Figure 3, and the cultivation parameters are summarized in Table 2. The bioreactor experiment chosen was that assigned experiment C4. In general, cell growth and AQ yield after 12 days of cultivation was better in the shake flask than in the bioreactor. The dry cell weight was 35.6 g/L in the shake flask but was only 24.6 g/L in the bioreactor. Whereas AQ content was ∼13−17 mg/g of DCW between days 6 and 12 in the shake flask, AQ content in the bioreactor remained below 10 mg/g of DCW throughout experimental period. There was no clear indication from the spent medium in the bioreactor to suggest that the bulk of anthraquinones had been released out of the cells. As shown by comparison between the bioreactor and shake flask systems, the overall cell growth rate, the maximum AQ yield, and the overall AQ production rate were 1.6, 2.4, and 2.2 times, respectively, lower in the former than in the latter.

The sugar uptake rate in the bioreactor was similarly lower than in the shake flask. Only fructose at 28.7 g/L and sucrose at a mere 0.8 g/L remained in the shake flask medium; in the bioreactor, not only was there a high concentration of fructose at 41.1 g/L remaining, but also both glucose and sucrose at 13.4 and 11.9 g/L, respectively, remained (Table 2). The overall rate of sucrose hydrolysis at 5.9 g/L·day in the bioreactor was significantly lower than in the shake flask at 8.1 g/L·day. High sugar content in the bioreactor implies that lesser amounts of glucose and fructose were consumed by the cells. In addition, a substantial amount of unutilized fructose indicated that sucrose was not yet fully hydrolyzed, and this can be attributed to insufficient invertase activities in the bioreactor. There was not much difference in the growth yield based on sucrose hydrolyzed between the shake flask and bioreactor systems (∼14%), but the AQ yield based on sucrose hydrolyzed was almost 60% higher in the shake flask. The presence of high amounts of sucrose, glucose, and fructose throughout the 12 day bioreactor cultivation may have imposed high osmotic conditions, which partly explain the slower cell growth rate. The lower sugar uptake rates could also explain the lower DCW attained in the bioreactor as this leads to lower endogeneous sugar pools. Cell sugar content has been shown to contribute significantly in the makeup of biomass constituents (Hagendoorn et al., 1994; van der Plas et al., 1995).

The slow growth rate in the bioreactor, however, does not result in high AQ content, as would be expected when growth is retarded in the shake flask. The differing performance between the bioreactor and shake flask could be explained by considering the hydrodynamic conditions and gas compositions in the two systems. By having a large surface area for the air−medium interface, the shake flask system provides an adequate amount of oxygen, whereas the rotary motion

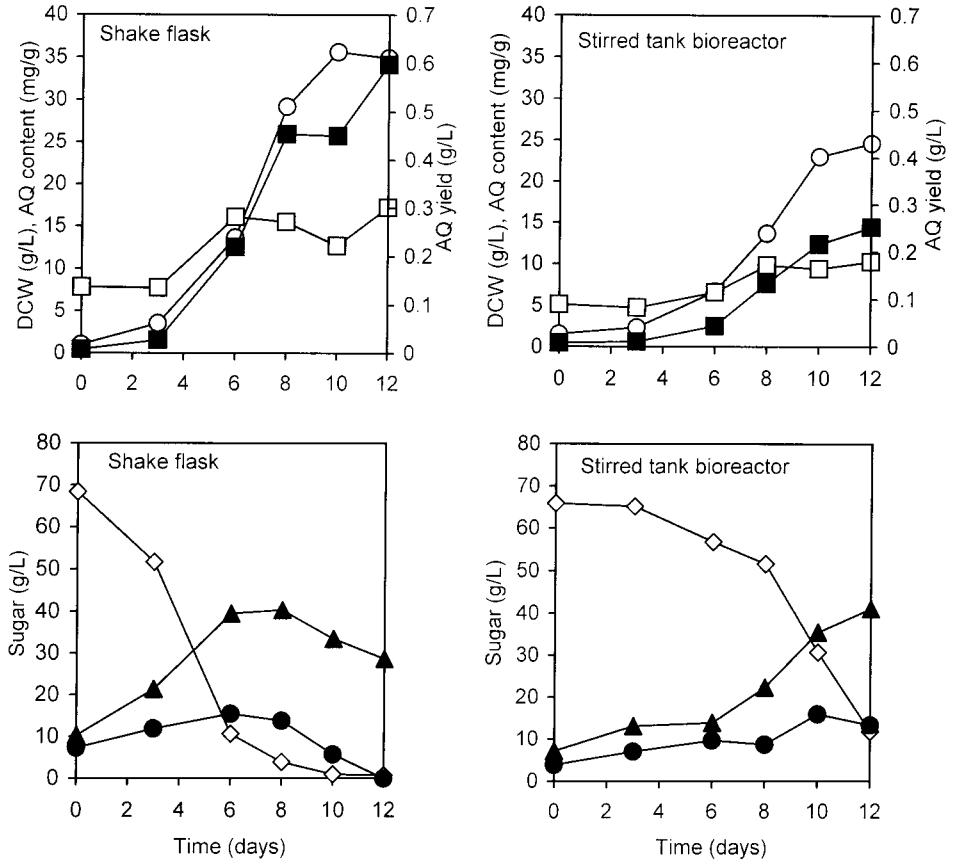

Figure 3. Comparison of cell growth, AQ yield, and sugar utilization of 36-month-old *M. elliptica* cell cultures in P medium under illumination of 500 lx, between shake flask and stirred-tank bioreactor (agitation speed = 75 rpm, aeration rate = 0.16 → 0.3 L/min, two paddle-type impellers with 45° downward paddle orientation): (○) DCW; (□) AQ content; (■) AQ yield; (◇) sucrose; (●) glucose; (△) fructose.

Table 2. Cultivation Parameters for 36-Month-Old *M. elliptica* Cell Supension Cultures in Shake Flask and Bioreactor Systems Using 80 g/L Sucrose as a Carbon Source in Production Medium Strategy for the Period of 12 Days[a]

parameter	shake flask	bio-reactor
max DCW (g/L)	35.6	24.6
max AQ content (mg/g of DCW)	17.2	10.3
max AQ yield (g/L)	0.60	0.25
max concn of sucrose (g/L)	68.4	65.9
sucrose remaining at end of cultivation (g/L)	0.8	11.9
glucose remaining at end of cultivation (g/L)	0	13.4
fructose remaining at end of cultivation (g/L)	28.7	41.1
amount of sucrose hydrolyzed (g/L)	67.6	54.0
amount of glucose utilized (g/L)	15.4	2.6
amount of fructose utilized (g/L)	11.7	0
overall cell growth rate (g/L·day)	4.59	2.96
overall AQ production rate (g/L·day)	0.067	0.031
overall rate of sucrose hydrolysis (g/L·day)	8.06	5.92

[a] Standard deviation of analysis in three replicates was in the range of 2−7% of the measured values.

of an orbital shaker platform provides adequate nutrient mass transfer without detrimental shear effects (Lee and Schuler, 1991). In the bioreactor, there is a higher shear effect as a result of stirring and forced aeration, and there are differences in gaseous exchange or concentration of gaseous compounds such as oxygen and carbon dioxide (ten Hoopen et al., 1994; Scragg, 1995). This entails further effort in improving bioreactor operating strategies for better cell growth and higher productivity such that the commercialization of useful products from plant cell cultures will come to fruition.

Conclusions. Growth and AQ formation of *M. elliptica* cell cultures were greatly affected by changes in the operating variables of the stirred-tank bioreactor. Continuous aeration may assist mixing by providing greater turbulence than intermittent air supply, although continuous high air flow rate may bring the culture into its early deceleration phase. A lower number of impellers could lead to higher growth rate, but a higher number of impellers could reduce only cell growth rate but not the AQ content and yield. Cultures in production medium or with higher growth rate and AQ production were less acidic than cultures in growth medium or with lower growth rate and AQ production. Despite some improvement with suitable bioreactor operating variables, DCW and AQ yield in the bioreactor were ∼45% and ∼140%, respectively, lower than in the shake flask.

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