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ADAPTIVE CONTROL OF BROMELAIN PRECIPITATION IN A FED-BATCH STIRRED TANK

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Abstract: In this work, bromelain is recovered from triturated pineapple stem and rind (usually kitchen waste) through the precipitation process with alcohol at low temperature. The temperature control is crucial to avoid the irreversible protein denaturation and consequently improving the precipitation yield. The process is carried out in a fed-batch system, so that its dynamic nature poses challenging control system design. Conventional and adaptive controllers are properly designed and on-line implemented through a fieldbus digital control system. Closed loop performance was improved under adaptive PID controller. Overshoot and response time decreased and no control action saturation occurred. *Copyright © 2002 IFAC.*

Keywords: Adaptive control, bromelain, enzyme precipitation, PID Controller, fieldbus.

1. INTRODUCTION

In many biotechnological industries, including food and pharmaceutical ones, the selective separation of a protein out of fermentation broths or vegetable sources has been a primary research interest for downstream processing operations. It is difficult and expensive to selectively recover a targeted protein from a broth due to the low protein concentration and the similarity of the physical properties between proteins present in the same solution.

Among the practical methods being applied to the large-scale recovery and purification of proteins from dilute solution, protein precipitation is regarded as a key operational process, which is used during the early stages of the downstream processing. Protein precipitation is frequently featured by the spontaneous fractionation and concentration as well as a low additive consumption and protein non-denaturation (Kim *et al*., 2002; Chen and Berg, 1993; Clark and Clatz, 1987). Protein precipitation usually produces insoluble protein by contacting precipitants, such as neutral salts, acids, organics solvents, or metallic ions, with the desired protein in a stirred tank.

The need for rapid monitoring in biotechnology has been highlighted by several authors (Paliwal *et al*., 1993; Ransohoff *et al*., 1990). The high proportion of the process cost attributed to the downstream processing steps for many products, means that ensuring that the purification sequence is performed within specified limits at high yield, and knowing rapidly when such limits are crossed is an extremely important consideration (Foster *et al*., 1986). Particularly, for enzyme precipitate products, quality control is mandatory to ensure structural authenticity. If, in addition, prior process knowledge allows feedback control using on-line information, then costly run-stoppage times or disposal of non-specification material may be avoided (Holwill *et al*., 1996).

While fermentation control problem is well addressed, the practical application of control in downstream processing has not been properly studied. Most of bioprocesses are carried out in batch or semi-batch systems, so that their dynamic nature poses challenging control system design. Non-linearity is usually found as well, therefore conventional feedback controllers are not supposed to be able to follow set point specifications.

The present work is concerned about the experimental control system development for fruit bromelain precipitation. Bromelain is the name of a group of powerful protein-digesting, or proteolytic, enzymes that are found in the pineapple plant (Ananas comosus). Discovered in 1957, and widely studied since then, bromelain is particularly useful for reducing muscle and tissue inflammation and as a digestive aid. Besides the pharmacological effects, bromelain is also employed in food industries, such as breweries and meat processing.

It is a fact that the majority of processes in the chemical industries can be satisfactorily controlled using conventional controllers. However, the conventional PID control method is inadequate for bioprocesses control in which processes dynamics will change in known ways during operation (time delays, process non–linearities and interactions). For these difficult problems, it is important to generate the initial settings with a model-based tuning strategy. Controller tuning involves the selection of the best values of Kc, Ti and Td. This is often a subjective procedure and is certainly process dependent. A number of methods have been proposed in the literature over the last 50 years. However, the most well-known tuning technique is the method of Ziegler and Nichols.

In this work, bromelain is recovered from triturated pineapple stem and rind (usually kitchen waste) through the precipitation process with alcohol at low temperature. The process temperature control is crucial to avoid the irreversible protein denaturation and consequently improving the precipitation yield. Conventional and adaptive controllers are properly designed and on-line implemented through a fieldbus digital control system.

2. THE PRECIPITATION PROCESS

The precipitation process aims to achieve separation by conversion of solutes to solids. Precipitation can result in both concentration and purification methods. The advantages of using precipitation for concentration and purification are: easy scale-up, involves simple equipments and can be based on a large number of alternative precipitants, some of them inexpensive or used in very low concentration. Precipitants can be chosen which do not denature biological products, and the precipitate form is often more stable than the solute material.

Solubility of Proteins

A larger number of water-miscible organic solvents like ethanol or methanol can be used to precipitate proteins. A typical globular protein presents to the solvent a surface consisting of regions of positive and negative charge, along with polar, but uncharged, hydrophilic regions and nonpolar, hydrophobic regions. The complex interactions between the protein surface and surrounding solvent determine the solubility. A protein is made insoluble by changing either the surface characteristics of the protein itself or by changing the solvent. The change in solubility that results is sufficiently great to be viewed as step change between soluble and insoluble. The resulting high level of supersaturation leads to rapid formation of an amorphous solid.

As in the case of salting-out, this phenomenon has been described in terms of removal of water from the hydration spheres of the protein allowing electrostatic forces to bring oppositely charged regions of the protein together. Water is removed both by bulk replacement by the organic solvent and by structuring of the water around the organic molecules. The solvent property affected is the dielectric constant. The hydrophobic area of the protein would tend to become more soluble, but the net result is decrease in solubility.

Fed-Batch Precipitation Tank

The bromelain precipitation process is carried out in a fed-batch stirred tank (500mL), according to Figure 1. Inside the jacked tank, the protein will be exposed to a range of operating conditions during the period in which the precipitant agent (ethanol 99.5%) is added. The first material precipitated will form at other than the final conditions. Overprecipitation is less likely to occur by adding precipitant slowly and well dispersing it. A micropump (pump 1) is employed to continuously feed the alcohol, at environmental temperature (about 25ºC), to the tank.

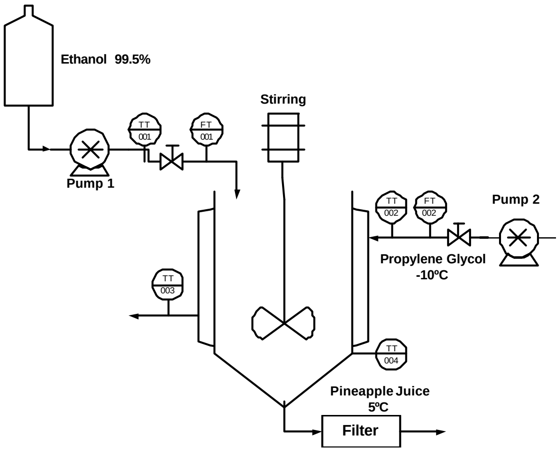

Fig 1. Fed-batch stirred precipitation tank.

Since the upper bound for bulk temperature is 10ºC, in order to avoid protein denaturation process, the cooling flow rate is manipulated through a variable speed pump (pump 2). Calibrated J-type thermocouples, located at precipitation mixture bulk and at the inlet and outlet of cooling fluid (propylene glycol), provide temperature measurements. The set point of bulk temperature is 5ºC.

3. THE DIGITAL CONTROL SYSTEM

The management of the digital control system is performed through a Foundation Fieldbus communication system, according to Figure 2. Four field devices compose the fieldbus network used to monitor and control the precipitation tank:

- Distributed Fieldbus Interface (DFI302): bridge to link different speed networks. It manages the communication between the Local Area Network (High Speed Ethernet) and the Fieldbus network (H1);

- Temperature Transmitters (TT302-1 and TT302-2): perform temperature data acquisitions and transmit them to the interface (DFI302);

- Fieldbus/Electric Current Converter (FI302): receives digital signal from DFI302 and converts to 4-20mA to operate the variable speed pump.

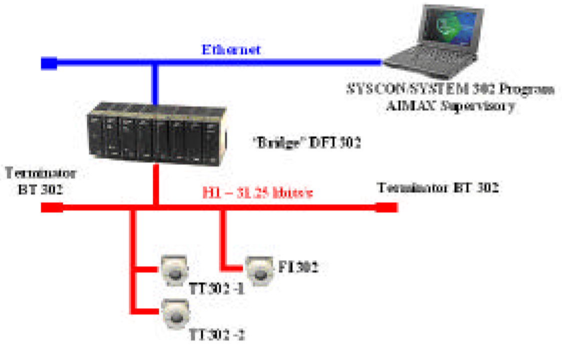

Fig. 2. Fieldbus network for bromelain precipitation tank monitoring and control.

The digital signal sent to the pump is computed by controllers (conventional or adaptive ones), which were implemented through the software Syscon (function blocks) and supervisory software Aimax (adaptive tuning equations).

4. MATERIAL AND METHODS

Pineapple juice preparation

Stem and rind of the pineapple fruit (species "Perola") are triturated and filtered. Distilled water is employed at dilution rate 1:1. The filtrate, called pineapple juice, contains the enzyme bromelain. Samples containing 100mL of pineapple juice are frozen at -18ºC (Cesar *et al*., 1999).

Controller tuning

Tuning PID systems is both a science and an art form. Proper parameters depend entirely on the system being tuned. Scaling conventions will differ depending on a particular PID implementation. When tuning a PID algorithm, generally the aim is to match some preconceived ideal response profile for the closed loop system.

It has long been recognized that first-order-plus-dead (FOPDT) approximations can adequately explain the behavior of a range of processes:

$$G(s) = \frac{K_p e^{-tds}}{\tau_p s + 1} \qquad (1)$$

Where Kp is the process gain, tp the process time constant and the td is the process time delay.

The Zeigler Nichols Open-Loop Tuning Method is a way of relating the process parameters to the controller parameters.

The process reaction procedure was carried out at five different tank levels. Pseudo-steady state was reached before step disturbances were implemented in cooling flow rate. Process parameters (gain, time constant and time delay) are then graphically obtained from the monitored tank temperature response (TT-004 – Figure 1). Due to the transient nature and process non-linearity, these parameters are not constant. In order to improve the design of the PID linear controller, Ziegler-Nichols tuning equations (Ogata, 1997) are applied for every pseudo-steady state studied.

Accurate tuning is hardly possible using trial and error method. The classical methods for controller settings can not be precise enough and it is expected the controller function can be improved considerably if they are better tuned.

From this methodology, an adaptive PID controller is obtained, implemented and compared to a well-tuned conventional PID.

A comprehensive summary of adaptive control was recently published by Sastry and Bodson (1994).

5. RESULTS

From the process reaction curve procedure, implemented at five different mixture volumes (100, 200, 300, 400 and 500 mL), and Ziegler-Nichols tuning equations, the scheduling relationships for PID controller parameters - Kc, Ti and Td - were determined (Figure 3).

The tuned and well tuned parameters obtained from Ziegler and Nichols method and fine tuned method (trial and error), respectively, are shown in Table 1.

Table 1. Tuned and well-tuned parameters (Kc, Ti and Td) obtained at different tank volume levels using Zigler and Nichols method and fine tuning method (trial and error).

Volume (mL)	Tuned			Well-tuned		
	Kc	Ti	Td	Kc	Ti	Td
100.0	433.1	26.0	6.5	101.6	156.0	3.3
200.0	223.1	44.0	11.0	52.8	264.0	5.5
300.0	159.9	74.0	18.5	37.4	444.0	9.3
400.0	144.8	78.0	19.5	34.2	468.0	9.8
500.0	110.9	112.0	28.0	26.7	672.0	14.0

From Figure 3, it is observed that the controller gain decreases as soon as tank volume increases. Indeed the process becomes more sensitive since the heat transfer area increases and consequently a small control action is required to regulate bulk temperature. Still according to PID Ziegler-Nichols tuning, the integral action must decrease and the effect of derivative term must increase as volume increases.

Experimental runs were carried out by loading the stirred tank with 100mL of pineapple juice at 5ºC and continuously adding ethanol 99.5%, at environmental temperature, until the tank volume reaches 500mL.

Since ethanol pump operates at fixed flow rate (0.18mL/s) and the batch time is known, the tank volume is computed and the obtained tuning relationships (Figure 3) are on-line applied in the adaptive PID controller.

Although similar behaviour is obtained from conventional PID controller application (Figure 5), undesirable control action saturation occurred. Fixed tuning parameters obtained at the early stage of the batch (volume: 100mL) were employed.

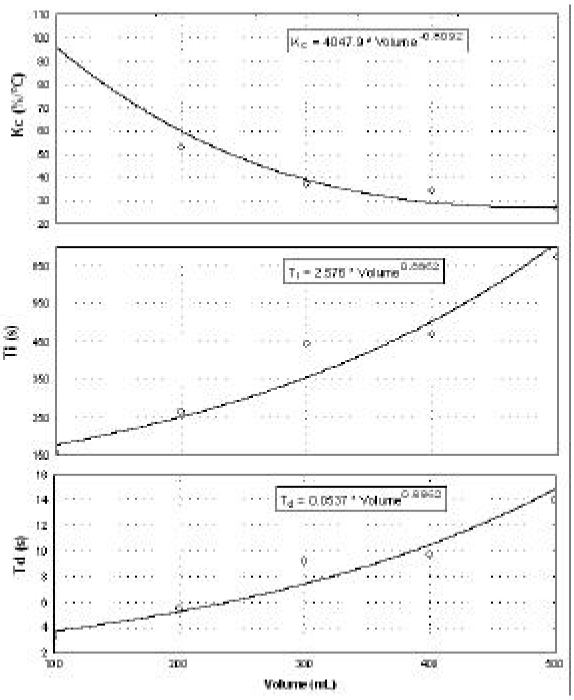

Fig. 3. Experimental values of controller gain (Kc), integral time (Ti) and derivative time (Td) obtained at different tank volume levels.

Figure 4 presents the results from the adaptive controller implementation. The behaviour of the controlled variable (bulk temperature deviation) and the manipulated variable (pump speed) is shown. An open-loop run was also carried out (pump speed equals to 50%). In the early moments of ethanol addition, there is a heat generation that immediately cause an increase on pump speed in the closed-loop system. Soon afterwards, the temperature decreases and pump speed leads to a minimum cooling flow rate. Besides precipitation heat generation, the ethanol inlet at environmental temperature adds heat continuously to the tank bulk mixture.

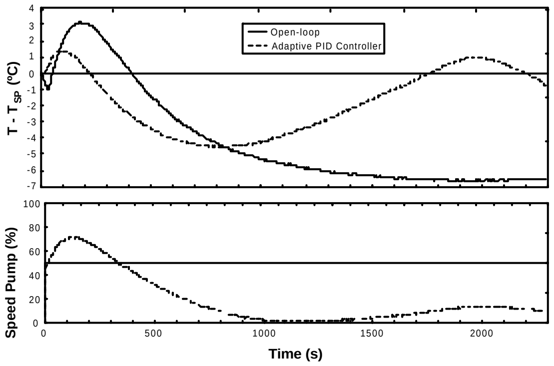

Fig. 4. Bulk temperature deviation (T-Tsp) and manipulated variable behavior obtained from experimental runs: open-loop and adaptive PID implementation.

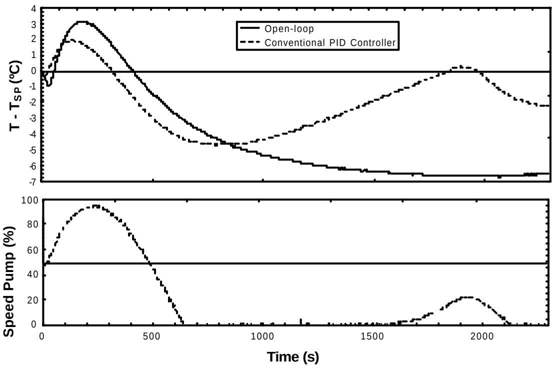

Fig. 5. Bulk temperature deviation (T-Tsp) and manipulated variable behavior obtained from experimental runs: open-loop and conventional PID implementation (Kc= 101.6 %/ºC, Ti= 156.0s, Td= 3.2s).

In order to compare conventional and adaptive PID controllers, performance criteria are computed from the experimental runs of Figures 4 and 5 and summarized on Table 2.

Table 2. PID and PID adaptive performance criteria from experimental runs.

Performance criteria	Controller	
	Adaptive PID	Conventional PID
ITAE	4,736,074	5,346,902
Overshoot (ºC)	1.3	1.9
Rise time (s)	220	320

From the results, it could be concluded that both controllers are suitable for the precipitation tank control. However, from Table 1, the adaptive controller showed better global performance criterion (ITAE) and reduced overshoot and rise time.

6. CONCLUSION

In the present work, the pineapple juice (raw material) is obtained from the stem and rind of the fruit, both considered as kitchen waste in most brazilian restaurants and industries. The precipitation process is usually the first step of the downstream processing, and this was the practical method adopted here in order to recover bromelain enzyme from the pineapple juice.

Looking forward to avoiding bromelain denaturation during the precipitation process, the temperature must be monitored and controlled. Due to the transient nature and non-linear features of bromelain precipitation fed-batch process, designing a temperature controller for the experimental apparatus is not a trivial task.

Conventional and adaptive controllers were properly designed and on-line implemented through a fieldbus digital control system. From the results, both controllers were considered suitable to control the temperature of the fed-batch tank. However, the performance was improved when adaptive controller was employed, since no saturation on control action occurred and the overshoot and rise time decreased.

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