MULTIPLICATION OF OIL PALM SUSPENSION CULTURES IN A BENCH-TOP (2-litre) BIOREACTOR

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ABSTRACT

Oil palm Elaeis guineensis *suspension cultures were multiplied in a B-Braun Biostat® B' 2L version 1.0 bioreactor. An initial experiment using the original system was not successful as most of the culture aggregates lodged between the baffle cage and inner wall of the vessel and also between the blades of the impeller. This damaged the aggregates. Some modifications were then made to the bioreactor by replacing the impeller, baffle cage and microsparger. After modification, the cultures showed good proliferation with about 10- to 14-fold weight increment after 50 to 80 days. Thus, the B-Braun bioreactor with slight modification has the potential for large scale multiplication of oil palm suspension cultures.*

Keywords: Oil palm suspension cultures, bioreactor, proliferation, multiplication, automation.

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INTRODUCTION

Oil palm *Elaeis guineensis* suspension cultures have been established using the shake flask system (De Touchet *et al*., 1991; Teixera *et al*., 1995; Wong *et al*., 1999; Tarmizi *et al*., 1999; Tarmizi, 2002) which was developed to produce a reliable supply of regenerable plant tissues. However, the system is inefficient for fast large scale proliferation of individual clonal embryogenic suspension cultures. The bioreactor, already widely used for the industrial production of microbial, animal and plant metabolites, would seem a good alternative system, especially as the bioreactor for plant shoot and embryo cultures would be essentially the same as that for microbial, animal or plant cell culture (Takayama and Akita, 1994).

The bioreactor has proven useful in the multiplication of suspension cultures of *Oryza sativa* L. (Okamoto *et al*., 1996), *Cyclamen persicum* mill (Hohe *et al*., 1999), *Oxalis triangularis* ssp., *Triangularis* (Teng and Ngai, 1999) and *Solanum tuberosum*, L. (Yu *et al*., 2000). As the potential of the bioreactor system is widely acknowledged, attempts were made to multiply oil palm suspension cultures using the method.

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MATERIALS AND METHODS

Plant Materials

The culture used for initial study was an embryogenic suspension with <2 mm aggregates from clone E34 (mature palm, ortet 0.189/2844). Further testing on the modified bioreactor system was conducted using clone E38 (mature palm 0.189/847), E68 (mature palm 0.189/168) and E80 (mature palm 0.189/195).

Bioreactor System

The bioreactor was a B-Braun Biostat® B 2L version 1.0 (*Figure 1*) - a bench top and batch culture fermentor with an autoclavable culture vessel. It is a complete system with built-in pumps, a vessel with a drive, digital measurements and controls. It can also be categorized as an aeration agitation bioreactor because of the incorporation of the sparger and impeller. The six-bladed disc impeller, stainless steel ring with pin hole sparger and baffle cage located near the internal wall of the glass vessel were to provide mechanical agitation for proper mixing of

the suspension culture being multiplied. The system supports both microbial and cell culture applications. However, in order to support plant cell culture, especially oil palm suspension culture, some modifications to the bioreactor system were needed. Thus, experiments were conducted to monitor the culture development before and after modification of the bioreactor.

Media

The liquid MS (Murashige and Skoog, 1962) medium with 1 mg litre^{-1} nicotinic acid, 0.1 g litre^{-1} myo-inositol, 0.1 g litre^{-1} L-glutamine, 3% sucrose (castor sugar brand MSM) was used (Rohani *et al.*, 2003). The medium was supplemented with 1 mg litre^{-1} dichlorophenoxyacetic acid (2,4-D) + 0.1 mg litre^{-1} 1-aphthaleneacetic acid (NAA). This hormonal combination was found effective in oil palm liquid culture systems (Tarmizi, 2002). The pH of the media was adjusted to 5.7 before autoclaving at 121°C for 25 min.

Inoculation of Culture

Approximately 4 g embryogenic suspension cultures were used to inoculate 1 litre MS medium [this is equivalent to 0.4% (w/v) friable calli for initiating the suspension culture in a shake flask]. The parameter settings for the bioreactor were a temperature of 28°C and agitation at 60 to 100 rpm. The pH and pO_2 were 5.7 and 20%-40%, respectively. The vessel was covered with black cloth to avoid light. As the bioreactor had only a single vessel, the experiments had to be done individually with two runs before and three runs after the modifications.

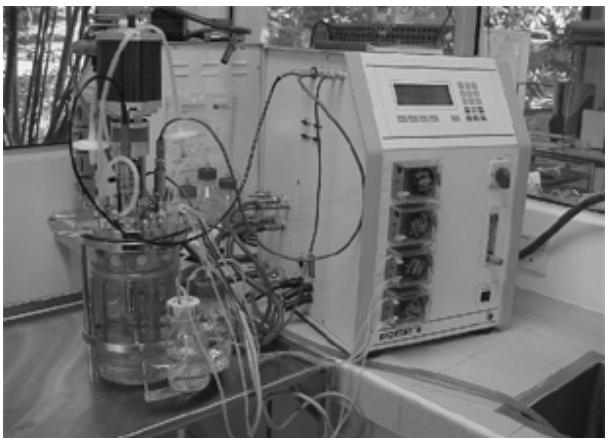
For comparison, suspensions from the same culture were transferred to 100 ml flasks with an inoculation of 0.4 g per 20 ml and incubated in darkness at 100 rpm on an orbital shaker. This shake flask system is used routinely for the proliferation of liquid cultures (Tarmizi, 2002).

RESULTS AND DISCUSSION

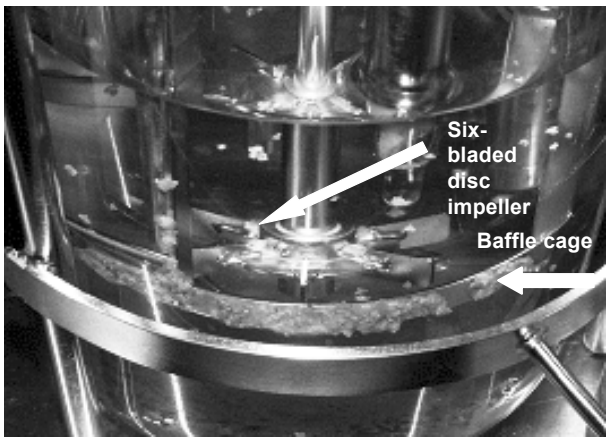
Before Modification

No significant weight increment of the cultures was observed after a month in the bioreactor (*Figure 2*). There was little proliferation of the cell aggregates as most of them were lodged in between the baffle cage and inner wall of the bioreactor vessel (*Figure 3*) and also between the blades of the impellers which resulted in severe shear damage during the stirring.

The damage was obviously more at the faster (100 rpm) than slower stirring speed further indicating the fragility of the culture and the requirement for more gentle agitation. The lower speed of 60 rpm was subsequently used to provide sufficient agitation for the aggregates so as not to settle at the bottom of the vessel. A build-up of pressure in the bioreactor vessel, indicated by less oxygen bubbling up from the sparger, was detected after one week. A modification was made to rectify this problem.



Bioreactor Biostat® B version 1.0.



Some components in the bioreactor vessel.

Figure 1.

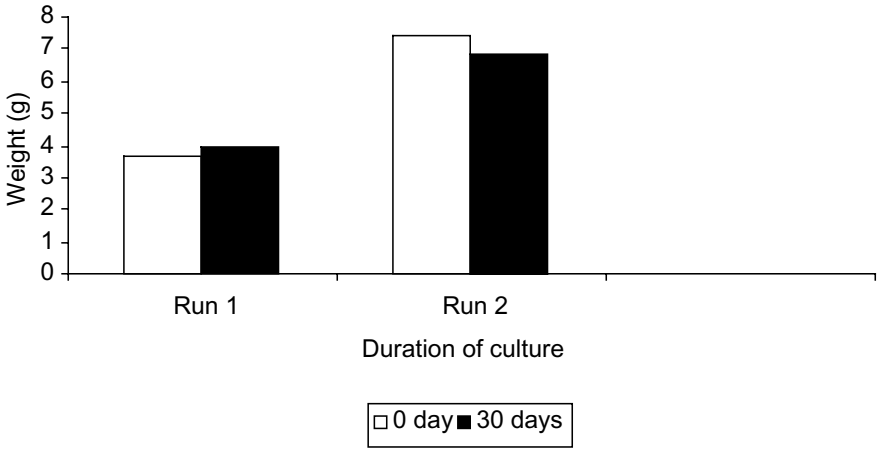


Figure 2. Fresh weights of the oil palm suspension cultures in the bioreactor before modification.

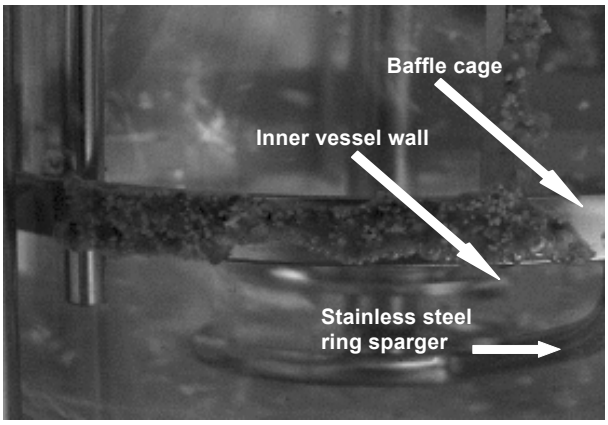


Figure 3. Aggregates lodged in between the baffle cage and inner vessel wall (one week culture).

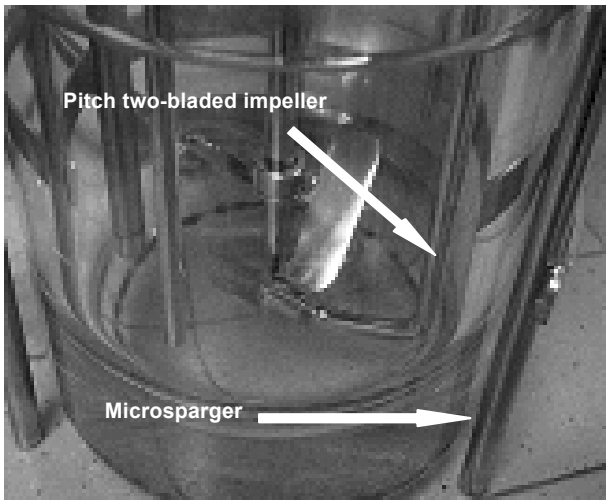


Figure 4. New impeller and microsparger.

Modification of the System

To overcome the problem of stuck aggregates and to reduce the high shear stress, the six-bladed disc impeller was replaced with a simple pitch two-bladed impeller. The blades were pitched at an angle of 45° on both sides to push the suspension cultures in a vertical flow along the tank axis. Axial mixing is considerably more efficient than radial mixing (Anon, 2001). It is also more effective at lifting the cell aggregates from the bottom of the tank. Axial flow impellers also impart a lower shear.

A microsparger (B-Braun, 0.2 μM) was installed to replace the stainless steel ring sparger (*Figure 4*). The microsparger produced smaller air bubbles which enhanced oxygen absorption by the culture (Takayama and Akita, 1994). A higher intake of oxygen would be necessary if the biomass is to increase faster. The one-head exhaust air filter (Sartorius 0.2 μm) was also replaced with a twin-head to reduce pressure build-up in the vessel (*Figure 5*).

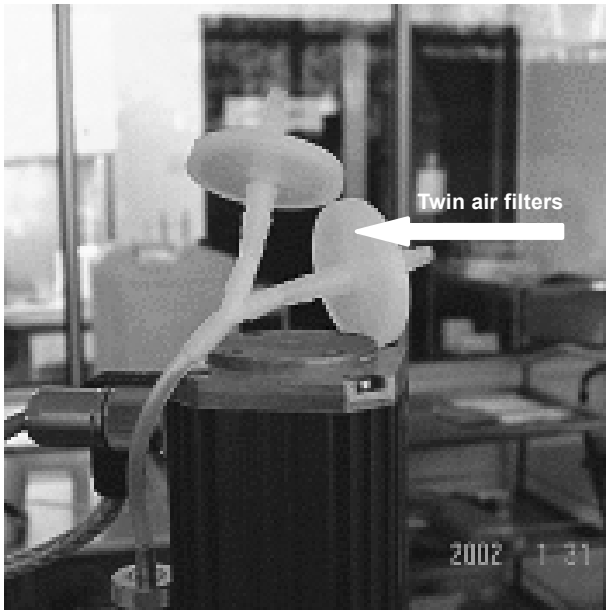


Figure 5. Twin exhaust air filters.

After Modification

The cell aggregates responded positively to the modifications. Morphologically, they appeared similar to suspension cultures in the shake flask system (*Figure 6*). A good proliferation rate was obtained with the fresh weight of the cultures from clone E34 increased 12 folds after 80 days in the medium treated with 1 mg litre^{-1} 2,4-D + 0.1 mg litre^{-1} NAA (*Table 1*). In contrast, the control cultures in the shake flasks only increased 2.5 folds in their fresh weight. Cultures from the bioreactor were able to regenerate when transferred to solid media (*Figure 7*). Further testing on the other clones showed that E68 and E80 increased their weights 9- and 14-fold, respectively, after about 50 days in the bioreactor. However, the cultures from clone E38 browned off after about 30 days with a weight increment of only 4.5 folds (*Figure 8*). This indicates that development in the bioreactor system varied between clones and may require different parameters.

Figure 6. Culture aggregates of clone E34 collected from bioreactor after 80 days of culture (1.1X).

CONCLUSION

There had been several reports in the literature on the proliferation of oil palm liquid suspension cultures but in all cases, the shake flask system was used (De Touchet *et al.*, 1991; Teixera *et al.*, 1995; Wong *et al.*, 1999; Tarmizi *et al.*, 1999). Therefore, this work on the bioreactor system shows a new approach to the proliferation of oil palm suspension cultures. The original six-bladed disc impeller and baffle cage were found to be unsuitable because of the damage caused on the cell cultures and had to be replaced. Shear damage is one of the major problems in using a bioreactor for multiplication of plant cultures (Glacken *et al.*, 1999). The present findings show the possibility of large scale production of embryogenic suspension cultures in a single run. Based on reports on the shake flask, monthly weight increments of three to seven folds can be obtained (de Touchet.,1991; Wong *et al.*, 1999; Tarmizi *et al.*, 1999). To get the same growth increase in culture as in this experiment (from 4 g to about 50 g), more shake flasks would have to be set up from the beginning and the cultures maintained for three months with monthly subcultures. Thus, time and space can be saved by using the more efficient bioreactor system. As stated by Wong *et al.* (1999), even the limited success of the shake flask for production of embryogenic calli has shown potential for automation. The present findings will indicate good potential towards a semi- or fully-automated process of oil palm clonal production.

TABLE 1. INCREASES IN FRESH WEIGHT (g) OF CLONE E34 LIQUID CULTURES IN THE SHAKE FLASK AND BIOREACTOR SYSTEMS (after modification)

System	Increase in fresh weight (g) time of culture			
	0 day	30 days	60 days	80 days
Shake flask (mean of 5 replicates±SE)	0.4	0.89±0.03	1.20±0.013	1.41±0.03
Bioreactor	4	17.63	31.56	53.29

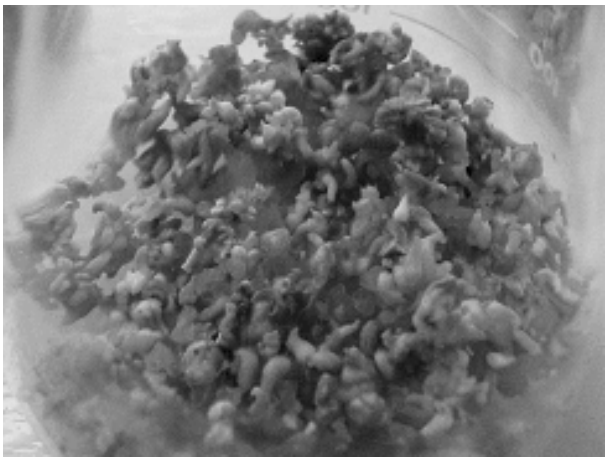


Figure 7. Cell aggregates of clone E34 from bioreactor starting to regenerate.

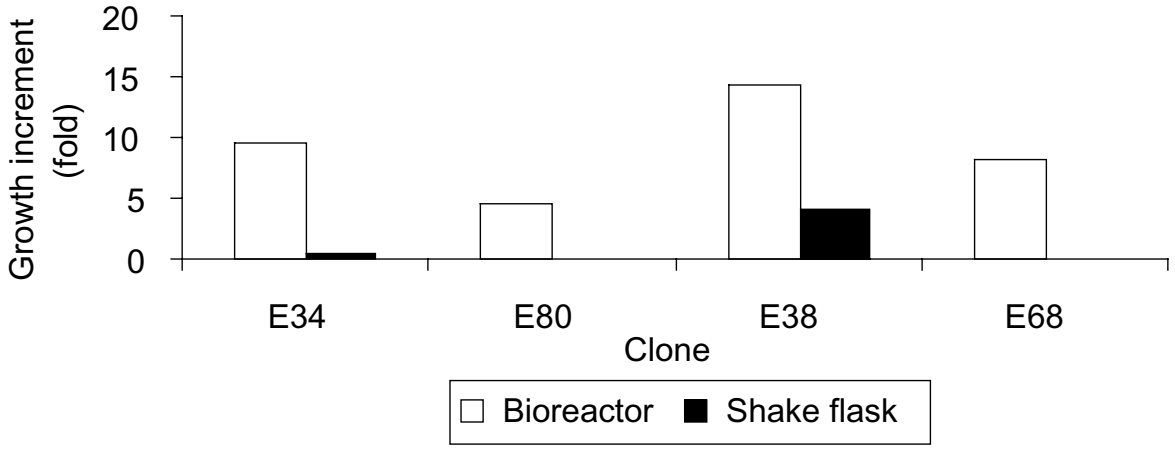


Figure 8. Growth comparison for oil palm clones after 50 days in the bioreactor and shake flask systems.

Further optimization of the protocol for the bioreactor system is currently ongoing. Vasil (1994) reported that although many of the somatic embryos formed in cultures give rise to normal rooted plants, there is still considerable variation in their development. There is a need for more in depth studies on the growth and development of somatic embryos of different clones in culture. Recent advances (Vasil, 1994) in the experimental synchronization of somatic embryo development, the nature and genetic control of the cell cycle, the identification and characterization of genes and molecular controls of embryogenesis, seed maturation and germination, will provide greater understanding on the biology of somatic embryogenesis. Vasil (1994) also concluded that the most difficult and intractable problems in the use of bioreactors for large scale somatic embryogenesis are in the biology of the system and not in the engineering.

At present, experiments are in progress to test more clones in the bioreactor system and to monitor their regeneration on solid culture media. This is important since some of the clones behave differently even in the same system and conditions. The clonal ramets produced would then be established in soil for evaluation of their clonal fidelity.

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