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Short Communication

The feasibility of growing cells of *Saccharomyces cerevisiae* for citronellol production in a continuous-closed-gas-loop bioreactor (CCGLB)

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ABSTRACT

The present work aims to address the gas-phase biotransformation of geraniol into citronellol using growing cells of *Saccharomyces cerevisiae* (baker's yeast) in a continuous-closed-gas-loop bioreactor (CCGLB). This study revealed that the gaseous geraniol had a severe effect on the production of biomass during the growing cell biotransformation resulting in the decrease in the specific growth rate from 0.07 to 0.05 h^{-1}. The rate of reaction of the growing cell biotransformation was strongly affected by agitation and substrate flow rates. The highest citronellol concentration of 1.18 g/L and initial rate of reaction of 7.06×10^{-4} g/min g_{cell} were obtained at 500 rpm and 8 L/min, respectively.

1. Introduction

Whole-cell biotransformation has become one of the outstanding synthetic processes to produce enantiopure organic compounds especially for process that involved reduction reaction. Reduction reaction requires co-substrates for cofactor regeneration, preferentially performed with living cells as they provide an *in situ* cofactor regeneration system. The whole cells of *Saccharomyces cerevisiae* (baker's yeast) is the most common microorganisms used as a biocatalyst in reduction of organic compounds including drugs and monoterpenoids as it offers high redox capacity and generally much less expensive and easy to handle (Perles et al., 2008; Carballeira et al., 2009; Khor and Uzir, 2011).

Previous works carried out for biotransformation of geraniol using baker's yeast had dealt with the resting cells instead of the growing cells (Doig et al., 1998; Valadez-Blanco et al., 2008). The resting cell system separates between cell growth and biotransformation reaction and thus, avoids interference of the cell growth to the reaction (Wang et al., 2005). Furthermore, product isolation in the growing cell system is rather complex due to a mixture of fermentation medium with the product formed from the biotransformation itself (Kieslich et al., 1984).

The current research aimed at studying a gas-phase biotransformation of geraniol using the growing cells of baker's yeast in a continuous-closed-gas-loop bioreactor (CCGLB). The gas-phase system avoids direct contact between substrate, product and the reaction medium and therefore, the use of growing cells will not be directly influenced by the system. In addition, it promotes better diffusion and thus, eliminates mass transfer limitation between gas and liquid phases (Lamare and Legoy, 1993). Such a system has been successfully applied for fermentation (Taylor et al., 2010) as well as for whole-cell biotransformation (Maugard et al., 2001; Goubet et al., 2002; Pescheck et al., 2009).

2. Methods

2.1. CCGLB configuration

The configuration of CCGLB system initially proposed by Steinig et al. (2000) was set-up with some modifications in order to fulfill the current biotransformation conditions. The system mainly consists of three reservoirs; substrate, whole-cell and product as shown in Fig. 1. The gaseous substrate was sparged continuously from the substrate reservoir (1) containing 200 mL liquid geraniol (Aldrich Chemical Co., USA) and subsequently flowed into the 5 L bioreactor vessel (2) (Labfors, Infors AG, Switzerland) through an air pump (Gast Manufacturing Inc., USA). The flow rate was adjusted by using a flow meter which was already integrated into the bioreactor system. The gaseous substrate further diffused into the medium which contained baker's yeast (Sigma Chemical Co., USA) and formed the corresponding gaseous product, citronellol. The product then left the bioreactor vessel via a condenser mounted on top of the bioreactor and later dissolved into a

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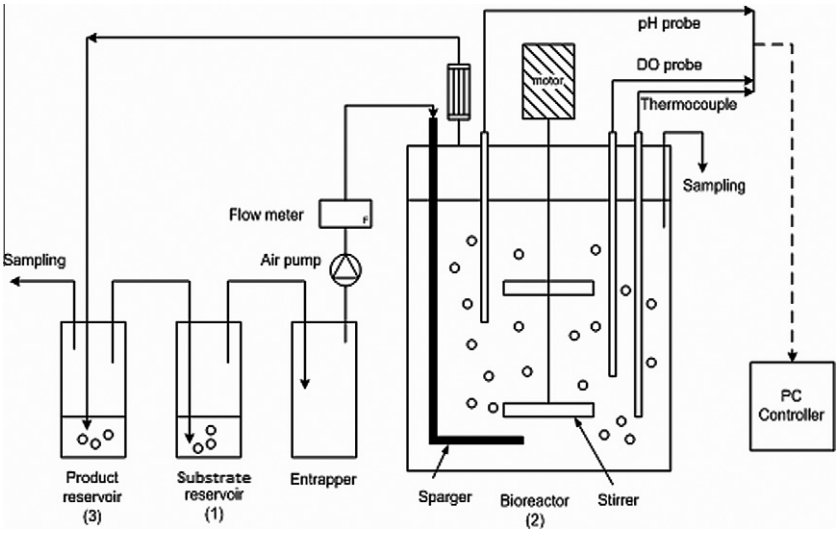

Fig. 1. Schematic diagram of the CCGLB system.

prepared 200 mL hexadecane (Aldrich Chemical Co., USA), which acted as a solvent in the product reservoir (3). The sample was ready to be analyzed using a gas chromatography.

2.2. Growing cell biotransformation

An anaerobic fermentation of yeast was carried out at pH 4 and 30 °C (optimized conditions). The composition of the media consisted of glucose (10 g/L), peptone (6 g/L) and yeast extract (3 g/L) (Fluka, USA). Using an aseptic technique, 1 g/L baker's yeast was added into the fermentation media. Biotransformation was carried out simultaneously by sparging gaseous substrate into the media for 36 h.

2.3. Analytical method

Biomass from the fermentation broth was measured using a spectrophotometer (Cecil 1000 series, UK) at 680 nm with appropriate dilution. The concentration of citronellol was measured using a gas chromatography (Perkin Elmer, Clarus 500, USA) equipped with a flame ionization detector (FID). A Supelcowax^{10} capillary column (30 m × 0.25 mm i.d × 0.25 μm film thickness; France) was used for the chromatographic separation. The injector and the detector were kept at 200 and 250 °C, respectively. The initial oven temperature of 160 °C was held for 3 min and then ramped at a rate of 8 °C/min to 230 °C. Helium was used as the carrier gas (Air Products, Malaysia) and the flow rate in the column was maintained at 2.5 mL/min. Hydrogen and air (Air Products, Malaysia) were supplied to the FID at 30 and 300 mL/min, respectively. Each sample was injected at least twice and the mean value was taken. An external standard method was used in sample analysis and calculations.

2.4. Determination of initial rate of reaction and specific activity

The initial rate of citronellol production was calculated for each batch experiment from plots of product accumulation against time (Doig et al., 1998). The ratio of citronellol produced per minute per gram of cell was measured to represent the corresponding initial rate of reaction. The specific activity of cells, (U/g_{cell}) or (μmol/min g_{cell}) was calculated from the ratio of the initial rate of reaction and molecular weight of citronellol (g/μmol).

3. Results and discussion

3.1. Effect of gaseous substrate (geraniol) on yeast growth

Fig. 2 represents the profile of yeast growth with and without the presence of gaseous geraniol. It shows that the growing cells were capable to perform the biotransformation in the CCGLB at which an accumulation of product was detected during the 36 h of reaction. About 0.77 g/L of total citronellol formation was obtained to give the initial rate of reaction and specific activity of 4.31×10^{-4} g/min g_{cell} and 2.76 U/g_{cell}, respectively. Growth of the cells however, showed a slight decline in the specific growth rate when the gaseous substrate started to accumulate in the fermentation medium. The cell growth without the presence of gaseous geraniol gives the specific growth rate of 0.07 h^{-1}, while with the presence of gaseous geraniol only gives 0.05 h^{-1}. This indicates that there was a competition of yeast cells to grow and simultaneously to catalyze the reduction reaction. Both processes directly compete for the same cofactor, which is regenerated in the glycolytic pathway (Perles et al., 2008). They may also compete for the same active sites of enzyme that responsible for the

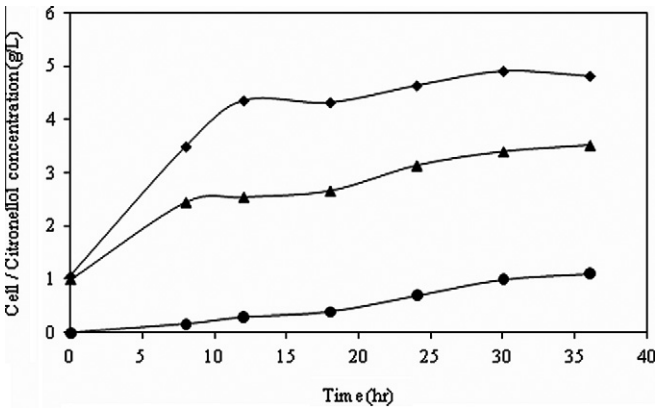

Fig. 2. Growth and biotransformation profiles during the course of geraniol reduction using growing cells of *S. cerevisiae* in CCGLB. The reaction was carried out for 36 h (conditions: pH 4, 30 °C, agitation rate of 350 rpm, substrate flow rate of 8 L/min, 10 g/L glucose, 1 g/L baker's yeast). '♦' Represents growth with free substrate (control); '▲' represents growth with biotransformation; '●' represents citronellol formation.

Table 1
Performance of growing cells of baker's yeast during biotransformation of geraniol in a CCGLB at different agitation and substrate flow rates.

Parameter	Value	Maximum product formation (g/L)	Initial rate of reaction (10^{-4} g/min g_{cell})	Specific activity (U/g_{cell})	Specific growth rate (h^{-1})
Agitation rate (rpm)	200	0.34	1.02	0.66	0.043
	350	0.77	4.31	2.76	0.056
	500	1.18	7.06	4.52	0.072
	800	0.85	5.03	4.14	0.063
Substrate flow rate (L/min)	4	0.56	1.82	1.16	0.052
	6	0.72	3.22	2.06	0.058
	8	1.18	7.06	4.52	0.072

reaction (Steinig et al., 2000). Even though the cell growth was severely affected by the substrate (geraniol), several strategies could be developed in order to increase the biomass production as well as the biotransformation productivity. The effects of agitation and substrate flow rates are believed to give a significant impact to both processes and they were further investigated in this study.

3.2. Effect of agitation rate

Study on the effect of agitation rate was carried out in order to identify the range of agitation rates that could promote a high specific growth rate as well as the rate of reaction in the CCGLB. A set of experiments was conducted by varying the stirrer speed between 200 and 800 rpm at a fixed substrate flow rate of 8 L/min, which is the maximum flow rate indicated on the flow meter. Table 1 shows the complete results from these experiments. By gradually increasing the agitation rate from 200 to 500 rpm, the biomass and citronellol production consequently increased, which implies that mechanical mixing helps to increase contacts between the cells and the nutrients in liquid media, and subsequently increased the cell metabolism and therefore the cell regeneration. Agitation rate at 800 rpm tends to decrease the specific growth rate as well as the initial rate of reaction with approximately 18% reduction of the total citronellol formation compared to that at 500 rpm. The decline in cell growth and citronellol productivity are probably due to the damage of one or more cellular component of yeast cells caused by the excessive mechanical stress (Saito et al., 2000; Bandaiphet and Prasertsan, 2006).

3.3. Effect of substrate flow rate

Substrate flow rate ranging from 4 to 8 L/min were investigated at constant agitation rate of 500 rpm. As indicated in Table 1, a lower substrate flow rate not only decreases the citronellol formation, but also the biomass production. Only 0.56 g/L citronellol and 3.52 g/L biomass were produced at the flow rate of 4 L/min at approximately a reduction of 47% and 14% respectively, than that determined at 8 L/min. The lower amount of citronellol produced at a lower substrate flow rate has led to a reduction in the initial rate of reaction. From these results, it can be deduced that, by accelerating the diffusion of geraniol into the aqueous phase seems to meet the demand of high biomass and citronellol production. The dispersion of the gaseous substrate may also help the movement of nutrients to diffuse from the liquid phase into the cellular compartment (Potumarthi et al., 2007; Garcia-Ochoa and Gomez, 2009).

4. Conclusions

A volatile compound of geraniol can be biologically transformed into citronellol by baker's yeast using the CCGLB system during its growth. Product formation was observed to be highly dependent on the cell growth. This information is believed to be the first encountered on such studies, since there are no previous investigations on biotransformation process during the exponential phase of cell growth. The growth of yeast was severely affected with the presence of substrate for biotransformation. However, strategies on varying parameters such as agitation and substrate flow rates were successfully introduced in order to enhance the overall productivity during the growing phase of a microorganism.

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References

Bandaiphet, C., Prasertsan, P., 2006. Effect of aeration and agitation rates and scale-up on oxygen transfer coefficient, k_La in exopolysaccharide production from *Enterobacter cloacae* WD7. Carbohyd. Polym. 66, 216–228.
Carballeira, J.D., Quezada, M.A., Hoyos, P., Simeó, Y., Hernaiz, M.J., Alcantara, A.R., Sinisterra, J.V., 2009. Microbial cells as catalysts for stereoselective redox reactions. Biotechnol. Adv. 27, 686–714.
Doig, S.D., Boam, A.T., Leak, D.I., Livingston, A.G., Stuckey, D.C., 1998. Optimisation of the kinetics of the stereoselective reduction of geraniol to citronellol in a two liquid phase system. Biocatal. Biotransform. 16, 27–44.
Garcia-Ochoa, F., Gomez, E., 2009. Bioreactor scale-up and oxygen transfer rate in microbial processes: an overview. Biotechnol. Adv. 27, 153–176.
Goubet, I., Maugard, T., Lamare, S., Legoy, M.D., 2002. Role of water activity and temperature on activity and stability of dried whole cells of *Saccharomyces cerevisiae* in a continuous solid–gas bioreactor. Enzyme Microb. Technol. 31, 425–430.
Kieslich, K., Rehm, H., Reed, G., 1984. Biotechnology vol. 6a: Methodology, 6a. Verlag–Chemie, p. 11.
Khor, G.K., Uzir, M.H., 2011. *Saccharomyces cerevisiae*: a potential stereospecific reduction tool for biotransformation of mono and sesquiterpenoids. Yeast 28, 93–107.
Lamare, S., Legoy, M.D., 1993. Biocatalysis in the gas phase. TibTechnol 11, 412–418.
Maugard, T., Lamare, S., Legoy, M.D., 2001. Gas phase biotransformation reaction catalyzed by baker's yeast. Biotechnol. Bioeng. 73, 164–168.
Perles, C.E., Moran, P.J.S., Volpe, P.L.O., 2008. Bioreduction of ethyl 3-oxobutyrate by *Saccharomyces cerevisiae*: a metabolic *in vivo* study. J. Mol. Catal. B: Enzyme 52–53, 1–6.
Pescheck, M., Mirata, M.A., Brauer, B., Krings, U., Berger, R.G., Schrader, J., 2009. Improved monoterpene biotransformation with *Penicillium* sp. by use of a closed gas loop bioreactor. J. Ind. Microb. Biotechnol. 36, 827–836.
Potumarthi, R., Subhakar, Ch., Jetty, A., 2007. Alkaline protease production by submerged fermentation in stirred tank reactor using *Bacillus lincheniformis* NCIM-2042: Effect of aeration and agitation regimes. Biochem. Eng. J. 34, 185–192.
Saito, I., Honda, H., Kawabe, T., Mukumoto, F., Shimizu, M., Kobayashi, T., 2000. Comparison of biotin production by recombinant *Sphingomonas* sp. under various agitation conditions. Biochem. Eng. J. 5, 129–136.
Steinig, G.H., Livingston, A.G., Stuckey, D.C., 2000. Bioconversion of hydrophobic compounds in a continuous closed-gas-loop bioreactor: feasibility assessment and epoxide production. Biotechnol. Bioeng. 70, 553–563.
Taylor, F., Marquez, M.A., Johnston, D.B., Goldberg, N.M., Hicks, K.B., 2010. Continuous high-solids corn liquefaction and fermentation with stripping of ethanol. Bioresour. Technol. 101, 4403–4408.
Valadez-Blanco, R., Ferreira, F.C., Jorge, R.F., Livingston, A.G., 2008. A membrane bioreactor for biotransformations of hydrophobic molecules using organic solvent nanofiltration (OSN) membranes. J. Membr. Sci. 317, 50–64.
Wang, Z., Zhao, F., Chen, D., Li, D., 2005. Cloud point system as a tool to improve the efficiency of biotransformation. Enzyme Microb. Technol. 36, 589–594.