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Simulation for Production of Syariah-Compliant Influenza Vaccine using Trypsin and Acutase in Batch Reactor via SuperPro Designer

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ABSTRACT

The halal status of the vaccine is being questionable among the Muslim community around the world. Some of them even took a risk not to take the vaccine when this issue is resurrected. This is due to the uses of porcine trypsin that comes from the prohibited animals which is pancreas (pig). The main aim of this study is to find the effectiveness of alternative enzyme (Acutase™) that comes from invertebrate sources in replacing the trypsin™ enzyme. Two modes of operation which are batch and continuous mode were compared to determine the high production rate of the vaccines. The production of syariah-compliant vaccine mainly focused on the bioreactor and simulated by using a software called SuperPro® Designer. The different reactions of time ranging from 1-5 days were tested to each enzyme. The hemagglutinin (HA) concentration gained will form the growth curves of the cell. The HA concentration data from the simulation indicates the number of cell growth in the cell culture process. Result shows a batch reactor can achieve until 60% of the yield production. The curves show the highest concentration of cell by using Acutase™ is at day 3 which is 1.17 X 10^6 cells/mL. Meanwhile, 0.98 x 10^6 cells/mL produced by trypsin enzyme at day 4. Total hemagglutinin produced is calculated by using the following standard condition, 1 HA unit corresponds to 10^4 cells per mL. Therefore, the hemagglutinin (HA) concentration produced by using acutase™ is 117 Unit HA compared to the trypsin™ which is 98.8 Unit HA. The results prove that the production of syariah compliant influenza vaccine can be produced in the future and enhance the uses of the vaccine among Muslim community.

Keywords: Batch Reactor; Influenza Vaccine Syariah Compliance; Acutase™; Trypsin™; SuperPro® Designer

INTRODUCTION

Vaccines are widely used in the global to against the widespread disease such as H1N1, H3N5, human papillomavirus infection and others. The uses of vaccine are increasingly important in the pharmaceuticals' industries. It can be seen that the capacity for the vaccines' production are really critical in the pharmaceutical industry. Vaccine is derived from Latin which is Variolae Vaccinae that invented by Edward Jenner for a disease knowns chicken pox (Baxby 1999). Edward Jenner successfully introducing a vaccine to cure chickenpox disease during 1796 which is this disease is very dangerous towards people (Lakhani 1992). In Vaccine Fact Book, Plotkin (2013) said that the vaccine is the term used that refer the medicine or component to enhance the immunization levels in human body to against the harmful diseases. There are many types of vaccine which is inactivated virus, using live but attenuated virus, specified nuclei or polysaccharides and inactivated toxin that produced by certain bacteria. Influenza is responsible for about 17,000 to 51,000 deaths annually in the United States and global pandemic death tolls reach the millions (Kang 2009).

Fathima et al. (2012) stated that influenza virus comes from Orthomyxoviridae family and can be classifies into three types which are Influenza A, Influenza B and Influenza C. The most dangerous influenza is A that causes pandemic and infects humans and animals such as pigs, birds and horses. The virus always undergoes genetic changes and form new types of viruses that cannot be recognized by humans' immune system. Hence, the production of influenza virus must be done annually and compatible to the new viruses (Wong & Webby 2013). Milián and Kamen (2015) discovered that there are many ways to produce a vaccine such as egg based, cell based and cell culture techniques. For example, Optaflu and Flucelvax are the vaccine that produced by cell culture by using Madin Darby Canine Kidney (MCDK) cell. In addition, Milian and Kamen (2015) also explained that by using a bioreactor to culture the cell can produce large quantity of virus. The influenza is then purified from the supernatant through several process which is filtration, centrifugation and chromatography to separate the impurities or cell debris within the virus. After that, the virus will be inactivated by using a chemicals such as B-propiolactone (B-PL) and cetyltrimethyl ammonium bromide (CTAB) and will be send to be tested.

Besides, enzyme is an essential substance required in the production of influenza vaccine in order to facilitate the process of penetration to the cell. Trypsin is one of the example of enzyme that commercially used in industry

nowadays. However, trypsin made from the pancreas (pig) which is prohibited used for Muslim. In the other hand, the uses of trypsin in the production is controversially discussed by the researchers as the Extraneous viruses were recently found in porcine trypsin preparations (Kekarainen et al. 2009) and animal derived products are generally considered a safety risk (Merten 2000; Audsley and Tannock 2008).The uses of proteolytic enzyme is to increase the concentration of the infectious virus toward cells. The enzyme will break the bond of hemagglutinins' head from the virus to facilitate the penetration of Ribonucleic Acid (RNA) viral into the cell that enables multicycle replication. Hence, the alternative enzyme is discovered (acutase™) that comes from invertebrate sources (A. Mohd Azmir Arifin 2010).

Vaccine also been discovered and practiced in Malaysia but the issue halal vaccine is being questionable and being a hot topic among the Muslim. Some of them even took a risk not to take the vaccine when this issue is resurrected. In surah al-Maidah verse 3 which means "…..Forbidden unto you (for food) are carrion and blood and swine flesh, and that which hath been dedicated unto any other than Allah, and the strangled, and the dead through beating, and the dead through falling from a height, and that which hath been killed by (the goring of) horns, and the devoured of wild beasts, saving that which ye make lawful (by the death-stroke), and that which hath been immolated unto idols. And (forbidden is it) that ye swear by the divining arrows…." This is due to the production process that involves blood consist of serum and cell components that may come from the prohibited animals such as pig and dog However, Irwan Bin Mohd Subri (2018) stated that the vaccine is purely intended to prevent the diseases from getting worse and Islamic law is concerned with the methods used in the treatment carried out. In Quran, there is an evidence that related to preventative measure which is in surah al-Baqarah, verse 195 which means "…and be not cast by your own hands to ruin…" This verse explained that we must not let and come near to anything harmful to us

METHODOLOGY

Production of vaccine involved several unit operation such as bioreactor, centrifugation, inactivation tank, shake flask and others. Basically, the main operation is at the bioreactor. This study will focus on the process in the bioreactor. Normally, mammalian cells are used and cultured at 37 °C. Then it will be treated with 5% carbon dioxide (Brown 1990). Oyeleye et al (2016) discovered that the cell cannot survive when the temperature is above 42 °C. Pressure in the bioreactor can be assumed 1.01325 bar or 101 kPa as bioreactor has air holes to release the gas produced. Optimal cell growth need neutral pH of 5.5 to 8.5 (Oyeleye et al, 2016). Without enough oxygen, the cell will produce acetic acid ant it is known as cultured anaerobically. By providing adequate ventilation, pH will remain at close to neutral. pH will be controlled in range of pH 6 to pH 8 in this study. Meghrous et al. (2009) recommended to set at time for the process to be 72 hours. SuperPro® Designer v9.5 software was used to develop a simulation model of bioreactor for production of syariah compliance influenza vaccine.

The bioreactor model was simulated using *hp* laptop with Windows 10 operating system and Intel Core i5. The software is used by following the steps in SuperPro® Designer User Guide. Basically, it starts with a new process file and filling in information to the process model by following the sequence showed in the guide. Then, it is required to build the process flow sheet and adding operations such as Charge, Agitation, Batch Heating, Batch Stoichiometric Reaction, etc., to each unit procedure. Once all operations that are necessary have been included, the process analysis is completed by solving several objective such as mass and energy balance, stream classification and etc. Charts and visualization of result can be generated once all the steps above are completed in Figure 1.

Two types of batch reactor will be tested by SuperPro® Designer which is batch reactor and continuous reactor to determine the high yield production, low cost and the advantages. The best reactor will be selected throughout the process.

ENZYME

Two enzymes which is trypsin™ and acutase™ will be simulated in the SuperPro® Designer with a different time of reaction (1-5 days) to investigate the cell growth of cultured cell in the bioreactor. The graphs between trypsin and acutase's activity will be plotted and the characteristics of the cell produced will be discussed according to each time. The graphs indicate the growth curves of the cells in the bioreactor. Result from the simulation will be compared with the experimental data for the validation of simulation. The total virus produced is calculated based on the total hemagglutinin produced, Total hemagglutinin produced is based in the mass per total cell and can be calculated by using the following standard condition, 1 HA unit corresponds to 10^4 particles per mL (Wang-Shick Ryu 2017).

RESULTS AND DISCUSSION

The result and discussion are from the finding of the journal and the simulation that had been stimulated by using the SuperPro® Designer. The simulation is set to 37°C at 1.01325 bar with the pH 6 to pH 8 in 72 hours of the retention time to select the suitable operation modes for the process.

BATCH PROCESS

Batch process is being simulated by the retention time about 72 hours for each batch. Figure 2 shows a batch process in the SuperPro® Designer. The supernatant produce contains the virus will be further purified in the downstream process. The production of the cell culture mainly operated with the batch cultivation mode. This mode is the simplest operation to grow the cell, hence to get a desire product. Currently,

80% of the industries using a commercial-scale of stainless steel equipment as this technology is a permanent fixture (Trazzino 2010).

However, the industry is moving to the new technology which is using a disposable bioreactor. It is more convenient compare to the stainless steel bioreactor. Disposable reactor also known as single-use bioreactor have a disposable bag instead of the culture vessel. Basically, it have three layer plastic foil which is made from Polyethylene terephthalate or LDPE, Polyvinyl acetate (PVA) and last layer is from Polypropylene. Each layer is to provide mechanical stability, act as gas barrier and as a contact layer, respectively.

The use of disposable bioreactor decreases the cleaning and sterilization process. Hence, Morrow K.J (2006) estimated the cost savings more than 60% compared to stainless steel bioreactors. The application of this bioreactor also minimizes the risk of the cross contamination and increase the process and biological safety. So, it is really suitable in any kind of biopharmaceutical product. But, some of the industries will just invest to the existing systems as to modify or replace the existing facility from stainless steel to disposable technology may be irreplaceable such as autoclave, heat-sterilized fermenter, large tank and others (Morrow 2006). Yet, this equipment is still proven and well trusted even it is expensive and need more labor intensive.

The production of influenza vaccine by using a batch reactor can achieve until 60% of yield productivity. However, with the comparison of disposable reactor is higher than the stainless steel reactor. These finding is supported by Howard L. Levine (2010) which is showing the disposable reactor have higher antibody titer which is 1000L compare to the stainless steel which is 30L. Figure 3 shows a productivity of disposable and stainless steel bioreactor (Howard L. Levine 2010).

CONTINUOUS PROCESS

The establishment of the continuous culture is starting from 1950s. It is one of the types of mode operation that been used in certain industry. The process also well-known advantages compare to the batch culture such as enhance process yield, steady-state operation and high volumetric efficiency. But, it only can be achieved with the uses of the STR operated in single which is chemostat or multi-stage STR configurations. The productivity of yield increased significantly by simulated. Although it will increase the productivity but it is not significant in the production.

As known, the doses of vaccine is low per produced but rapidly in a year. Hence, many vaccine are typically low volume products. Fencl et al. (1972) claimed that the stable operation with chemostat might fail when the production is in small amounts. It is caused by the inhibition of the cell growth by product or the cell deteriorate happen within the operation such as lytic viruses. In addition, Frensing & T. Heldt (2013) discovered that the continuous is significant reduction of virus yield by formation of the defective interfering particles (DI). DI are spontaneously generated virus mutant that been lost as a defective replication or non-

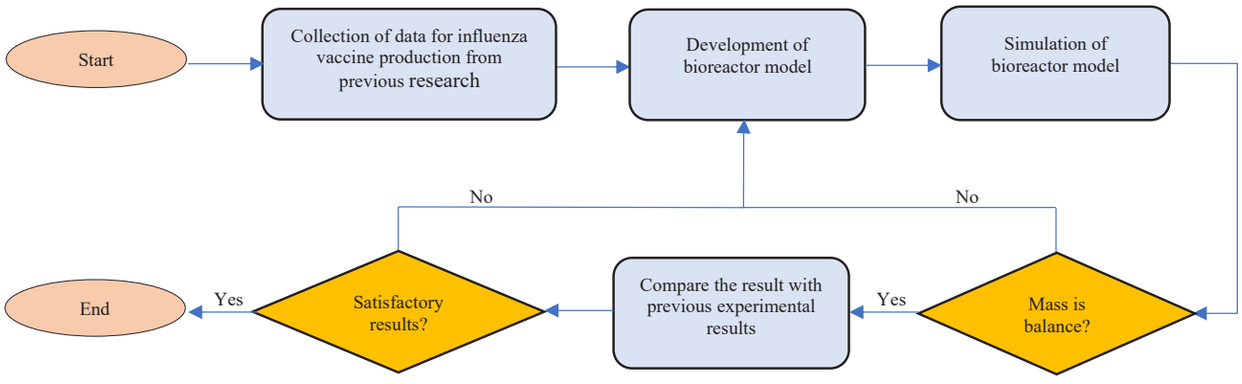

FIGURE 1. Flow Chart Process in SuperPro® Designer

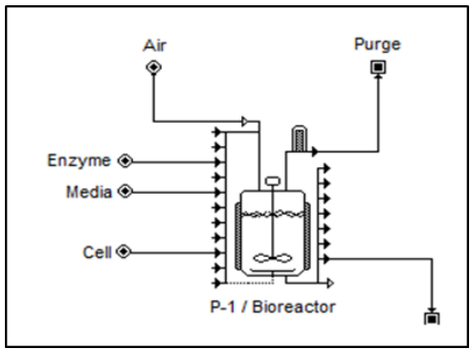

FIGURE 2. Batch Process in the SuperPro® Designer

homologous recombinant. Figure 4 shows the continuous influenza vaccine production.

Although the continuous process is more desirable as the production will significant high but industries might have and face several challenges. Hoskisson and Hobbs (2005) claimed that the fast advanced in modifying the genetic engineering also will be required when changing to the continuous modes. Therefore, the establishment industries will focus more on the batch mode cultivation as it have more advantages regarding ease of the operation and process robustness.

COMPARISON OF THE MODE OPERATION

There are two modes of operation which is batch culture and continuous. Each modes have their own advantages and disadvantages. Table 1 shows a comparison between batch and continuous (Gaurab Karki 2017).

ENZYME

Trypsin enzyme is commercially used in the production of cell culture-based influenza vaccine production to promote the virus infection by proteolytic activation of viral hemagglutinin that enables multicycle replication. (Claudius Seitz 2012). 4.5 of enzyme is inserted in the simulation with the different time of reaction (1-5 days). Figure 5 shows the cell concentration by using acutase and trypsin with a different times. The uses of trypsin in the inoculation process is highly and promising the population of cells to still alive (Claudius Seitz 2012). The growth of the cells for trypsin start with day 1 which is 1.81 X 10^5 cells/mL. The cells start to growth until reaching the maximum concentration in day 4 which is 9.88 X 10^5 cells/mL. Cell concentrations increase from the lag phase to the log phase within day 1 to day 4. Lag phase is the condition where the cells been introduced to the new environment which is media (Mahesh Bule 2017). The cell growth is just minimal. The log phase indicates that the cells are already adjusted to the new environment and the rapidly growth will take place. It start from the Day 2 until reaching the maximum concentration in day 4 before entering the death phase.

Besides, acutase enzyme can be obtained from the invertebrate animal such as crab (A. Mohd Azmir Arifin 2010). Based on the result shown in Figure 3.3, the highest concentration of the cell is on the day 3 which is 1.17 X 10^6 cells/mL. The cell concentration start with 1.74 x 10^5 in Day 1, 3.60 x 10^5 in day 2, 1.27 x 10^6 day 3 and start to fluctuate in day 4 and 5 which is 9.87 x 10^5 and 5.79 x 10^5, respectively as reaching the death phase. This result is supported by the lab scale experiment done by Irwan (2019) who found that the high yield of cells happen at day 3 which is 1.34 x 10^6 cell/mL. However, the rate of cell duplicate for the trypsin enzyme is a bit late which is at day 4 (0.98 x 10^6 cells/mL). The concentration of acutase cultivation reach the exponential phase at the day 3 meanwhile trypsin enzyme is still in the lag phase.

This proves that the cell growth of culture by using acutase is faster than trypsin. This is because the trypsin enzymes require longer time to reform the cell cultures as the digestive properties of trypsin is stronger than acutase (Claudius Seitz 2012). Trypsin is the most crucial and effective way to break the bond between the host cells during cultivation. Seitz et al (2012) claimed that the trypsin is commercially used in the vaccine production industry even though it require a long time to reach a high cell concentrations. Therefore, trypsin is commonly used during the inoculation of the vaccine in the vaccine production industry as its effectiveness produces high concentrations of viral infections against host cells. Nonetheless, the indigestion of the trypsin may damage the host cell structure (Ting Lee & Chen Lee 2015).

This research also show that the acutase enzyme have the ability to produce effective cell proteolytic. Hence, the effect are almost similar to the trypsin. Ting Lee, Chen Lee (2015) discovered the advantages of using the acutase is can minimize the damaging to the cellular structures but it will take longer to dissolve cells than trypsin. Irwan (2019) also studied about the host cell viability for the each cultures. Both enzyme are not significantly different as the survival cells from the first day until the fifth day reach until 80% in both culture. The uses of different enzymes does not significantly affect cell viability. The results show that each

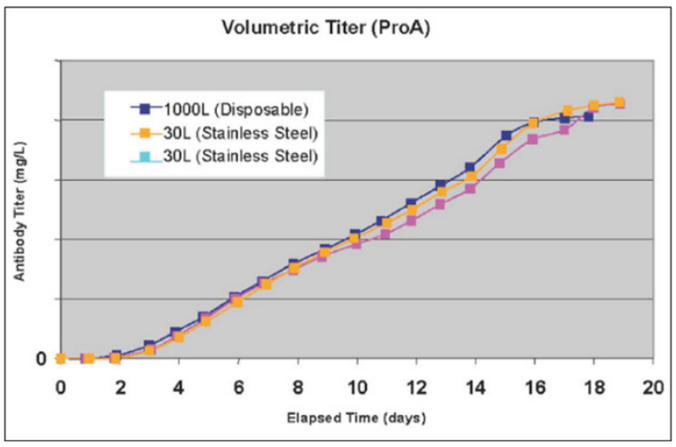

FIGURE 3. Productivity of Disposable and Stainless Steel Bioreactor (Howard L. Levine, 2010)

proteolytic enzymes have a different growth and process of cell multiplication.

HEMAGGLUTININ CONCENTRATION

Determination the amount of virus that has been infected is by measuring the hemagglutinin protein. This protein can be found on the surface of the influenza virus which is responsible to the process of binding between viruses to the infected cells (SinoBiological 2019). Meghrous et al (2009) stated that the average of 9-10 mg HA can be produced per 10^9 cells. However, there is a standard condition to calculate the HA theoretically which is 1 HA unit corresponds to 10^4 cells per mL (Wang-Shick Ryu 2017). Furthermore, the amount of HA also can be generated based on the effective yield estimated by SuperPro® Designer. Figure 6 below shows the amount of HA that have been produced by using a standard condition's calculation.

Based on the result, the production of the HA for acutase is higher than trypsin which is 117 unit HA to 98.8 unit HA. The data obtained shows acutase can produce more virus. However, this results is not significant as the procedure of the lab scale is not simple as the simulation and the data generated by simulation is not same. Irwan (2019) discovered that the HA for the trypsin is likely high compared to the acutase which is 256 HAU to 32 HAU. There is a possibility that the difference in concentration and activity of the enzyme results in the differentiation of proteolytic and host cell growth. The HA assay is the method to produce influenza virus titer based on their ability to tie the red blood cells through glycoprotein and its superficial proteins have been used to measure the

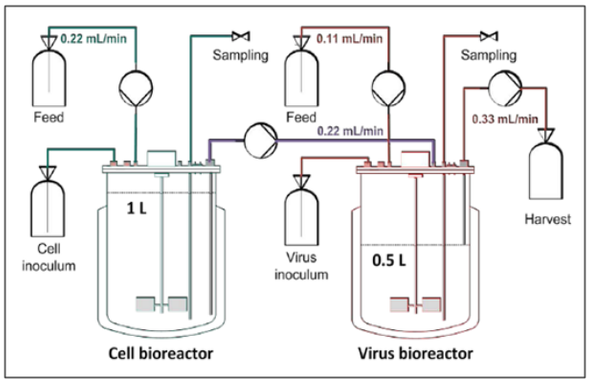

FIGURE 4. Continuous Influenza Vaccine Production (Frensing & T. Heldt, 2013)

TABLE 1. Comparison of Batch and Continuous Cultivation

	Batch	Continuous
Cultivation System	Closed	Open
Addition of fresh nutrition	No	Yes
Removal of waste	No	Yes
Chance of contamination	Low	High
Growth Phase	Lag, log, stationary and decline phase	Lag and log phase
Product Yield	Low	High
Volume of cultural	Constant	Constant
Compatible to production of vaccine	Very Compatible	Still in development

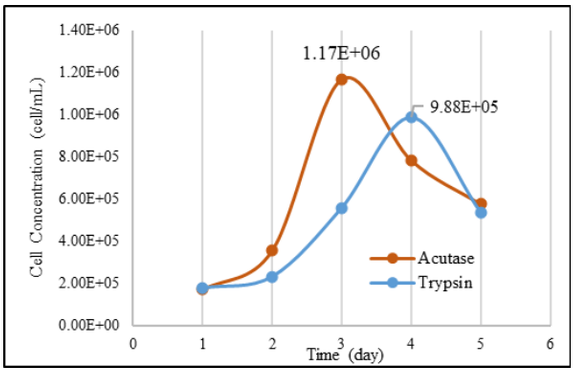

FIGURE 5. The Cell Concentration by Time using Acutase and Trypsin

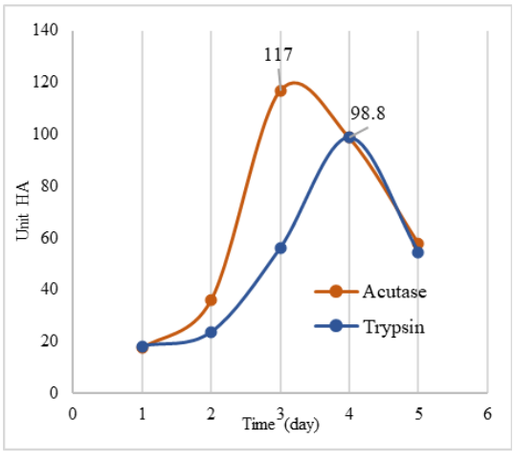

FIGURE 6. The Hemagglutinin Number by Time for Acutase and Trypsin Enzyme

concentration (Killian 2014). Simulation process only can make a prediction based on the theoretical and calculation. Other than that, virus infectivity test also can be tested by using serial dilution method. It is the crucial test to know the exact number of HA produced.

CONCLUSION

The production of influenza vaccine is compatible and suitable with the batch operation. Hence, the yield production is about 60%. Although it is lower compared to the continuous mode but it is more significant and effective. The artificial intelligence (AI) will be added to ensure the components of the vaccine is syariah compliant. The process of cultivation of the cells for the each enzyme achieves the cell concentration of acutase ($1.17X10^6$ cell/mL) and trypsin ($9.88X\ 10^5$ cell/mL). Each cultivation needs 3 and 4 days to reach a maximum of cell concentration, respectively. The concentration of hemagglutinin (HA) by using acutase can achieve up to 117 unit HA compare to the trypsin which is 98.8 unit HA with different time of cultivation in 3 to 4 days respectively. This study proves that the syariah-compliant influenza vaccine can be produced by replacing the trypsin enzyme to acutase. However, it can be predicted that the costing of the vaccine will be more expensive from current technology

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DECLARATION OF COMPETING INTEREST

None.

REFERENCES

Audsley J. M. & Tannock G. A. 2008. Cell-based InfluenzaVaccines Progress to Date. Drugs 68(11):1483–1491

Brown, I. R. 1990. Induction of heat shock (stress) Genes in the mammalian brain by hyperthermia and other traumatic events: A current perspective. *Journal of Neuroscience Research* 27(3): 247–255.

Claudius Seitz, Britta Isken, Björn Heynisch & Maria Rettkowski, Timo Frensing & Udo Reichl. 2012. Trypsin promotes efficient influenza vaccine production in MDCK cells by interfering with the antiviral host response. *Appl Microbiol Biotechnol* 93: 601–611.

Derrick Baxby, Edward Jenner. 1999. Inquiry bicen- tenary analysis. *Journal of Vaccine* 17: 301-307.

Ernest Milián and Amine A. Kamen. 2015. Review Article: Current and Emerging Cell Culture Manufacturing Technologies for Influenza Vaccines. *BioMed Research International* Article ID 504831

Fathima, S., Lee, B. E., May-Hadford, J., Mukhi, S. & Drews, S. J. 2012. Use of an innovative web-based laboratory surveillance platform to analyze mixed infections between Human Metapneumovirus (hMPV) and other respiratory viruses circulating in Alberta (AB), Canada (2009–2012). *Viruses* 373(June): 2754–2765.

Fencl et al. Appl. Chem Biotechnol. 1972. 22, 405

Frensing, T, Heldt, S., Jordan, I., Genzel, Y., Krober, T., Hundt, B., Wolff, M., Seidel-Morgenstern, A., Reichl, U. 2013 Max Planck Institute for Dynamics of Complex Technical Systems, Magdeburg/ Germany Chair for Bioprocess Engineering, Otto-von-Guericke University, Magdeburg/ German.

Gaurab Karki. 2017. Difference between batches,fed-batch and continuous culture technique. Bacteriology, Microbiology.

Hoskisson PA1, Hobbs Continuous culture--making a comeback G. Microbiology. 2005 Oct; 151 (Pt 10):3153

Howard L. Levine. 2010. Vaccine Manufacturing Facilities of the Future. Slide.

Irwan Mohd Subri. 2018. Vaksinisasi sebagai kaedah pencegahan menurut Islam - USIM

J. Trazzino. 2010. Vaccine Manufacturing Facilities of the Future. Slide

Kekarainen T, Martinez-Guino L, Segales J. 2009. Swine torque teno virus detection in pig commercial vaccines, enzymes for laboratory use and human drugs containing components of porcine origin. J Gen Virol 90 (Pt 3):648–653

Killian, M. L. 2014. Chapter 1 Hemagglutination Assay for Influenza Virus 1161: 3–9.

Mahesh Bule. 2017. Process Kinetisc. Slide

Meghrous, J., Mahmoud, W., Jacob, D., Chubet, R., Cox, M. & Kamen, A. A. 2009. Development of a Simple and high-yielding fed-batch process for the production of influenza vaccines. *Vaccine* 28(2): 309–316.

Merten OW. 2000. Safety for vaccines. *Cytotechnology* 34(3): 181–183.

Mohd Azmir Arifin, Maizirwan Mel, Mohamed Is-mail Abdul Karim, and Aini Ideris. 2010. Research Article: Production of Newcastle Disease Virus by Vero Cells Grown on Cytodex 1-Microcarriers in a 2-Litre Stirred Tank Bioreactor

Morrow K. J. 2006. Disposable bioreactors gaining favour: New component and system improve process reliability and reduce cost. *Genetic Engineering.* 2006. *News* 26 (12): 42–45.

Muhammad Amirul Irwan & Nurina Anuar. 2019. Effect of Different Proteolytic Enzymes on The Infectivity of influenza B Virus in Mammalian Cell Culture for Production of Syariah Compliance Virus Vaccine. Unpublished Thesis UKM.

Oyeleye, O. O., Ogundeji, S. T., Ola, S. I. & Omitogun, O. G. 2016. Basics of animal cell culture: Foundation for modern science. *Biotechnology and Molecular Biology Reviews* 11(2): 6–16.

Plotkin. 2013. *Vaccine Fact Book.* Sino Biological.

Influenza Hemagglutinin (HA) Protein Function.2019.https://www.sinobiological.com/Influenza-Hemagglutinin-Function-a-147.html

S. Lakhani. 1992. Early clinical pathologists Edward Jenner (1749-1823).

Seitz, C., Isken, B. & Heynisch, B. 2012. Trypsin promotes efficient influenza vaccine production in MDCK cells by interfering with the antiviral host response 601–611

Ting Lee, Chen Lee, C. Z. 2015. Effect of acutase or trypsin dissociation on the apoptosis of human striatum-derived neural stem cells. *Chinese Academy of Medical Science* 37(2): 185–194.

Wang Shik Ryu. 2017. *Molecular Virology of Human Pathogenic Virus*es. 1st edition

Wong Sook-San & Richard J. Webby. 2013. Traditional and new influenza vaccine. *Journal ASM*.