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CELL IMMOBILIZATION FOR CYCLODEXTRIN PRODUCTION: MINI REVIEW

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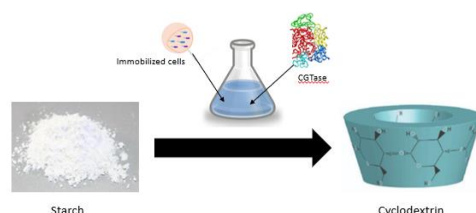
Graphical abstract



Abstract

Cell immobilization has been applied in various industries, including chemical manufacturing, food, pharmaceutical, and textile. Recently, innovations in cell immobilization techniques and support materials have been put forward for application in high value-added chemical biosynthesis, such as cyclodextrin (CD). The techniques, support materials, and process parameters of cell immobilization play important roles in achieving high CD yield. This review should help one choose the correct cell immobilization technique and support for a CD biosynthesis setup. Previously, CD biosynthesis utilized free cells, even though they present difficulties such as the low product yield, cell lysis, unstable plasmid, and non-reusable cells. This review highlights how the problems that arise from free-cell bioreactors could be mitigated by cell immobilization. The process conditions of cell immobilization for CD production are also presented.

Keywords: Cell immobilization, cyclodextrin, cyclodextrin glucanotransferase, immobilization supports, immobilization techniques

Abstrak

Imobilisasi sel telah digunakan dalam pelbagai industri, termasuk pembuatan kimia, makanan, farmaseutikal, dan tekstil. Baru-baru ini, inovasi dalam teknik imobilisasi sel dan bahan sokongan telah dikemukakan untuk aplikasi dalam biosintesis kimia bernilai tinggi, seperti cyclodextrin (CD). Teknik, bahan sokongan, dan parameter proses imobilisasi sel memainkan peranan penting dalam mencapai hasil CD yang tinggi. Ulasan ini akan membantu memilih teknik imobilisasi sel dan sokongan yang betul untuk persediaan biosintesis CD. Sebelum ini, biosintesis CD menggunakan sel bebas, walau bagaimanapun mereka menunjukkan kesukaran seperti hasil produk yang rendah, lisis sel, plasmid tidak stabil, dan sel yang tidak boleh digunakan semula. Ulasan ini

menekankan bagaimana masalah yang timbul daripada bioreaktor sel bebas dapat dikurangkan oleh imobilisasi sel. Kondisi proses imobilisasi sel untuk pengeluaran CD juga dibentangkan.

Kata kunci: Imobilisasi sel, siklodekstrin, siklodekstrin glukanotransferase, sokongan imobilisasi, teknik imobilisasi.

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1.0 INTRODUCTION

Research interest in cell immobilization has been growing in recent years. Conventional free-cell fermentation has been plagued with inherent shortcomings, such as low product yield, high cell lysis, unstable cell plasmid, and non-reusable cells. Cell immobilization has been proposed as a solution for these issues. It presents many advantages, including cell stability, lower recovery costs, enhanced fermentation productivity, and a feasible continuous process.

Cell immobilization is defined as the physical containment or position of whole cells to protect their catalytic activities in a certain defined region of space [1]. Immobilized cell systems offer various benefits over free-cell systems, such as increased growth rate and adhesion properties, resistance to inhibitors, and higher biocatalytic efficiency [2]. They are also more resilient to shifting environmental conditions and have a lower risk of contamination [3], while providing continuous use, improved cell stability, and higher cell density [4]. Other than that, cell immobilization offers easy handling, which is beneficial for industrial operations [5]. Theoretically, using immobilized cells in a continuous process would result in a smaller reaction volume, hence reducing expenses [6]. Cell immobilization can also enhance reactor productivity, facilitate separating cells from the bulk liquid, and allow for long-term continuous operation [7].

Despite the advantages outlined here, immobilized cell systems do have some limitations. The high metabolic activities of the chosen cells need to be maintained and the selection of supports must be industry-friendly [4]. The immobilization supports and the high cell density may also present issues. Natural and synthetic supports have been studied in the course of yield optimization.

CD is composed of glucopyranose units linked together by α-1,4-glycosidic bonds, forming a ring or cyclic structure [8]. CD is soluble in water, non-toxic, and can form inclusion complexes via its hydrophobic interior [9]. The complexed molecule may even change its physical and chemical properties, translating to the high market demand for CD in various industries such as pharmaceutical, textile, and chemical manufacturing [10]. The CD market is expected to record a 2.8% compound annual growth rate in sales over the next five years, with the global market size reaching USD 210 million by 2024, an increase from USD 180 million in 2019.

CD is produced via enzymatic conversion from starch, catalysed by the enzyme cyclodextrin glucanotransferase (CGTase). This enzyme can be found in many strains of *Bacillus*, *Thermoanaerobacter*, *Brevibacterium* and *Thermoanaerobacter bacilli*, but most CGTase is naturally produced by *Bacillus*. However, these microbes only produce a small amount of this enzyme, and the limited supply has driven its market price sky-high. Recombinant enzyme technology has been used to increase enzyme synthesis throughput. The cells must excrete the enzyme into the extracellular space. The most popular gram-negative bacteria for synthesizing heterologous proteins is *E. coli*. *E. coli* is highly sought-after as a production host in biotechnology due to its quick cell growth, well-characterized genome, and voluminous protein expression [11]. However, free whole-cell biocatalysts, especially *E. coli*, frequently experience cell lysis and instability, which reduces biotransformation efficiency. In the present study, cell immobilization is explored as an approach to overcome the bottlenecks mentioned.

CD production by free cells presents technical, biological, and economic challenges that are summarised in Figure 1. A free-cell bioreactor could only be operated in batch fermentation mode, which requires more idle time for the difficult task of sterilisation. A continuous fermentation mode is more time efficient since it does not require the same mandatory idling period. Free cells also tend to have inconsistent CD output. The cell strains and supports have different performances, which result in variations in their product yield. Table 1 depicts the different types of cells and support materials used to produce CD and CGTase. Choosing the suitable support and cell type, optimizing the process parameters, and applying essential fermentation strategies are several approaches to overcome these challenges.

Table 1 Different cell types and support materials used to produce CD and CGTase

Microbe	Support Material / Technique	CD / CGTase production	Ref.
B. sphaericus	Loofa sponges (Adsorption on solid support)	94.2 U/ml of CGTase	[9]
B. firmus	Loofa sponges (Adsorption on solid support)	22.93 mg/ml of CD	[12]
B. firmus	Silica–titania (Adsorption on solid support)	13.28 mg/ml of CD	[13]
Recombinant *E. coli*	Activated charcoal (Adsorption on solid support)	520 U/ml of CGTase	[14]
B. sphaericus	Chitosan (Flocculation)	98.29 g/ml of CD	[15]
Bacillus agaradhaerens	Polyvinyl alcohol (PVA) cryogels (Entrapment within porous matrix)	5 mg/ml of CD	[16]
Bacillus subtilis	Micro-encapsulated Tributyrin (Mechanical containment behind a barrier)	1.62 mg/ml·h of CD	[17]
Recombinant *E. coli*	Polyvinylidene fluoride hollow fibre membrane (Mechanical containment behind a barrier)	831.74 U/ml of CGTase	[18]
B. pseudalcaliphilus	Polysulphone membranes (Mechanical containment behind a barrier)	1.59 mg/ml of CD	[19]
B. firmus	Bovine bone charcoal (Mechanical containment behind a barrier)	0.77 U/ml of CGTase	[20]

Cell lysis reduces fermentation efficiency, and cell immobilization purports to protect against it. Ahmed *et al*. [21] reported a 67% increase in pectinase production by *Geotrichum candidum* immobilized on corncob. The risk of microbial contamination is also mitigated by utilising a genetically modified microorganism such as recombinant *Escherichia coli* *(E. coli)*, as it produces less contamination and has a high growth rate during fermentation.

Economically, free-cell systems incur high substrate and operation cost. A higher percentage of substrate is required to produce CD due to the high substrate-enzyme binding that contributed to the high CD production [22]. However, the medium turns viscous with a high substrate concentration, which could be detrimental to CD production. Furthermore, in a process that involves microbes, it can cause microbial pollution and requires a pre-treatment process. These factors translate to high production cost from the separation processes required to obtain the CD.

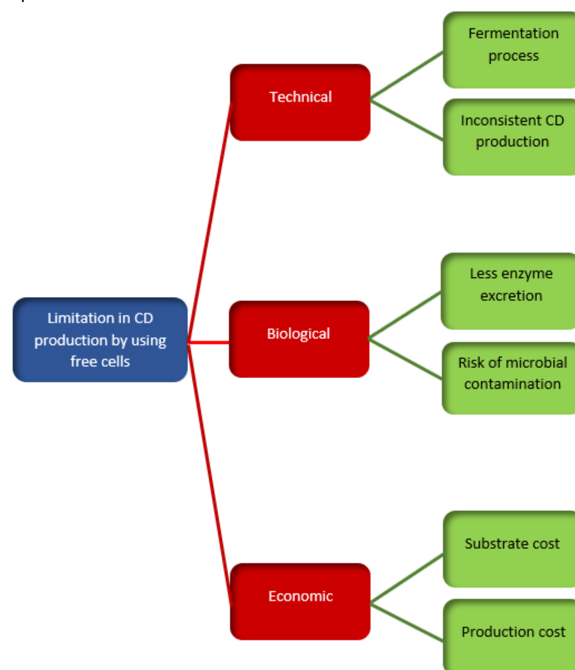

Figure 1 Technical, biological, and economic limitations of CD production by free cells

Progressing CD production systems from the lab-scale experimental setups to full-scale production facilities entail weighing some factors, including the microbe's metabolic parameters, growth rate, substrate choice, and substrate loading, with the aim of maximising microbial growth and CD production while resisting contamination and poisoning by foreign organisms and chemical compounds [23]. Cell morphology may also be modified for increased enzyme excretion.

Various immobilization techniques and supports have been employed in recent studies for improved product yield, but there are still problems caused by the toxicity of the chemical compounds used and the risk of microbial contamination and death, which may negatively impact the fermentation process. The parameters applied throughout the fermentation process may also be a limitation, such as agitation. The agitation could disrupt the microbial cells and lead to a low yield [24]. If possible, a system that does

not require excessive agitation and can generate less shear stress should be implemented.

2.0 CELL IMMOBILIZATION TECHNIQUES

Cell immobilization techniques include adsorption on a solid support, flocculation, entrapment within a porous matrix, and mechanical containment behind a barrier, which differ in the types of support, microorganism, and application. Various supports are evidently appropriate for CD production, including cellulose, chitosan, cotton fibre, calcium alginate, and hollow fibre membrane. Figure 2 presents some cell immobilization techniques.

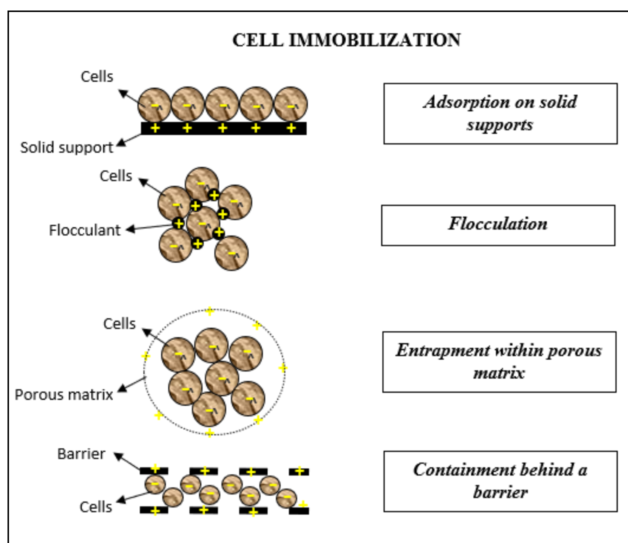

Figure 2 Cell immobilization techniques

2.1 Adsorption on Solid Support

Adsorption is commonly used in cell immobilization using various types of supports. It is the simplest cell immobilization method, as it entails moving the cells from the bulk phase to the solid support surface, followed by cell adhesion and support surface colonization [25]. The cell membrane and the support are held together by electrostatic forces or covalent bonds [26,27]. Cellulosic materials like cellulose and sawdust, as well as porous porcelain and porous glass, are examples of solid supports. Chitosan can be used with the solid supports to enhance adsorption [27]. Using an inexpensive support can reduce the cost of cell adsorption [9]. However, this technique relies on relatively weak bonds, and the adsorbed cells can detach themselves from the support under certain conditions. Nevertheless, an efficient binding process can still be achieved [28]. Various operating parameters, such as medium composition, pH, and environmental conditions can influence cell adsorption [25]. A good selection of parameters lead to a highly efficient cell adsorption. Kilonzo *et al*. [29] investigated the adsorption of recombinant strain C468 of *Saccharomyces cerevisiae* on cotton cloth for glucoamylase production. The immobilization experiments were performed on different fabrics, including 100% heavy bleached cotton (woven), 100% polyester (PE), 65% PE/35% cotton (woven), 100% nylon (woven), 100% polyurethane foam, and cellulose-reinforced polyurethane. The analysis of total cell density revealed that the average immobilized cell density was the highest on cotton fibre (100% heavy bleached cotton) at 92 g/L.

2.2 Flocculation

Flocculation is the aggregation of cells to form flocs or flakes [30]. Flocculation can be classified as an immobilization method because the large aggregates have potential applications as biocatalysts. The ability to form aggregates is mainly observed in moulds, fungi, and plant cells. Artificial flocculating agents like chitosan-based cationic polymers can be used to induce aggregation in cultures of cells that do not flocculate naturally. Rehn *et al*. [31] stated that chitosan flocculation is a simple and effective technique for fixing *Escherichia coli* into functional biocatalysts. They achieved immobilization by raising the medium pH to slightly alkaline, resulting in the formation of large fast-sedimenting flocs. The highly stable and fast-sedimenting flocs owe their characteristics to the high molecular weight of chitosan. However, analysis revealed that as the cell-to-chitosan ratio increased, the sediment volume decreased. Diffusion limitation was minimal even though cell concentration were very high in the flocculation preparation.

Another example of a flocculation technique is yeast flocculation (e.g., *S. cerevisiae*). Jin and Speers [32] studied the flocculation of a strain of *S. cerevisiae* (*NewFlo* strain LCC125). The suspending buffer's pH was maintained in the range of pH 3.8–5.8. Raising the pH buffer promoted the flocculation of the *NewFlo* strain LCC125. The study also demonstrated the effect of the culture conditions (e.g., temperature) on flocculation in the brewing industry. The temperature of the *NewFlo* strain LCC125 was examined from 5 °C to 25 °C, and the strain showed improved flocculation. the *NewFlo* strain LCC125 evidently flocculated better at higher pH and temperature.

2.3 Entrapment within the Porous Matrix

The process of entrapment involves trapping immobile cells inside of a support. A membrane is created around the immobilized microorganisms to guarantee their survival through processing and storage in the polymers [33]. This technique has achieved high mechanical strength, although with some accompanying issues such as cell leakage, high immobilization costs, and diffusion limitation [34, 35]. Calcium alginate and other natural polymers are

commonly used for gel trapping. Synthetic polymers like polyvinyl alcohol (PVA) have also gained attention due to their non-toxicity, high stability, high durability, and industrial scalability [36]. Gel entrapment has also been performed by combining several polymers for beads formation. PVA and sodium alginate is one such combination used for ammonia oxidation [37]. Orrego *et al*. [38] immobilized *S. cerevisiae* cells in calcium alginate beads to ferment soluble sugars in coffee mucilage into ethanol. Different bead sizes with different alginate concentrations were tested. Beads 3 mm in diameter and a 2% alginate concentration resulted in the best output of ethanol/g sugar after 18 hours. Entrapment also enabled the reuse of the immobilized cells with stable ethanol production. Smaller beads, with their larger surface-volume ratio, would boost the mass transfer of substrates from the medium to the immobilized cells, resulting in a higher rate of ethanol production and sugar conversion.

2.4 Mechanical Containment behind a Barrier

One of the fundamental methods of cell immobilization is the containment of cells behind a barrier, which can be accomplished by using microporous or ultra-porous membrane filters, trapping the cells in a microcapsule, or interacting with two immiscible liquids on the surface [26]. This technique is useful when a minimal transfer of compounds or free-cell products is desired. However, this method will result in cell loss during mass transfer and membrane biofouling may occur. Börner *et al*. [39] immobilized *Clostridium acetobutylicum* DSM 792 as macroporous aggregates through cryogelation for butanol production. Cell-based cryogels have a highly porous and elastic structure with walls consisting of packed cross-linked cells. The immobilized cells exhibited improved butanol production compared to free cells. Cell-based cryogels are successful alternatives for improving the acetone-butanol-ethanol process as cells are immobilized in a macroporous structure with low mass transfer limitations for high production yield.

Membranes have also been used for cell mechanical containment. Ouyang *et al*. [40] stated that the development of cell immobilization techniques using hollow fibre membranes has many advantages. One of these is a high surface-to-volume ratio that allows more cells to be immobilized on the membrane. This approach is cost-effective as the membranes are readily available and cheap compared to other supports.

The immobilization of *E. coli* cells on hollow fibre membranes was employed to increase enzyme excretion while suppressing cell lysis [41]. Large surface area, high flexibility, and self-supporting capability are the benefits provided by hollow fibre membrane supports. The large surface area contributes to a higher productivity. Hollow fibre membranes can be applied in various fields, including water treatment, medicine, pharmaceutical, and food processing due to their unique structures [42]. Hollow fibre membranes also offer high mechanical strength, a high surface-to-volume ratio, operational durability, non-toxicity, excellent chemical resistance, and biodegradability [43].

Porous ceramic hollow fibre membranes could support immobilized *E. coli* due to the presence of pores and lumen in its structure [44]. Oxygen and nutrients from the exterior can freely infiltrate through the fibre wall and enter its lumen, thereby nourishing the *E. coli* cells. Simultaneously, *E. coli* cells cannot pass through the wall and are trapped in the space. Markov *et al*. [45] carried out the immobilization of cyanobacteria on hollow fibre membrane in a photobioreactor for hydrogen production. The hydrogen production was maintained at steady state for three months. They concluded that the exclusive features of hollow fibre membranes contribute more advantages than disadvantages. With low prices in the market, hollow fibre membrane is a good choice as a support in cell immobilization. Many microorganisms have been studied for CD production. For small-scale production, the most commonly used bacteria species are *Bacillus licheniformis*, *Bacillus subtilis*, and *Bacillus circulans* [17]. The *Bacillus* species are popular choices because they are hardy organisms that can survive even in the poorest environmental conditions. They are fast growing and naturally produce the enzyme for CD synthesis [46].

CD production by immobilized cells has many benefits as the process is becoming more efficient and cost effective. The literature suggests that there are various types of cells, immobilization techniques, and supports that have different advantages and give different CD yields. This may be because of the different cell morphologies, techniques, and supports used.

3.0 PRODUCTION OF CD BY CELL IMMOBILIZATION

Various techniques and supports have been proposed to achieve high CD production. Mazzer *et al*. [13] used the adsorption method to immobilize *Bacillus firmus* strain 37 on two different supports: silica-titania (SiO_2/TiO_2) and silica-manganese dioxide (SiO_2/MnO_2). The supports were prepared via the sol-gel method. The immobilization was most efficient at 60 °C in five days. The CD yields were 11.7 mM and 11.2 mM for the cells immobilized on SiO_2/TiO_2 and SiO_2/MnO_2, respectively. The immobilized cells achieved higher yields than the free cells (8.3 mM). At the end of the first cycle (5 days per cycle), the cells immobilized on SiO_2/TiO_2 and SiO_2/MnO_2 showed the best results in the production of CD. However, production rate decreased thereafter due to cell desorption after the second cycle, caused by the weak bond between the cells and the supports.

The results showed that adsorption is compatible with continuous use, but less so with repetitive cycles, for which another method such as entrapment in gel appeared to be superior. For the support materials, many organic and inorganic materials are compatible with adsorption. Overall, adsorption is easy to operate and very cost-effective, while desorption and poor reusability are the major disadvantages.

Delani *et al*. [15] immobilized two different species of alkalophilic *Bacilli* on different support materials via flocculation using chitosan as the flocculant. The support materials used were loofah sponges for *Bacillus firmus* and synthetic sponges for *Bacillus sphaericus* on a loofah sponge. The immobilized *B. sphaericus* achieved 86.6 mM of CD after 20 days, compared to only 32.8 mM of CD produced by the free *B. sphaericus* over the same period. Flocculation is a simple method that is easy execute, but it has high operational costs and the flocculant may be toxic. The supports chosen in the study, loofah and synthetic sponge, allow stable production of CD. Loofah sponge is also cheap and renewable. Meanwhile, synthetic sponges are resistant and hydrophobic due to their polyurethane composition.

Martins *et al*. [16] immobilized *Bacillus agaradhaerens* cells entrapped in polyvinyl alcohol (PVA) cryogels for CD production. The highest CD yield of approximately 92%–94% was achieved at pH 9 and 50 °C. They commented that the entrapment technique produced biocatalysts with high mechanical strength. The PVA cryogel support had high micro- and macro-porosity. The internal structure and porosity of the PVA cryogel beads were important to reduce diffusional barriers. The PVA cryogel beads retained their activity for up to one month when stored in proper conditions. It was proven that the immobilization techniques and the support material used can enable cell reuse.

To date, there is no specific report on the immobilization of cells via mechanical containment behind a barrier for CD production, although there is one for CGTase production. Silva *et al*. [47] studied the immobilization of *Bacillus firmus* strain 37 on bovine bone charcoal for the production of CGTase. Bovine bone charcoal was used as the support material because it has hydroxyapatite (calcium phosphate) and traces of calcium carbonate, which makes it an alkaline buffer [48]. These characteristics are critical since the growth pH of *B. firmus* and CGTase production was between 9 and 11. Bovine bone charcoal has a significant role in the immobilization of cells, leading to increased product yield. Its high porosity lets microorganisms to adsorb and develop on its surface and within its crevices. The maximum CGTase activity was achieved after 120 hours of fermentation (2.48 U/mL). This technique could promote mass transfer by allowing the transmission of oxygen, nutrients, and cellular products for preserving the cell viability. Moreover, it also protects the cells from mechanical and environmental stresses while sustaining some growth and metabolic activities for extended periods.

Man *et al*. [49] used mechanical containment behind a barrier as a method of cell immobilization. For CGTase excretion, recombinant *E. coli* was immobilized on a hollow fibre membrane, chosen due its low mass transfer resistance, operational durability, and high mechanical strength. It is also non-toxic, easily accessible, and reasonably priced. Additionally, the liquid medium in the membrane's intra-capillary area lessens diffusional limitation experienced by the immobilized cells. As a result, the immobilized cells excreted CGTase profusely, 2.8–4.6 times more than free cells.

In conclusion, cell immobilization techniques and the types of support depend on the production system and the type of microorganism, as these have different characteristics which may give different results in terms of the reaction and production of desired product. Table 2 shows the advantages and disadvantages for cell immobilization techniques.

Table 2 Techniques of cell immobilization and their advantages and disadvantages.

Techniques of Cell Immobilization	Advantages	Disadvantages
Adsorption on solid support	- Suitable for continuous process - Compatible with many support materials - Easy to operate - Cost effective	- Cell desorption - Poor reusability
Flocculation	- Simple technology - Easy to perform	- High operational cost - Toxic
Entrapment in the porous matrix	-High mechanical strength	-Diffusion limitation
Mechanical containment behind a barrier	- Promotes mass transfer - provides protection against mechanical and environmental stresses	- Cell and enzyme leakage

4.0 FACTORS AFFECTING CELL IMMOBILIZATION FOR CD PRODUCTION

Cell immobilization for CD production may be influenced by many factors, chiefly the immobilization method and the support material. Other pertinent process parameters include reaction time, temperature, substrate concentration, and pH.

4.1 Reaction Time

Reaction time affects CD production by providing sufficient opportunity for the substrate to bind with the enzyme, thus increasing the number of enzymatic reactions in the medium. The optimal reaction time would allow the substrate to bind with the enzyme active sites with sufficient time to produce CD. Reaction times that are too short or too long may cause low CD production [50,51]. Moriwaki *et al*. [9] demonstrated that immobilized cells (*B. firmus* strain 37) on a loofah sponge reached its maximum CD yield after 72 hours. The immobilized *B. firmus* strain 37 produced 12.89 mg/mL of CD, whereas the free cells of the same strain produced only 2.00 mg/mL. In this case, too short a time may not provision enough time for the substrate to bind with the enzyme.

Pazzetto *et al*. [52] evaluated CD production by immobilizing *B. firmus* strain no. 37 on a loofah sponge. The CD yield reached 20 mM over 144 hours of reaction. The researchers also measured the yield from *B. firmus* strain 37 immobilized on a loofah sponge and a synthetic sponge for different immobilization times (5, 10, 20, and 30 days). The average CD yield was 18.14 mM at 10–30 days. They concluded that increasing reaction time did not alter β-CD production. This phenomenon may be because of the CD accumulating on the enzyme active sites over time, which induced product inhibition. In conclusion, studies have shown that a moderate reaction duration is advisable, as long as it is sufficient for the substrate to bind more extensively on the enzyme active site and convert into CD. However, the effect of reaction duration on CD production may also differ depending on the microbe species and the immobilization support.

4.2 Temperature

One of the key factors influencing cell adhesion to the matrix, as well as the effectiveness of the cell immobilisation method and CD production, is temperature [53]. Muria *et al*. [54] found that increasing the temperature from 45 °C to 65 °C raised the final concentration of CD from 10.93 mM to 14.13 mM. However, the final concentration decreased to 11.52 mM as the temperature was further raised to 70°C, due to enzyme denaturing.

Pazzetto *et al*. [52] discovered that CD yield from *B. firmus* strain no. 37 immobilized on loofah sponge increased from 6.46 mM to 8.83 mM as the temperature was raised from 40 °C to 60 °C. Muria *et al*. [54] and Pazzetto *et al*. [52] demonstrated that the optimal temperature range lied between 40 °C and 65 °C. At a lower temperature, the molecules have a lower kinetic energy, leading to fewer collisions and fewer interactions between the substrate and enzyme, leading to a low CD yield. At a higher temperature, cell disruption and enzyme denaturation occur, preventing the cells from attaching properly to the support and hindering the substrate from binding to the enzyme active site. Hence, a moderate temperature (30–50 °C) is advisable to achieve high CD production.

4.3 Substrate Concentration

In cell immobilization for CD production, the optimum concentration of substrate is required. A dilute substrate is not sufficient to bind with the enzyme's active sites, whereas a concentrated solution tends to be very viscous, causing difficulty in stirring the reaction mixture. It also oversaturates the support surface with substrate, which could reduce CD production. On top of that, the choice of substrate concentration depends on the type of substrate used. Most studies, especially those in the field of bioprocess, varied the substrate concentration to observe its effect on the cell immobilization system for CD production. For example, Sakinah *et al*. [55] varied the substrate concentration in the range of 2%–20%. The result showed that at a high substrate concentration (20%), the CD production was also high (90% of total CD). Another study conducted by Muria *et al*. [54] also varied the substrate concentration (5, 10, 20, and 30 g/L) and found that the CD production was the highest (14.13 mM) at a high substrate concentration (30 g/L).

Schöffer *et al*. [56] obtained the maximum CD production of 1.32 g/L by using a 4% soluble starch solution, in which *Thermoanaerobacter sp.* was immobilized on chitosan. The starch could act as a reaction inhibitor at a high enough concentration (10%). Pazzetto *et al*. [52] observed that the production of CD by *B. firmus* immobilized on loofah sponge increased to 19.26 mM, 19.14 mM, and 20.35 mM at 10%, 15%, and 18% dextrin concentration, respectively. This study recommended 10% dextrin for economic reasons, and the result also displayed very small differences in the values of CD produced. A low substrate concentration is advisable to reduce the cost and to allow for adequate mixing, so that the substrate can bind with the enzyme active site in the medium, hence promoting a high level of CD production.

4.4 pH

The pH of the buffer solution in producing CD is also one of the crucial and important factors, as it can influence the strength of the bonding between the cells and the support surface. CD production prefers an acidic medium if the cells used are gram negative and the support used is positively charged. In an alkaline solution, CD production is reduced due to the excess hydroxyl ions (OH^-) which confers a negative charge to both the cells and the support surface, causing them to repel each other [57]. If the cells are gram positive, the support must be negatively charged, and this combination can produce more CD in an alkaline medium, In which they will have different surface charges and thus attract each other. The attachment of *S. cerevisiae* on fibres such as cotton, nylon, polyurethane, and

polyester depends on the medium pH [58]. For cotton, cell attachment increased from 0.0763 to 0.2006 h^{-1} as the pH value decreased from pH 9 to pH 2. Nylon, polyurethane, and polyester also showed the same pattern as cotton. Electrostatic repulsion between the cell surfaces and fibres prevents attachment due to the negative charges in both the yeast cells and fibres. in an acidic medium, the charge is less negative, hence reducing the electrostatic repulsion between the yeast cells and fibres and promoting the attachment of the cells to the fibres. High cell attachment to the matrix resulted in high cell concentration, which in turn translates to a high CD formation.

However, Delani *et al*. [15] showed that flocculated alkalophilic *Bacilli* prefers an alkaline medium (pH 8) for CD production. The type of cell used affected the cell immobilization system and the production of the desired product. In summary, researchers may choose between an alkaline or acidic medium for the bioreactor, taking into consideration the microbial cell and the support used.

5.0 CONCLUSION

Various cell immobilization methods and supports have been developed for CD production, and they exhibited clear advantages over free-cell systems in terms of product quality, product yield, and operation cost. Designing a CD production process utilizing immobilized cells requires balancing parameters including reaction time, temperature, substrate concentration, and pH. We recommend optimizing these process parameters to improve CD production.

Large-scale CD production faces many challenges, particularly in the operational cost needed to maintain good mass transfer rate within the medium. A membrane bioreactor with immobilized cells presents a promising alternative for industrial CD production with its high specific surface area for cell immobilization, low maintenance, high mass transfer, and direct oxygen supply to the bioreactor without an agitator (shear-free environment).

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