ejpmr, 2018,5(5), 66-71

SJIF Impact Factor 4.897

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Review Article

ISSN 2394-3211

EJPMR

SENSORS, PRECURSORS AND MEDIUM OPTIMIZATION OF FERMENTATION PROCESS

***Sikander Ali, Shanza Ashraf, Ayesha Akram, Arfa Jabbar**

Institute of Industrial Biotechnology, Government College University, Lahore, Pakistan.

Corresponding Author: Sikander Ali

Institute of Industrial Biotechnology, Government College University, Lahore, Pakistan.

Article Received on 03/03/2018 **Article Revised on 24/03/2018** **Article Accepted on 14/04/2018**

ABSTRACT

Industrial fermentation is the process of fermentation by use of microorganisms such as bacteria, fungi and eukaryotic cells to make useful products for humans. Different factors are important to optimize prior to the fermentation. These include the sensors, precursors as well as medium optimizing techniques. Different sensors are present to control the respective parameters that are essential for successful process of fermentation. Fermenters are designed according to condition of microbes. At small scale or lab level small fermenter are used while at pilot scale large fermenters are used. The review article focuses on the different precursors such as aroma, flavor, odor and antibiotic precursors. Also, various optimizing techniques including OFAT, PBD as well as TD.

KEYWORDS: One-factor-at-a-time (OFAT), Plackett Burman Design (PBD), Taguchi Design (TD).

INTRODUCTION

The process of fermentation has been used since ancient times. It is known that products have been produced by the use of fermentation technology from the very early ages but now the modern era is focusing on products that are of high quality and cheap as compared to the previous ones.[1] Industrial fermentation is the process of fermentation by use of microorganisms such as bacteria, fungi and eukaryotic cells to make useful products for humans. Some chemicals are also made by the process of fermentation. The fermentation rate depends on concentration of microorganisms, enzymes, temperature, pH and also oxygen requirement for aerobic fermentation. The process of fermentation is divided into four types i.e, production of biomass, production of enzymes, production of extracellular metabolites and transformation of substrate.

Sensors of an Industrial Fermenter

Sensors are used to monitor the physical, biological and chemical parameters such as dissolved oxygen, concentration of carbon dioxide, pressure, potential of hydrogen and turbidity control. Sensors are classified on the basis of primary input, transduction principles, property and application. The laboratory scale includes feedstock and heater i.e, nutrient and temperature control agents by peristaltic pump.[2] Sensors used in fermenters and bioreactors are as follows.

pH Sensor

Fermenters are normally operated very efficiently. The process is done at constant pH. The pH changes as metabolic products are formed in the fermentation media. So, pH control is essential for the process of fermentation. pH has a main role in the growth of cell and formation of product by breakdown of substrate and transport the product via cell wall. The function of pH sensor is to maintain pH level during the process. pH meter is used for this purpose to measure the alkalinity and acidity of liquid.[3]

Temperature Sensor

Temperature is also an important parameter and needs to be monitored according to conditions of microorganisms present in fermenter. So, there is a need to control temperature by temperature sensor or thermo well with temperature probe. It control temperature of wort instead of ambient temperature inside the fridge. It is usually located on side of fermenter and wrap it on the top of probe for insulation. A thermometer works only to measure the ambient temperature indoor and outdoor. A liquid filled thermometer is also used because liquid reacts slower to change than air outside present. It also measures the exact temperature while the door is opened for a short time.

Dissolved oxygen sensor

The dissolved oxygen concentration in medium i.e, proportional to pO2 that is partial oxygen pressure known as DO, is an important parameter of gas metabolism. The rate of air flow and stirring speed is monitored by the process control system (PCS). During the process of fermentation oxygen concentration declines. The purpose of process control system (PCS) is to monitor the speed and rate of air flow at about 50% saturation.[4]

Amperometric Sensors

These sensors are used to analyze the lactate content during the process of fermentation and two methods are most commonly used.

1. Physical adsorption
2. Electrochemical polarization

Sensor immobilized in lactate oxidase by physical adsorption is characteristic of narrow range and have great response as compared to biosensor immobilized in poly (3,4-ethylenedioxythiophene) PEDT lactate oxidase by electrochemical polarization. Immobilization method does not affect stability.[5] Different matrixes are used for immobilization on membrane. Recently 2,6-d-dihydroxynaphthalene polymerized with 2-(4-aminophenyl)-ethylamine gives high sensitivity to lactate. Nylon matrixes are also used with high sensitivity and based on lactate oxidase immobilization. PEDT has low charge alteration, weak conductivity and high stability.

Chlorine Dioxide Amperometric Sensor

Chlorine dioxide diffuses across silicone membrane of sensor between electrolyte solution and cathode. When potential is applied reduction occurs at gold cathode and silver anode is oxidized. The removal of electrons at cathode and at anode it is accepted generates current flow that is in direct proportion to CLD concentration outside the sensor in the medium.

Dissolved Carbon Dioxide Sensor

Carbon dioxide is the product of fermentative metabolism and respiratory pathway of microorganisms. During process of fermentation emission of carbon dioxide relates with consumption of substrate and concentration of biomass. The concentration of carbon dioxide have prominent effect on metabolism of microorganisms. For example, high concentration of CO2 may lead to delayed budding process in yeast. The formation of products in medium also depends on CO2 concentration. Very low CO2 may lead to yield loss.[6]

Turbidity sensor

In turbidostat method the medium contains all the nutrients essential for the growth of microbes. It consists of a photoelectric cell also known as turbidity sensor that measures the changes in turbidity and then controls the medium amount fed to fermenter. This type of sensor is free of surface outputs and are covered with window to prevent the sensor fouling and protection of fiber in the presence of abrasive particles.

Optical Density Sensor

Optical density is measured to calculate the growth and metabolic activity of cells. It is logarithmic function and light absorption is increased, intensity of light is decreased while passing through the sample. The problem is that OD also measures dead cells. If too many dead cells are present metabolic activity will decrease. Optical sensor is used to measure cell biomass and also monitor the filtration of medium during the process of fermentation. Optical probes that are dipped in liquid culture have been developed on basis of fluorescence, transmission, reflectance and particle analysis. Biomass range can be measured by path length between detector and source. These sensors have advantages of fouling sensor and eliminate the contamination.[7]

Foam Sensor

During fermentation process it is very important to reduce the foaming. If foaming becomes more then due to wet filters contamination may occur. Tapping may develop leading to loss of contents present in fermenter. In some cases antifoams also causes problems with downstream processing and aeration. Foam breakers are also used that breakdown the foam by certain rotating mechanisms inside the fermenter. 'Turbosep' is developed in which foam is placed on the stationary turbine vanes and as a result liquid part is moved back to fermenter.[8]

Precursors of Fermentation

Precursors are the substances added before or during the process to increase the yield and quality of fermentation product without any key change in the original molecule of the end product.[9]

Aroma Precursors

Microorganisms play an important part in generating natural compounds, particularly as precursors of food aromas. The short chain fatty and keto acids are used as precursors for the production of characteristic fruit type aroma compounds. Cell suspension cultures of strawberry produce low concentrations of ethyl and butyl butyrate and alter keto valerate to butanal and butanol. The esterase, decarboxylase, and alcohol dehydrogenase are may be present in cell cultures of strawberry.[10]

In koji fermentation, formation of volatile compounds by *Aspergillus oryzae* is increased in oxygen limiting conditions. The yeast *Zygosaccharomyces rouxii* has wide importance in the generating furanone, a quality of the aroma of a traditional fermented food, miso. Amino acids and reducing sugars are chief components aroma precursors in chocolates. The amino acid degradation by reducing sugars during the process of roasting has a little effect on the production of supplementary aromas and flavors in chocolates.[11]

Malolactic fermentation, a biochemical process, carried out in wine subsequent to alcoholic fermentation. It converts L-malic acid into L-lactic acid and carbon dioxide, and results in the de-acidification and enhanced microbiological stability of wine. The capability of certain bacteria to make moderately important enzymes, capable of hydrolysing glycosides and esters as their substrate, is a cause of changes in aroma as a significant amount of these compounds in grapes and wine occurs as non-volatile and odorless glycosides.[12]

Wheat bran, cassava and sugar cane bagasse are plenty substrates for the growth and aroma production by the mold *Ceratocystis fimbriata*. In nutrient media, sugar cane bagasse with a synthetic medium having glucose gives a fruity aroma whereas leucine and valine containing medium gives sturdy banana like aroma. Aroma formation is dependent on growth and utmost aroma level is attained at maximum respirometric activity. When leucine or valine is added in the substrate, the production of overall volatiles reaches tenfold higher than the ripe bananas.[13]

Table 1: Subtrates and precursors of aroma by *Ceratocystis fimbriata.*

Substrate	Precursors
Wheat bran	Leucine
Wheat bran	Urea
Cassava bagasse	Leucine
Cassava bagasse	Valine
Sugar cane bagasse	Leucine

Flavor Precursors

The importance of terpenoid and C13 norisoprenoid glycosides to the flavor of aromatic wines has been known as precursors of fruity and floral flavors. The flavor precursors in grapes are structurally diverse from that of wine. They range from glycosides, conjugates of amino acids, volatiles with no odor, hydroxycinnamic acids to several others. For making wine they are mostly derived from the grape berry, oak and other materials. Flavors are obtained from precursors during crushing and production. The processes may include enzymatic and non-enzymatic transformations of microbial enzymes like glycosidases, esterases, decarboxylases, acid-catalyzed hydrolysis and various chemical rearrangements. Flavors are also extracted from glycosides and amino acid conjugates of mouth microflora. It gives that flavor precursors participate in retronasal aroma development by discharge in mouth during consumption.[14]

Allium cepa (onion), *A. sativum* (garlic) and other Alliums are significant because of the cookery importance for the flavors and odors. They are the characteristics of all species and are formed by chemical conversions of a chain of volatile sulphur compounds released by the breakdown of somewhat stable, odorless, S-alkyl cysteine sulphoxide flavor precursors by the enzymes (i.e, alliinase and lachrymatory synthase factor). The secondary metabolites of Alliums are S-methyl cysteine sulphoxide, S-allyl cysteine sulphoxide, S-trans-prop-enyl cysteine sulphoxide and S-propyl cysteine sulphoxide. The synthesis these precursors involves (thio) alkylation of cysteine otherwise of precursor such as O-acetyl serine.[15]

Meat flavor is feature of volatiles produced as a result of reactions of non-volatile components which are induced thermally. The water soluble compounds, low in molecular weight, and lipids are important precursors of cooked meat flavor. The Maillard reaction and vitamin degradation are important reactions in cooking which build up meat flavor from uncooked meat with slight aroma and bloody taste. The pre-slaughter and postmortem conditions such as animal breed, sex, age and cooking circumstances are vital in flavor improvement of cooked meat.[16]

Odor Precursors

Various types of thiols play an important function as odorant in Sauvignon blanch wines. Particularly, following three thiols participate in the tropical fruit aromas of the wine.

1. 4- mercapto-4-methylpentan-2-one (aroma of black cat pee)
2. 3-mercaptohexanol (aroma of grapefruit)
3. 3- mercaptohexyl acetate (aroma of passion fruit)

For the fabrication of these aromatic compounds, yeast is required during fermentation of wine from precursors present in grape juice. Somehow, cys-teinylated and glut-athionylated conjugates are shown as thiol precursors. Cys and glut precursors in grape juice are used by yeast and converted into the particular thiols. Afterwards yeast acetylates a portion of 3-mercaptohexanol to yield 3-mercaptohexyl acetate.[17]

Table 2: Important Odorants and their Precursor in Wheat Bread.

Odorant in Wheat bread	Precursor	Odor
2-acetyl-1-pyrroline	Ornithine, Proline	Roasty, cracker like
3-Methylbutanal	Leucine	Malty
(E)-2-Nonenal	Lipids	Fatty
2-Phenylethanol	Phenylalanine	Flower
3-Methylbutanol	Leucine	Fermentation Like
1-Octen-3-one	Lipids	Mushroom like

Precursors in oil based Fermentation

Methylmalonyl-CoA is among the common precursors utilized by modular type I polyketide synthase. All the six polyketide accumulation steps in erythromycin biosynthesis make use of precursor whereas seven steps in monensin B biosynthesis employ the precursor. The presence of adequate intracellular methylmalonyl-CoA signifies a preventive factor for production of considerable amount of polyketide products in fermentation, both in natural and engineered hosts. Corn oil and corn meal acquired from high oil corn are involved in useful products. Fermentation based products include amalgamation of corn meal left after the extraction of oil from high oil corn with water and an enzyme.[18]

Antibiotic Precursors

The addition of precursors to the antibiotics is the earliest method for improving yield and efficiency of that particular antibiotic. A range of side chains are added o

the penicillin molecule such as the addition of phenylethylamine from the corn steep liquor resulted in yield of penicillin G (Benzyl penicillin). Similarly the addition of phenylethyl acetic acid to the penicillin increased its yield by three folds.[19]

Table 3: Important antibiotics, their precursors and Microorganisms.

Antibiotics	Precursor	Microorganism
Penicillin G	Phenylacetic acids related compounds	*Penicillin chrysogenum*
Griseofulvin	Chloride	*Penicillium griseofulvin*
Nikkomysins	Nucleosides and bases	*Streptomyces tendae*
Organomysin A and B	*p*-hydroxycinnamate	*Streptomyces organonesis*
Cyclosporin A	DL-α-Amino butyric acid	*Tolypocladium inflatum*
Cyclosporin C	L-Threonine	…

The biosynthesis and regulation of 6-aminopenicillanic acid (6-APA), benzylpenicillin (BP) and phenoxymethylpenicillin (PMP) by the strains does not differentiate significantly. In the absence of precursor both strains synthesize 6-APA (Aminopenicillanic acid). While, in phenylacetic acid and phenoxyacetic acid controlled biosynthesis: the fungus produces BP or PMP on the basis of nature of precursors used. In case of insufficient amount of precursors 6-APA is synthesized along the penicillins. PAA is demonstrated to be an additional energetic precursor than POAA. If both precursors are there in fermentation broth, only BR is manufactured. A significant characteristic of strain 316A is its improved sensitivity to PAA particularly in the early period.[20]

Dichloroacetam is a disinfection byproduct which is present in drinking water. The chloramphenicol (CAP) and its analogues (i.e, thiamphenicol, TAP; florfenicol, FF) occur in wastewater affected waters. CAPs have greater DCAcAm formation potential than two respective amino acid precursors. In drinking waters ng/L levels of CAPs do not participate enough as to DCAcAm development as microgram per litre levels of amino acids. Thus the CAPs ought to be measured as potential precursors of DCAcAm, particularly in greatly wastewater contaminated water.[21]

Medium optimization

Prior to the formation of any of these metabolites, the process of optimizing medium is done. In the 19th century, the process of medium optimization was performed my using conventional methods that were costly, slow and less efficient. These methods involve alteration of one factor or variable whereas remaining all the other factors constant. However, the latest methods that are now being used for optimizing medium are cheap, efficient and give results rapidly. In these methods, different factors are altered.[22]

The most important factors that can be optimized involve nutritional components like carbon and nitrogen, osmolality, pH, temperature.

Systems for optimization

The systems which can be used for medium optimization are of two types including both the closed and open systems. The difference between these systems is based on the number of factors that can be optimized. In closed system, limited amount of factors are optimized whereas in open system, infinite number of factors can be optimized.[23] The closed system has the disadvantage that all the factors necessary for optimization cannot be considered.

Criteria for successful optimization

The following table shows the criteria of successful medium optimization.

Table 4: criteria for optimization.

Factors	Effects
Yield of desired product	Maximum
Biomass concentration	Maximum
Product production	Maximum
Yield of unwanted products	Minimum
Quality	Consistent and high
Problems during optimization	minimal

Nutritional control of metabolite production

The most important nutrients that have great effect on the formation of metabolite is carbon as well as nitrogen.[24]

Carbon source

The most vital nutrient that the microbes require for their growth is the carbon source. The growth as well as the compounds produced as a result of metabolic reactions depends on the degree at which this source is consumed. As antimicrobial substances are produced in the secondary metabolism, therefore, their concentration is increased by the consumption of galactose that is a major carbon source. The production of penicillin is decreased in the presence of glucose.[25]

Nitrogen source

The presence and amount of nitrogen containing nutrients in the fermentation process has an essential role in the formation of products. Microbes have the ability to consume different types of nitrogenous nutrients.[26] For example, when tryptophan is incorporated for the synthesis of actinomycin, its production is increased. On the other hand, incorporation of tryptophan inhibits the synthesis of candicidin.[27]

Phosphate

The plasma membrane of the microorganism is composed of various components. One is the phospholipid which provides selective barrier in the movement of molecules. The phosphate molecules are capable of increasing the synthesis of various antibiotics

such as cephalosporins and tetracyclines. As discussed earlier, levels of phosphate in medium has a major effect in the formation of metabolic products. If its level surpasses the normal required value then it shows inhibitory effects.

pH of media

Carbon dioxide bicarbonate system is widely used in the fermentation technology but it has a poor buffering capacity that can be upgraded by using zwitter ions. Na2CO3 as well as NaOH can be incorporated into the medium for adjusting and maintaining the optimal pH.[28]

Strategies for medium optimization

The strategies required for the fermentation medium optimization is largely based on OFAT that is one factor at a time. Some statistical techniques that can be used for this purpose include Plakett Burman design, Central composite design and Taguchi Design.

OFAT

OFAT stands for one factor at time. As the name indicates, in this technique, a single factor is altered whereas the remaining components remains the same. The levels of the constituents that are chosen for the medium optimization are altered according to the requirements. The advantage of this strategy involves its ease to operate and results can easily be interpreted. Apart from its advantages, one major disadvantage of this strategy is that is a slow process and time consuming as well as hard work is required. One factor at a time is divided into some of the groups which include removal experiments, replacement experiments and supplementation experiments.[29]

Statistical medium optimization

In order to overcome the problems that were encountered in OFAT, new statistical methods were developed.[30] These methods are more efficient, rapid, cheap and less hard work required. Statistical methods involve the following.

Plakett Burman Design

PBD is based on the fact that it is not necessary that the constituents that can be added into the medium can support the growth of microbial cells. Therefore, those constituents must be removed or excluded from the medium in the initial phases. Two kinds of variables are designed for this purpose. One is named as dummy variable and the other one is known as real variable. Real variable are those whose level or amount can be altered whereas in dummy variable, level and amount is kept fixed.[31]

Taguchi design

This design was first discovered by Genichi taguchi. The purpose of developing this method was to solve and reduce the risk of problems that were encountered in PBD.[32] The basic mechanism of TD is to check all the components while conducting limited experiments. 3 important phases that are involved in this method involves the system planning, design of parameters and tolerance design stage. In the system planning, the characteristics that are designed are checked for their experimental points. Design of parameter involves the checking the consequence of different factors on the experiment whereas in the last stage, the factors that have a great consequence on the end product are improvised.[33]

CONCLUSION

Prior to the fermentation process, an important factor to be considered is the optimization of medium. The latest methods that are based on statistics are move efficient and valuable because of less time consumption. Moreover, these methods make the products cheap. New researches and studies are in progress to develop such methods which are best suited for the fermentation process. Moreover, the sensors present in the fermentation vessel include pH, temperature, dissolved oxygen, dissolved carbon dioxide, turbidity and foam sensor.

REFERENCES

1. Dubey, K.K, A. Ray and B. Behera. 2008. Production of demethylated colchicine through microbial transformation and scale-up process development. *Process Biochem*, 43: 251-257.
2. Montague, G. A, A. J. Morris and A. C. Ward. 2013. Fermentation Monitoring and Control: A Perspective. *Biotechnol. Gen. Eng. Rev*, 7(1): 147-188.
3. Venetka, I. A. and D. R. Walt. 1993. Fiber-optic Sensor for Continuous Monitoring of
4. Fermentation pH. *Nature*, 11: 726-729.
5. Phillips, D. H. and M. J. Johnson. 1961. Measurement of dissolved oxygen in fermentations. *Biotechnol. Bioeng*, 3(3): 261-275.
6. Shkotova, L. V, T. B. Goriushkina, C. Tran-Minh and A. P. Soldatkin. 2008. Amperometric biosensor for lactate analysis in wine during fermentation. *Materials sci. eng*, 28(5): 943-948.
7. Neil, M. and D. B. Kell. 1989. The control and measurement of CO2 during fermentations. *J. Microbiological Methods*, 10: 155-176.
8. Kangwa, M, V. Yelemane, A. N. Polat and M. Fernandez. 2015. High-level fed-batch fermentation with optical density measurement. *AMB Express*, 5(1): 70.
9. Toledo, J, G. Ruiz-Diez, U. Schmid and J. L. Sanchez-Rojas. 2018. Flow through sensor based on piezoelectric MEMS resonator for the in-line monitoring of fermentation. *Sensors and Actutators*, 254: 291-298.
10. Bernard, H. A. 2009. *Microbial Production of Biopolymers and Polymer Precursors: Applications and Polymer Precursors.* Caister Academic Press Norfolk, UK.

11. Christen, P, J.C. Meza and S. Revah. 1997. Fruity aroma production in solid state fermentation by Ceratocystis fimbriata: influence of the substrate type and the presence of precursors. *Mycol. Res,* 101(8): 911-919.
12. Hong, Y. C, L.C. Huang, G. A. Reineccius, S.K. Harlander and T.B. Labuza. 1990. Production of aroma compounds from strawberry cell suspension cultures by addition of precursors. Plant Cell, Tissue and Organ Culture, 21: 245-25.
13. Kinghorn, J. K. and G. Turner. 1992. *Applied Molecular Genetics of Filamentous Fungi.* (1st Ed) Blackie Academic and Professional, UK.
14. Lancani, G. and R. Lorenzetti. 1993. *Biotechnology of Antibiotics and other Bioactive Metabolites.* (1st Ed) Plenum Press, New York.
15. Li, C, G. Florova, K. Akopiants and K.A. Raynolds. 2004. Crotonyl-coenzyme A reductase provides methylmalonyl-CoA precursors for monensin biosynthesis by Streptomyces cinnamonensis in an oil-based extended fermentation. Microbiology. 150(10): 3463-3472.
16. Moyer, A. J. and R. D. Coghill. 1946. Penicillin. IX. The laboratory scale production of penicillin by submerged culture by *Penicillium notatum* Westling. *J. Bacteriol,* 53: 79-93.
17. Martin, F. P, P.M. Canas, E.G. Romero and M. L. Palop. 2015. Aromatic compounds released from natural precursors by selected Oenococcus oeni strains during malolactic fermentation. *Eur. Food Res. Technol,* 240: 609–618.
18. Mi, K, J. C. and M. R. Tariq. 2015. Meat flavor precursors and factors influencing flavor precursors. Meat Sci, 110: 278-84.
19. Ough, C. S, E. A. Crowell and L. A. Mooney. 1988. Formation of Ethyl Carbamate Precursors During Grape Juice (Chardonnay) Fermentation. I. Addition of Amino Acids, Urea, and Ammonia: Effects of Fortification on Intracellular and Extracellular Precursors. *Am. J. Enol. Vitic,* 39: 243-249.
20. Parker, M, D. L. Capone, I. L. Francis and M. J. Herderich. 2017. Aroma Precursors in Grapes and Wine: Flavor Release during Wine Production and Consumption. *J. Agric. Food Chem.*, 1017-1023.
21. Rohan, T. A. and M. Connell. 1964. The Precursors of Chocolate Aroma: A Study of the Flavonoids and Phenolic Acids. *J. Food Sci*, 29(4): 460-463.
22. Santiago, M. and R. C. Gardner. 2015. Yeast genes required for conversion of grape precursors to varietal thiols in wine. *Yeast Research,* 15: 3-9.
23. Tikhonova, O, V, M. V. Surorova, E. Bartoshevich and L. M. Lure. 1992. Effect of penicillin precursors on antibiotic biosynthesis in various strains. *Antibiot. Khimioter.*, 37(1): 3-6.
24. Rajeswari, P, P.A. Jose, R. Amiya and S.R. Jebakumar. 2014. Characterization of saltern based Streptomyces sp. and statistical media optimization for its improved antibacterial activity. *Front. Microbiol,* 5: 753.
25. Shih, I, Y. Van and Y. Chang. 2002. Application of statistical experimental methods to optimize production of poly (γ-glutamic acid) by *Bacillus licheniformis* CCRC 12826. *Enzyme Microb. Technol*, 31: 213-220.
26. Rokem, J.S, A.E. Lantz and J. Nielsen. 2007. Systems biology of antibiotic production by microorganisms. *Nat. Prod. Rep*, 24(6): 1262-1287.
27. Marwick, J.D, P.C. Wright and J.G. Burgess. 1999. Bioprocess Intensification for Production of Novel Marine Bacterial Antibiotics through Bioreactor Operation and Design. *Mar. Biotechnol*, 1(5): 495-507.
28. Singh, V, M. Khan, S. Khan and C.K. Tripathi. 2009. Optimization of actinomycin V production by Streptomyces triostinicus using artificial neural network and genetic algorithm. *Appl. Microbiol. Biotechnol*, 82(2): 379-385.
29. Sanchez, S. and A.L. Demain. 2002. Metabolic regulation of fermentation processes. *Enzyme Microb. Technol*, 31: 895-906.
30. Stanbury, P. F, A. Whitaker and S.J. Hall. 2017. *Principles of fermentation technology*. Oxford, U.K: Elsevier Butterworth-Heinemann, 110-116.
31. Gonzalez, R, L. Islas, A.M. Obregon, L. Escalante and S. Sanchez. 1995. Gentamicin formation in Micromonospora purpurea: stimulatory effect of ammonium. *J. Antibio*, 48(6): 479-483.
32. Tripathi, C, V. Praveen, V. Singh and V. Bihari. 2004. Production of antibacterial and antifungal metabolites by *Streptomyces violaceusniger* and media optimization studies for the maximum metabolite production. *Med. Chem. Res*, 13: 790-799.
33. Vaidya, R, P. Vyas and H. Chhatpar. 2003. Statistical optimization of medium components for the production of chitinase by *Alcaligenes xylosoxydans*. *Enzyme Microb. Technol*, 33: 92-96.
34. Castro, P.M, P.M. Hayter, A.P. Ison and A.T. Bull. 1992. Application of a statistical design to the optimization of culture medium for recombinant interferon-gamma production by Chinese hamster ovary cells. *Appl. Microbiol. Biotechnol*, 38(1): 84-90.