Biomass conversion - Production of bio-fuels and chemicals from starch and cellulose

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Display of novel enzymes on the yeast cell surface is very powerful method to develop the efficient whole cell biocatalysts, because the diffusion problem of substrate and product is circumvented [1]. Yeast has shown to have a potential to display large molecular weight enzymes. Moreover, rigid structure of yeast cells would make them practically appropriate as microbial whole cell biocatalysts. In this study, we have developed the various new methods to display novel enzymes and their applicability to the practically important reactions, namely, productions of biofuels and chemicals from biomass was studied.

The plasmids for cell surface display of amylolitic enzymes, cellulolityic enzymes and lipases by fusing with cell wall anchoring proteins α-agglutinin and flocculin flo1p with various lengths were constructed and introduced into yeast cells. The ethanol production from starchy and cellulosic materials was investigated by yeast cells displaying amylolytic enzymes and cellulolytic enzymes, respectively. In addition, biodesel fuel production from plant oil and methanol was investigated by yeast cells displaying lipase.

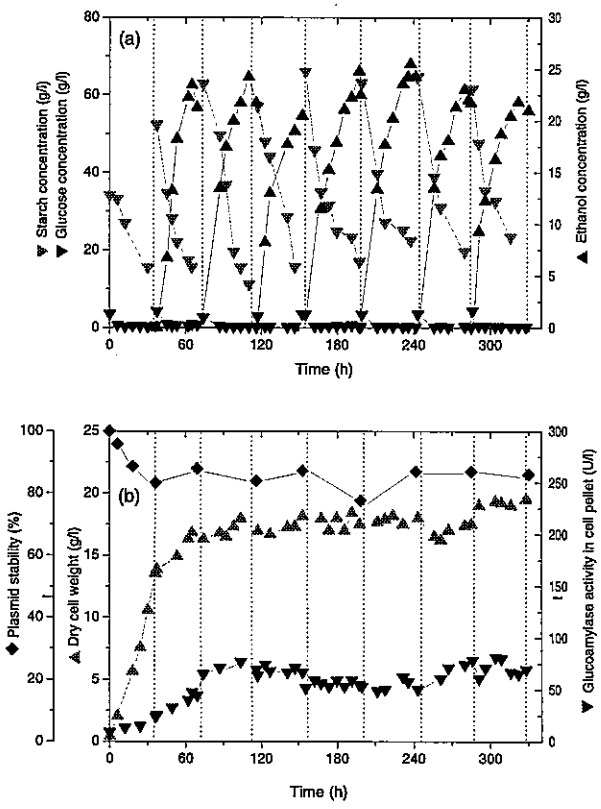

Figure 1. Repeated-batch ethanol production from Starch by glucoamylase surface displayed flocculent yeast cells

Repeated batch fermentation of soluble starch to ethanol by yeast displaying *Rhizopus oryzae* glucoamylaseshowed that the cells maintained high ethanol production rates from soluble starch over 300 h **(Fig.1)** **[2]**.To further improve ethanol productivity from starchy materials, both co-display and secretion of *Bacillus stearothermophilus* α-amylase were found to be effective in glucoamylase-displaying yeast cells [**3,4**].

In addition, yeast displaying *Trichoderma reesei* endoglucanase II, cellobiohydrolase II and *Aspergillus*

aculeatus β-glucosidase directly fermentbarley β-glucan and amorphous cellulose to ethanol (**Figs.2 and 3**) **[5-8]**.

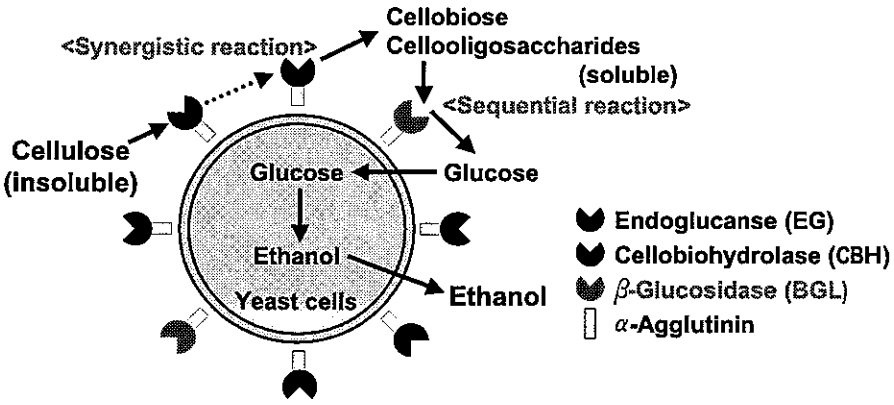

Figure 2. Schematic diagram of direct ethanol production from cellulose using yeast strain co-displaying three types of cellulolytic enzyme on the cell surface.

The above results showed that yeast cells displaying cell surface amylolytic and cellulolytic enzymes are very effective for direct fermentation of starchy and cellulosic materials to ethanol. On the other hand, yeast cells displaying *R. oryzae* lipase successfully synthesize methyl esters (MEs) from the plant oil and methanol in a water-containing system without an organic solvent. ME content reached over 80% by stepwise addition of methanol to the reaction mixture **[9,10]**.

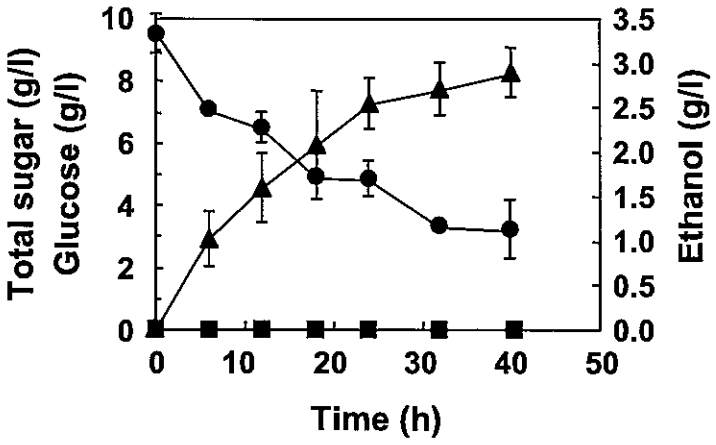

Figure 3. Time course of production of ethanol from amorphous cellulose as the sole carbon source using yeast strain displaying three types of cellulase. Symbols: ▲, ethanol; ●, total sugar; ■, glucose in culture broth. The data points represent the average of seven independent experiments.

By displaying novel enzymes on the cell surface, efficient whole cell biocatalysts were constructed. The displayed enzymes are regarded as a kind of self-immobilized enzyme on the cell surfaces. As shown in the case of ethanol production, a combination of cell surface displayed enzyme system and intracellular metabolic system is a very effective approach to develop cells with novel metabolic ability for industrial applications. In addition, success of bioconversion by lipase displayed yeast cells shows that they are applicable to the various bioconversions in organic compounds. Since various methods to display active enzymes are now available, this approach will be applicable to various bioconversion processes. By combining surface displayed enzymes, intracellularly overexpressed enzyme and intracellular metabolic system, various types of novel whole cell biocatalysts could be prepared (**Fig. 4**). They will open up the various new applications of whole cell biocatalysts to the industrially important processes.

References

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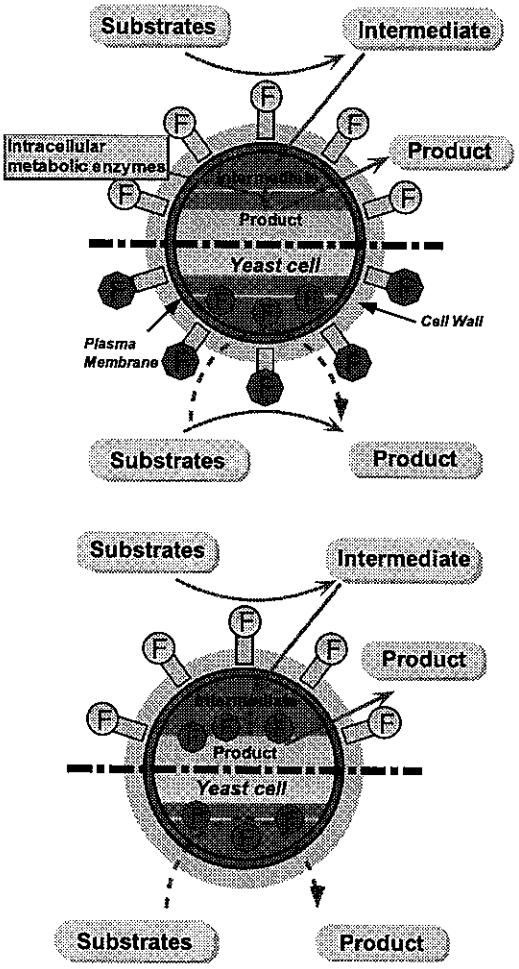

Figure 4 Novel whole cell biocatalysts by combining surface displayed enzyme and intracellular enzymes.

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