Stirred Bioreactor 2015

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Submission date: 05-Oct-2019 08:33AM (UTC+0700)
Submission ID: 1186394309
File name: Stirred_Bioreactor_2015.pdf (367.96K)
Word count: 3050
Character count: 16812

Studii şi Cercetări Ştiinţifice
Chimie şi Inginerie Chimică, Biotehnologii, Industrie Alimentară
2015, **16** (3), pp. 245 – 252

Scientific Study & Research
Chemistry & Chemical Engineering, Biotechnology, Food Industry
ISSN 1582-540X

ORIGINAL RESEARCH PAPER

STIRRED BIOREACTOR FOR THE ROBUSTNESS PRODUCTION OF RECOMBINANT GST.VP28 IN FED-BATCH CULTIVATION OF *ESCHERICHIA COLI*

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Received: September, 01, 2015
Accepted: September, 30, 2015

Abstract: *Escherichia coli* is the most popular platform for the production of recombinant proteins as vaccine candidates. One important factor that may influence the quantity and quality of the expressed proteins using the bacterial host is a bioreactor. Thus, this study was aimed at comparing the influence of two different bioreactors, conventional (Sakaguchi flask) and stirred bioreactors on the growth of *E. coli* BL21 as a host cell and production of GST.VP28 recombinant protein in the host. The result showed that total wet biomass of *E. coli* BL21 harvested from the stirred bioreactor was significantly higher, compared to the total biomass from the conventional bioreactors. In addition, SDS-PAGE results also indicated that the amount of the GST.VP28 recombinant protein collected from the stirred bioreactor was higher than the quantity of recombinant protein from *E. coli* cultured in the conventional bioreactor. Furthermore, the stirred bioreactor also produced larger soluble fraction and lower inclusion body. This result suggested that the stirred bioreactor is very suitable for improving the production of recombinant protein using *E. coli*.

Keywords: *conventional bioreactor, E. coli BL21, recombinant protein, Sakaguchi flask, shrimp vaccine candidate*

INTRODUCTION

Escherichia coli is the most popular and well-established expression platform for screening or generation of recombinant protein as vaccine candidates for animals or humans. It is used as a cell factory that allows generation of the functional protein in large quantity with cheap cost in industrial scale. It was the first host used to generate human insulin in 1982 and bovine growth hormone in 1994 with a production cost of less than 5 $/g, showing the versatility and economic potential of *E. coli*-based production [1, 2]. Therefore, the gram-negative bacteria have been used as a host to produce heterologous protein both in laboratory scale and industrial capacity [3 – 5].

Many strategies have been introduced and designed in cultivation for the high-level and cost-effective production of heterologous proteins using the host. Bioreactor design, media composition, and temperature optimization are the most prospective variables that have been studied to fulfill the above objectives. From bioreactor design point of view, adequate aeration and agitation are important factors in the success of protein expression using the bacterial expression system in liquid medium [6]. It is because the primary function of the bioreactor is to provide a suitable environment for the growth of the host to obtain the optimal amount of desired product. Moreover, the bioreactor should meet the optimal milieu for native conformation of the generated protein.

Previous studies have reported the significant effects of oxygen level supplied through aeration on the growth rate and final cell number of bacteria [7 – 10]. Shaking of bacterial culture increased the growth rate and reduced the size of *Brevundimonas diminuta* [7]. In addition, the shaking gave a higher final yield and increased rate of DNA synthesis in *Streptococcus faecalis*. The aeration of bacteria cultured in liquid media with shaking increased the growth rate and final cell number [9].

Agitation of culture is necessary to maintain a uniform environment throughout the bioreactors contents [11]. In a bioreactor without agitation, it is commonly found that bacterial cells are aggregating near the bottom of the bioreactor due to the fact that their cell mass is higher than that of the liquid medium. Thus, media content in a region above the aggregate cells is richer in nutrients than in the bottom of the reactor. Since the aggregate cells accumulated in the bottom part, the richer part media is not accessible for the cells. Therefore, sufficient agitation speed of culture is crucial for maintaining a suspended state of media and bacteria that make the resources available for the host [6].

In this research, a large surface bioreactor (Petri dish) was compared with conventional bioreactor using Sakaguchi flask to generate recombinant GST.VP28 as shrimp vaccine candidate [12 – 16], using *E. coli* BL21 as a host cell. Adequate aeration and agitation in the large surface bioreactor was obtained using stirrer during cultivation time, whereas shaking was performed at 110 rpm in the Sakaguchi flask. The results indicated that the use of large surface bioreactor (Petri dish) equipped with stirrer produce a higher amount of biomass of GST.VP28-expressing *E. coli* BL21 comparing to conventional small surface bioreactor (Sakaguchi flask) with shaking. In addition, the bioreactor produces a larger amount of soluble protein comparing to Sakaguchi bioreactor, indicating that the stirred bioreactor is very suitable for the robustness production of recombinant protein in batch culture of *E. coli*.

MATERIALS AND METHODS

Conventional and stirred bioreactor

In this research, two bioreactors were used for generation of recombinant GST.VP28 protein in fed-batch of *E. coli*. A conventional bioreactor, Sakaguchi flask, was used as a control of the fed-batch culture with shaking at 110 rpm. As a comparison, a large surface bioreactor, Petri dish with 15 cm of a diameter which usually used for large agar plate, was used for liquid culture. A magnetic stirrer was put inside of this bioreactor for aeration and agitation. The stirrer speed was adjusted to 2.5 scales based on the code in the speed adjustment button. These bioreactors were set up as shown in Figure 1.

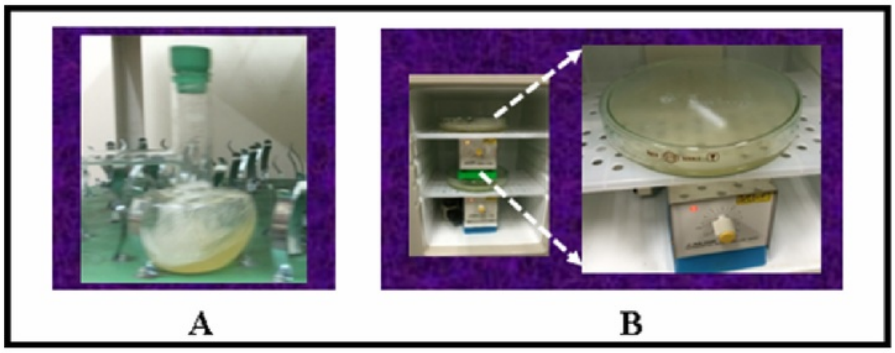

Figure 1. *Bioreactor used in this study. A = conventional bioreactor (Sakaguchi flask) with shaker, B = large surface bioreactor (Petri dish) equipped with magnetic stirrer*

Production of GST.VP28 on conventional and stirred bioreactors

For the production of GST.VP28, glycerol stock of *E. coli* BL21 cell carrying pGEX.VP28 plasmid was streaked into LB-agar plate containing 50 µg·mL^{-1} of ampicillin and incubated at 37 ºC for overnight. The grown single colony was inoculated into 2 mL LB broth supplemented with 50 µg·mL^{-1} of ampicillin and incubated with shaking at 110 rpm at 37 ºC for overnight. Then, 1 mL of the saturated culture was inoculated into 100 mL LB broth in Sakaguchi flask and Petri dish supplemented with 50 µg·mL^{-1} of ampicillin and incubated at 37 ºC until OD600 = 0.5. After that, 0.5 mM isopropyl-β-D-thiogalactopyranoside (IPTG) was added to these cultures and continued for 6 hours cultivation. Cells were then harvested by centrifugation at 6,000 g at 4 ºC, and the obtained pellet was diluted with 10 mL of phosphate-buffered saline (PBS) for sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis.

Analysis of GST.VP28 quantity and solubility

For the protein quantity and solubility analysis, 1 mM phenylmethylsulfonyl fluoride (PMSF) was added to the diluted pellet, homogenized using a vortex, and lysed by sonication (10 x 30 s pulses with 45 s interval). The lysate obtained is centrifuged at

12,000 g for 15 min. After centrifugation, the resulting supernatant is transferred to a fresh tube and subsequently compared the fusion protein contents in the supernatant with the pellet using SDS-PAGE.

RESULTS AND DISCUSSION

Oxygen is a critical substrate in anaerobic bioprocesses such as aerobic fermentation [10]. Since oxygen has low solubility in water (approximately 6000 times less soluble than glucose), the hydrodynamic condition of bioreactor influences the oxygen transfer from the gas phase to aqueous media. Therefore, aeration and agitation are necessary to solve the oxygen to media [6, 11, 17].

Small surface bioreactor such as Sakaguchi flask or conical flask is widely used for laboratory scale suspension culture of a microorganism to generate some biotechnologically metabolite products [11, 18]. Aeration and agitation of culture in these bioreactors are facilitated by shaking in various speeds (100-200 rpm). Since the surface of these bioreactors is small and long, spoiling of the liquid medium could be avoided.

In this research, Sakaguchi flask was used to produce recombinant GST.VP28 recombinant protein in a fed-batch cultivation of *E. coli* BL21 with shaking at 110 rpm. As shown in Figure 1, the flask was filled with 80 mL of LB broth medium inoculated with *E. coli* BL21 carrying pGEX.VP28 recombinant plasmid. The used of a porous sponge as a cap of the flask would permit oxygen transfer to meet the microbe requirement.

Shaking of the conical bioreactor at 110-130 rpm is necessary for obtaining a good aeration and agitation which are needed not only to provide dissolved oxygen for cells but also to homogenize culture media in the bioreactors [17]. However, the shaking may also give an adverse effect on the microbial physiology, especially the cell metabolism and growth.

To prove the above assumption, the use of Sakaguchi bioreactor with shaking was compared with the use of large surface bioreactor (Petri dish), in which the agitation and aeration process were performed by magnetic stirrer with adjustable speed (Figure 1B). By using the magnetic stirrer, hydrodynamics of the medium would be more comfortable than shaking and finally would give a positive effect on the production of the protein target. In succinate generation, the lower aeration experiment has higher productivity in the generation of the product than the use of higher aeration [19].

Observation during cultivation showed that the use of magnetic stirrer in Petri dish provide excellent mixing of media and bacteria which was indicated by a uniformity of culture color as occurred in shaking culture using Sakaguchi flask. The stirring also facilitates heat transfer from the culture to the environment which is necessary for the comfortable environment for growth, maintenance and production of microorganism.

On the physical point of view, the use of stirrer is more suitable for nutrient uptake for microorganism than the use of shaking which then affect the *E. coli* growth. This assumption was proved by the higher amount of final wet biomass of *E. coli* BL21 grown in the Petri dish with stirrer comparing to in Sakaguchi flask with a shaker (Figure 2).

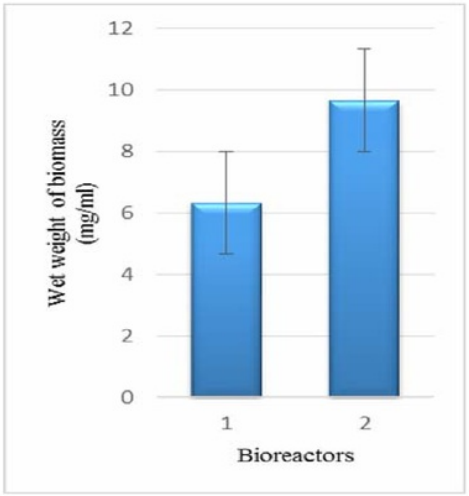

Figure 2. *Wet weigh of biomass (mg·mL^{-1}) of E. coli BL21 producing GST.VP28 generated using conventional (1) and stirred bioreactor (2)*

A larger amount of final wet biomass of *E. coli* grown in stirred Petri dish was followed by the higher amount of GST.VP28 expression in the bioreactor. As shown by SDS-PAGE result in Figure 3, induced *E. coli* BL21 grown in stirred Petri dish produce higher band intensity of 45-kDa protein comparing to those produced in Sakaguchi flask with shaking, indicating that the stirred Petri dish produced a higher yield of GST.VP28. This result is consistent with the antibody expression result at lower shaking speed [17].

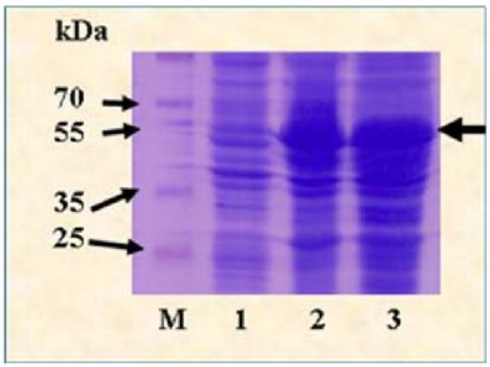

Figure 3. *SDS-PAGE analysis of recombinant GST.VP28. M = marker, lane 1 = uninduced cells, lane 2 = IPTG Induced cells in conventional bioreactor, lane 3 = IPTG Induced cells in stirred bioreactor*

Two possible factors might contribute to the higher concentration of GST.VP28 in the stirred bioreactor. First, a greater amount of biomass generated in the stirred bioreactor

could be a contributing factor to the higher amount of expressed protein. Second, enough oxygen level and a better environment are provided in media during cultivation using magnetic stirrer affecting cell metabolism and thus synthesis of the target protein. Furthermore, the effect of stirred Petri dish on the quality of the obtained protein was also evaluated. As shown in Figure 4, the intensity of 45-kDa soluble protein band generated by stirred bioreactor (lane 4) was higher than produced by shaking bioreactor (lane 2). These results indicated that the amount of the soluble protein produced by stirred bioreactor was higher.

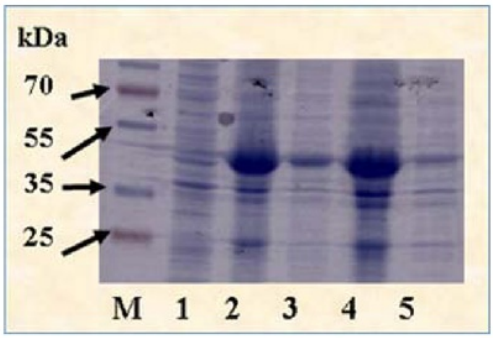

Figure 4. *SDS-PAGE analysis of recombinant GST.VP28*
M = marker, lane 1 = uninduced cells, lane 2 = IPTG induced supernatant using conventional bioreactor, lane 3 = IPTG induced pellet using conventional bioreactor, lane 4 = IPTG induced supernatant using stirred bioreactor, lane 5 = IPTG induced pellet using stirred bioreactor

Moreover, Figure 4 also shown that the band intensity of IPTG induced pellet using conventional bioreactor (lane 3) was higher than the band intensity of IPTG induced pellet using stirred bioreactor (lane 5). These results indicated that the amount of inclusion body in the protein generated by conventional bioreactor was higher than using stirred Petri dish. A higher speed agitation in the Sakaguchi flask seems to be responsible for aggregation of the recombinant protein in the conventional bioreactor. Another possibility is a higher speed of shaking, which can increase the temperature during cultivation, triggering the protein denaturation and reducing the amount of native protein [20].

CONCLUSIONS

The total amount of cell biomass and recombinant proteins-expressing *E. coli* cultured in a stirred bioreactor were significantly higher compared with the total cell biomass and the amount of protein being collected from *E. coli* cultivated in a conventional fermenter (Sakaguchi flask or Erlenmeyer). In addition, the stirred bioreactor produced a larger amount of soluble protein comparing to the other fermenter, indicating that the fermenter design is very suitable for improvements of the robustness of recombinant protein in batch culture of *E. coli*.

ACKNOWLEDGMENTS

The authors were grateful to Prof. Hideo NAKANO, Assist. Prof. Takaaki KOJIMA, and Assist. Prof. Jasmina DAMNJANOVIC (Laboratory of Molecular Biotechnology Nagoya University, Japan) for useful advice and helpful discussion. This work was supported by Grants from Directorate General of Higher Education, Indonesian Ministry of Research, Technology, and Higher Education, Republic of Indonesia.

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