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Response Surface Method as an Efficient Tool for Medium Optimisation

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ABSTRACT
Medium optimisation can be carried out in several ways. Most popular is the one-variable-at-a-time approach. This approach is however extremely inefficient in locating the true optimum when interaction effects are present. To overcome the problems with interaction effects, efficient medium will be achieved when mathematical optimisation techniques in-terms of Response Surface Methodology (RSM) were applied. RSM as an efficient tool has a long history in the literature. There has been an embarrassment of theoretical and empirical work to show the efficiency of RSM. The microorganisms when inoculated in appropriate, suitable and selective medium using RSM will enhance the concentration of target product used for commercial and industrial application.

Key words: Response surface method, optimization, medium composition

INTRODUCTION
The formulation of a given medium from the different manufacture does vary slightly and may have been modified from the original description of the medium in the literature. Any medium for microbial growth should contain, at minimum, specific elements in the appropriate proportions.

There are two possibilities approach to research designs; one is the change of one variable at a time (Classical method), it is necessary if one seeks to understand the fundamental relationship between cause and effect and is the bases on which theories of equilibrium and kinetics have been founded. The second approach is to change one or more variables from one test to the next (statistical design), it is the empirical one of directly measuring the effect of various changes in conditions without necessarily concerning oneself with the precise nature of the mechanism producing the observed effects (Davis, 1993).

Recent research efforts have focused on further process development (optimisation) and scale-up of a target product. As we all known, medium components play a very important role in enhancing cell growth and increase the target product accumulation, therefore, medium optimisation study is very important. In general, medium optimisation by the traditional 'one-factor-at-a-time' technique was used (Gokhade *et al*., 1991). This method is not only laborious and time consuming but also often leads to an incomplete understanding of the system behavior, resulting in confusion and a lack of predictive ability.

However, little work had been published regarding the variables that affect cell growth for production of target product focusing on optimisation of medium composition and physical environments (Gawande *et al*., 1998). To achieve high product yields, it is a prerequisite to design an efficient medium that will help in rendering the process to be more economical.

Response Surface Methodology (RSM) is a powerful and efficient mathematical approach widely applied in the optimisation of fermentation process, e.g., media components on enzymes production (Adinarayana and Ellaiah, 2002; Park *et al.*, 2002; Puri *et al.*, 2002), production of other metabolites (Zhang *et al.*, 1996; Sunitha *et al.*, 1998; Sadhukhan *et al.*, 1999; Hujanen *et al.*, 2001), spore production and biomass production optimisation. It can give information about the interaction between variables, provide information necessary for design and process optimisation and give multiple responses at the same time.

MEDIUM COMPOSITION

The formulation of the culture media is of critical importance because the composition will affects product concentration, yield and volumetric productivity. It is also important to reduce the cost of the medium as this may affect the overall process economics (Souza *et al.*, 2006). Growth medium or culture medium is a liquid or gel designed to support the growth of microorganisms or cells. An important distinction between growth media types is that of defined versus undefined media (Madigan and Martinko, 2005). A defined medium will have known quantities of all ingredients. For microorganisms, they consist of providing trace elements and vitamins required by the microbe and especially a defined carbon source and nitrogen source. Glucose and glycerol are often used as carbon sources and ammonium salts or nitrates as inorganic nitrogen sources. An undefined medium has some complex ingredients, such as yeast extract or casein hydrolysate, which consist of a mixture of many chemical species of unknown proportions. Undefined media are sometimes chosen based on price and sometimes by necessity, some microorganisms have never been cultured on defined media.

The basic components necessary in medium are constantly being developed or revised for use in the growth and identification of the microorganism that are of interest for research and in the microbiological clinical laboratory, specifically in bacteriology. To support microbial growth, medium must provide an energy source, as well as source of carbon, nitrogen, sulfur, phosphorus and many organic growth factors that the organism is unable to synthesize. To perform such a microbiological assay a growth medium must contains all the growth requirements for the bacterium.

Why we should optimise the medium?: Medium had been adjusted to meet the standard performance and must be soundly based in theory and practice and applicable to our purpose, also the whole process to occur with efficiency and dispatch. As a first step in the medium design, an examination of cell's needs and requirements provides important information.

OPTIMISATION TECHNIQUES

Classical techniques: The traditional one-at-a-time optimisation strategy is simple and useful for screening and the individual effects of medium components can be seen on a graph without the need to revert to more sophisticated statistical analyses. Unfortunately, this simple method frequently fails to locate the region of optimum response because the joint effects of factors on the response are not taken into account in such procedures. It was reported that the complexities and uncertainties associated with large-scale fermentation usually come from a lack of knowledge of the sophisticated interactions among various factors affecting fermentation (Liu *et al.*, 2005).

STATISTICAL MATHEMATICAL TECHNIQUES

Simulation optimisation technique: Simulation optimisation can be defined as the process of finding the best input variable values from among all possibilities without explicitly evaluating each possibility. The objective of simulation optimisation is to minimize the resources spent while maximizing the information obtained in a simulation experiment (Carson and Maria, 1997).

Stochastic optimisation technique: Stochastic optimisation is the problem of finding a local optimum for an objective function whose values are not known analytically but can be estimated or measured. Classical stochastic optimisation algorithms are iterative schemes based on gradient estimation. More, recently Andradottir (1990) proposed a stochastic optimisation algorithm that converges under more general assumptions than these classical algorithms. Leung and Suri (1990) reported better results with the Robbins-Monro algorithm when applied in a finite-time single-run optimisation algorithm than when applied in a conventional way.

Gradient based search methods: In this category estimate the response function gradient f (Ñ) to assess the shape of the objective function and employ deterministic mathematical programming techniques.

Heuristic methods: Heuristic methods represent the latest developments in the field of direct search methods (requiring only function values) that are frequently used for simulation optimisation. Many of these techniques balance exploration with exploitation thereby resulting in efficient global search strategies.

A-teams: An A-team (asynchronous team) is a process that involves combining various problem solving strategies so that they can interact synergistically. De Souza and Talukdar (1991) viewed an A-team as a process that is both fast and robust.

Response surface methodology (RSM): Response surface methodology is a procedure for fitting a series of regression models to the output variable of a simulation model (by evaluating it at several input variable values) and optimizing the resulting regression function.

Statistical deigns developed specifically for optimisation purposes are not so structured as to give insight into what is happening in the chemical process; they are aimed simply at achieving a desired result with maximum economy. They are said to provide empirical feedback.

The selection of appropriate condition is usually based on a combination of the different variable, which involves the conventional techniques of optimising one factor at a time. This approach is tedious, time consuming and expensive, especially for a large number of variables. Moreover it does not guarantee the determination of optimum conditions among the variables (Summer *et al*., 1979). Conventionally the practice of a single factor optimisation by maintaining other factors involved at an unspecified constant level does not portray the combined effect of interactions of factors involved. The method is time consuming and requires a large number of experiments to determine the optimum points (Box *et al*., 1978). The limitations of a single factor optimisation process can be eliminated by optimizing all the contributing process parameters collectively using statistical experimental design in particularly Response Surface Methodology (RSM). The experimental design constitutes an efficient tool and is well adopted for treating problems with large number of variables and allows simultaneous, systematic and efficient variables of all components (Box *et al*., 1978). Statistical experimental design is one way to increase the amount of information-rich data and the use of software packages has made this technique increasingly popular (Gawande and Patkar, 1999) and can be applied to optimise the process condition for target product.

Statistically designed experiments are highly efficient in that they give a fixed amount of information with much less effort than the classical one-variable at-a-time approach and many of them give additional information about interaction as a bonus. It will be shown that significant interaction are an important clue in the search for optimum conditions and substantial interactions mean that careful control will have to be exercised if a reproducible process is to ensure (Haines, 2010).

There are other techniques, specially aimed at the optimisation of a process or a product, which are essentially based on or derived from factorial experiments, but in which the total design is evolved in stages rather than being fully preplanned. These are known by such names as method of steepest ascent and Evolutionary Operations (EVOPS).

RSM as an economic and efficiency for optimisation: Response Surface Methodology (RSM) is concerned with the modeling of one or more responses to the settings of several explanatory variables. The nature of the function relating the responses to the variables is assumed to be unknown and the function or surface is modeled empirically using a first-or a second-order polynomial model. The broad aims of RSM are to investigate the nature of the response surface over a region of interest and to identify operating conditions associated with maximum or minimum responses. RSM is generally conducted in three phases, as emphasized in Myers and Montgomery (2002). Phase 0 involves the screening of explanatory variables to identify those which have a significant effect on the responses, phase 1 is concerned with the location of optimum operating conditions by conducting a sequence of suitable experiments and phase 2 involves the fitting of an appropriate empirical model, usually a second-order polynomial model, in order to examine the nature of the response surface in the vicinity of the optimum. The fundamentals of RSM are set out in the seminal papers of Box and Wilson (1951) and Box and Draper (1959) and in the books by Box and Draper (1987), Khuri and Cornell (1996) and Myers and Montgomery (2002). Further developments are drawn together in three key review articles, namely those of Hill and Hunter (1966), Myers *et al.* (1989) and Myers *et al.* (1989, 2004). It is clear from these articles that research into RSM within academia continues to flourish and that the associated techniques are used extensively in industry. RSM is a design that has been described as the most powerful statistical techniques in technological research.

In order to begin to explain how experimental design and analysis can help to optimise medium in a very economical and productive way. Table 1 summarized the data from literature that shown the percentage improvement of enzyme production compared to un-optimised medium.

The results indicate that when a submerged culture in shake flasks was operated at 28°C, initial pH 5.5 and rotation speed 105 rpm, the biomass and triterpenoid content in dry basis could be increased to 3.20% (w/w) and 31.8 mg g^{-1}, respectively. Hao *et al.* (2006) strongly support the use of RSM for medium optimisation. The optimisation of the medium resulted not only in increasing FPA up to 10.6 IU mL^{-1} but also in reducing the cost of the medium. The invented plate for screening mutants and the chosen method of the optimisation of medium composition were efficient, relatively simple, time and material saving. Hao *et al.* (2006) optimised a medium composition using RSM and cellulose production increased from 7.2 to 10.6 IU mL^{-1}. It is stated that application of RSM in medium formulation increased CGTase production 231% compared with basal medium.

Response Surface Methodology (RSM), which is a collection of statistical techniques for designing experiments, building models, evaluating the effects of factors and searching for the optimum conditions, has successfully been used in the optimisation of bioprocesses.

Table 1: Summary of reported data using RSM for maximization of enzymes production and minimization of the cost

Target product	Microorganism	Optimisation technique	Result	References
Xyloketal	Fungus *Xylaria* sp. 2508	RSM	1.32-fold	Xiaoboa *et al.* (2005)
L-glutamine		Desirability function approach (DFA) together with response surface methodology (RSM)	Production cost decreased by 7.1 concentration of glutamate increased by 53.6	
Pyruvic acid	*Torulopsis glabrata* TP19	RSM with Plackett-Burman design	Production increased from 42.2 to 42.4 g L^{-1}	Zhang and Gao (2007)
Lactic acid	*Lactobacillus amylophilus* GV6	RSM	100% increase in lactic acid production	Naveena *et al.* (2005)
Actinorhodin	*Streptomyces coelicolor* A3(2)	RSM	32% higher than that of the unoptimised medium	Elibol (2004)
Alkaline protease	*Bacillus* sp.	RSM	maximum alkaline protease production was 4,98,123 PU L^{-1}	
Thermal stable β-glucanase	*Bacillus subtilis* ZJF-1A5	RSM	1.4 times higher than that in original medium	Tang *et al.* (2004)
Citric acid	*Aspergillus niger* MTCC 281	RSM	Maximum	Ambati and Ayyanna (2001)
Xylanase	*Aspergillus fischeri*	RSM	1.9-fold increased	Senthilkumar *et al.* (2005)
Polysaccharide	*Cordyceps sinensis*	RSM	Maximum polysaccharide production was 3.05 g L^{-1}	Hsieh *et al.* (2007)
Alkaline lipase	*Burkholderia multivora*	One-variable-at-a-time" and the statistical approaches	12-fold enhanced production	Gupta *et al.* (2006)
CGTase,	*Bacillus* G1	RSM	CGTase production increased 231%	Ibrahim *et al.* (2005)
Cellulose	*Trichoderma reesei* WX-112	RSM	Cellulase activity increased from 7.2 to 10.6 IU mL^{-1}	
Triterpenoid	*Antrodia cinnamomea* AC0623	RSM	Triterpenoids content could be increased from 16.7 to 31.8 mg g^{-1}	
CGTase,	*Bacillus clausii* E16	RSM	This yield was 68% higher	Alves-Prado *et al.* (2007)
Nisin	*L. lactis* Lac2, a mutant strain	RSM	Doubled the yield	Zhou *et al.* (2008)
Phytase	Fungus	RSM		Dahyia *et al.* (2009)

Li *et al.* (2007) stated that the fermentation media for MTGase production has been almost the same in every published work since Ando *et al.* (1989). In his work he have modified and optimised the fermentation media for MTGase production using RSM., it is evident that the use of a FFD statistical design and CCD can be used to determine the significant variables and the optimum conditions for MTGase production, respectively. The optimisation of the medium resulted not only in an 86% higher MTGase activity than in the media previously cited in the literature, but also in a reduction of the constituents costs and an improvement in repeatability. It is important to observe that the value of 1.4 U mL^{-1} of MTGase activity obtained in this study is significant when compared with values previously reported in the literature that ranged from 0.25 to 2.5 U mL^{-1} (Zhu *et al.*, 1995). In addition, the relevance of these results is that the factors leading to the increased activity were identified and are important for further studies.

Application of RSM in different areas rather than medium optimisation was also studied by many researcher. Ibrahim *et al.* (2003) used RSM in optimizing of the physical environment for CGTase enhancement in terms of temperature and pH and stated that CGTase production was increased by 163.9% in optimised conditions. While Benyounis *et al.* (2005) optimises keyhole parameters (i.e., maximize penetration (P) and minimize the heat input, width of welded zone (W) and Width of Heat-Affected Zone (WHAZ) in CW CO_2 laser butt-welding of medium carbon steel using RSM which resulted in improve the weld quality, increase the productivity and minimize the total operation cost. In addition to that, superimposing the contours for the various response surfaces produced overlay plots.

CONCLUSION

From the extensive literature review presented here, it can be concluded that the field of RSM is well-researched and established within the industrial context and researchers in medium composition and related disciplines could well draw with advantage on the broad framework provided by this methodology in order to design and analyze their experiments. The success of this work was mainly due to the systematic analysis, the suitability of the mathematical methods and the accuracy of the RSM while suitable mathematical methods could be found and the accurate of the specific condition could be ensured.

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