Astaxanthin production by *Phaffia rhodozyma* using a pH-stat control

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Abstract: - Fed-batch essays using yeast *Phaffia rhodozyma* were performed to obtain high astaxanthin concentrations. The yeast was grown over an inexpensive media obtained from the *Yucca fillifera* date. Feeding was carried out through implantation of a pH-stat control system. Biomass (28.7 mg/l) and pigment (18 mg/l) concentration three folded those that were obtained by previous batch cultures.

Key words: Fed batch, Astaxanthin, *Phaffia rhodozyma*, Control, pH-stat, Biomass, pigment.

1 Introduction

In a fermentation process microorganisms consumes sugars and salts from a culture medium using these for growth and/or product formation. When fermentation behavior is known, important strategies can be created to manipulate the process obtaining the desired product. A control system is a necessary tool that allows to eliminate disturbances which affects the process fermentation. In a Batch fermentation the reactor is prepared with medium which includes nitrogen, carbon sources and the microorganism. Fermentation elapse until sugars are consumed and the fermentation ends, in a fed-batch fermentation medium (salts and sugars) are fed to the bioreactor trying to keep product formation in a constant way. Many control techniques has been used for fermentation processes, when a sensor measures the key variables involved in the process a simple control system could be implemented. When there is no data available from the key variable new approaches has to be taken, such as open loop strategies where control is performed based on historical data from fermentations. Indirect fermentation control is based on estimation from mass balance equation, calculations gotten through other variables who gave useful information about the process like pH, dissolved oxygen, this kind of control are called pH-stat, DO-stat. Another type of estimations could be done with Kalman filters, neural networks, fuzzy logic, adaptive control [1]. Astaxanthin (3,3'-dihydroxy-, β-carotene –4,4' dione) is a pigment which gives the red orange characteristic color to lobster, shrimp, and crabs [2]. Many microorganisms like yeast, bacteria and algae produce this pigment. Yeast *Phaffia rhodozyma* have shown to be a promising production source. This pigment has been used to add color to trout and salmon which are grown in culture farms, flesh from these fishes are colorless, because they can not synthesize pigment, instead they got it from their natural food source. An additional advantage is that although pigment is intracellula is not necessary to use expensive extraction methods to get astaxanthin, because the yeast is mixed with the fish food. Some companies have begun to sell feeding supplement that include astaxanthin as a natural antioxidants source. In this work a fed strategy over a fed-batch fermentation, for astaxanthin production by the *Phaffia rhodozyma* yeast was performed using a pH-stat control system.

2 Problem Formulation

In a fermentation is wanted to produce high biomass or product concentrations, nevertheless this is not a simple task due to microorganism are alive with a changing and poorly predictable metabolism. A deeply understanding of the microorganism must be developed so a strategy can be planned. Methodology used usually on microorganism study is to begin with shake flask experiments, later reactor essay are performed. In a batch culture the bioreactor is set up with the media necessary for growth and product formation, along the fermentation is only added the solutions that keeps pH at setpoint, antifoam and some gases.

batch fermentation has the drawback that is not possible to obtain high biomass or product concentrations due that high sugar concentrations inhibit growth and product formation [3]. Another drawback is the dead time every time that the reactor has to be prepared and sterilized among fermentations.

3 Problem solution

A 3 liter Applikon bioreactor was used, a Cole Parmer pH on/off meter controller 5652-00, Cole Parmer dissolved oxygen meter controller 0197200, Cole Parmer antifoam controller 01973-00 were coupled to a personal computer with a 90 Mhz microprocessor using a PCL-711S data acquisition and some external hardware. The computer was used to register fermentation data. *Phaffia rhodozyma* mutant strain M-18 derived from the strain ATCC 24202 was used. Culture media contained 0.5 g/l urea, 0.5 g/l potassium phosphate, 2 g/l magnesium sulfate, carbon source was date juice derived from the *Yucca fillifera*, that has demonstrated to be an inexpensive and efficient medium for growing the yeast *Phaffia rhodozyma* and for astaxantin production [4]. pH was controlled to 6 adding acid (HCL) and base (NaOH), also foam formation was controlled adding antifoam to the culture medium. Agitation speed was kept to 900 rpm, air flux was kept to 50 ml/min. Dissolved oxygen and temperature were registered on line.

3.1 Batch cultures

Batch experiments were carried out looking for the best growth and pigment production conditions. Working volume for the reactor was 1500 ml and fermentation lasted 72 hours. From this essays it was found that yeast acidify medium for 25 to 30 hours, suddenly medium pH changes and gets basic as it is shown on figure 1. pH was controlled to 6 using an on / off controller, low limit was set to 5.9 and high limit was set to 6.08, figure shows clearly the two phases involved in the fermentation. This pH change is associated with the end of the exponential phase and the begin of the stationary phase, it also coincide with the maximum dissolved oxygen consumption and the maximum temperature reached in the fermentation, and it could be associated with the carbon and nitrogen source consumption, this increase in pH was used as a signal for feeding sugars in the fed-batch fermentation.

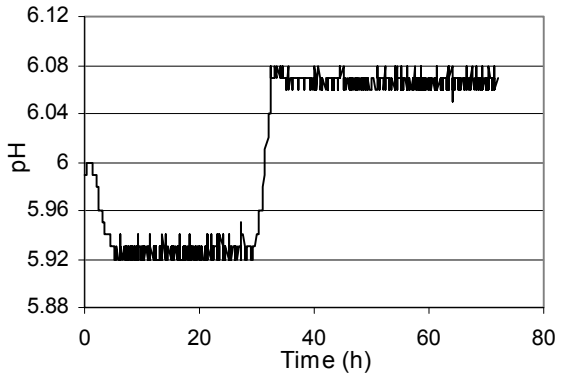

Figure 1. pH changes presented on a batch fermentation.

3.2 Fed-batch cultures.

Fed-batch fermentations began with 20 g/l of carbon source concentration, an initial working volume of 1200 ml, ml and fermentation lasted 96 to 144 hours, other conditions were the same as the batch fermentations. Date juice was concentrated to 300 g/l and mixed with 10 g/l of urea, Carbon source was concentrated avoiding increase in the bioreactor volume. At the beginning of the fermentation NaOH was added to avoid that medium decreased its pH below setpoint, along this phase yeast tends to produce compounds that acidify the medium, when pH began to turn base and increase its value the program written in the computer activated the feeding pump and an amount of date juice concentrated was added to the medium reestablishing fermentation to its previous state, this was performed until all the programmed additions has been carried out, on figure 2 can be seen a graph where feeding is responding to pH changes.

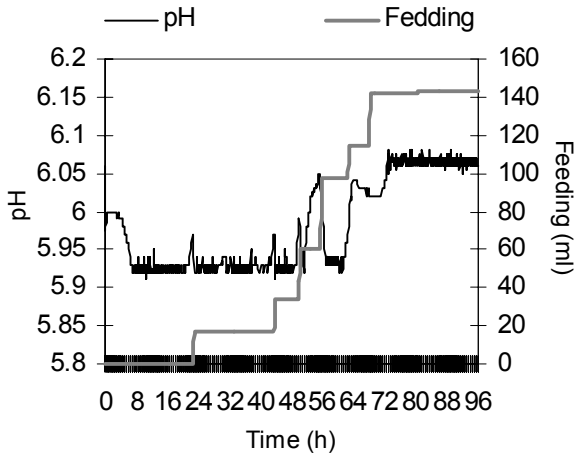

Figure 2. pH behavior and date juice additions based on pH changes.

After all feeding additions has been performed fermentation is left to end, in this phase due to pH tends to increase HCL must be added to the medium. Different essays were done until it was found the optimal feeding quantity. On figure 3 it can be seen a diagram showing the program logic.

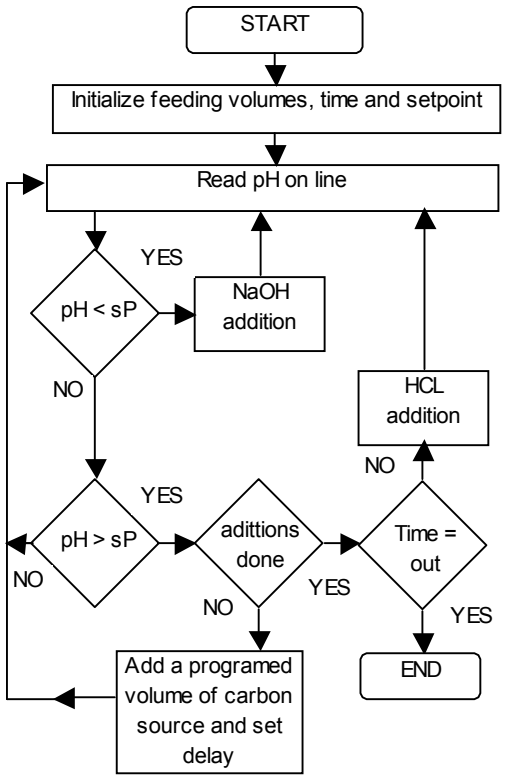

Figure 3. Control diagram.

On first and second addition pH returns to its original acidifying state easily, but on the next additions this can not be accomplished quite easy, pH stays only a minimal amount of time acidifying so volume added to the bioreactor must be increased this is due that yeast is doubling its population and consumes sugars faster than before. Care must be taken because a large amount of sugars accumulated could inhibit growth or product formation, that's why after a series of large volume additions a short one was followed. An unexpected problem detected along fermentations consisted in pH changes that were not considered, so the program could not avoid to add date juice saturating the media with sugars, to overcome this problem additions were followed by a fixed time delay, this approach eliminated the problem. Eight fed-batch fermentation were carried out, on each one addition volumes were adjusted to obtain the best response for growth and product formation as well. As it can be seen on Figure 4 best results are shown. Final volumes added were 16, 16, 32, 32, 16, 32 ml.

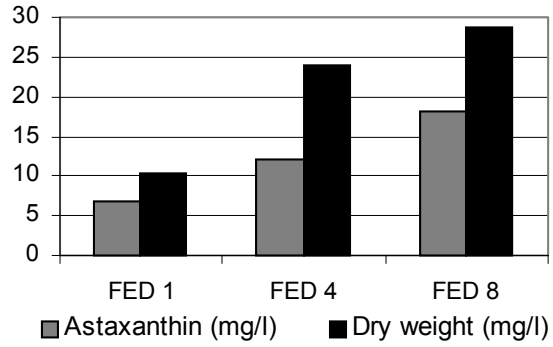

Figure 4. Data from different fermentations.

4. Conclusion

High astaxanthin concentrations have been obtained using a simple pH-stat control feeding strategy, 28.7 g/l on dry weight and 18 g/l from Astaxanthin. Although good concentrations has been gotten, better approaches are encouraged to be search on future works.

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