Penicillin fermentation and role of strain improvement on efficiency

Introduction

Penicillin is one of the most vital drugs known to the medical world. Its discovery by Alexander Fleming in late 1920's changed the face of the world by bringing hope in peoples' lives as penicillin was effectively used against diseases such as syphilis and staphylococcus infections. Penicillin's discovery was an unexpected accident carried out while isolating staphylococcus aureus by growing bacteria on petri dishes. It was discovered that on one of the dishes a contaminating mould named penicillium notatum had no bacteria around it. Fleming noticed this behavior and obtained a small amount of the secreted antimicrobial product and named it penicillin. However Fleming was not able to commercialize the product into starting a large scale production process and preserved the cultured organism. As penicillin became more popular among other researchers, fermentation processes were being developed to improve its yield. [3]. Using these fermentation techniques, researchers started to carry out the production of penicillin in labs at a smaller scale. This process required a considerable amount of time and effort. One of the first methods used to produce penicillin came not too soon after its discovery. The first production procedure resulted in penicillin being produced by fermentation using surface method. In this method, penicillium mould was grown on top of a quiescent medium and bottle plant techniques were used to yield penicillin. This was a descent breakthrough in producing the antibiotic drug but it took very long growing cycles of penicillium mould. [3]. The demand for antibiotics really went up during and after the Second World War. At this

time penicillin was well known around the world, however its production at larger scale with better efficiency was needed to make it a household name.

The challenge of producing penicillin at larger scale was very daunting for researchers and engineers. It was at that time when fermentation research on corn steep liquor at a laboratory in Illinois, USA allowed in producing 2. 3 million doses through development of deep tank fermentation [5]. As the demand for penicillin was rising, different ways of obtaining the antibiotic drug increased. Such production methods involved the use of fermenters with buffers and separation funnels, the property of liquid-liquid extraction, distribution ratios and finally the use of a membrane in a countercurrent extraction column.

Penicillin Production Procedures

Since the discovery of penicillin as an antibiotic drug, there have been many different methods developed to produce penicillin efficiently in order to achieve maximum yield. For instance, the recovery of penicillin using an emulsion liquid membrane in countercurrent extraction column is very successful method yield a highly qualitative product. The use of a countercurrent extraction column requires both a dispersed phase and an aqueous phase. The aqueous phase is made of penicillin potassium salt which is dissolved in a citrate buffer solution while the dispersed phase is made of a mixture of internal aqueous solution; sodium carbonate present in de-ionized water and organic solution, a mixture of a secondary amine, and nonionic polyamine in kerosene [1]. In order to process this method, the continuous phase is first fed through the top of the column at unsteady state, and when the flow of this phase reaches steady state, the dispersed

phase is injected through the bottom of the column, producing a countercurrent extraction with the help of a nozzle. While the process is extracting, time samples are taken from the top and the bottom to monitor the concentration of penicillin. Once the separation of the continuous phase and dispersed phase occurs with the help of a filtration process, the penicillin that was present in the continuous phase is recovered. The effectiveness of the removal of different types of penicillin can be removed at a greater value at lower pH. This is because at a lower pH the impurities are left behind and can be removed, but at a higher pH, the impurities will remain with the organic phase, and thus will be hard to separate. This is the major reason why the aqueous extract is acidified again, so that we are able to lower the pH and obtain a better amount of penicillin and remove most of the impurities present [4].

Although methods like Emulsion liquid membrane in countercurrent extraction column can be very productive and economically feasible, some other measures are still required to produce penicillin in industry at a larger scale.

Industrial Procedure

The industrial process of the recovery of penicillin is in fact more complex. It requires the use of a fermenter, a filtration system and 3 buffer units, as shown in figure below.

The industrial process uses the same principle of distribution ratio, which determines the measure of purity in the extracted product. The distribution ration is equal to the concentration of a solute phase in an organic phase

divided by its concentration in aqueous phase. This principle is effectively used in removing the level of impurities from the final product. In this process, corn-steep liquor, which is a waste product in the wet milling of corn, is entered as the feed. When it enters the fermenter, additional nutrients such as metabolism modifiers and antifoaming agents can also be added. The pH is then adjusted and the contents in the fermenter are seam sterilized. As the temperature reaches a value of about 25oC, the fermenter is inoculated with a pure and high yielding mutant strain of Penicillium [2]. At this point, it comes in contact with sterile air with the help of turbine agitation to provide proper air medium interface and the constant addition of extra nutrients and antifoaming agents [2]. This whole process is carried out for approximately one week. After the fermentation period, the solution is then sent to a filtration unit where impurities such as mycelium are separated from the aqueous solution that contains penicillin. As the separation takes place, the aqueous solution goes through three extraction units to obtain the final product in solid form. First the solution enters the primary extraction unit where its pH is reduced to a value of two by using sulfuric acid and amyl acetate, these results in penicillin to be extracted. This organic solution is then sent into the secondary extraction unit where it comes into contact with an aqueous buffer solution with a pH of six. This results in the formation of rich penicillin aqueous solution. Finally this solution is sent to the third and final extraction unit. Here the solution is re-acidified and either contacted with the same organic solvent used in primary extraction or with another organic solvent such as methyl isobutyl ketone. This second contact with organic solvent is used to prevent the transfer of

any soluble impurities [2]. After continuous treatment, the final product of penicillin is recovered in solid form.

Conclusion

Penicillin is world renown antibiotic, its importance came in the early 1900's were it was used as a healing agent for bacterial infections. Till today its importance plays a vital role in society. Penecillin process is carried out with the help of new ways being developed in the recovery of penicillin. To conclude, it was noted that there are many different ways to recover penicillin using different agents. However, the quality and quantity of the product plays an important role in determining the best fermenting process. Using the principles of distribution ratios recovered penicillin in an almost pure form. While the use of countercurrent extraction column gave a more modernized method, but was not always able to give similar results as the use of distribution ratios.

References

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