Preliminary Screening Of Media Formulation Through One Variable At A Time Methodology

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Abstract: Optimization of media component through changes in one variable at a time was used to enhance the biomass and antioxidant activity of selected microorganism (Escherichia coli MTCC no. 40) on different formulated media. For the present study, different types of carbon, nitrogen and mineral sources were used to formulate media composition. On the basis of absorbance, components were selected to formulate new medium and finally formulate a new medium by using all variables in one medium. It was reported that unaltered media showed 0.126 ±0.001 absorbance, media 1 (altered carbon source) showed 0.143 ±0.001, media 2 (altered nitrogen source) showed 0.150 ±0.001, media 3 (altered mineral source) showed 0.124 ±0.012 and media 4 (Altered medium) showed 0.090 ±0.005 absorbance at 492 nm wavelength. The component comparison and analysis was based on changes in one factor in medium. Significant result i.e. increase in biomass was reported in altered nitrogen and carbon medium compared to the unaltered medium. These components will be further used in the formulation and optimization of medium component for response surface methodology which are the primary steps involved in bioprocess technology to enhance the biomass of the particular microorganism.

Key words: One variable analysis, Medium Optimization, Response Surface Methodology, Altered Medium, Biomass.

Introduction

E. coli is the most powerful host vector system in genetic engineering and widely used in pharmacy due to fast multiplication, easily genetic manipulation and high rate of production of recombinant protein. Drug discovery and therapeutic recombinant protein development processes are carried out in E.coli and used to screening target protein for the discovery of novel drug. (Rodriguez et al, 2014; Sahdev et al, 2008). So it is necessary to enhance the biomass production of microorganism such as E. coli. Many researchers worked on nutrient requirements, growth conditions and studies on new medium development for enhancement of biomass production that could lead to more economical production of any type of product. Component of medium optimization is mainly done by Response surface methodology (RSM), which is a group of many statistical and mathematical techniques. RSM is very useful technique for designing and scrutiny of the medium component. It is applicable in many interested variables like increase in biomass production. Biomass production is enhanced by components used in medium formulation. The objective of this response is to optimize medium components through Response surface methodology (Rogosa et al, 1961; Myers et al, 1995). Medium cost, production of a large number of cells and cost of harvesting method are the factors that should be considered to optimize medium composition. (Oh et al, 1995). RSM is a type of screening design method which is used to design economic experimental plans. It requires fewer primary experiments and filters out desired effects and further used to design an experiment which is based on the statistical variation. (Montgomery, 1991). RSM is used by many researchers to modeling the experiments, evaluating the responses and interactions between medium components throughout the study. At present many researchers have used RSM technique to design experiments, regression modeling techniques and as optimizing tool to envisage the maximum bio-product yield. (Dasari et al, 2009; Mohamed et al, 2013; Tan et al, 2010). Optimization of medium at industrial level is usually a major concern to maximize the growth of microorganism and increase the end product (Para et al, 2005; Schmidt, 2005). Quantity of end product is based on different parameters and composition of nutrient component. It Influences the final yield or productivity of particular micro-organism which affect the overall process of economics (Schmidt, 2005). A study on the different variables and interactive effects of a different component in a simple medium suggested that simple medium will help in efforts to optimize the mass production of the selected microorganism. The objectives of this study were to determine the most significant variables and optimize the medium composition which is the primary step to performed response surface methodology.

Materials and Method

Experimental design for One-factor-analysis-at-a-time is based on standard method of media formulation without modification from previous paper. (Aneja, 2015; Tomar et al, 2019). All broth culture was incubated at 28^0C for 48 hours. After the incubation period, the growths of bacterial cells were observed on the basis of turbidity. Preliminary efficiency observed on the basis of turbidity and screening of all media component was done. We selected three substrates with combination of yeast for further optimization of new medium (Table 1). On the basis of absorbance one substrate chosen from each category (carbon, nitrogen and mineral) and used with yeast to formulate new composition. Escherichia coli (MTCC no. 40 l) tested strains were cultivated on the optimized medium under laboratory conditions. All experiments were done in triplicates form. The result of biomass production was calculated in the form of absorbance at 490 nm.

Result and Discussion

Escherichia coli is an expression prokaryotic host - vector system, which is widely used to synthesized any type of therapeutic products and recombinant heterologous proteins (non-glycosylated) because of its fast multiplication property and easy scale up process. Around 30% of the approved therapeutic recombinant proteins are produced through E. coli as an expression system for therapeutic uses. (Mohammed et al 2015). Medium optimization is an essential before production of any large scale secondary metabolite and still one of the most critical phenomenons. In past (before 1970s), optimization of media was accomplished by using different traditional methods, which were tedious, costly and time taking. Traditional methods were involving lots of experiments to

obtain accurate result. After discovery of statistical and mathematical techniques through computer, formulation and optimization of medium component has become more easy, efficient and economic. For any type of medium designing pH, temperature and medium components (e.g., carbon, nitrogen, mineral etc.) are the important factor which must be optimized through RSM. It could be easy to achieve maximum product concentration in form of biomass and other activity through preliminary optimization of above parameters (Wang et al, 2011; Franco-Lara et al, 2006; Gupte and Kulkarni, 2003). Stepwise one variable approach such as ‘‘one factor-at-a-time’’ is difficult and lengthy process, especially for large scale production because a large number of factors contributes in the production of desired metabolites. (Xu et al. 2003). The main application of media optimization in bioprocess industries is to maximize the biomass production. Increased biomass can improved product yields, reduced cost and overall time (Rao et al, 2000). On the basis of absorbance, Glycerol (altered carbon source), ammonium chloride (altered nitrogen source) and inositol were used with yeast extract as a new combination of medium composition named media 1, media 2 and media 3 respectively. Altered media were made from combination of these three new sources of carbon, nitrogen and mineral components with yeast. Table 2 showed the absorbance of all these media was compared with unaltered media (Nutrient agar). According to absorbance, alternative medium gives significant results, which showed the potential to enhance the growth of industrial important microorganism.

Conclusion

In this research, we optimized medium formulation in terms of their utility, maximum yield of metabolite produced by the pre-optimization process. Various recent studies have shown that E. coli strains can be modified specifically for each therapeutic protein to achieve high product yields as well as high-quality products. Statistical design of experiments can be employed to model the relationship between certain variables and one or more responses in a single process. Since the optimization has a remarkable effect on the mass production of microorganism, the optimization of growth conditions, cost and effectiveness of culture medium.

References

[1]. Aneja KR. 2015. Experiments in microbiology, plant pathology and biotechnology. New age international (P) limited: ISBN: 978-81-224-1494-3.

[2]. Dasari VRRK, Donthireddy SRR, Nikku MY. 2009. Optimization of medium constituents for Cephalosporin C production using response surface methodology and artificial neural networks. J Biochem Technol. 1: 69–74.

[3]. Franco-Lara E, Link H, Weuster-Botz D. 2006. Evaluation of artificial neural networks for modelling and optimization of medium composition with a genetic algorithm. Process Biochem.41, 2200–2206. 10.1016/j.procbio.2006.06.024.

[4]. Gupte M, Kulkarni P. 2003. A study of antifungal antibiotic production by Thermomonospora sp MTCC 3340 using full factorial design. J. Chem. Technol. Biotechnol.78, 605–610. 10.1002/jctb.818

[5]. Mohamed MS, Tan JS, Mohamad R. 2013. Comparative analyses of response surface methodology and artificial neural network on medium optimization for Tetr aselmis sp. FTC209 grown under mixotrophic condition. Sci World J.1–14.

[6]. Mohammed N, Baeshen1, Ahmed M, Al-Hejin1, Roop S, Mohamed MM, Hassan AI, Kulvinder S, Nabih A. 2015. Production of Biopharmaceuticals in E. coli: Current Scenario and Future Perspectives J. Microbiol. Biotechnol. 25(7), 953–962 http://dx.doi.org/10.4014/jmb.1412.12079

[7]. Montgomery D.C., Design and Analysis of Experiments.1991. New York: John Wiley and Sons Inc.

[8]. Myers RH, Montgomery DC. 1995. New York: Response Surface Methodology: Process and Product Optimization Using Designed Experiments, John Wiley & Sons.

[9]. Oh S, Rheem S, Sim J, Kim S, Baek Y. 1995. Appl. Environ. Microbiol. 61 3809-3814.

[10]. Parra R, David A, Naresh M.2005. Medium optimization for the production of the secondary metabolite squalestatin S1 by a Phoma species combining orthogonal design and response surface methodology. Enzyme Microbiol Technol. 37: 704–711.

[11]. Tomar RS, Sharma NS, Banerjee S. 2019. Pre optimization and one factor analysis of media composition for Response surface methodology. Journal of Current Science, Vol 20, Special Issue. 04. Available Online: https://journal.scienceacad.com.

[12]. Rao JK, Chul-Ho K, Sang-Ki R. 2000. Statistical optimization of medium for the production of recombinant hirudin from Saccharomyces cerevisiae using response surface methodology. Process Biochem. 35:639–647.

[13]. Rodriguez V, Asenjo JA, Andrews BA. 2014. Design and implementation of a high yield production system for recombinant expression of peptides. Microb. Cell Fact. 13: 65.

[14]. Rogosa M, Franklin JG, Perry KD. 1961 J. Gen. Microbiol. 24 .473-482.

[15]. Sahdev S, Khattar SK, Saini KS. 2008. Production of active eukaryotic proteins through bacterial expression systems: a review of the existing biotechnology strategies. Mol. Cell. Biochem. 307: 249-264.

[16]. Schmidt FR. 2005. Optimization and scale up of industrial fermentation processes. Appl Microbiol Biotechnol. 68:425–435.

[17]. Tan JS, Ramanan RN, Ling TC. 2010. Comparative of predictive capabilities of response surface methodology and artificial neural network for optimization of periplasmic interferon-a2b production by recombinant Escherichia coli.Minerva Biotechnol. 22:63- 73.

[18]. Wang JC, Hu SH, Liang ZC, Yeh CJ. 2005. Optimization for the production of water- soluble polysaccharide from Pleurotus citrinopileatus in submerged culture and its antitumor effect. Appl. Microbiol. Biotechnol. 67, 759–766. 10.1007/s00253-004-1833-x

[19]. Xu CP, Kim SW, Hwang HJ, Choi JW, Yun JW. 2003.

Optimization of submerged culture conditions for mycelia growth and exobiopolymer production by [20].

Paecilomyces tenuipes C240. Process Biochem. 38:1025–1030

Conflict of interest: The authors declare no competing or conflicts of interest.

Table 1: Formulation of media by different C, N, and mineral sources

S. No	Name of medium	Nitrogen Source (5gm/liter)	Carbon Source (1.50gm/liter)	Mineral Source (5gm/liter)	Yeast (1.50 gm/liter)
1	Unaltered media	Peptone	Beef	Nacl	Yeast
2	Media 1 (Altered Carbon)	Peptone	Sodium citrate	Nacl	Yeast
3	Media 2 (Altered Nitrogen)	Ammonium Chloride	Beef	Nacl	Yeast
4	Media 3 (Altered Mineral)	peptone	Beef	Inositol	Yeast
5	Media 4 (Altered Medium)	Ammonium Chloride	Sodium citrate	Inositol	Yeast

Tables and figures

Table 2: Comparison between absorbance of different altered medium with unaltered medium

S. No.	Name of medium	Absorbance (at 492 nm)
1	Unaltered media	0.126 ±0.001
2	Media 1 (Altered Carbon)	0.143 ±0.001
3	Media 2 (Altered Nitrogen)	0.150 ±0.001
4	Media 3 (Altered Mineral)	0.124 ±0.012
5	Media 4 (Altered Medium)	0.090 ±0.005