# Module – 13: Medium Optimization

#### Introduction

Fermentation industry require particular product from given organisms. Only particular product is not important but it should be produce in large quantity. For the production of huge amount of particular product, either medium formulation is proper or there should be improvement in organism.

Medium optimization is a process where components of medium or different conditions either varied in concentration or changed so that we can get better growth of the organisms for high productivity.

Different combinations and sequences of process conditions need to investigate to determine the growth conditions, which produce the biomass with the physiological state best, constituted for product formation. There may be asequence of phases each with a specific set of optimal conditions.

#### **Different Methods for Fermentation Process Optimization**

#### Borrowing

It is an open-ended system where all the medium components used for analysis of product formation taken from the different authors. It is very easy to see the ingredients of particular media from various authors, but there are so many options and one has to select the appropriate medium for particular fermentation process.

#### **Component Replacing**

It is also an open-ended system for process optimization.Ingredients of the different fermentation medium compared and others replace few of the components. With this method, we cannot get idea about interaction of different medium components, but different medium component like carbon, nitrogen and other sources screened for medium optimization.

#### **Biological Mimicry**

In this method, different elemental composition required by the microbes for its best growth is studied. Medium formulationbased on the composition and exact amount of components required by microorganisms. Such type of medium gives best growth and high yield of product but this process is time consuming and expensive. It is not easy to analyze the elemental composition of microbes. It is a close-ended system, where component interactions cannot studied.

### **One Factor At-A-Time**

It is also a close-ended system for medium optimization process. In this method, optimization of medium carried out by changing any one of the ingredients of the medium while keeping all other parameter as it is. It is time consuming and difficult to study the interactions among the medium ingredients. This method is useful for study of only few medium ingredients or parameters, as it requires large number of experimental sets which is again time consuming and expensive. This method is very popular as it is easy and suitable for given medium at a time.

## **Factorial Design**

Factorial design is a close-ended system for optimization of medium. This method involves variation in factors or parameter at two or more levels. Factorial design is highly efficient in providing interaction among various factors and allow us to study effect if each factor and its interactions, giving maximum yield.

In this process equation is used that will provide information regarding particular factors like strain, medium components and other physical parameters and their interactions; that can change yield.

To make a full factorial search, which would examine each possible combination of independent variable at appropriate levels, could require a large number of experiments,  $x^n$ , where x is the number of levels and n is the number of variables. This may be quite appropriate for three nutrients at two concentrations (2<sup>3</sup> trials) but not for six nutrients at three concentrations.

When investigation is for more than five independent variables, the Plackett-Burman design used to find the most important variables in a system, which is then optimized in further studies.

#### **Plackett-Burman Design**

This procedure will identify the important variables and allow them to be rank in order of importance to decide which to investigate in a more detailed study to determine the optimum values to use.

Plackett-Burman gives a series of designs for up to one hundred experiments using an experimental rationale known as balanced incomplete blocks. This technique allows for the evaluation of X-1 variables by X experiments. X must be a multiple of 4, e.g. 8, 12, 16, 20, 24, etc. Normally one determines how many experimental variables need to be included in an investigation and then selects the Plackett-Burman design which meets that requirement most closely in multiples of four.

Any factors not assigned to a variable designated as a dummy variable. Alternatively, factors known to not have any effect may be included and designated as dummy variables; the incorporation of dummy variables into an experiment makes it possible to estimate the variance of an effect (experimental error).

This procedure will identify the important variables and allow them to rank in order of importance to decide which to investigate in a more detailed study to determine the optimum values to use.

The next stage in medium optimization would be to determine the optimum level of each key independent variable, which identify by the Plackett-Burman design using response optimization techniques, which is introduced, by Box and Wilson.

#### **Response Surface Methodology**

Not only different variables but also their quantity is important in formulating medium for optimal growth of organisms giving high yield. It is a Statistical experiment design, which provides information regarding quantity of various variable used in Plackett-Burman method. Here response is the yield of product when particular quantity of various variables is used. Here mathematical calculations used for the combinations of quantities of various parameters and its effect, which gives result, which is plotted, and model is prepared by which one can predict the amount of variable s for the medium optimization.

#### **Evolutionary Operation**

It is method for obtaining high yield by using factorial design serially; while changing variables of media used in factorial design until improvement in the result is greater than the estimated values.

#### **Evolutionary Operation Factorial Design**

Thismethodology is a hybrid of evolutionary operation and factorial design technique. This methodology is multi variable sequential search technique; where the effects of n variable factors are studied and response is analyze statistically, which enable the selection of optimum conditions for individual parameters for planning of following experiments.

#### **Artificial Neural Network**

Various Experiments performed regarding medium optimization, data generated due to such experiments are plot in mathematical equations, and model created. Artificial neural network is the model in which set of experimental data are used which is used to predict new data with the help of mathematical equation.

This model tool fails or confused when data are not in particular, sequence and different data obtained for the same reading feed in different manner but it can give average value for such data, which can solve the problem.

#### **Genetic Algorithms**

It is non-statistical method, based on principle of genetic manipulation, which lead to desired organisms producing high yield. With the help of mutations, crossing over or recombination organisms with unique organisms are produced which can give better yield in particular medium formulation conditions. After obtaining such organisms,replication of such strain produce high yield strains. However, its main disadvantage is that it is not capable of storing the information generated at each stage of the optimization process.

#### Conclusion

Media formulation or optimization is tedious and never-endingprocess. Each technique discussed above has its own merits and demerits. However, only one method is not useful in designing media formulation or optimization. Nowadays hybrid techniques used for the medium optimization as well as process optimization.

## References

- **Principles of Fermentation Technology:** (2<sup>nd</sup> edition, by Peter F. Stanbury, Allan Whitaker and Stephen J. Hall, Butterworth-Heinemann, An imprint of Elsevier Science.)
- Industrial Microbiology: (By Casida L. E.New Age international (P) ltd publications)
- A Text Book of Industrial Microbiology: (2<sup>nd</sup> edition By WulfCrueger&AnnelieseCrueger)
- **Biotechnology:** Food Fermentation Microbiology, Biochemistry & Technology Vol. 1 & 2:(By V.K. Joshi & Ashok Pandey)
- **Manual of Industrial Microbiology and Biotechnology:** (2<sup>nd</sup> Edition by Arnold L. Demain and Julian E. Davies, Ronald M. Atlas, Gerald Cohen, Charles L. Hershberger, Wei-Shou Hu, David H. Sherman, Richard C. Willson and J. H. David Wu)
- Industrial Microbiology-An introduction: By Michael J. Waites, Neil L. Morgan, John S. Rockey and Gary Higton)
- Comprehensive Biotechnology-The Principles, Applications and Rugulations of Biotechnology in Industry, Agriculture and Medicine: (By Mrray Moo Young)
- Fermentation Technology : Up Stream Fermentation Technology- Vol-I: (By H. A. Modi-Pointer Publications)
- **Fermentation Technology :** Down Stream Fermentation Technology- Vol-II: (By H. A. Modi-Pointer Publications)
- **Industrial Microbiology by Prescott and Dunn's:** (4<sup>th</sup> edition, edited by Gerald Reed, CBR publications)
- Fermentation Technology: (By M.L. Srivastava, NAROSA publications)
- Industrial Microbiology: (By A.H. Patel)
- International student edition: Microbiology- A laboratory Manual: (4<sup>th</sup> edition. By James G. Chappuccino& Natalie Sherman)
- Bacteriological Techniques: (By F.J. Baker)
- Introduction to Microbial Techniques: (By Gunasekaran)
- Mannual of Industrial Microbiology and Biotechnology: (2<sup>nd</sup> Edition by Arnold L. Demain and Julian E. Davies, Ronald M. Atlas, Gerald Cohen, Charles L. Hershberger, Wei-Shou Hu, David H. Sherman, Richard C. Willson and J. H. David Wu)

## Web references

- <u>http://www.homebrew.net/ferment/</u>
- <u>http://www.soyinfocenter.com/HSS/fermentation.php</u>
- <u>http://www.ensymm.com/pdf/ensymm\_fermentation\_abstract.pdf</u>
- <u>http://scialert.net/fulltext/?doi=jm.2007.201.208</u>
- http://aem.asm.org/content/7/1/57.full.pdf
- <u>http://www.slideshare.net/yongkangbirdnest/lecture-4-sterilization</u>
- <u>http://www.ars.usda.gov/research/publications/publications.htm?seq\_no\_115=140721</u>
- http://www.scribd.com/doc/30706834/Fermentation-Design
- http://www.wiley-vch.de/books/sample/3527318194 c01.pdf
- <u>http://www.engineersirelandcork.ie/downloads/Biopharmaceuticals%2020Jan09%20-%202%20-%20Ian%20Marison%20DCU.pdf</u>
- <u>www.yobrew.co.uk/fermentation.php</u>
- <u>http://bioscipub.com/journals/bbb/pdf/19-24.pdf</u>
- http://gertrude-old.case.edu/276/materials/web/immobilizedenzymereview.pdf
- <u>http://download.bioon.com.cn/upload/month\_0902/20090223\_b809d1c59ba2a6e2abfdJtWiJOiFDm02.att</u> <u>ach.pdf</u>
- http://bioprocess-maulik.blogspot.in/2007/07/design-of-industrial-fermentation.html

- http://hsc.csu.edu.au/biology/options/biotechnology/3051/biotechnologyPart3.html
- <u>http://www.rsc.org/ebooks/archive/free/BK9780854046065/BK9780854046065-00001.pdf</u>
- <u>http://www.biotech.upm.edu.my/academics/On%20Line%20Note/Bioprocess/BTK%205301/Lect6%28In</u> oculum%20Preparation%20and%20Development%29.pdf
- <u>http://www.biotechresources.com/services-strain.shtml</u>
- <u>http://www.idosi.org/wjc/4%281%2909/14.pdf</u>
- <u>http://cheserver.ent.ohiou.edu/Paper-gu/DualFeed.pdf</u>