ESTABLISHMENT OF OIL PALM SUSPENSION CULTURE IN THE SIXFORS BIOREACTOR

By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Master of Science

July 2006

Especially dedicated to all MMBPP' ist.....

Abstract of the thesis presented to the Senate of University Putra Malaysia in fulfillment of the requirement for the Degree of Master of Science

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Chairman: Professor Madya Badlishah Sham Bahrain

Faculty: Food Science and Technology

In vitro propagation is an important part of the oil palm industry's approach towards clonal propagation of high-yielding materials. Oil palm suspension cultures have been established using the shake flask system which was developed for production of a reliable supply of regenerable plant tissues. However, this system is inefficient for fast large scale proliferation of embryogenic suspension cultures.

Bioreactors have been used for the industrial production of microbial, animal and plant metabolites. However, it's used was not well known in oil palm suspension culture. During the development of oil palm suspension culture in the sixfors multibioreactor, nutrients and extra cellular metabolites were monitored where kinetic parameters and nutrients to biomass conversion yield were calculated to better characterise the behaviour of oil palm suspension culture. It was observed that the amount of biomass of all the cell lines was at an average of 2-3 fold higher than the original inoculums weight with an incubation period of 30 to 60 days.

The carbon source, which is sucrose, was hydrolysed to glucose and fructose in the first 10 days and both were completely utilised after the 25th day. The sugar to biomass conversion yield was low and the mainly linear growth showed that the growth of the cell was limited by the culture conditions. Nitrogen sources from the MS media remained in excess until the end of the growth period where only 30% of ammonia and 15% of nitrates were utilised which resulted in the cell being toxic and thus limiting cell growth.

The growth was exponential in the first 10 days with a maximum specific growth rate of 0.07 day^{-1} which corresponded to a doubling time of 10 days. The cells then entered a period of linear growth until Day 25 to reach the maximum dry weight of 4 g/l, after which the cells began to die off causing the dry weight to fall to 2.8 g/l at Day 45.

The pH profile was an indication of the nitrogen and sugar uptake by the cells. The pH decreased rapidly from 5.6 to 4.0 in the first 9 days and then increased gradually to 4.4 at the 25th day. At this point, the cell growth had stagnated, and the pH quickly increased to 5.5 before declining again to the end of the culture at Day 45. The initial pH decrease was partly due to the uptake of ammonium. After this, however, the great increase was due to the uptake of nitrate ions to the ammonium stored in the vacuoles of the cell.

This study provides a better understanding of oil palm suspension culture in a bioreactor with regards to the growth, nutrient uptake and metabolite production. This information will further enhance the progress of oil palm clonal materials development towards mass propagation production.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PEMBIAKKAN KULTUR AMPAIAN SAWIT DI DALAM BIOREAKTOR SIXFOR

Oleh

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Julai 2006

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Pembiakkan secara *in vitro* adalah kaedah utama industri sawit kearah pengeluaran klon yang mempunyai hasil yang tinggi. Kultur ampaian sawit telah berjaya dilaksanakan dengan menggunakan sistem kelalang goncang di mana ianya dilakukan secara berterusan untuk penghasilan anak klon sawit. Walaubagaimanapun, sistem ini kurang efisen untuk pengeluaran berskala besar kultur ampaian sawit embriogenik.

Bioreaktor telah digunakan didalam industri pengeluaran bagi sektor mikrobiologi, haiwan dan metabolit tumbuhan. Walaubagaimanapun, ianya jarang dilaksanakan pada kultur ampaian sawit. Di dalam kajian menggunakan bioreaktor sixfor, nutrien dan pengeluaran metabolit telah diselidik. Di samping itu parameter kinetik dan penggunaan nutrien berbanding dengan penghasilan biomass telah dikira untuk menggambarkan keadaan sebenar kultur ampaian sawit. Semasa proses inkubasi 30 ke 60

hari, biomass telah meningkat secara purata sebanyak 2-3 kali lebih tinggi daripada berat asal.

Pada hari yang ke sepuluh sumber karbon iaitu sukrosa telah dihidrolis sepenuhnya kepada glukosa dan fruktosa. Kedua-dua sumber tersebut thabis digunakan selepas hari yang ke 25. Hasil pengeluaran biomass berbanding dengan gula adalah kecil dan pertumbuhan sel secara linear menunjukkan ianya agak terbatas disebabkan keadaan gula yang tidak mencukupi di dalam media. Sebaliknya, sumber nitrogen di dalam media MS adalah berlebihan di mana pada peringkat akhir eksperimen hanya 30% ammonia dan 15% nitrat yang telah digunakan. Ini menyebabkan sel bertoksid serta mengakibatkan pertumbuhannya terbantut. Di samping itu, terdapat keputusan eksperimen yang memberi tindak balas serta keputusan yang berbeza di antara satu sama lain. Ini adalah disebabkan sumber ortet yang pelbagai.

Pertumbuhannya di hari kesepuluh adalah pada tahap eksponential dengan kadar pertumbuhan spesifik sebanyak 0.07/hari yang berkadar sama dengan pengandaan sebanyak 10 hari. Sel kemudian melalui fasa pertumbuhan linear sehingga hari ke 25 untuk sampai ke tahap berat kering maksima 4 g/l. Ianya kemudian menurun ke 2.8 g/l di hari ke 45 bilamana sel mula mati.

Profil pH menunjukkan tahap pemakanan sumber nitrogen dan gula.. Di hari yang ke sembilan, pH menurun secara drastik dari 5.6 ke 4.0 dan

meningkat secara perlahan sehingga hari ke 25 kepada 4.4. Di masa ini pertumbuhan sel terbantut serta kemudiannya pH menaik secar drastic ke 5.5 sebelum menurun balik diperingkat pengakhiran kultur dihari ke 45. Penurunan pH dipemulaan adalah sebahagiannya disebabkan pemakanan ammonia. Selepas itu, peningkatan pH yang tinggi adalah disebakan pemakanan nitrat ion ke pada ammonia yang disimpan didalam sel vakuol.

Kajian ini telah memberikan penambahan pengetahuan terhadap kultur ampaian sawit dari segi pertumbuhan, pengambilan nutrien dan pengeluaran metabolit. Bagi tujuan pengkomersialan, adalah diharapkan segala hasil kajian yang didapati akan dapat mempercepatkan proses pengeluaran klon sawit secara besar-besaran.

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to Professor Madya Badlishah Sham Baharin, the Chairman of my Supervisor Committee, for his kind assistance and guidance through the course of my study till the preparation of this thesis. I am also very grateful to the other Supervisory Committee, Dr Ahmad Tarmizi Hashim and not least Professor Dr ChoKyun Rha.

Gratitude was also expressed to Professor Anthony J. Sinskey for his guidance and moral support in giving all the comments and his remarks that “Honeymoon is over let’s work” and “Did you learnt any new things” was still in my memory.

My sincere appreciation was extended to the Prof Chokyun Rha (Biomaterial and Engineering Department) and Prof Anthony Sinskey (Biology Department) Labs of Masssachusetts Institute of Technology USA especially to Dr. Phil Lessard, Dr T.G.Sambandan, Dr. Daniel Chooi and Dr Laura Willis for their encouragement during my 2 years benchwork at MIT. Special thanks to Dr. Nathalie Gorret for her kind assistance and guidance througout the project. Also to all MMBPP trainees for their encouragement and support.

Not the least but most important is my deepest appreciation to my wife Shahriah Md Khamis for her sacrificed and understanding in taking care of our childrens especially during our stayed at Apartment 6, 27 Whiting Street, 01902 Lynn, Massachusetts, USA. Also all this is not going to be meaningful

with the coolness of my children Farhah, Muhammad Harisah, Abu Hurairah, Nurshazana, Hanan, Dhamirah, Ahmad Danial and Luqman Hakim.

I also would like to acknowledge Director General Of MPOB and Massachusetts Institute of Technology for their support in coordinating the MMBPP trainees programmed. Thanks are also due to BIOTEK Malaysia, Ministry of Science, Technology and Innovation for successfully implementing MMBPP.

I certify that an Examination Committee has met on 13th July 2006 to conduct the final examination of Samsul Kamal Bin Rosli on his Master of Science thesis entitled "Establishment of Oil Palm Suspension Culture in the Sixfors Bioreactor" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

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