MONITORING OF pH AND TEMPERATURE EFFECTS IN FERMENTATION PROCESS OF ITACONIC ACID

Siti Rozaimah Sheikh Abdullah, Jamaliah Md. Jahim, Norliza Abd. Rahman, Norhayati Ismail, Idayu S. M. and Saleha Atan
Department of Chemical & Engineering Process, Faculty of Engineering, Universiti Kebangsaan Malaysia, 43600 UKM, Bangi, Selangor.

ABSTRACT

The environmental conditions such as temperature and pH have great impact on microbial growth kinetics and product formation during fermentation process. Temperature can be related to activation energy that affects the growth rates and death parameters while pH influences the activity of the enzyme and consequently affects the microbial growth rate and product formation. In this study, four different conditions were used to investigate the effects of temperature and pH on the formation of itaconic acid. The experiments were conducted using local strain Aspergillus terreus IMI 282743 in a 2.5 L laboratory fermenter (KF2.5L KoBiotech, Korean) for production of itaconic acid. The temperature and pH were changed for various fermentation conditions such as: (1) the temperature was controlled in the range of 30.0 $\pm$0.1°C and the pH was controlled in the range of 3.1-3.2, (2) the temperature was controlled in the range of 30.0 $\pm$0.1°C and the pH was increased to 7.0 before it was allowed to decrease in a natural way, (3) the temperature was allowed to change naturally but the pH was controlled in the range of 3.48-3.50, and finally, (4) both temperature and pH were allowed to change naturally throughout the fermentation cycle. From the four conditions above, the maximum itaconic acid production of 1.0 mg/mL was observed in the second state, in which the temperature was controlled in the range of 30.0 $\pm$0.1°C and the pH was initially increased to 7.0 before it was allowed to decrease naturally during the fermentation process.

Keywords : *Aspergillus Terreus, Itaconic Acid, Temperature Control, pH Control*

INTRODUCTION

With the new interest in sustainable development, the chemical industry is making many attempts to replace petrochemical-based monomers with natural ones. Itaconic acid is one of the promising substances within the group of organic acids. Itaconic acid is a white crystalline unsaturated dicarbonic acid with carboxyl group conjugated to the methylene group. It can be easily incorporated into polymers and may serve as a substitute for petrochemical-based acrylic or methacrylic acid. The first method of producing itaconic acid was by pyrolysing citric acid and hydrolysing the anhydrides. Another early method was the carboxylation of itaconic acid. Chemical synthesis is mainly performed by dry distillation of citric acid and subsequent treatment of the anhydride with water [1]. Alternative approaches are the oxidation of mesityl oxide and subsequent isomeration of the formed citric acid, or the oxidation of isoprene. But none of these processes can really compete with fermentation by fungi and therefore none are practiced commercially. The widespread applications in polymer manufacturing field have resulted in increased demand for itaconic acid. It is used at 1-5% as a comonomer in resins and also in the manufacture of synthetic fibres, in coatings, adhesive, thickeners and binders.

Itaconic acid is known to be produced on a commercial scale using only *Aspergillus terreus*. However, several attempts have been made to find other microorganisms that are not as sensitive to particular fermentation conditions (e.g. substrate impurities) or which have a more favourable product composition. Among the filamentous fungi, some ustilaginales species also produce itaconic acid. The screening process has produced itaconic acid in the fermentation broth of an Ustilago zeae strain. In a further screening of this species, one strain was found to produce about 15 g itaconic acid/L. As growing filamentous fungi may cause particular problems in bioreactors, yeasts were also tested for itaconic acid production [1-2].

The best yields of itaconic acid are achieved with glucose or sucrose as substrate, but other carbon sources like starch, molasses, hydrolysates of corn syrup or wood, and many combinations thereof were also tested. The most frequently used substrates are beet or sugarcane molasses, pretreated by ion exchange or ferro-cynide; hydrolysed starch; or simply sugars (sucrose, glucose). A very new approach is the use of citric acid as precursor in a membrane reactor. With the price of citric acid at about one tenth of that of itaconic acid, this method should prove to be economical [2].

The itaconic acid fermentation process works optimally under phosphate-limited growth conditions at sugar concentrations between 100 and 150 g/L. Once the fungal biomass is established, the phosphate level should be kept rather low to prevent growth. During fermentation, the pH decreases to about 2 and itaconic acid becomes the main product. The temperature normally is kept at around 37˚C, but some investigators have tried to increase the optimum temperature. An adequate oxygen supply is essential because anaerobic conditions will irreversibly damage the biomass. Itaconic acid is strongly affected by several medium components including Fe, Mn, Mg, Cu, Zn, P and N. This means that, in order to get reproducible high productivities, the substrate quality has to be controlled either by using refined quality starting materials or by pretreatment of raw materials before or during fermentation [3-4].

Due to the high price, the use of itaconic acid is restricted. At present, the production rates do not exceed 1 $gL^{-1}h^{-1}$, accompanied by product concentrations of about 80 g/L. Some new biotechnology approaches such as immobilisation

techniques, genetic engineering and screening programmes, could lead to higher productivity.

The main developments in itaconic acid production (batch fermentation, free suspended biomass) took place before 1966. Over the next 15 years, the interest in itaconic acid production declined. However, since the early 1980s, there has been increasing concern regarding sustainability, environmental conservation, renewable resources and rising energy costs. Therefore, the development of new fermentation technologies and more sophisticated bioprocess control has led to renewed interest in improving itaconic production. As mentioned above, itaconic acid production is very sensitive to several medium components, oxygen supply, pH and temperature range. To ensure the fermentation process held completely, the requirement to such a smart control system is important in monitoring and controlling these parameters. The smart system such as fuzzy logic control has been used in various fields and has been found to be successful in controlling the non-linear system [5].

Several approaches have been proposed to handle the non-linear response of pH via a fuzzy logic controller. A fuzzy controller applies qualitative and fuzzy knowledge, not mathematical equations, to describe a process. A fuzzy control system consists of a set of fuzzy rules that makes use of *if-then* conditions whose true values lie between 0 and 1. This allows us to derive a conclusion whose true values lie between 0 and 1 [5].

This study finally aims to develop a fuzzy logic control technique in order to control the fermentation process of itaconic acid. However, this paper will only describe a preliminary study that was initiated to investigate any effects of pH and temperature in order to obtain the most suitable process conditions for the fermentation of itaconic acid, before any control experiment being conducted. The fermentation was conducted using *Aspergillus terreus* from local strain with glucose as its main carbon source. Temperature and pH parameters were taken as the controlled parameter as both these factors would affect the fermentation process.

MATERIALS AND METHODS

Microorganism, growth conditions and inoculum development

Aspergillus terreus IMI 282743 from local strain was grown and maintained on *Potato Dextrose Agar* (PDA) medium at 30˚C for 7 days. Following that process, *Aspergillus terreus* was subcultured in a Petri dish at 30˚C for 4 days and subsequently at room temperature for 3 days. A fermentor with a capacity of 2.5 L Model KF2.5L (KoBiotech, Korean) was used with the working volume of 2.0 L. A loopful of the culture was inoculated into 200 mL growth medium containing 60 g/L D-glucose, 4 g/L ammonium nitrate, 0.950 g/L magnesium sulfate, 0.004 g/L copper sulfate, 0.088 g/L potassium di-hydrogen–phosphate and 1.000 g/L itaconic acid. The growth medium was then incubated at 30˚C under shake flask conditions for 3 days in a rotary shaker (150 rpm). After that period, it was inoculated into 2 L of production medium which has the same composition as that of the growth medium.

Analytical Methods

The fermentation process was repeated four times under different conditions of pH and temperature in order to gain the best condition for the fermentation process. The growth medium compositions were the same as above in all fermentation throughout the experiment. The pH and temperature conditions were varied according to: (1) controlled temperature at 30.0 ±0.1 ˚C and pH at 3.10 - 3.20, (2) controlled temperature at 30.0 ± 0.1 ˚C and initial pH was increased to pH 7.0 before it was allowed to change naturally, (3) temperature was allowed to change naturally and controlled pH at 3.48 - 3.50, and lastly, (4) both temperature and pH were allowed to change naturally. After the fermentation process, the final concentration of itaconic acid was analysed using HPLC Agilent 1100 (Agilent Technology, Germany), with column Zorbax SB-C18, 4.6 x 250 mm (Agilent Technology, Germany). The mobile phase was 0.02 M phosphoric acid, operated at a flow rate of 1.2 mL/min.

RESULTS AND DISCUSSION

Phases of Batch Growth-Cycle

The sample was taken every 3 hours during the fermentation process that was conducted for 24 hours. It was more frequently taken if the fermentation process took a longer time in order to determine the concentration of itaconic acid and fungal biomass formed. Four stages of analysis under different conditions has resulted the growth profiles in Figures 1-4.

From all the *A.terreus* growth profiles, a general batch growth-cycle that started with lag phase, followed by

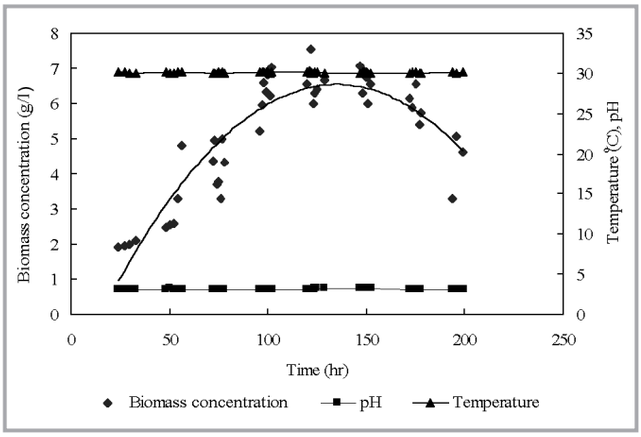

Figure 1: Growth profile of A.terreus with controlled temperature at 30.0 ±0.1°C and pH at 3.10-3.20

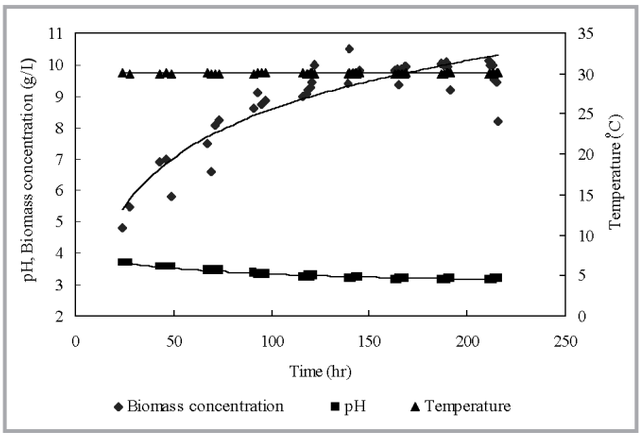

Figure 2: Growth profile of A.terreus with controlled temperature at 30.0 ±0.1°C and initial pH was increased to pH 7.0 before it was allowed to change naturally

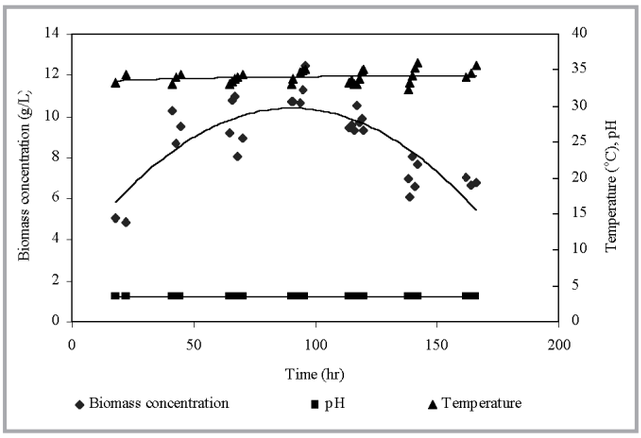

Figure 3: Growth profile of A.terreus with temperature was allowed to change naturally and controlled pH at 3.48-3.50

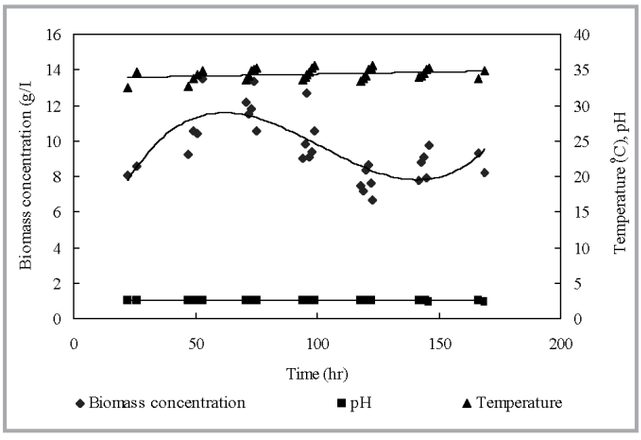

Figure 4: Growth profile of A.terreus when both temperature and pH were allowed to change naturally

exponential phase, stationary phase and finally death phase, were observed. The first phase, the initial lag phase, which was of variable duration while the exponential growth phase showed the cell number (dry weight), increased exponentially. This was also referred to as the logarithmic phase. Following this was a stationary phase, where the cell numbers were the highest. Finally the cell numbers declined during the death phase [6].

Comparison of product concentration

Table 1 showed the comparison of the highest product concentration and the total product concentration for each fermentation condition conducted. From the results obtained, the best fermentation condition was the second condition, where the temperature was controlled at 30.0 ±0.1 ˚C and initial pH was increased to pH 7.0 before it was allowed to change naturally, showed by the highest product concentration obtained. The poorest product concentration can be observed in first condition, where temperature and pH controlled at 30.0 ±0.1˚C and 3.10-3.20, respectively. The study showed that the fermentation process was extremely affected by pH change, and not significantly affected by temperature factor. This finding was in agreement with the work carried out by Rychtera and Wase [7].

Table 1: Comparison of product concentration

Fermentation Condition	Highest Product Concentration, mg/mL	Total Product Concentration, mg/mL
Controlled temperature at 30.0±30.1°C and pH at 3.10-3.20	0.79	20.54
Controlled temperature at and initial pH was increased to pH 7.0 before it was allowed to change naturally	1.00	40.83
Temperature was allowed to change naturally and controlled pH at 3.48-3.50	0.92	27.03
Both temperature and pH were allowed to change naturally	0.94	27.15

CONCLUSIONS

In this study, the batch fermentation process under a condition in which the temperature was controlled at 30.0 ±0.1˚C and initial pH was increased to pH 7.0 before it was allowed to change naturally, has been proven to produce the highest product concentration. The highest product concentration obtained under this condition was 1.00 mg/mL and total product concentration was 40.83 mg/mL. The initial pH was increased to pH 7.0 in order to start the production of itaconic acid. Naturally pH decreased without a need of control during fermentation process is indeed an attractive discovery. This will encourage the fermentation of itaconic acid in industrial scale from the view of control and chemical cost. This finding will initiate further work to develop rule-based information for a fuzzy logic control system to monitor and control the pH and temperature during the fermentation process.

ACKNOWLEDGEMENT

Financial support for this research from the Malaysian Government under IRPA 09-02-02-0011-EA066 is greatly acknowledged. ■

REFERENCES

[1] T. Willke, and K. D. Vorlop, *B*iotechnological production of itaconic acid, *Applied Microbiology* Biotechnology, 56: 289-295, 2001

[2] C. S. K. Reddy and R. P. Singh, Enhanced production of itaconic acid from corn starch and market refuse fruits by genetically manipulated *Aspergillus terreus* S.K.R 10, *Bioresource Technology*, 85: 69-71, 2002.

[3] M. H. Gyamerah, Oxygen requirement and energy relations of itaconic acid fermentation by *Aspergillus terreus* NRRL 1960, *Applied Microbiology Biotechnology*, 44: 20-26, 1995.

[4] M. L. Shuler and K. Kargi, *Bioprocess engineering : Basic concepts*, Prentice Hall, New Jersey, 1992.

[5] O. P. Yadav, N. Singh, R. B. Chinnam and P. S. Goel, A fuzzy logic based approach to reliability improvement estimation during product development, *Reliability Engineering and System Safety*, 80(1): 63-74, 2003.

[6] W. B. Harvey, and S. C. Douglas, Biochemical Engineering, Marcel Dekker, Inc., New York., 1997.

[7] M. Rychtera, and D. A. J. Wase, The growth of *Aspergillus terreus* and the production of the itaconic acid in batch and continuous cultures: The influence of pH, *Journal Chemical Technology Biotechnology*, 85: 509-521, 1981.

PROFILE

Dr Siti Rozaimah Sheikh Abdullah received her M.Eng (1992) from the University of Nottingham, U.K. and Ph.D (1999) from Universiti Kebangsaan Malaysia. She is a senior lecturer/researcher in the Department of Chemical and Process Engineering, UKM. Her current interests include wastewater treatment, biological process plus neural and fuzzy-based control systems.

Jamaliah Md. Jhim received both B.Eng and M.Sc from Universiti Kebangsaan Malaysia and Ph.D (2000) from the University of Bradford, U.K. She is a lecturer/researcher in the department of Chemical and Process Engineering, UKM. Her current interests include biochemical separation and optimisation of fermentation process.

Norliza Abd. Rahman received the B.Eng (1993) from the University of Glamorgan, U.K. and M.Sc from Universiti Kebangsaan Malaysia. She is a lecturer/researcher in the department ofChemical and Process Engineering, UKM. Her current interests include biological process and process control systems.

Norhayati Ismail received her B.Eng (2003) from Universiti Kebangsaan Malaysia. She is currently a Masters student in the Department of Chemical and Process Engineering, UKM.

Saleha Atan received her B.Eng (2003) from Universiti Kebangsaan Malaysia. She is currently a research student in the Department of Chemical and Process Engineering, UKM.