

EFFECT OF AGITATION AND AERATION ON YIELD OPTIMIZATION OF OIL PALM SUSPENSION CULTURE

DAE-SUNG CHOI*; MARIA HELENA CAÑO ANDRADE*; LAURA B WILLIS*; CHANGWON CHO*;
JOERG SCHOENHEIT**; PAOLO BOCCAZZI*; RAVIGADEVI SAMBANTHAMURTHI*; ANTHONY
J SINSKEY* and CHOKYUN RHA*

ABSTRACT

Oil palm is an oil producing tropical plant. While it is the highest yielding oil producing plant, it takes three years to yield. Therefore there is an urgent need for controlled production for oil palm suspension cells in a bioreactor to support the clonal propagation of elite planting materials. The propagation of the cells is affected by various culture conditions. In this study, we examined the effect of two operating parameters - agitation rate (80 to 335 rpm) and aeration (10% to 80% dissolved oxygen) - on the proliferation of suspension cells. The sugar consumption, production of biomass, amino acids and organic acids were monitored. At 43% dissolved oxygen, two of the agitation rates (120 rpm and 225 rpm) resulted in more than 200% increase in biomass compared to the initial inoculum. This study indicates that the bioreactor operating parameters between 120-300 rpm and 20%-80% dissolved oxygen are acceptable for the culture of oil palm cells.

Keywords: bioreactor, agitation rate, dissolved oxygen, cell mass, oil palm.

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INTRODUCTION

Oil palm (*Elaeis guineensis* Jacq.) is an oil producing plant, indigenous to Africa and cultivated in tropical climates, mostly in Southeast Asia. Because of the considerable nutritional value of the vitamin E and carotenoid-rich oil, and the high agricultural oil yield (around 6000 kg ha⁻¹), palm oils has become one of the most important edible oils in the world. For clonal multiplication of elite plants, with improved

agricultural traits, micropropagation of oil palm by tissue culture is a most promising approach (Gorret *et al.*, 2004; De Touchet *et al.*, 1991).

Cell suspension cultures have been extensively utilized in oil palm micropropagation. The potential for commercializing clonally propagated oil palm accessions via plant suspension culture depends on a high and reliable production rate of polyembryos (Teixeira *et al.*, 1995). To accomplish this, it is necessary to optimize the culture conditions by controlling the most important variables related with the embryo production, such as pH, DO, agitation, temperature and media composition (Jay *et al.*, 1992; Shimazu and Kurata, 1999; 2003). Although flask cultivation is a very effective method for growing cells on a laboratory scale, its usefulness is limited by inter-flask variation and the difficulties in controlling the culture conditions.

A major advantage of bioreactor propagation is the ability to control various operational parameters such as the medium composition (Teixeira *et al.*, 1995), temperature, cell density (Shigeta *et al.*, 1996), dissolved oxygen (Kessel and Carr, 1972; Liu and Zhong, 1997; Nuutila *et al.*, 2000; Padgett and

* Biomaterials Science and Engineering Laboratory,
Massachusetts Institute of Technology,
77 Massachusetts Avenue, Cambridge,
MA 02139, USA.
E-mail: ckrha@mit.edu

** Department of Biology,
Massachusetts Institute of Technology,
77 Massachusetts Avenue, Cambridge,
MA 02139, USA.

+ Malaysian Palm Oil Board,
P. O. Box 10620,
50720 Kuala Lumpur,
Malaysia.

Leonard, 1996), pH (Jay *et al.*, 1994) and agitation rate (Jay *et al.*, 1994; Tarmizi *et al.*, 2004; Kieran *et al.*, 1997; Rodriguez-Monroy and Galindo, 1999; Sanchez *et al.*, 2002; Trejo-Tapia *et al.*, 2001; Wongsamuth and Doran, 1997). Bioreactor processes also permit the scale-up of conditions that were found optimal for culture performance in the initial small scale. Bioreactor production of somatic embryos has been reported for at least 14 plant species (Ibaraki and Kurata, 2001). In general, the tissue culture process in flask culture may take about 15 months, while the bioreactor system may decrease the time by several months.

However, few studies have reported the use of bioreactors for micropropagation of oil palm (Gorret *et al.*, 2004; Tarmizi *et al.*, 2004). We have described the culture of oil palm suspension cells in a bioreactor, and our findings suggest that more biomass can be obtained by optimization of the growth conditions (Gorret *et al.*, 2004). The objective of this work was to carry out optimization studies on two of the bioreactor conditions for culturing oil palm cells - the agitation rate and dissolved oxygen.

EXPERIMENTAL

Materials

Oil palm cell line E90L1 was provided by the Malaysian Palm Oil Board (MPOB) (Bangi, Malaysia). The oil palm cell suspension cultures were propagated via sequential subculture in flasks as described (Gorret *et al.*, 2004) to generate sufficient inocula for the bioreactors. Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) was prepared using Sigma-Aldrich products (St. Louis, MO, USA) and used for all the experiments. The MS medium was supplemented with 1 mg litre⁻¹ 2, 4-dichlorophenoacetic acid (2, 4-D) and 1 mg litre⁻¹ of alpha-naphthalene acetic acid. Cells in Erlenmeyer flasks were incubated on a rotary shaker at 26°C and agitated at 125 rpm. The medium in the flask cultures was changed every 10 days and conditioned medium added as described (Gorret *et al.*, 2004).

Oil Palm Bioreactor

Cultures were carried out in Sixfors bioreactors (Infors, AG CH-4103 Bottmingen, Switzerland). The glass vessels were custom fitted at the bottom with a large bore port at a 15° angle slant toward the top to allow accurate sampling of the biomass. The working volume was 350 ml or 500 ml depending on the experimental design, and 8.2 or 12.0 g fresh weight (FW) of cells were used to inoculate each bioreactor. The temperature was regulated at 26°C. The %DO was controlled with a discontinuous air

flow regulated by a solenoid valve. The bioreactors were equipped with two 2.5 cm diameter marine-type impellers. The pH was not controlled.

Determination of Fresh Weight

Ten millilitre samples of cells and medium were harvested from the bioreactor cultures every seven days and centrifuged at 3500 x g for 10 min. The cells were then washed with cold distilled water and collected by centrifugation. The cell pellets were weighed for their FW measurements (Gao *et al.*, 2000).

Chemical Analysis

To measure the consumption of reagents and production of primary metabolites, sugars and organic acids were quantified by high pressure liquid chromatography (HPLC) analysis (Gorret *et al.*, 2004). Ten millilitre samples were collected regularly during the run and centrifuged at 3500 xg for 10 min. The supernatants were filtered through 0.2 µm Acrodisc filters. Sugar and organic acid concentrations were determined by HPLC (Agilent model 1050, Waldbronn, Germany) using an Aminex® HPX-87H column (Bio-Rad, Hercules, CA, USA). Sample analysis was performed at 40°C using 5 mM sulphuric acid as the mobile phase at a flow rate of 0.6 ml min⁻¹. Sugars were determined by a refractive index detector (Agilent Model 1047A) and organic acids with the UV detector at 210 nm (Agilent Model 1050). Amino acids were analysed as orthophthaldialdehyde (OPA) derivatives by reverse-phase chromatography using a C18 AminoQuant column with an Agilent Series 1050 HPLC system. Nitrate and nitrite were determined using a colorimetric method (Cat No. 1 746 081, Roche, Mannheim, Germany). Ammonia was analysed using an enzymatic method (Cat No. 171-UV, Sigma, St Louis, MO, USA). Total phenolics were determined by a modified Folin-Ciocalteu colorimetric method (Gao *et al.*, 2000).

Experimental Design

The evaluation of the effects of agitation rate and aeration on biomass production was conducted in three phases. In the first experiment, we compared the growth rate in flask cultures versus in two bioreactor conditions: the previously reported benchmark conditions, indicated as BR1, of 150 rpm and 20%DO, and condition BR2 in which we decreased the agitation rate and dissolved oxygen to 80 rpm and 10%DO. Fermentations BR1 and BR2 were conducted in duplicate, simultaneously, for 52 days. In parallel, seven cultures in Erlenmeyer flasks containing 70 ml medium inoculated with 1 g (fresh weight) cells were incubated in an Infors Multitron

shaker (ATR, Laurel, MD) at 150 rpm in air (*i.e.* 20%DO). These flask cultures were incubated continuously for 110 days with replacement of the medium every 10 days. During medium replacement, cells were harvested and re-inoculated into flasks to minimize cell loss in the supernatant. The 10% (v/v) of the conditioned medium were used from the previous culture medium.

In the second experiment, we evaluated the influence of agitation at 150 and 300 rpm and aeration at 40%, 60% or 80% DO (conditions BR3 through BR8 in *Table 1*). Cells and medium were harvested from the vessel at regular intervals for analysis as described in the sections on Determination of Fresh Weight and Chemical Analysis.

In the third experiment, we evaluated five agitation rates from 120 to 335 rpm and five aeration rates from 11% to 74%DO (BR9 to BR17 in *Table 1*). In this experiment, as opposed to the sampling in the first and the second experiments, we took samples only of the medium supernatant, and not the cells, during the incubations. Instead, we harvested all the cells at the end of the incubation to minimize cell loss and sampling error. A culture volume of 350 ml was used in this experiment (*Table 1*).

Microscopy

Oil palm cells cultured in shake flasks, and those from BR3 through BR8, were observed with an inverted microscope (Nikon TE300, Japan) and Haemocytometer (Sigma Chemical Co., St. Louis, MO). The 10 μ l samples were loaded and observed under 400x magnification.

RESULTS AND DISCUSSION

Growth of Oil Palm Tissue Culture: Bioreactor *vs.* Flasks

In our earlier work, we established baseline conditions for the growth of oil palm cells in the bioreactor: 150 rpm and 20%DO (Gorret *et al.*, 2004). In this work, we evaluate the effect of different agitation rates and different levels of aeration (%DO) on growth, nutrient uptake and metabolite production, in three experimental phases. The first experimental phase compared growth in the bioreactor *vs.* flask culture under the previously established baseline conditions, and evaluated the effect of decreasing the oxygenation and agitation in the bioreactor.

We compared the growth of oil palm cells in the bioreactor under the baseline conditions of 150 rpm and 20%DO, referred to as BR1, with growth under reduced agitation and aeration levels of 10% DO and 80 rpm, referred to as BR2 (*Table 1*). In parallel, we compared the growth of oil palm cells in the bioreactor condition BR1, conducted as a batch culture, with the growth of oil palm cells in flask cultures that underwent medium replacement every 10 days and grown under atmospheric oxygen (20% DO) and shaken at 150 rpm. The bioreactor culture conditions BR1 and BR2 were carried out in duplicate and results reported as averages. The seven replicate flask cultures were grown for 52 days.

The nutrient uptake, pH and biomass of BR1 and BR2 were monitored every week (*Figure 1*). Both cultures converted the disaccharide sucrose to the

TABLE 1. EXPERIMENTAL CONDITIONS FOR OIL PALM BIOREACTOR CULTURE

	Batch	RPM	%DO	IFW (g)	Volume (ml)
Experiment 1	BR1	150	20.0	12.0	500
	BR2	80	10.0	12.0	500
Experiment 2	BR3	300	40.0	12.0	500
	BR4	300	60.0	12.0	500
	BR5	300	80.0	12.0	500
	BR6	150	40.0	12.0	500
	BR7	150	60.0	12.0	500
	BR8	150	80.0	12.0	500
Experiment 3	BR9	120	43.0	8.2	350
	BR10	225	43.0	8.2	350
	BR11	335	43.0	8.2	350
	BR12	150	65.0	8.2	350
	BR13	225	11.0	8.2	350
	BR14	225	43.0	8.2	350
	BR15	225	74.0	8.2	350
	BR16	300	20.0	8.2	350
	BR17	300	65.0	8.2	350

Note: RPM: revolutions per minute, %DO: % dissolved oxygen, IFW (G): initial fresh weight of inoculum in grammes, BR: bioreactor.

monosaccharides glucose and fructose within the first week of cultivation. Although the overall shapes of the curves were similar, the kinetics of sugar consumption between BR1 and BR2 were different (Figures 1a, b and c). In fact, cells cultivated under BR1 conditions depleted the sugar one week earlier (45 vs. 52 days) than those cultivated under BR2 conditions, indicating that BR1 conditions were more favourable to sugar consumption and growth.

The slight increase of pH (from 4.8 to pH 5.4) indicated that the sugar was being consumed and the maintenance of this value was an indication also that the bioreactor remained free of contamination (Figure 1d). No significant amounts of organic acids were found. Small quantities of acetate were

detected (data not shown); this may suggest that biomass production did not suffer the competition of secondary metabolites for organic acids production.

Cell growth was monitored by measurement of the final FW of the cells in the reactor, expressed as grammes FW produced per day. The results from the comparison of final FW under these three conditions (BR1, BR2 and flask culture) indicated that the decreased agitation and aeration condition (BR2) were disadvantageous compared to the benchmark BR1 conditions (Figure 2). Therefore, we conducted a second experiment to examine the effect of increased aeration and agitation as described in the following section.

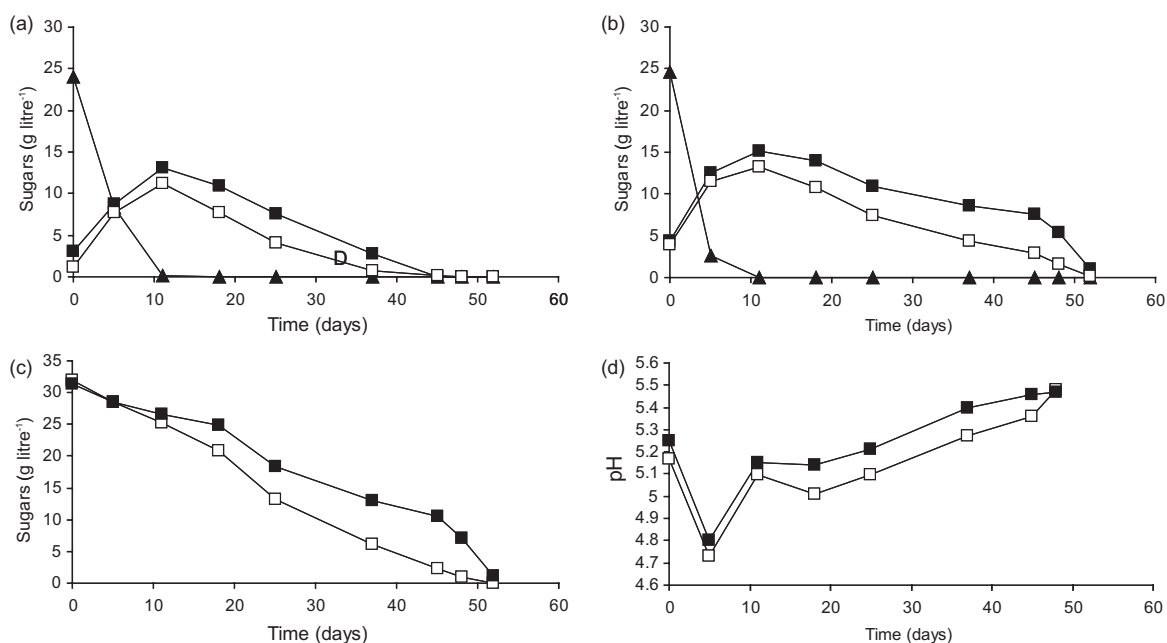


Figure 1. Nutrient uptake and extracellular pH of oil palm suspension cultures cultivated in batch bioreactor conditions, BR1 (20% DO, 150 rpm) and BR2 (10% DO, 80 rpm). (a) Extracellular sugars in BR1; -▲- sucrose, -■- glucose, -□- fructose. (b) Extracellular sugars in BR2; -▲- sucrose, -■- glucose, -□- fructose. (c) Data from panels (a) and (b) expressed as total sugar consumption in BR1 (□) and BR2 (■). (d) Extracellular pH in BR1 (□) and BR2 (■). In all panels, data are averaged from replicate bioreactor runs.

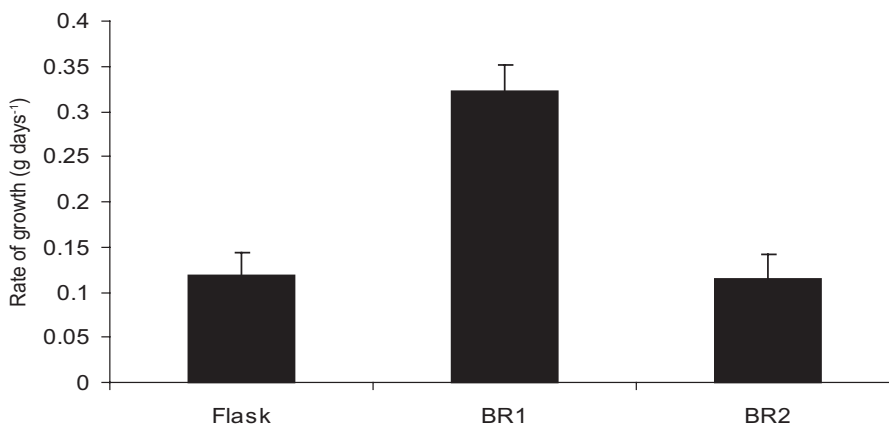


Figure 2. Biomass (fresh weight) of oil palm suspension cultures cultivated in flasks vs. batch bioreactor conditions, BR1 and BR2. Seven flask cultures were incubated for 110 days. Relative ratio of growth (RG) is expressed as (final fresh weight - initial fresh weight) / incubation days.

Optimizing Bioreactor Operation: Agitation and Aeration

In this experiment, agitation at 150 rpm and 300 rpm, each at 40%, 60% and 80% DO were tested. In each bioreactor operation, metabolites in the extracellular medium were monitored. Samples of the culture medium were withdrawn from the bioreactor and filtered, and the concentrations of sucrose, fructose, glucose, citrate, acetate, pyruvate and lactate determined.

The fructose consumption in bioreactors BR3 to BR8 is shown in *Figure 3*. We also analysed the total sugar consumption and glucose depletion; the glucose consumption showed a similar pattern to the fructose depletion (data not shown). The fastest

fructose consumption was in bioreactors BR6 (40%DO and 150 rpm) and BR8 (80%DO and 150 rpm). Cells grown with 40%DO and 300 rpm (BR3) showed the slowest sugar consumption. These results indicated that nutrient consumption was influenced by both processing variables: agitation and aeration.

Nitrate, ammonia, and total phenolic compounds were measured (*Figure 4*). Bioreactor BR3 produced more ammonia and nitrate compared to BR4 and BR5. The accumulation pattern of total phenolic compounds was similar among the three bioreactors. The content of primary metabolite production during the bioreactor operation was also analysed. Eight amino acids (alanine, valine, methionine, isoleucine, leucine, lysine, homoserin and threonine)

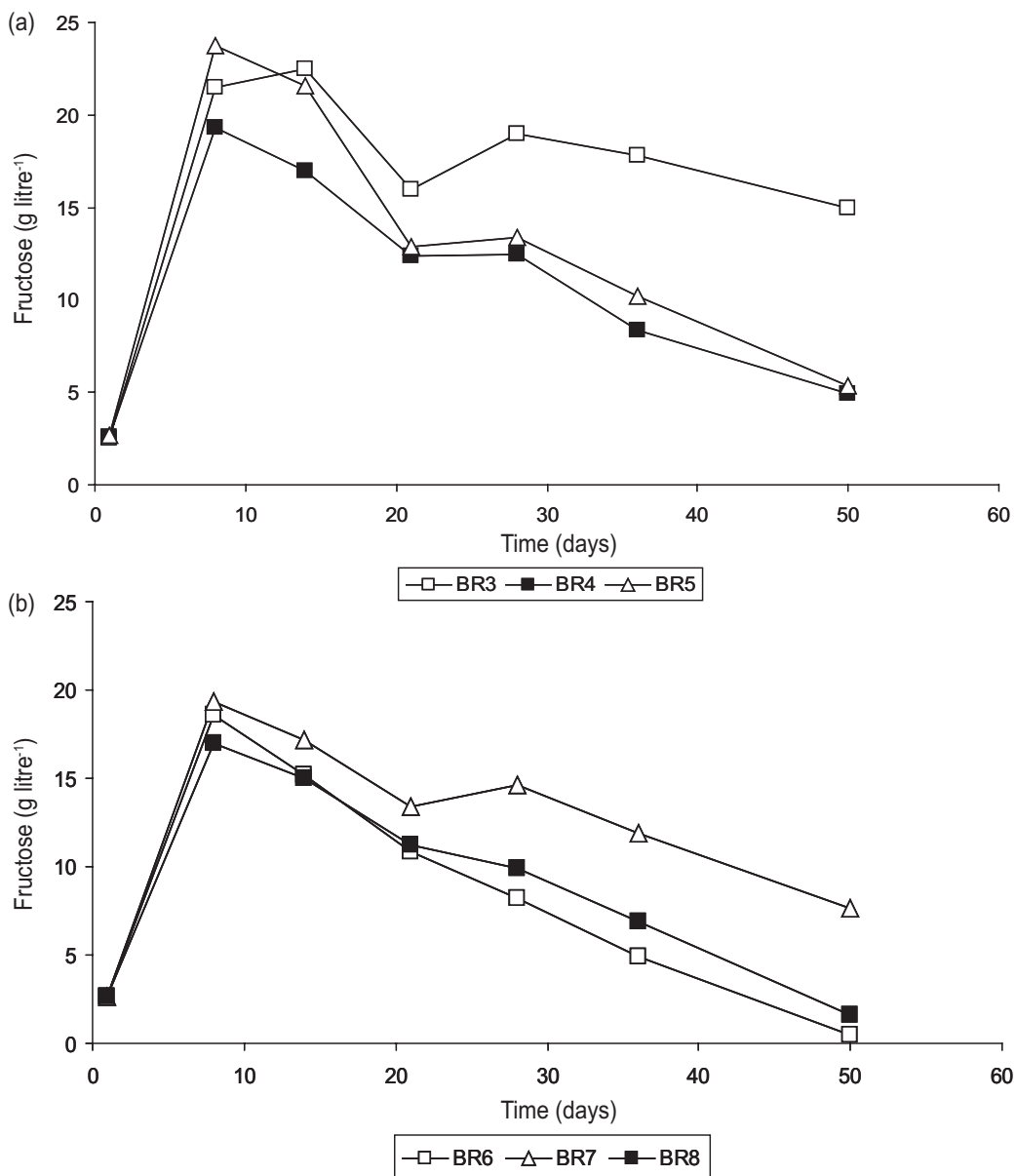


Figure 3. Nutrient uptake during suspension culture of oil palm cells in batch bioreactors. (a) Content of extracellular fructose in BR3 (300 rpm, 40% DO, □); BR4 (300 rpm, 60% DO, ■); BR5 (300 rpm, 80% DO, △). (b) Content of extracellular fructose in BR6 (150 rpm, 40% DO, □); BR7 (150 rpm, 60% DO, △); BR8 (150 rpm, 80% DO, ■).

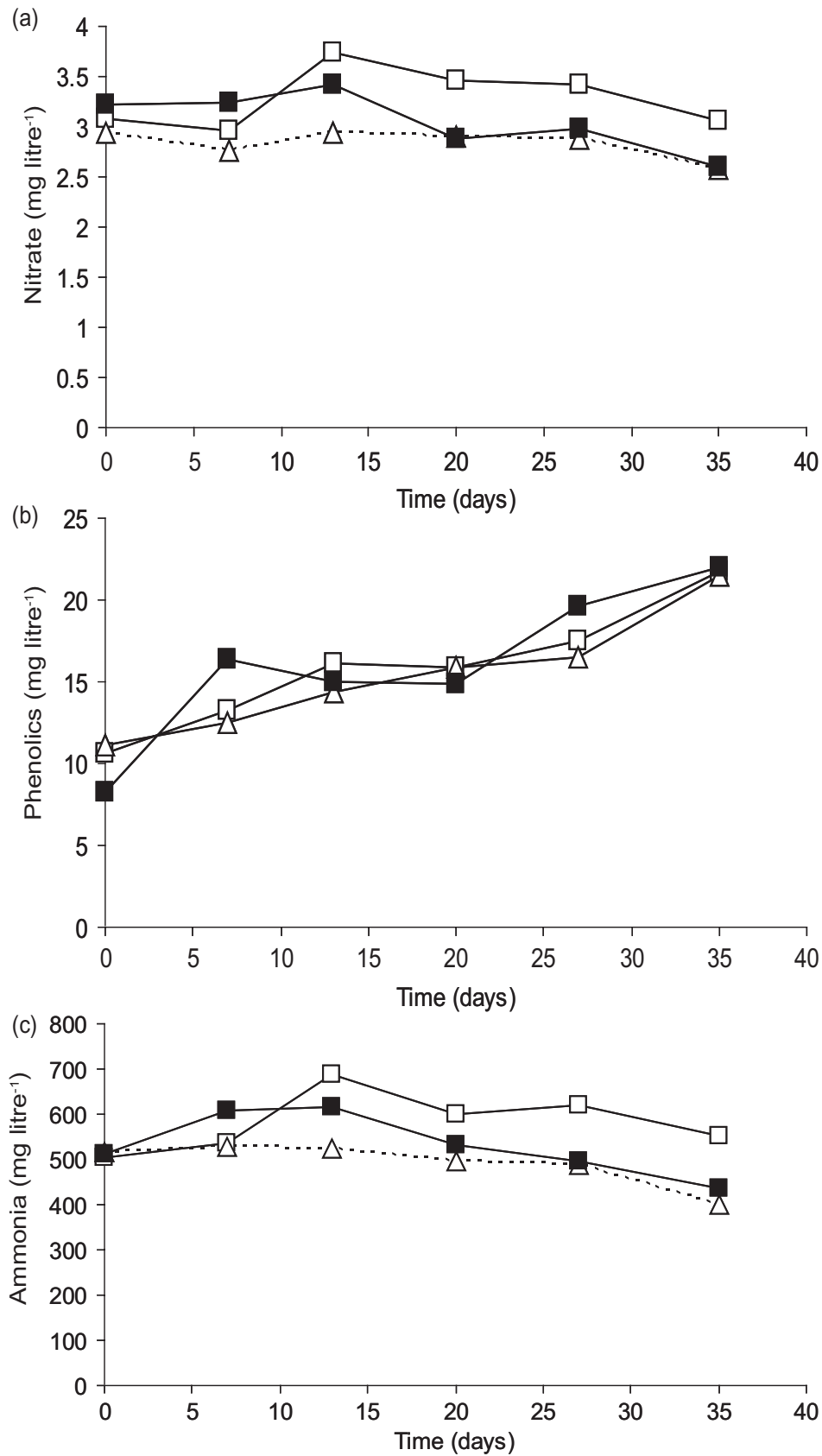


Figure 4. Extracellular metabolite accumulation during suspension culture of oil palm cells in batch bioreactors. (a) Nitrate, (b) total phenolics (expressed as mg litre⁻¹ eq. Gallic acid), (c) ammonia, in bioreactor conditions BR3 (300 rpm, 40% DO, □), BR4 (300 rpm, 60% DO, △), BR5 (300 rpm, 80% DO, ■).

TABLE 2. YIELDS OF OIL PALM BIOREACTOR CULTURES

	Batch	RPM	%DO	IFW (g)	FFW (g)	% Biomass increase
Experiment 1	BR1	150	20.0	12.0	28.8	140
	BR2	80	10.0	12.0	18	50
Experiment 2	BR3	300	40.0	12.0	32.8	173
	BR4	300	60.0	12.0	29.6	147
	BR5	300	80.0	12.0	31.7	164
	BR6	150	40.0	12.0	29.2	143
	BR7	150	60.0	12.0	23.0	92
	BR8	150	80.0	12.0	32.2	168
Experiment 3	BR9	120	43.0	8.2	25.6	212
	BR10	225	43.0	8.2	27.6	237
	BR11	335	43.0	8.2	8.8	7
	BR12	150	65.0	8.2	19.6	139
	BR13	225	11.0	8.2	13.9	70
	BR14	225	43.0	8.2	19.7	140
	BR15	225	74.0	8.2	23.8	190
	BR16	300	20.0	8.2	16.5	101
	BR17	300	65.0	8.2	24.4	198

Note: RPM: revolutions per minute; %DO: % dissolved oxygen; IFW (g): initial fresh weight of inoculum in grammes; FFW (g) final fresh weight of inoculum in grammes; % biomass increase: $\{[(FFW-IFW)/IFW]*100\}$.

were measured. The extracellular levels of four of these are shown in *Figure 5*. Production of leucine was similar in BR3, BR4 and BR5. Bioreactor BR3 produced the highest levels of extracellular homoserine, 73 mg litre⁻¹, after 36 days incubation. This was 3.6-fold higher than those observed in bioreactors BR4 and BR5 (*Figure 5*). Homoserine is a key intermediate in the biosynthesis of aspartate-derived amino acids, and while extracellular homoserine levels were increased, extracellular lysine and methionine levels were decreased under BR3 conditions. This could be due to inhibition of one or more enzymes in the biosynthesis of lysine, methionine and threonine. BR3 conditions, which had the highest homoserine and lowest lysine and methionine levels, also had the slowest sugar consumption of conditions BR3-BR8. Another explanation for the decreased growth under BR3 conditions could be the phenomenon often described as 'general amino acid inhibition' in which exogenous amino acids caused inhibition of growth, as has been observed in suspension cultures of *Nicotiana glauca* (Bonner and Jensen, 1997).

Effects of Agitation and Aeration on Oil Palm Cell Growth

In the first and second phases of the experiment, we evaluated the effect of agitation and content of dissolved oxygen on biomass production and production of extracellular metabolites. To expand on the results, we selected a new range of values to test for agitation (120-335 rpm) and aeration (20%-75%DO) for oil palm cultivation.

In this third phase of the experiment, yield was evaluated as end point measurements of biomass. In contrast to the earlier fermentations, samples of the oil palm cell mass were not taken during the bioreactor run. Rather, all the cells were harvested at the end of the run. This procedure was employed in order to eliminate a possible source of loss during sampling. *Figure 6* shows a summary of the increase in biomass for all three experiments, with 17 bioreactor operating conditions.

We found that a broad range of operating conditions is suitable for the cultivation of oil palm suspension cells. Thirteen out of seventeen bioreactions yielded at least a doubling in biomass; *i.e.* >100% increase in biomass over the initial inoculum. Both of the highest yielding bioreactions, which increased the biomass more than 200% compared to the initial inoculum, were conducted at 43%DO: BR9 (120 rpm, 43%DO) and BR10 (225 rpm, 43%DO). However, a second reactor conducted with the same setpoints as BR10 (BR14) had an intermediate yield of 140% biomass increase compared to the 237% achieved with BR10. Additional replicate experiments will be required to determine the range of variation in biomass increase for this cell line grown under identical setpoints.

Agitation is a key bioreactor operating parameter that is required for adequate mixing and which contributes to mass and heat transfer. However, excessive agitation may be detrimental to the cells and their growth. The highest agitation condition that we tested, 335 rpm in BR11, resulted in only a 7% increase in biomass over the initial inoculum. This was the smallest increase in biomass that we

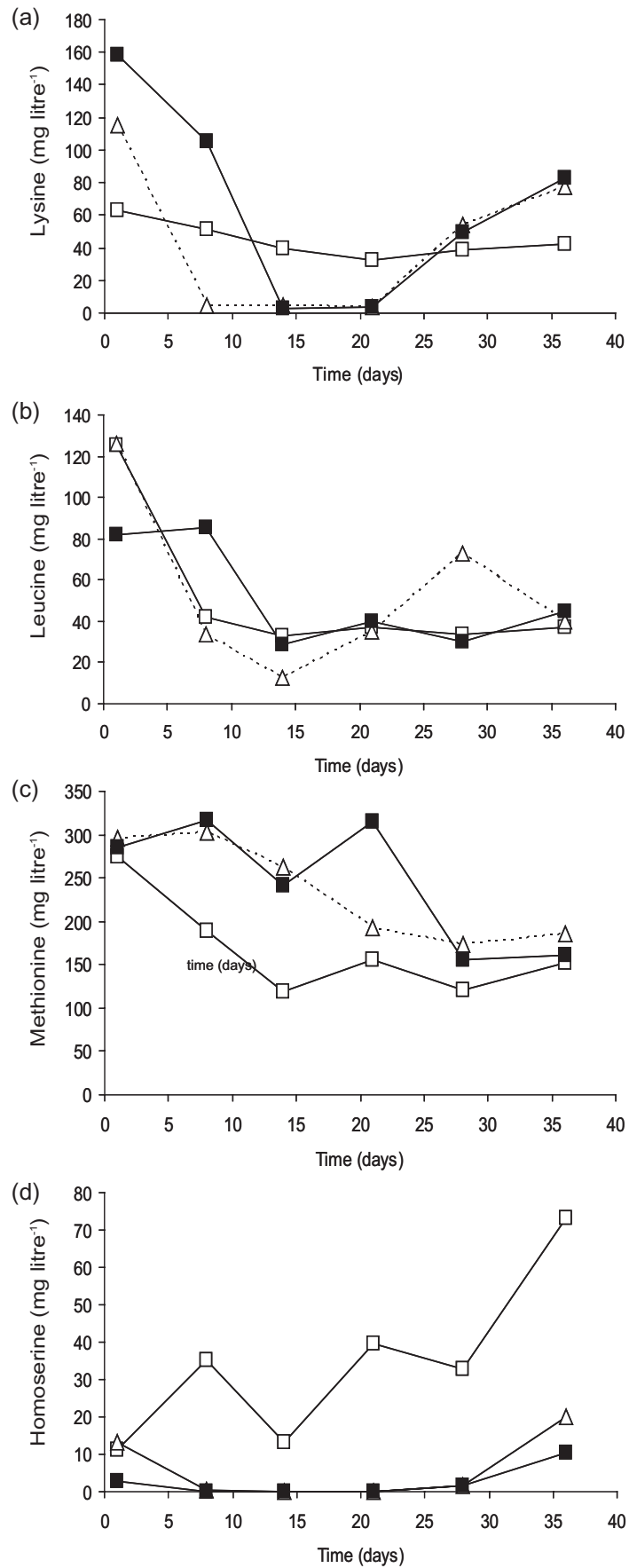


Figure 5. Extracellular amino acid accumulation during suspension culture of oil palm cells in batch bioreactors. (a) Lysine (mg litre⁻¹), (b) leucine (mg litre⁻¹), (c) methionine (mg litre⁻¹), and (d) homoserine (mg litre⁻¹) production in BR3 (300 rpm, 40% DO, □), BR4 (300 rpm, 60% DO, △), BR5 (300 rpm, 80% DO, ■).

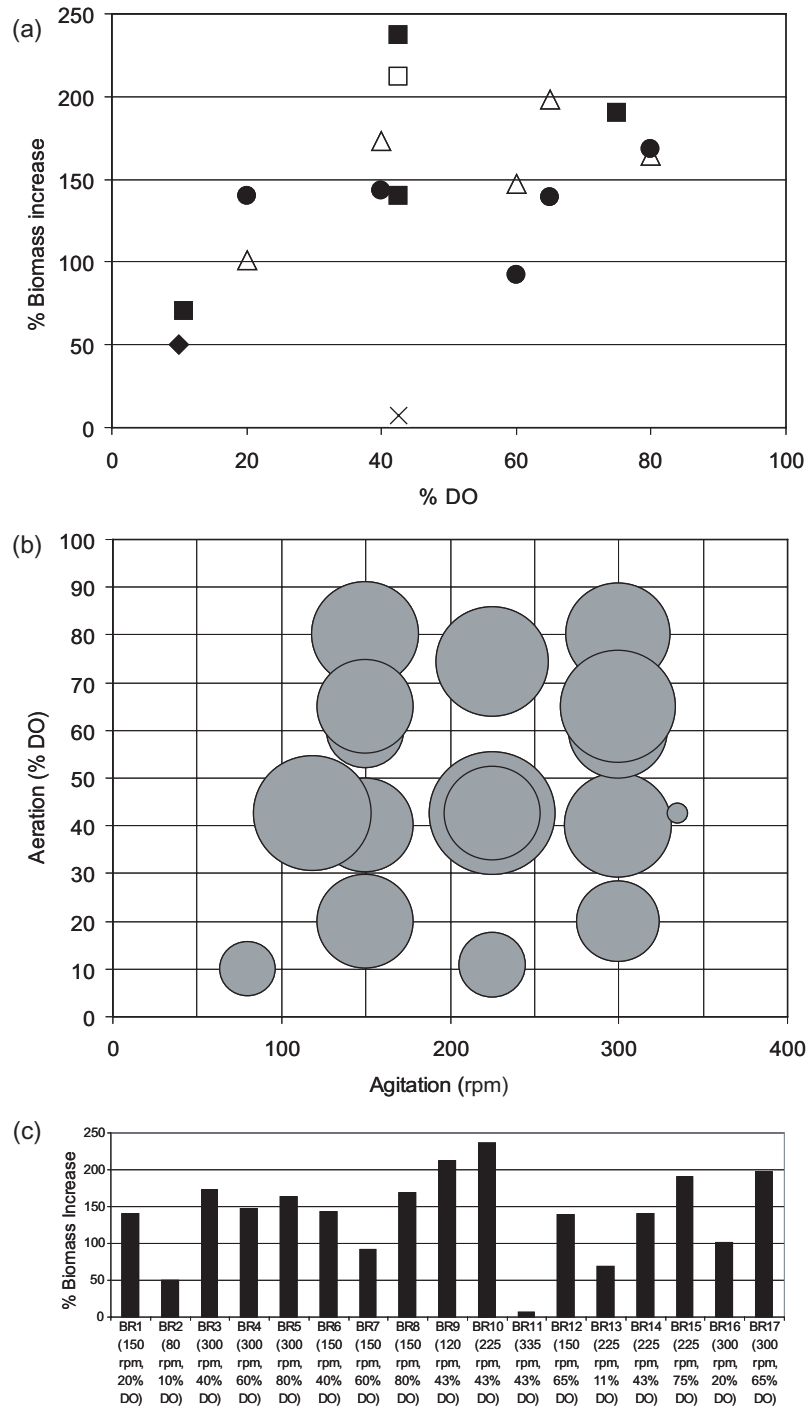


Figure 6. Effects of agitation and aeration on the growth of oil palm bioreactor cultures. (a) Percent biomass increase, calculated as $\{[(\text{final fresh weight}-\text{initial fresh weight})/\text{initial fresh weight}]*100\}$, plotted as a function of % DO.

Symbols indicate agitation rate: ◆, 80 rpm; □, 120 rpm; ●, 150 rpm; ■, 225 rpm; △, 300 rpm; x, 335 rpm.

(b) Biomass increase as a function of agitation and aeration. The areas of the circles show percent biomass increase, calculated as in (a). (c) Bar chart of percent biomass increase [calculated as in (a)] for bioreactor cultures.

observed, suggesting that this agitation rate is too high for the cultivation of oil palm suspension cells.

The other main variable we tested for bioreactor micropropagation for oil palm culture was aeration. The lowest oxygenation conditions that we tested, 10% and 11% DO in BR2 and BR13, resulted in the second and third smallest increases in biomass. Normal atmospheric conditions are approximately

20% DO; decreasing oxygen below atmospheric levels was correlated with decreased growth. Oxygen is an essential factor for plant cells growth, required for energy generation and the biosynthesis of primary and secondary metabolites of the cell. The concentration necessary for somatic embryogenesis in plant suspension culture is not clear in the literature and the values vary from 7% to 80% DO

for different cells (Gorret *et al.*, 2004). For example, there is disagreement in the literature regarding somatic embryogenesis of carrot cells: some have reported that > 70%DO is optimal (Jay *et al.*, 1992; Shimazu and Kurata, 1999; 2003) while others suggested 16%DO (Kessel *et al.*, 1972). The assessment of the optimal %DO is complicated by the fact that flushing nitrogen and oxygen may remove other gaseous plant growth regulators. Fisichella and Morini (2003) found that flushing the headspace in order to control oxygen levels reduced the occurrence of somatic embryogenesis: they hypothesized that another gas was influencing this process.

Morphology of Oil Palm Suspension Cells

To gain additional insights into the effects of agitation and aeration on the growth of oil palm suspension cells, we examined the morphology and integrity of the cells. In the literature, there are several reports on the proliferation of oil palm liquid suspension culture in shake flasks (De Touchet *et al.*, 1991; Teixeira *et al.*, 1995; Tarmizi *et al.*, 2004). In flask cultures, oil palm cells have a tendency to grow as aggregates (Figure 7a), an attribute that has necessitated pressing cells through a sterile metal sieve during the flask subculture process (Gorret *et al.*, 2004). We assessed the viability of oil palm cell

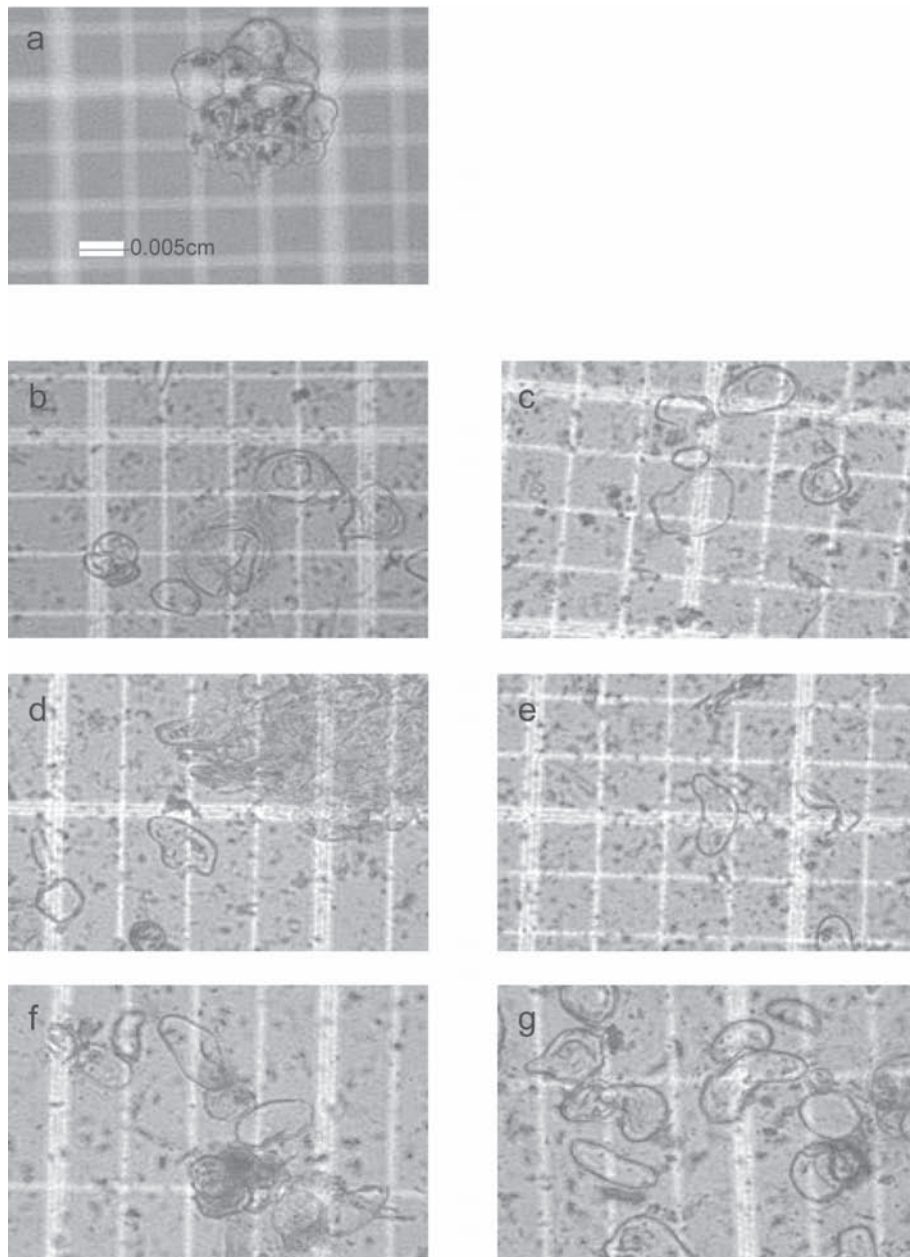


Figure 7. Microscopic images of oil palm suspension cells (400x with inverted microscope. Scale bar: 0.005 cm). (a) Cells grown in flask culture at 150 rpm under atmospheric conditions (20%DO); (b) BR3 (300 rpm, 40%DO); (c) BR6 (150 rpm, 40%DO); (d) BR4 (300 rpm, 60%DO); (e) BR7 (150 rpm, 60%DO); (f) BR5 (300 rpm, 80%DO); (g) BR8 (150 rpm, 80%DO).

aggregates using a standard assay, in which the reduction of 2,3,5-triphenyltetrazolium chloride (TTC) by viable cells gave water-insoluble red formazan. Only the outer surface of the cell clump showed viability with the TTC assay (data not shown). If the diffusion of the TTC is not hindered through the cell aggregates, then it may indicate that the cells inside the aggregate are not viable; the inner portion of the cells may be non-viable due to the lack of nutrients and oxygen.

Optimizing the agitation conditions in the bioreactor could help break up the aggregates, permitting the free cells to grow faster and become more embryogenic than the multicell clumps. We examined the morphology of oil palm cells cultured in bioreactors BR3 to BR8 (Figure 7). We observed that increasing agitation to 300 rpm did not offer significant advantages in cell morphology. As shown in Figure 7, panels (b), (d) and (f) samples cultivated under conditions of 300 rpm agitation contained significant cell clumps. Maintaining 150 rpm agitation and increasing aeration to 40%, 60% and 80%DO did not noticeably reduce the number of cell aggregates observed (Figures 7c, 7e and 7g). Cells growing under all the bioreactor conditions observed (BR3-BR8) appeared to contain more cell debris than those cultivated in shake flasks (Figure 7a). Further studies of the effect of agitation on cell morphology and cell viability will be required to determine which reactor conditions maximize the maintenance of integrity of the cells while reducing clumping.

CONCLUSION

Taking into account the sugar consumption and increase in biomass production, there appears to be an optimal range of conditions for rpm and %DO for bioreactor cultivation of oil palm suspension cells: agitation at 120-300 rpm and aeration of 20%-80% dissolved oxygen. Aeration lower than atmospheric conditions (20%DO) was detrimental to biomass production. Excessive agitation (335 rpm) was correlated with poor growth. The two highest yielding bioreactions in these experiments were conducted at 43%DO.

This work demonstrates that the key factors of agitation and aeration affect the growth of oil palm cells in a bioreactor system, as assessed by the cell mass increase, amino acid production and total phenolic compound production. Our experimental observations indicate that the *E. guineensis* cell line is responsive to agitation (rpm) and content of dissolved oxygen (%DO).

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