UNIVERSITY OF CAPE TOWN
DEPARTMENT OF MOLECULAR AND CELL BIOLOGY

MCB304S **Paper Two** **OCT/NOV 2001**
Time: 3 hours **Total marks 100**

Answer FOUR out of FIVE questions from Section A, and BOTH questions from Section B
In the interest of economy, please write on both sides of the exam script.

Section A

Answer **4 out of 5** questions: **(60 marks in total)**

1. Discuss the advantages of studying biodegradation of xenobiotics by microbial communities as opposed to their degradation by a single, purified bacterial strain. Include examples in your answer. [15]

2. The Cape Town City Council has commissioned your company to formulate a herbicide to control the water weeds which clog Zeekoevlei and Zandvlei each summer. However, since the country has become very conscious of environmental issues, your product must be biodegradable. Write an essay, in the form of a document to the Cape Town City Council, in which you describe the evidence you have concerning the safety and biodegradability of your product in order to reassure the authorities. Take note of the location in which your product will be used. [15]

3. You are part of a team in a biotechnology industry which produces enzymes. It is your responsibility to design a program to purify a cell extract containing these enzymes.
   a) Outline the strategy you might consider to effect the purification of the enzymes and discuss the factors that you would take into account when selecting the appropriate strategy(ies).
   b) Briefly list and define processes which could be considered specifically to fractionate the different enzymes in the extract.

   [15]

4. Using the balances for biomass and substrate, derive the equations defining the concentrations of biomass and substrate at steady state in a continuous biomass reactor, as a function of dilution rate and feed substrate concentration. [15]

5. The following steady state data were obtained for the growth of *Klebsiella aerogenes* on a glycerol-limited growth medium in a continuous bioreactor

D h^{-1}	c_X mg/ml	c_S mg/ml
0.04	3.15	0.02
0.07	3.10	0.03
0.10	3.15	0.05
0.12	3.10	0.08
0.14	3.05	0.11
0.16	3.00	0.17

Estimate the kinetic constants: K_S , μ_{max} and Y_{SX} if the feed substrate concentration is 10 mg/ml.

[15]

Graph paper required

Section B

Answer **BOTH** questions: **(40 marks in total)**

1. Please answer the following questions in the context of your group's project for the Entrepreneurship Module, i.e. the Agarase or Indigo project.

   a. Did your 'product idea' (i.e. improved agarase or environmentally-friendly Indigo) have unique features? If so, what were they? (4)

   b. What benefits does your product offer to potential customers? (4)

   c. Did you identify market segments (i.e. customer types, or customers with similar preferences) for which your product may be attractive? If so, why do you think your product might be attractive to them? (If possible, please estimate how much your product may be worth to them?) (8)

   d. What problems do you foresee in persuading such customers to switch to your product? (4)

   e. What are the most important factors to bear in mind when searching for information on markets? (2 bonus marks)

[20]

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2. The extracts "Enzyme & Microbial Technology 25 (1999) 23-30 (attached), concern the integration of foreign genes into the yeast chromosome in order to enhance the production of ethanol from biomass material. Interpret the results given in Fig. 7 with respect to the aims and achievements of the authors.
   Figure 1 is supplied for information of plasmid and strain construction.
   Figs 3, 5 and 6 may be useful in the discussion of the findings in Fig. 7. **[20]**

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Enzyme and Microbial Technology 25 (1999) 23–30

enzyme and microbial technology

δ-Integration of endo/exo-glucanase and β-glucosidase genes into the yeast chromosomes for direct conversion of cellulose to ethanol

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Abstract

For the direct conversion of cellulose to ethanol, recombinant yeast having a full set of genes for the cellulolytic enzymes was developed. Using the δ-sequences of the Ty1 retrotransposon as target sites for homologous recombination, heterologous genes of endo/exo-glucanase and β-glucosidase were integrated into the chromosomes of *Saccharomyces cerevisiae*. The number of both integrated genes was found to be approximately 44. This newly constructed yeast, *S. cerevisiae L2612δGC*, successfully expressed and secreted the cellulolytic enzymes. Expression levels of cellulolytic enzymes, cell growth, and ethanol production in cellulose-containing media were significantly increased in comparison with plasmid-based expression. Maintenance of the integrated genes was also perfect up to 50 generations even in nonselectable media. © 1999 Elsevier Science Inc. All rights reserved.

Keywords: δ-Integration; *Saccharomyces cerevisiae*; Cellulose; Ethanol; Endo-glucanase; Exo-glucanase; β-Glucosidase

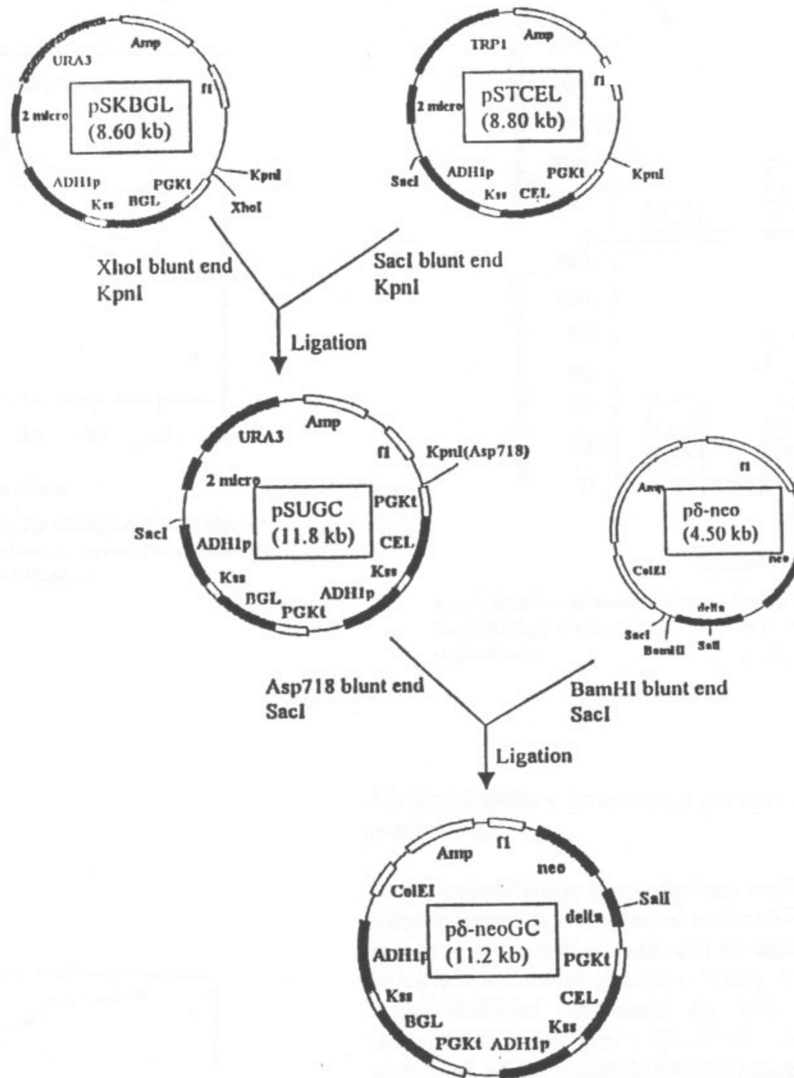

Fig. 1. Construction of the plasmid vector, pSUGC, and δ-integrative vector, pδ-neoGC, for both cellulase and β-glucosidase expressions in *S. cerevisiae L2612.*

3.2 The new cellulose-utilizing recombinant yeasts

By introducing pSUGC and pδ-neoGC into *S. cerevisiae L2612*, two cellulose-utilizing recombinant yeasts were developed. The plasmid (pSUGC)-harboring yeast was named *S. cerevisiae L2612GC*. The conditions of transformation and selection did not affect the characteristics of this recombinant yeast remarkably. However, these conditions greatly affected the characteristics of the δ-integrated recombinant yeast. After cutting with *Sal*I, linearized pδ-neoGC was transformed into *S. cerevisiae L2612*. Selection of transformants was made on YPD-G418 (0.5 g/l) plates on which the host strain could not survive. The resulting recombinant yeast was named *S. cerevisiae L2612δGC*. The conditions of transformation and selection such as electric capacitance and G418 concentration were optimized to get a good recombinant yeast strain. As shown in Table 1, optimal G418 concentration was 1.0 g/l. The optimum capacitance of electroporation was obtained at 25 μF with the pulsing time of 1.54 ms. However, the integrated copy number of genes was revealed to be higher in the transformant obtained at 50 μF than that of the transformant obtained at 25 μF through the Southern hybridization analysis, as shown in Fig. 2. By using these results, we isolated a recombinant yeast strain, which showed the highest enzyme activities and integrated copy number of the genes.

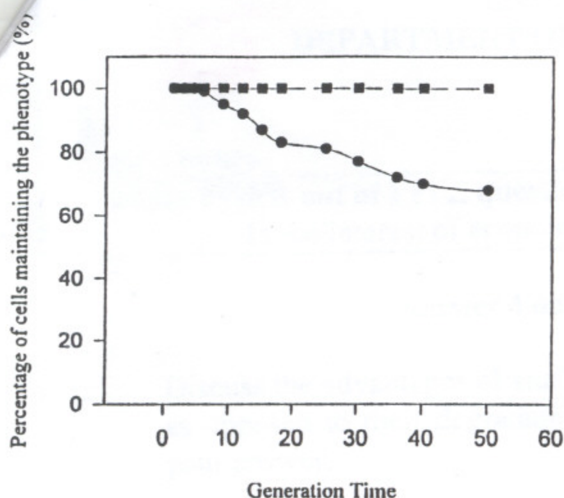

Fig. 3. Maintenance of the recombinant genes transformed with plasmid (pSUGC) and δ-integrative (pδ-neoGC) vectors in recombinant yeasts. ●: *S. cerevisiae L2612GC*; ■: *S. cerevisiae L2612δGC*.

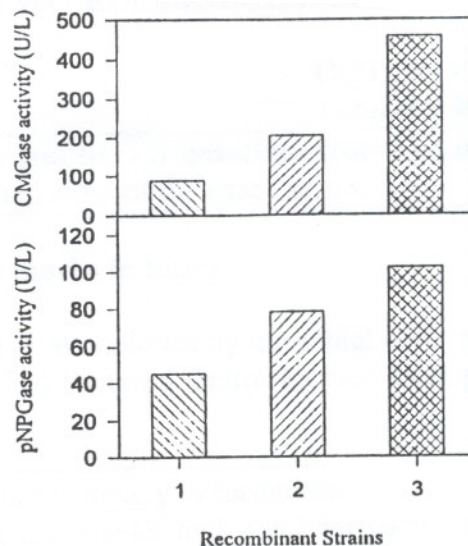

Fig. 5. Relation between δ-integrated copy number of genes and expression level. Colony numbers 1, 2, and 3 have 4, 8, and 40 δ-integrated genes, respectively.

3.5. Batch culture dynamics of the recombinant yeast strains

The recombinant yeast showing the highest cellulolytic enzyme activities was selected to examine recombinant protein expression, cell growth, and ethanol production in cellulodextrins-containing media (YPC). The cellulolytic enzyme activities expressed by this new δ-integrated recombinant yeast, *S. cerevisiae L2612δGC*, was compared with those of the plasmid (pSUGC)-harboring recombinant yeast, *S. cerevisiae L2612GC*.

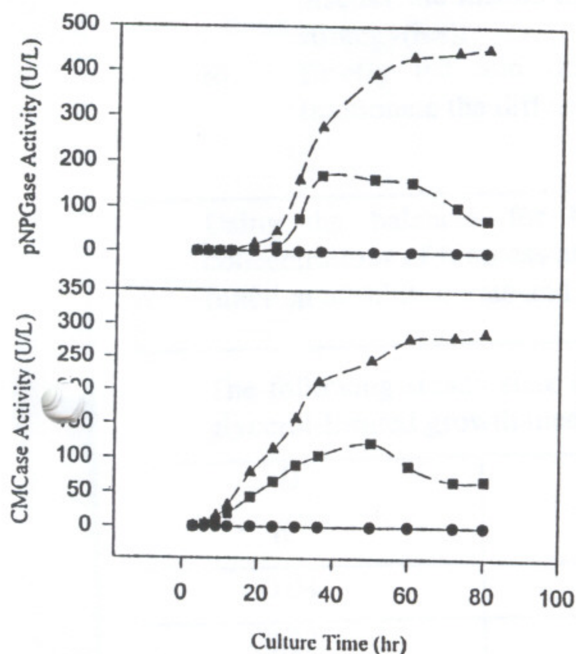

Fig. 6. Time courses of endo/exo-glucanase and β-glucosidase expression. ●: *S. cerevisiae L2612*; ■: *S. cerevisiae L2612GC*; ▲: *S. cerevisiae L2612δGC*.

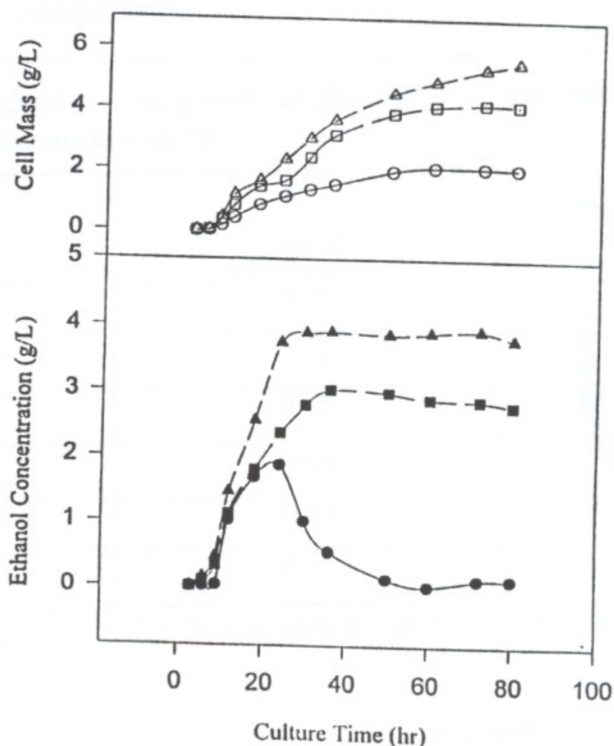

Fig. 7. Time courses of cell growth and ethanol production. ●, ○: *S. cerevisiae L2612*; ■, □: *S. cerevisiae L2612GC*; ▲, Δ: *S. cerevisiae L2612δGC*.