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Effect of process parameters on immobilization of recombinant *Escherichia coli* on pineapple peel

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ABSTRACT

Cyclodextrin can be produced from the degradation of starch using the enzyme cyclodextrin glucanotransferase (CGTase). Its favorable characteristics have seen its use in industries such as cosmetics, personal care, textiles, and pharmaceuticals. The production of CGTase by wild-type *Bacillus* sp. is low, so recombinant *Escherichia coli* has been used for higher enzyme yields. Cell lysis and plasmid instability are among the challenges that emerge during recombinant enzyme excretion that hinder the production of recombinant CGTase in *E. coli*. In this study, a cell immobilization technique using pineapple peel was employed to overcome this problem. The effects of changing process parameters such as pH, contact time, and temperature on the immobilization of recombinant *E. coli* were studied, one parameter at a time. The optimal conditions for the production of cyclodextrin were pH 8 leading to a 55.95% immobilization yield, a contact time of 24 h for a 55.16% immobilization yield, and a temperature of 25 ℃ for 53.11% immobilization yield. In brief, pineapple peel was determined to be a suitable supporting matrix and optimized process parameters increased the immobilization of recombinant *E. coli*, improving CGTase production while maintaining low cell lysis.

Keywords: cell immobilization; recombinant *E. coli*; cyclodextrin glucanotransferase; pineapple peel; process parameter

1. INTRODUCTION

The cyclodextrin molecule is cylindrical in shape with a hydrophobic interior and hydrophilic exterior (Řezanka, 2016). This allows it to form inclusion complex adducts with a large range of molecules via host-guest interactions, in many cases altering the solubility of the hydrophobic interior in an aqueous medium. Cyclodextrin glucanotransferase (CGTase) is one of the enzymes that has been used for production of cyclodextrin and its demand is increasing in many industries including pharmaceutical, textile, and food.

Generally, the reported yields of CGTase through *Bacillus* sp. production is low, typically less than 50 U/mL of CGTase activity before the optimization of the process parameters and culture medium (Mahat et al., 2004; Mora et al., 2012; Rahman et al., 2004). The CGTase gene used in this study was previously isolated from the *Bacillus lehensis* G1 genome, and its CGTase production was reported to be a relatively low ~ 20 U/mL (Mahat et al., 2004).

Recombinant *E. coli* has been used for its superior CGTase production properties compared to its wild-type counterpart through recombinant technology, which generally shows a significantly improved way of producing enzymes in bulk solution. Excretion of recombinant enzyme into culture medium offers several benefits compared to cytoplasmic expression; protein purification is simplified and product stability can be improved (Nor Ashikin et al., 2022). However, cell lysis and plasmid instability are among the challenges that emerge during recombinant enzyme excretion. To overcome these issues, cell immobilization is utilized to minimize environmental stress and to improve recombinant CGTase excretion.

Cell immobilization involves physical or chemical fixation of cells onto or into a support, for example, adsorption of cells on a surface, cell flocculation, covalent binding of cells to a carrier, cell entrapment, and cell encapsulation (Abdul Manaf et al., 2020; Boura et al., 2022). Cell immobilization can improve cell stability, allowing for easier cell separation and reuse, and can protect the cells from environmental stresses such as changes in temperature, pH, or solvent (Eş et al., 2015; Guzik et al., 2014; Lapponi et al., 2022). Cell immobilization by adsorption is gaining popularity because it allows direct contact between the cells and the nutrients in the medium, thereby diminishing or removing mass transfer limitation. A study conducted by Abd Rahman et al. (2020) showed that the immobilization of recombinant *E. coli* on untreated multi-walled carbon nanotubes (MWCNTs) via adsorption resulted in the highest xylitol yield (5.47 g/L). The adsorption reduced mass transfer limitations, hence contributing to enhanced bioconversion of xylose to xylitol.

Many materials have been utilized as cell immobilization supports with the aim of improving product yield and cell performance, such as alginate–calcium hydrogel (Cao et al., 2022), activated charcoal (Pachelles et al., 2021), algaroba gum (de Souza et al., 2022), and hollow fiber membrane (Che Man et al., 2015). Kyriakou et al. (2020) reported an ethanol yield of 63 g/L and a productivity of 7.9 g/L·h from *Saccharomyces cerevisiae* immobilized on pistachio nut biochar. The process also significantly improved the cell reusability for up to six repeated batch experiments compared to the unsupported cell culture.

Biomass waste such as pineapple peel, coconut husk, and sawdust are suitable supports for cell immobilization (Chwastowski and Staroń, 2022). In this study, pineapple peel was selected as the support for recombinant *E. coli* immobilization. Pineapple (*Ananas comosus*) is a popular tropical fruit in Malaysia and it is classified as a major fruit because it could potentially generate significant income for the agricultural sector. While the pineapple flesh is carved out and either eaten raw or made into a variety of sweets, pastries, juices, and concoctions, the peel and the crown are discarded as waste. Typically, about half of a pineapple's mass ends up as waste, in the form of residual pulps, peels, and crowns, which are disposed of as domestic waste. However, pineapple peels could be added to animal feed, or used as a reducing agent, antioxidant, fertilizer, or substrate for bio-ethanol production (Badrulzaman et al., 2021; Erukainure et al., 2012; Saraswaty et al., 2017).

Pineapple peel is mostly composed of hemicellulose and cellulose (Aditiya et al., 2016), which means it can bind cells for immobilization purposes. Moreover, pineapple peel is environmentally benign and inexpensive. Given these features and characteristics, pineapple peel could be used for cell immobilization support delivering for high immobilization yield of the recombinant *E. coli* with easy separation for batch or continuous processes.

In the present study, the immobilization of recombinant *E. coli* on pineapple peel via the simple *in situ* adsorption technique, without treatment with other chemicals, was studied. The effect of process parameters such as pH, contact time, and temperature on the immobilization process were investigated. To date, no studies have been performed on the immobilization of recombinant *E. coli* through adsorption using untreated pineapple peel.

2. MATERIALS AND METHODS

2.1 Materials

Pineapple peel was obtained from Tunas Manja Sdn Bhd, Kuantan, Pahang. Glycine, tryptone, glycerol, sodium chloride, yeast extract, ampicillin, magnesium chloride, potassium chloride, phenolphthalein, and sodium were purchased from Friendemann Schmidt (Parkwood, Australia). Dibasic sodium phosphate (Na_2HPO_4), sodium hydroxide (NaOH), and monobasic sodium phosphate (NaH_2PO_4) were purchased from Merck Sdn Bhd (Selangor, Malaysia).

2.2 Recombinant *E. coli* immobilization on pineapple peel

Recombinant *E. coli,* carrying CGTase gene from *Bacillus lehensis* G1, was produced by the method outlined by Jonet et al. (2012) and it was obtained from the Genetic Laboratory, Universiti Teknologi Malaysia. *E. coli* JM109 was used as the cloning host and *E. coli* BL21 (DE3) was used for expression. The pET-21a (+) system (Novagen) was employed as a vector backbone for cloning. Ampicillin, an antibiotic, was used at a concentration of 100 µg/mL.

The pineapple peel was dried at 90 °C and sterilized at 121 °C for 15 min, then weighed on an analytical balance before the immobilization process. In a 250 mL flask, a strip of sterilized dried pineapple peel [3 cm (width) × 3 cm (length) × 0.5 cm (thickness)] was combined with 50 mL Luria Bertani medium (10 g/L tryptone, 5 g/L yeast extract, 5 g/L NaCl) and ampicillin. The pineapple peel was cultivated with the recombinant *E. coli* from the glycerol stock at 37 °C, for 18 h at 200 rpm for the cell immobilization process. Then, the pineapple peel was rinsed with sterilized distilled water to remove loose cells. The peel was placed on a weighing boat and dried in an oven until constant weight was achieved. Lastly, the weight of the dried peel was recorded.

2.3 Effect of process parameters on cell immobilization

2.3.1 Effect of pH

The effect of pH on the immobilization of recombinant *E. coli* on pineapple peel was studied by conducting the immobilization process with the medium pH values set at pH 5, 6, 7, 8, 9 and 10. Phosphate-citrate buffer was used to adjust the medium to pH 5 and pH 6, sodium phosphate buffer for pH 7 and pH 8, and glycine-NaOH buffer for pH 9 and pH 10. Each of the immobilization runs was conducted at 25 °C with 200 rpm agitation for 24 h. The immobilization yield was then calculated as described in Equation 1.

$$X\ (\%) = \frac{(W_1 - W_2)}{W_2} \times 100\% \quad (1)$$

where *X* is the immobilization yield, while W_1 and W_2 are the weight of pineapple peel before and after the immobilization process, respectively.

2.3.2 Effect of contact time

The effect of contact time on the immobilization of recombinant *E. coli* on pineapple peel was studied by conducting the experiment at varying contact times of 12 h, 15 h, 18 h, 21 h, 24 h, and 27 h. Each of the immobilization runs was conducted at 25 °C, pH 8, and 200 rpm agitation. Afterward, the immobilization yield was calculated using Equation 1.

2.3.3 Effect of temperature

The immobilization procedure was performed at 20 °C, 25 °C, 30 °C, 35 °C, and 40 °C to study the influence of temperature on the immobilization of recombinant *E. coli* on pineapple peel. Each run was performed at pH 8 with 200 rpm agitation for 24 h. The immobilization yield was determined as per Equation 1.

2.4 Analytical methods

2.4.1 Cell Density

The density of immobilized cells was determined using Equation 2:

$$\mathrm{X(mg/mL)} = \frac{(\mathrm{W_1 - W_0})}{\mathrm{V}} \times 1000 \qquad (2)$$

where X is the cell density, W_1 is the weight of the dry cell and the pineapple peel (mg), W_0 is the weight of the dry pineapple peel (mg), and V is the total volume of the pineapple peel (4.5 mL).

The cell density of free cells was determined using Equation 3.

$$X(mg/mL) = \frac{W_1 - W_0}{V_s} \times 1000 \qquad (3)$$

where X is the cell density, W_1 is the weight of the dry cell and the dry filter paper (mg), W_0 is the weight of the dry filter paper (mg), and V_s is the sample volume (mL).

2.4.2 Scanning electron microscopy (SEM)

The micrographs of the pineapple peel were taken using SEM. The pineapple peel was fixed by immersing it in a 2.5% glutaraldehyde solution overnight at 4 °C. After that, it was thoroughly washed with distilled water and dehydrated by successively immersing it in 50%, 70%, 80%, 95%, and 100% ethanol, for 10 min per immersion. The pineapple peel was then dried at the critical point. The completely dried pineapple peel was coated with gold, and its SEM image was taken using a HITACHI TM3030 instrument.

3. RESULTS AND DISCUSSION

3.1 Effect of pH on immobilization of recombinant *E. coli*

In this study, recombinant *E. coli* was immobilized on pineapple peel at various pH values (pH 5, pH 6, pH 7, pH 8, pH 9, and pH 10) to determine the pH level for the highest immobilization yield. Each immobilization was conducted at 25 °C, with 200 rpm agitation, for 24 h. As shown in Figure 1, the highest immobilization yield of 55.95% was obtained with the medium at pH 8. Adsorption highly depends on the surface properties of both the cell and the support, such as surface charge, hydrophobicity, and hydrophilicity, thus these factors must be taken into consideration when optimizing cell immobilization on a support. The outer coating of *E. coli* is hydrophilic and net negatively charged (Baek et al., 2015; Bugg et al., 2011). The surface of the pineapple peel contains an abundance of hydroxyl ions (OH^-) and behaves as a hydrophilic support. Therefore, one possible mechanism for the effective adsorption of recombinant *E. coli* on pineapple peel is hydrophilic interaction; a second possible mechanism is the electrostatic interaction between the negatively charged (recombinant *E. coli* and OH^- at pH 8) and positively charged domains on the surface of recombinant *E. coli*. The positively charged domains consist of the unique combination of proteins, carbohydrates, polysaccharides, lipids, and DNA (Pachelles et al., 2021; Steinberger and Holden, 2004) that form a biofilm. The electrostatic interaction between the pineapple peel and the biofilm could induce the recombinant *E. coli* to adsorb onto the pineapple peel, which would explain the high cell immobilization yield detected at pH 8. Similarly, a study conducted by Li et al. (2019) showed that pH 8 was the optimal pH for the immobilization of *E. coli* expressing recombinant glycerol dehydrogenase on mannose-functionalized magnetic nanoparticles, resulting in an 82.4% immobilization yield. The formation of hydrogen bonds and hydrophobic interactions between the cells and the support was pH-dependent.

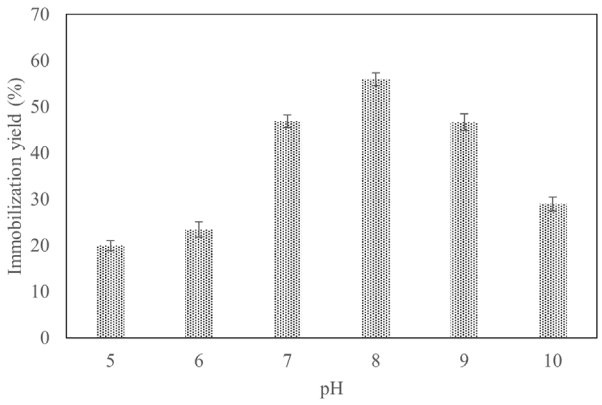


Figure 1. Effect of pH on immobilization yield

The cell immobilization conditions were set at 25 °C and 200 rpm agitation. The immobilization yield was calculated after 24 h of contact time. The highest immobilization yield was detected at pH 8. When the medium was too acidic (pH 5) or too alkaline (pH 10), the attachment of recombinant *E. coli* on the pineapple peel was not favorable. These conditions were not conducive to cell growth, thus reducing the number of cells detected on the support at the conclusion of the experiment. The immobilization yield was only 19.93% at pH 5 and 28.96% at pH 10, as shown in Figure 1. A contradictory result was obtained by Susilowati et al. (2019), who observed that pH 12 was optimal for the immobilization of bacteria-alginate-chitin for crack remediation. This difference was due to the enhanced stability of the immobilized cells that may have protected against cell damage after entrapment in the alginate-chitin

3.2 Effect of contact time on immobilization of recombinant *E. coli*

One of the vital factors influencing the immobilization of recombinant *E. coli* onto the pineapple peel is contact time. In this study, the effect of contact time was investigated by varying it from 15 h to 27 h. Each immobilization was conducted with a pH 8 medium and 200 rpm agitation at 25 °C. As shown in Figure 2, when the contact time was extended from 15 h to 24 h, the immobilization yields also increased. At 15 h of contact time, only 30.71% of the cells were successfully immobilized onto the pineapple peel. At 24 h of contact time, 55.16 % immobilization yield was obtained. The extra contact time allowed more recombinant *E. coli* to bind to the surface of the peel. A similar result was obtained by Utomo et al. (2019) in the immobilization of *Zymommonas mobilis* on silica derived from rice husk ash, which observed the immobilization yield increase from 24% at 15 min to 31% at 30 min. This result demonstrated that adequate time was required for the cell coating to interact with the silica's surface functional group. Thus, when the contact time increased, the number of cells attached to the support surface also increased.

The cell immobilization conditions were set at 25 °C, pH 8, and 200 rpm agitation. The highest immobilization yield was detected at 24 h of contact time.

When the contact time was prolonged from 24 h to 27 h, the immobilization yield decreased to 35.30%. This was due to the detachment of the recombinant *E. coli* from the peel surface. Cell detachment likely occurred because the support was submerged in the medium for too long (Pang et al., 2016). The cell density of detached cells was 2.45 mg/mL, compared to 0.173 mg/mL for the immobilized cells, which showed that more cells detached from the pineapple peel with longer contact time. Ta et al. (2016) performed the immobilization of *Saccharomyces cerevisiae* cells on water hyacinth stem pieces and showed that 20 h was the optimal contact time, achieving 41.6% immobilization yield. When the contact time was extended further, the immobilization yield decreased to 38% due to cell desorption from the surface of the water hyacinth stem pieces into the culture medium. In this study, it was determined that 24 h was the optimum contact time for the highest yield of the immobilization process of recombinant *E. coli* on pineapple peel.

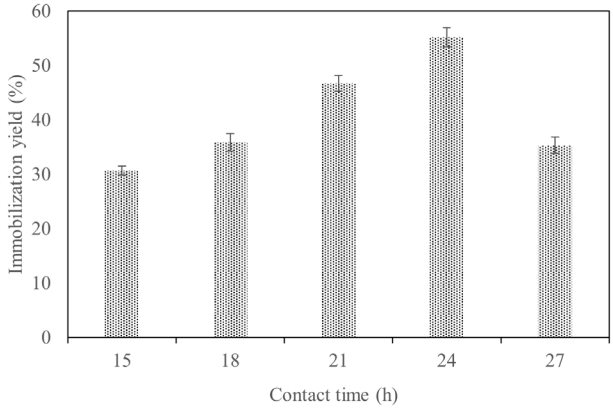


Figure 2. Effect of contact time on the immobilization yield

3.3 Effect of temperature on the immobilization of recombinant *E. coli*

Temperature is the third crucial parameter that can affect cell immobilization. The immobilization of recombinant *E. coli* on pineapple peel was conducted at 20 °C, 25 °C, 30 °C, 35 °C, and 40 °C to determine the optimal temperature. Each run was conducted at pH 8, 200 rpm agitation, and 24 h of contact time. As shown in Figure 3, the optimal temperature was 25 °C, resulting in an immobilization yield of 55.24%. Che Man et al. (2015) also found that the growth of recombinant *E. coli* was favored at this temperature, because the number of cells bound on the pineapple peel increased, leading to a higher cell immobilization yield. In this study, SEM was used to observe the attachment of the recombinant *E. coli* onto the pineapple peel at 25 °C, as shown in Figure 4, which confirmed that recombinant *E. coli* was favored at 25 °C and resulted in a higher immobilization yield. A contradictory result was observed by Li et al. (2019) in the immobilization of *E. coli* on mannose-functionalized magnetic nanoparticles, who found that the optimal immobilization temperature was 40 °C for a 82.4% immobilization yield. This result might be due to the accelerated movement of cells at 40 °C which then resulted in a higher immobilization yield.

The immobilization yield decreased significantly as the temperature increased from 25 °C to 40 °C. The immobilization yields at 30 °C, 35 °C, and 40 °C were 51.99%, 24.32%, and 19.11%, respectively. The cell densities declined and cell deaths mounted at high temperatures (Dai et al.,

2019), thus affecting the cell immobilization yield. A result obtained by Rochex et al. (2004), in which the attachment of *Pseudomonas putida* on cellulose fiber was the highest at 40 °C. Higher temperatures may promote the adhesion capacity of the bacteria on the support surface. An increase in temperature led to an increase in the number of bacterial collisions with the support surface, and consequently, an increase in opportunities for attachment of cells.

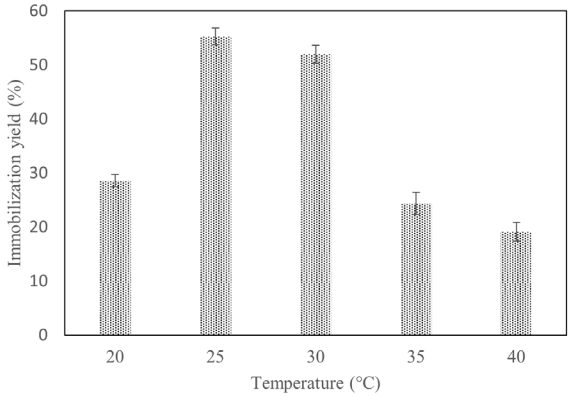
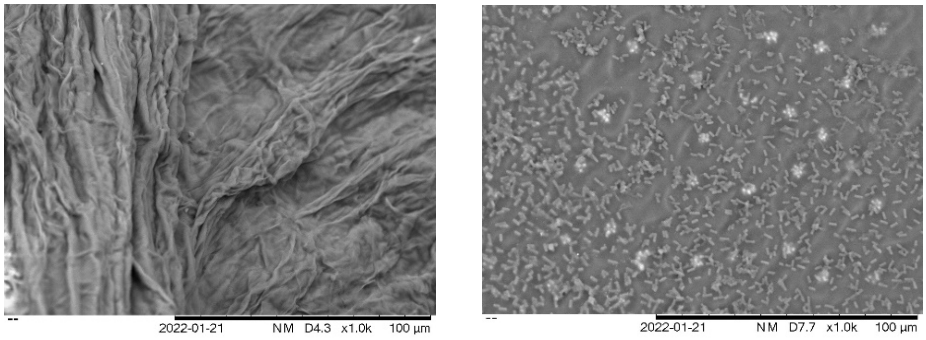


Figure 3. Effect of temperature on the immobilization yield



(a) (b)

Figure 4. Scanning electronic microscopy showing (a) the pineapple peel before the cell immobilization process and (b) the recombinant *E. coli* immobilized on the pineapple peel

4. CONCLUSION

The process parameters pH, contact time, and temperature in a cell immobilization process were optimized to obtain the highest possible immobilization yield. The most favorable pH for recombinant *E. coli* immobilization on pineapple peel was pH 8. The optimum contact time for the highest immobilization yield was 24 h. The ideal temperature for cell immobilization was 25 °C. Pineapple peel proved to be a good matrix for cell immobilization, serving to enhance the production of CGTase.

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