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Comparison of Thermostable Xylanase Production by Escherichia coli Immobilised onto Different Nanoparticles

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Immobilisation process can be applied for both whole cells and enzymes to optimise the operational performance system for industrial applications. A successful immobilisation process leads to the development of economically and ecologically available biocatalyst such as xylanase. However cell lysis becomes one of the biggest problems in the enzyme excretion when E. coli is used as a host. In this study, the effects of different nanoparticles on xylanase excretion and cell lysis of immobilized E. coli were examined. For protein expression, the cells were cultured in various immobilized matrices on graphene oxide treated, graphene oxide untreated, carbon nanotube treated and carbon nanotube untreated with 100 mg/mL IPTG concentrations at 30 °C temperature and 200 rpm agitation rate for 24 h. The immobilised cells demonstrated a 7 % increase in xylanase excretion and a 39 % reduction of cell lysis compared with free cells using untreated graphene oxide. Consequently, the immobilisation of E. coli using nanoparticles was verified to increase xylanase excretion and cell stability.

1. Introduction

The creation of heterologous biocatalyst by employing recombinant DNA innovation has drawn a great interest in the industrial process of biotechnology. Some of them have even now been produced at the manufacturing level (Man et al., 2015). The production of recombinant enzyme and protein mounts up in the microorganisms. For that reason, the excretory production of the recombinant biocatalyst might be prepossessing, with concern to the steadiness of the protein excretion and advance downstream processing (Mergulhão et al., 2005). Excretion of recombinant proteins to the periplasm or culture medium has certain preferences over intracellular production. For instances, improved biological activity, uncomplicated downstream processing, and N-terminal validity of the expressed peptide and better solubility of the product as well as stability. Thus, protein excretion is crucial, as it facilitates stable and active biocatalyst production (Man et al., 2016).
In industrial microorganism, Escherichia coli (E. coli) is turning out to be the utmost commonly used prokaryotic expression systems for recombinant proteins considering to its easier growth requisite, short doubling period and well-known genome. The utilisation of E coli has several downsides, for instances, subsequent protein inactivity, improper folding and inconstancy of vectors throughout large-scale fermentations (Morowvat et al., 2015). Nevertheless, E. coli still becomes a preferred host for heterologous gene expression, considering to its variation of mutant strains, recombinant fusion partners as well as attainable plasmids that made E. coli as an operative mechanism in the biotechnology industry (Zajkoska et al., 2013).
Naturally, E. coli does not discharge high measures of enzymes or proteins, recovery of a recombinant gene product such as proteins can be significantly reduced by an excretion procedure that reduces contamination from host proteins. If the excreted proteins are extracellular protein, cell disturbance is not necessary for recovery and, even on account of periplasmic translocation, cell wall permeabilisation or osmotic shock can be

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utilised to acquire the protein without the discharge of contaminants cytoplasmic protein (Mergulhão et al., 2005).

Thus, in order to improve the system performance of the recombinant E. coli for recombinant protein excretion, immobilisation is one of the efficient strategy. Immobilisation has numerous benefits over the conventional fermentation process of a free cell system (Nguyen et al., 2009). The benefits include reducing the plasmid instability and cell lysis issue, improving the productiveness of the recombinant cells, low sensitivity to pH and temperature when compared with free biocatalyst (Thirumarimurugan, 2016), lowering the possibility of contamination, decreasing the fermentation time, advancing the rate of substrate uptake, and resultant in estimated concentration of volatile component for product (Man et al., 2016). E. coli in the form of immobilised enzyme has been utilised mostly in laboratories, catalyses various interesting reactions and also on an industrial scale, prominent to the manufacture of beneficial substances (Zajkoska et al., 2013).

Enzyme expression by immobilised cells is thoroughly interrelated to bioconversion because of their easier separation from products for potential reutilisation, competent effectiveness in catalysis as well as improved operational stability compared to free cells (Howard et al., 2003). The advancement of enzymes in industry has depended intensely on the utilisation of microbial sources. Microorganisms are beneficial since they can be produced economically in inexpensive media and short fermentations (Sánchez and Demain, 2011). Xylanases can act synergistically with other hemicellulases to yield commercial xylooligosaccharides (Jiang et al. 2009). Plant cell wall consists of hemicellulose that is tight correlate and high branching with other biopolymers (Farrah and Mahdeda, 2015). One of the main hemicelluloses constituents of plant cell wall is xylan, which comprises of â-14 linked xylopyranose residues backbone made up of branches that consist of acetyl, glucuronosyl residues and arabinofuranosyl (Farrah and Mahdeda, 2015). Plant biomass such as xylan fits the requirement as an economical and sustainable feedstock for feasible production of numerous value-added compounds and biomolecules.

Xylanase is one of the enzymes classification which degrade the linear polysaccharide beta-1,4-xylan into xylose, thus breaking down hemicelluloses, which is one of the major components of plant cell walls. Xylanases are extracellular enzymes that produced by microorganisms such as bacteria (saprophytic and phytopathogenous), Mycorrhizic fungi and some yeasts. This enzyme also found in protozoa, insects, crustaceans, snails, seaweed and also seeds of plants during the germination phase in the soil (Kanimozhi and Nagalakshmi, 2014). A complete and efficient enzymatic hydrolysis of this complex polymer depends mainly on two types of enzymes, which are endo-1,4- β-xylanases and β- xylosidases. Both enzymes hydrolyse the xylanopyranose of the central chain and hydrolyze xylobiose as well as other xylooligosaccharides resulting from the action of endoxylanases (Kanimozhi and Nagalakshmi, 2014).

Immobilisation matrices could be categorised based on their chemical configuration as inorganic or organic supports. Usually, the inorganic supports always provide the whole protection contrary to unstable conditions. This is because, they are really constant in a variation of solvents (Srividya, 2014), as well as over a wide-ranging pH, satisfying hydraulic properties. Besides, they are also lower disposed to bacterial attack. Meanwhile, organic polymers are elastic, flexible and comprised of larger reactive groups for biocatalyst attachment that could be cast within all geometry including beads and membrane (Elnashar, 2010).

One of the most generally used matrices for the cell immobilisation are calcium alginate beads since they can compromise certain advantages. The advantages of using calcium alginate beads include its ease of obtainability, ease of preparation, economy benefits as well as satisfying biocompatibility. Even so, there are certain limitations that are disclosed to their usage. For examples, calcium alginate beads have big pore size, lower mechanical strength that will cause the whole cells to be detached from the matrix. There are also limitations in the mass transferal and gel deterioration (Duarte et al., 2013).

On the other hand, with the development of nanotechnology, the nanoparticles create potential and novel material due to their specific physico-chemical properties. This material can be used as immobilisation matrix for a wide range of industrial applications such as, the production of recombinant protein from immobilised bacteria (Ahmad and Sardar, 2015). Nanoparticles are the suitable material to fix diffusion problems when the process is dealing with the macromolecular substrates. In addition, the large surface-to-volume proportion presented by nanomaterial results in the concentration of the immobilised cells or biocatalyst that is significantly advanced than that given by other materials (Saifuddin et al., 2016). Thus, nanoparticles such as graphene oxide and carbon nanotube are key modules in the future market of advance innovation that are greatly impact the material's mechanical properties. For instance, the elasticity and stiffness and afford biocompatible environs for immobilisation method (Ahmad and Sardar, 2015).

Graphene oxide (GO) can afford the good potential for various applications because it has the exceptionally large particular surface region with the two operative sides that has the sufficient oxygen comprising surface functionalities. For instances, hydroxyl, carboxylic groups, epoxide and greater water solubility element that afford an optimal substrate for immobilisation process. Zhang et al. (2010) have demonstrated that the immobilisation of biocatalyst on the GO sheets may possibly take place easily without any supplementary

surface modification and cross-linking agents (Zhang et al., 2010). A graphene sheet, as a biocompatible material is also useful in enfolding microorganisms to isolate and/or keep them from the surroundings, as the aggregating of graphene sheets manage to trap the microbes within them and to separate them from the culture medium of surroundings (Akhavan et al., 2015).
Hence, they can be utilised for biocatalyst immobilisation as it has abounding functional groups also wide particular surface region. These characteristics give an ideal substrate for the biocatalyst immobilisation application and may possibly take place readily out of utilising any agents for cross-linking and further surface alteration or modification (Zhang et al., 2010). Graphene oxide can also be applied for immbolisation of the whole cell system.
Another type of nanoparticles, known as Multiwall carbon nanotubes (MWCNTs), are materials containing carbon with a tube-shaped. MWCNTs have a diameter measuring in the nanometer scale that shows remarkable mechanical, structural, electrical, thermal and electrochemical feature (Lee et al., 2010). The nanoparticles could contribute to the large surface region for higher biomolecule loading, minimise the diffusion limits and also can act as a biocompatible microenvironment that aids biocatalysts to preserve its catalytic properties (Saifuddin et al., 2016). They are progressively drawing attention as the nano-scale substantial for the possible biological purpose. For examples they are used as a nanoparticle matrix for enzyme immobilisation that is leading to nano-biocatalysts and biosensors application. Current studies have proven that the immobilised biocatalyst onto multiwall carbon nanotubes exhibit higher biocatalyst activity as well as stability relative to the solution counterparts (Lee et al., 2010).
From the literature reviews, immobilised cell using nanoparticles is highly recommended and is a convincing technique for xylanase excretion with fewer occurrences of cell lysis. In this paper, the effects of different nanoparticles on xylanase excretion and cell lysis from immobilised E. coli are studied and the result is to be compared to a free cell culture medium. Graphene oxide and carbon nanotubes are two nanoparticles chosen as cell culture matrices in this study.

2. Material and methods

This section discusses the materials preparation (Section 2.1) and step-wise analysis (Section 2.2 and 2.3) designed to carry out the experimental work on xylanase production by E. coli immobolised onto different nanoparticles.

2.1 Escherichia coli strain and cell immobilisation

The recombinant Escherichia coli strain carrying a xylanase gene from Aspergillusfumigatus af293 was used in this study. The strain was constructed before by Wahabet al. The xylanase gene (xyng) from A. fumigatus af293 was cloned into expression host, E. coli BL21 (DE3) holding vector pET21a (+). This vector comprises a signal peptide M5 that can direct the protein expressed into extracellular space. E. coli BL21 (DE3) functioned as the host for heterologous expression while E. coli JM109 was utilised for storing function. The producing recombinant plasmid was labelled as pM5xyng and the E. coli from positive resultant mutant were cultured with nanoparticle matrices overnight in Luria Bertani broth medium containing ampicilin (50mg/mL) at 37 °C 200 rpm. The nanoparticle matrices were washed with sterile distilled water after 18 h to remove the detached cells. The immobilised cells were then transferred to 250 mL flasks comprising of 50 mL expression medium.

2.2 Analytical procedures

2.2.1 Assay for xylanase activity

The enzyme activity was analysed by incubating the diluting xylanase enzyme in sodium acetate buffer with pH 5.0 and temperature 50 °C for 10 min time by using a 1 wt%/vol beech wood xylan (Merck), a substrate solution. The reducing sugars were analysed by adding 500 µL of DNS (2-hydroxy-3, 5 dinitrosalicylic acid) reagent, boiling for 5 min cooling and reading the absorbance at 540 nm. One unit of xylanase activity was represented as the total of enzyme releasing 1 µmol/min of reducing sugar (xylose equivalent) under the assay conditions.

2.2.2 B-galactosidase activity (cell lysis)

Cell viability was measured by determining the quantity of β-galactosidase in the extracellular medium using 0-nitrophenyl-β-d-galactopyranoside (ONPG). A total of 1 mL of substrate buffer comprising 4 mg/mL of ONPG in 0.1 M phosphate buffer (pH 7.4) was added to 0.1 mL of sample, prior to incubation in a 37 ˚C water bath for 10 min. The reaction was stopped by adding 0.5 mL of 1 M sodium carbonate, and the absorbance was read at 420 nm. One unit of xylanase enzyme activity was defined as the quantity of enzyme that forms 10−8 mol of ONP per minute under the assay conditions.

2.3 Treatment of multiwall carbon nanotube

The impurities of untreated multiwall carbon nanotubes (MWCNTs) were performed by using 37 % hydrochloric acid. In a 500 mL of round bottom flask containing 200 mL of hydrochloric acid solution, 1.0 g of multiwall carbon nanotube was stirred for 2 h, before diluted with deionised water to remove the acid content until it retained in pH 7 and filtered using vacuum pump. The resulted MWCNT was dried in a 67 °C vacuum oven for overnight.

3. Results and discussion

This section reveals the results from the experimental work done as described in Section 2, together with the discussion on the results displayed.

The effects of the graphene oxide and multiwall carbon nanotube on xylanase activity and β-galactosidase activity of the immobilised cells were investigated. Figure 1 shows the xylanase activity and β-galactosidase activity verified for the different types of nanoparticles and free cells. The uppermost excretion of enzyme activity was perceived in untreated graphene oxide (0.060 U/mL), followed by free cells (0.056 U/mL), untreated multiwall carbon nanotube (0.053 U/mL), treated graphene oxide (0.052 U/mL) and treated multiwall carbon nanotube (0.047 U/mL). As shown in Figure 1, the β-galactosidase activity of immobilised and free cells. The highest cell lysis activity was observed in treated graphene oxide (50.68 U/mL), followed by untreated multiwall carbon nanotube (65.83 U/mL), treated multiwall carbon nanotube (65.19 U/mL), free cells (50.68 U/mL), and untreated graphene oxide (30.76 U/mL).

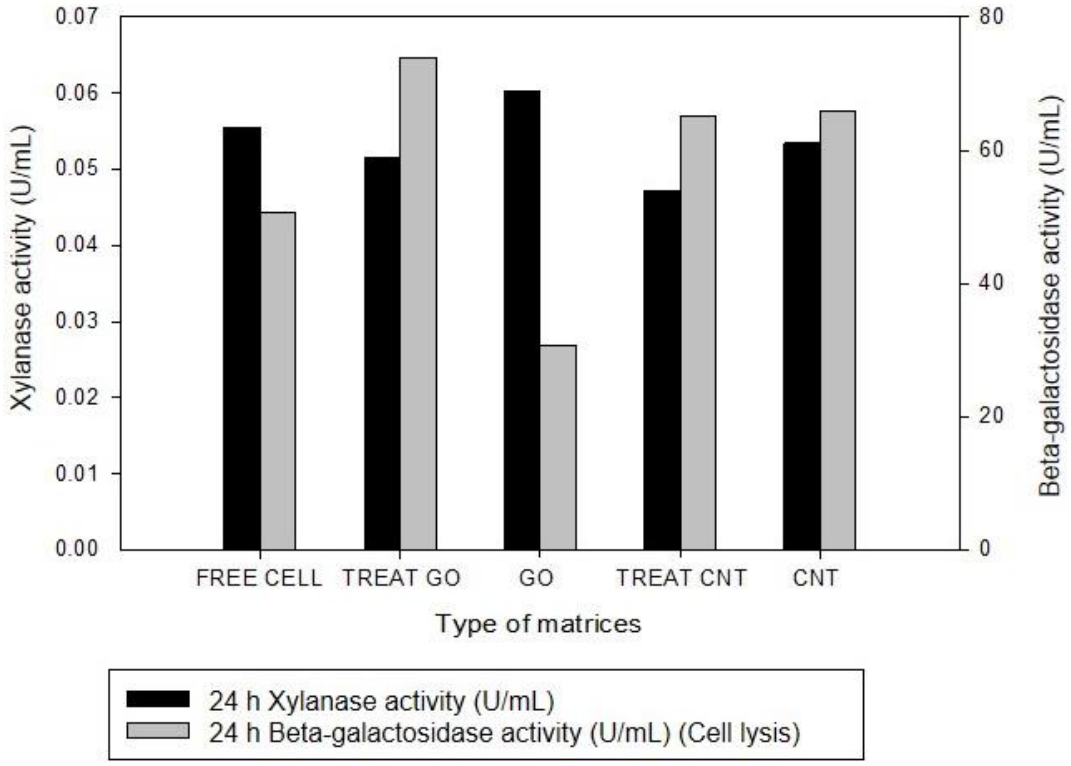

Figure 1: Effect of the nanoparticles on xylanase activity and β-galactosidase activity of the immobilised cells

The immobilised cells onto untreated graphene oxide were associated with uppermost xylanase activity with the lowermost occurrence of cell lysis compared with other matrices tested, probably for the reason that the biocatalyst throughputs of immobilised cells were advanced than those of free cell culture as the concentration immobilised cells onto the graphene oxide matrix is higher than free cells and the reduced cell lysis most probably be caused by cell mass transfer restriction and compartmentalisation (Zajkoska et al., 2013). The high xylanase activity perceived in the immobilised cells of graphene oxide may have caused by the capability of these cells to more readily incorporate the nutrients than in free cell culture; for instance, through attaining nutrients adsorbed at the solid-liquid boundary (Man et al.,2015). Moreover, graphene oxide is conceivably enhanced the attainment of hydrogen, H_2 and oxygen, O_2 penetrability and has a high hydrophilicity property

that made it competently dissolve in culture medium or other hydrogen bonding polar solvents, hence opening the way for cell attachment to the support matrix (Ammar et al., 2016).
Whereas, the xylanase activity attained by immobilised cells onto treated graphene oxide, untreated multiwall carbon nanotube and treated multiwall carbon nanotube was lower, conceivably due to the diffusion restrictions and this limitation possibly will be conquered by increasing the loading of immobilised cells onto a support (Zajkoska et al., 2013). The multiwall carbon nanotubes were correlated with lower xylanase excretion and higher cell lysis probably due to the chemical inertness of MWCNTs that obstruct their process proficiency and this has inhibited their full potential for the process efficiency. One more drawback of the MWCNTs regarding their usage in biomedical and biochemistry utilisation is that they are very hydrophobic and usually form unsolvable aggregates. Considering the solubility limitation of MWCNTs in any solvents, it is also quite hard to separate one carbon nanotube from the other and thus, appropriate amounts of stabilisers are necessary to prevent phase separation and flocculation (Saifuddin et al., 2016).

4. Conclusion

Interest in the enzyme application based strategies for industrial application processes are gradually more preferred over the conventional chemical methods, as the technique is environmental friendly, potentially saving the cost and highly competent for industrial scale production. To overwhelm the recent problems in immobilisation techniques such as cell lysis, further studies should focus on the development of stable cell immobilisation methods that could pull down this drawback and reduce the cost of the immobilised cells process. This comparison designated that recombinant E. coli immobilised onto graphene oxide increases xylanase production as well as reduces the occurring of cell lysis in comparison to a free-cell system. The optimal measure of xylanase excreted by immobilised cell was 0.060 U/mL, in comparison with 7 % advanced than production in free cell culture medium (0.056 U/mL). Immobilised cell performs a 39 % reduction in cell lysis with 30.76 U/mL, compared to free cell with 50.68 U/mL. Thus, this study presents crucial remark to demonstrate graphene oxide as a convenient immobilisation matrix for recombinant E. coli.

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