CONTINUOUS FERMENTATION PROCESSES IN THE PRODUCTION OF ALCOHOL

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ABSTRACT

The production of ethanol in Brazil has increased over the last decade and is presently around 12 billion liters annually. It is estimated that about one quarter of this production is obtained through continuous fermentation. The technologies currently applied in the fermentation procedures are reviewed, a mathematical model of cascade fermentation reactors and the practical experience gathered in terms of different processes are also discussed.

INTRODUCTION

The importance of ethanol as a motor fuel either alone or mixed with gasoline is well known. Besides, there are a wide range of applications of ethanol in the chemical, pharmaceutical and personal care products industry, among others.

The production of ethanol in Brazil nowadays is around 12.5 billion liters yearly. More than 90% of this alcohol is used as motor fuel. When the Alcohol Program was established in Brazil, 16 years ago, all new plants were erected for the conventional batch Melle-Boinot process, which was developed in France about 60 years ago. The main characteristic of this process is the use of disk-bowl centrifuges for separation of the yeast cream, and then yeast cream is diluted with fresh water and treated with sulfuric acid to pH 2.6-2.8 and maintained in agitation for about 3 h before a new batch is started. It is a fed-batch process, where the substrate is fed under controlled conditions until reaching the volume of the fermenter. It is important to emphasize that in spite of being an old process, the Melle-Boinot process is very convenient and satisfactory, when considering operational aspects and efficiency of conversion of sugar to alcohol (yield) that most of the new continuous processes developed had so many disadvantages that they have been abandoned.

In the last decade, the interest for continuous fermentation has been resumed to reduce the cost of production, this being particularly important in the face of reduced petroleum prices (when the Alcohol Program was established in Brazil petroleum prices were close to U$ 30/barrel and later, dropped to U$ 15). It has to be pointed out also that the overcoming of initial priorities for the implementa-
tion of large scale production (more than three hundred new plants) the Program has led to the technological majority with logical consequences for the development of new technologies. A great endeavor has been made among alcohol producers and research institutions to develop new production procedures. Technical support from overseas universities has also contributed to reach such objectives.

CONTINUOUS FERMENTATION

Continuous fermentation processes for alcohol production have been used over the years and the Guillaume-Egrot process (Marille?) developed in France near 1950 was probably the first successful process used in the world (Figure 1). This process is the base for the so-called cascade fermentation procedures. The change from batch to continuous fermentation in most of the well-operated distilleries in Brazil was at first considered a risky decision due to the fact that in these distilleries the batch Melle-Boinot process was performing quite well. In spite of this from 360 existing distilleries in Brazil around 60 have already been converted to continuous fermentation.

When one considers the possibility of switch-over from batch to continuous fermentation the following considerations arise: possibility of infections, deposits of solids in the bottom of fermenters, beer alcohol content and stoppages.

FIGURE 1. Guillaume cascade fermentation.

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The consequences of fermentation infections are nowadays reasonably well known and bacterial counts of $10^6$/ml are common and not considered relevant provided other operational conditions are satisfactory. When cane juice is used as substrate for the fermentation it should be heated up to 105°C and clarified as usual for sugar production, to destroy most of bacteria and wild-yeast strains that occasionally emerge, after that it should be cooled down to about 32°C. Molasses whether final molasses or non-exhausted molasses coming from sugar production usually causes no difficulties for fermentation. It is well known that when exhausted molasses is the only substrate for the fermentation the high level of non-ethanol inhibitors hinders fermentation rate and high retention time is necessary for the conversion of sugar to alcohol. In this case it is generally accepted that molasses should be treated to precipitate calcium sulfate. A faster fermentation rate and higher beer ethanol content can then be expected.

High concentration of viable yeast cells is the most important consideration as far as continuous fermentation is concerned, high fermentation rate and therefore high yields can be expected. Yeast cells concentration should be in the range of 3.5-4.0%w. The resulting yeast cream coming off the yeast separator must be as concentrated as possible to overcome most of resulting infections since most of the bacteria can be drawn out together with the yeast-free beer. The Brazilian experience gathered on the operation of various systems for continuous fermentation indicates that before recycling the yeast cream to the fermenters it is necessary to dilute it with fresh water and to treat it with sulfuric acid to pH 2.4-2.8 according to infection level and after that to hold it for one or two hours before pumping back to the fermentation. Sulfuric acid treatment for the yeast milk is a sound control method for infections in most cases. Recent trials have been made to reduce or eliminate the use of sulfuric acid. It has been shown that it is possible to operate without sulfuric acid where good conditions are exercised, but this practice is very doubtful and it is not recommended.

As a result of precipitation of insoluble solids that have been formed during the fermentation process or due to suspended matters (mainly yeast cells) that settle down as fermentation proceeds, large quantities of deposits in the bottom of the fermenters can be found if fermenters are not properly designed. This build-up material causes stagnant areas resulting in poor conditions for yeast metabolism and a good environment for bacteria development. This is considered one of the main causes of continuous fermentation failures. A stirred fermenter in this case is advisable to prevent sedimentation.

It is generally believed that continuous fermentation processes cannot attain high levels of beer ethanol content. The Brazilian experience indicates that high beer ethanol content (10% v.) in continuous fermentation can be maintained for long periods without loss of yield provided the proper procedure for the continuous fermentation is followed.
Finally, another concern is related to stoppages. The interruption of operation, whether batch or continuous fermentation is practiced, is always disturbance for the process. Brazilian experience indicates that short stoppages, around 24 h or less, usually do not produce a deleterious effect on fermentation itself, provided a good fermentation procedure is being utilized.

In Brazil alcohol production is made only by yeast fermentation. Various procedures for continuous fermentation have been tried and some of them are no longer used. The main continuous processes that have been utilized can be summarized as follows:

- One-step fermentation: the entire procedure takes place in one fermenter with constant alcohol content.
- Cascade fermentation: the individual fermenters are connected in cascade fashion and are passed through consecutively.

**ONE-STEP FERMENTATION**

Three different procedures for one-step continuous fermentation have been tested in Brazil:

- **BIOSTIL** process developed by Alfa-Laval and implemented through Codistil.
- **Hoechst-Uhde** process based on the application of flocculant yeast.
- **FERCEN** process developed by Engenho Novo.

**BIOSTIL Process**

The process was developed by Alfa-Laval/Sweden and during 1983 came to full scale operation in Brazil through Codistil. The flowsheet of the process is shown in Figure 2. It was developed for the processing of high solids products and recirculated slops were used for wort preparation. A specific yeast strain was used for this high osmotic pressure medium. Yeast cream was separated and recycled to the fermenter without dilution. Sulfuric acid was used to maintain pH on 4.5.

The fermenter was a cylindrical tank without stirring, provided with a cylindrical coil at the bottom for air injection. Heat released during fermentation was removed through a plate heat exchanger. Nutrients were added to the fermenter and air was injected to maintain cell propagation. Low alcohol content was maintained in the range of 5.5-6.5% v. due to the high osmotic pressure. Low sugar concentration was maintained to prevent sugar losses in the outflow.
The BIOSTIL process was designed to produce very low volumes of slops. Although the process had been in operation for four years in two distilleries, there were severe difficulties to maintain steady conditions. Finally, due to poor yields the process has been abandoned.

Hoechst-Uhde Process

The Hoechst-Uhde process attempted to eliminate the use of centrifugal separators and so a flocculating yeast strain was used. It was operated by two distilleries in Brazil. The process flowsheet is shown in Figure 3. Basically the fermenter was a tower with a top diameter larger than normal body which is intended to serve as a yeast separator. The overflow of the tower was sent to a sedimentation tank for further separation of yeast which was pumped back to the main fermenter. The overflow from the sedimentation tank again was sent to a second sedimentation tank and from this one the overflow was sent to distillation. The yeast separated in the second sedimentation tank was either recirculated or separated as a by-product. Sulfuric acid and eventually phosphoric acid were added to maintain pH 4.0 during fermentation. Liquid ammonia was used as nutrient as well. Continuous air injection was used in order to maintain yeast propagation. Like in all one-step fermentations sugar concentration was maintained at low levels to minimize losses of unfermented sugar.
The difficulties experienced by the distilleries which have operated this process were related to unexpected non-floculation of yeast, erratic performance, low alcohol content and low yields. The use of this process have been discontinued.

FERCEN Process

The FERCEN procedure for continuous fermentation operates with individual fermenters connected in parallel where fermentation takes place in stirred tanks (Figure 4). Sugar concentration is maintained below 1 g/l. From the outflow of fermenters yeast is separated by centrifugal separators and no regular dilution or sulfuric acid addition is practiced. Eventually, sulfuric acid is added to the yeast cream using an in-line mixer. The process is being used for some autonomous distilleries where the only substrate is cane juice. At one distillery where the substrate was a blend of cane juice and molasses the performance of the process was poor and it was discontinued.

CASCADE FERMENTATION

There are at least four main cascade fermentation procedures operating successful in Brazil: Vale do Rosario, Copersucar, Codistil and BIOES.
All cascade fermentations in essence are similar and all are based on the original Guillaume process as described. The main differences seem to be on the strategies to overcome accumulation of deposits of sediments matter in the bottom of the fermenters and the number of stages utilized. Where good operation is practiced in general the cascade fermentation shows a very active behavior especially in the first and second stages where high carbon dioxide production can be observed. All cascade fermentation processes in Brazil are carried out with yeast recycling to attain a fast fermentation rate and high alcohol content (10% v.). Yields are normally around 90% or more. Due to the very active fermentation, in the first stage and to a minor extent in the second stage there are usually foaming problems and sometimes high consumption of antifoaming agents. The strategy in this case is to connect consecutively the fermenters using a gutter-pipe and in this way foam flows from the first to the second stage and as it goes forward it is reduced, thus minimizing the use of antifoam.

The Vale do Rosario Factory process consists of four stages of fermenters of the same size. Wort is fed to the top of the first fermenter and comes out through the bottom to the next stage. Flow is by gravity. Distinct lines of four fermenters are placed to attain flexibility when rate of production changes.

The Copersucar procedure consists of three stages of fermenters of different sizes where the volume of the first fermenter is 50% of the total volume and the
other two have the same size, i.e., 25% of total volume each (Figure 5). The fermenters have a 60° sloped bottom to prevent accumulation of deposits of insoluble solids, in some circumstances the first fermenter is about 30 m high. The flow from one stage to the other is by gravity through the bottom of one stage to the top of the next stage. To get some agitation air is injected at the bottom of the fermenters. Air is also intended to maintain some aerobic condition. Yeast is separated from the last stage and after that diluted and treated with sulfuric acid to pH 2.0-2.5 and then held in agitation for about two hours before being pumped back to the first stage.

![Diagram of Copersucar cascade fermentation process]

**FIGURE 5.** Copersucar cascade fermentation.

The Codistil procedure consists of four (sometimes five) stages usually the first two stages of the same size corresponding each one to about 30% of the total volume and the last two fermenters of the same size corresponding each one to about 20% of the total volume required (Figure 6). The bottom of the fermenters is sloped at about 10°. No stirrers are provided to the fermenters. The flow is by gravity from the top of one stage to the bottom of the other. Yeast is separated from the overflow of the last stage and the cream is diluted and treated with sulfuric acid similar to the Copersucar procedure.

The BIOES procedure is a continuous stirred cascade fermentation process which was implemented at Ester Factory after four years of development. During pilot plant tests and semi-industrial scale operation the influence of the main factors on fermentation was investigated, like number of stages, product inhibition, sugar concentration and the role of agitation in the fermentation process to achieve optimum conditions for fast fermentation rate, high ethanol content and high yields. These investigations have led to the adoption of four stages of fermenters.
FIGURE 6. Codistil cascade fermentation.

The first fermenter is operated without stirring and the others are operated with stirrers in which specific power consumption is increased from the second to the fourth stage (Figure 7). The flow from one stage to the other is by gravity from the bottom of one fermenter to the top of the other. The fermenters are basically of the same size but two fermenters are operated in parallel forming the first stage and another two are operated in the same way as the second stage to achieve flexibility when production rate is changed; in this way, retention time is maintained relatively constant. Yeast is separated from the last stage and then diluted with fresh water and treated with sulfuric acid to pH 2.5-3.0, after that it is held in a continuous stirred tank for about one hour before being recycled back to the first stage. Normally, no nutrients are used in this procedure.

EXPERIMENTAL DATA AND MODELING

The fermentation reaction can be expressed as:

\[ \text{Glucose (S) + nutrients} \rightarrow \text{Ethanol (p) + Cells (X) + by-products cells} \]

During the course of alcohol fermentation, the accumulated ethanol in the broth and its relevant enzymes, alcohol dehydrogenase and hexokinase can inhibit the metabolic activities such as the specific growth rate, specific ethanol production rate, cell viability, sugar consumption and so on (Chang and Wang).
The fermentation processes can be compared on identical bases using a single fermentation kinetic model for the yeast *Saccharomyces cerevisiae*. Based on the work of Lee et al. who utilized the Monod form, representing the decrease in fermentation activity under low substrate conditions, with a second term representing the inhibitory effect of ethanol at high concentrations according to the studies of Levenspiel and finally a third term representing the inhibition effect due to the cell mass concentration according to Cysewski & Wilke, the model can be given as:

$$\mu = \mu_{\text{max}} \left( \frac{S}{K_s + S} \right) \left( 1 - \frac{P}{P_m} \right)^{n} \left( 1 - \frac{X}{X_m} \right)^{m}$$

Where $\mu$ is the specific cell growth rate (g cell produced / g cell.h); $\mu_{\text{max}}$ is the maximum specific cell growth rate; $S$ is the substrate concentration (g/l); $K_s$ is the substrate saturation constant or Monod constant (g/l); $P$ is the product (ethanol) concentration (g/l); $P_m$ is the ethanol concentration limit; $n$ is the product inhibition power; $X$ is the yeast cell concentration (g/l); $X_m$ is the yeast cell concentration limit (g/l); and $m$ is the cell inhibition power.

Under Brazilian industry conditions Andrietta & Stupiello studied the kinetic parameters of the model in laboratory scale. They calculated the main parameters which are shown in Table 1.
TABLE 1. Kinetic parameters for batch ethanol fermentation.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ks</td>
<td>1.6 g/l</td>
</tr>
<tr>
<td>n</td>
<td>3.0</td>
</tr>
<tr>
<td>m</td>
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<tr>
<td>Pm</td>
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</tr>
<tr>
<td>Xm</td>
<td>100 g/l</td>
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<tr>
<td>μ max</td>
<td>0.41 l/h</td>
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</tbody>
</table>

The same model was used for continuous ethanol fermentation. Using simulations Andrietta & Stupiello studied the optimum design parameters for continuous processes in operation in Brazil. Figure 8 shows the rate of substrate consumption according to the substrate concentration. By definition, the residence time for a plug flow reactor is the area under the curve between the initial and final concentration of substrate and the residence time for backmix reactor is the rectangular area under the same extremes. It can be observed that the residence time required for a specific conversion is lower for a plug flow reactor.

The utilization of plug flow reactors for ethanol fermentation is affected by the agitation caused by carbon dioxide evolution and so it is difficult to realize in industrial operation. On the other hand backmixing decreases the reaction rate for an end-product inhibited reaction Maiorella et al. so a single backmix reactor (continuous stirred tank reactor) is not convenient for ethanol fermentation. However, the use of CSTR arranged in series can be used to overcome end-product inhibition effects, resulting in a substantial reduction in reactor volume as compared to the simple CSTR Maiorella et al. By using the kinetic model it was concluded that a four stages of CSTR fermenters behave reasonably equivalent to a theoretical plug flow fermenter.

**Kinetic Parameters for the BIOES Fermentation**

By using the kinetic model described it was possible to calculate the substrate saturation constant (Ks) and the product inhibition power (n) for the BIOES fermentation procedure. The substrate saturation constant can be explained as the affinity level of the yeast strain for the substrate and the product inhibition power (n) as the tolerance of the yeast strain for the ethanol concentration. By using an optimization subroutine it was calculated the parameters Ks = 9.0 g/l and n = 2.17 for the BIOES fermentation procedure. The substrate constant (Ks) calculated under industrial scale was higher than that calculated under laboratory scale, and this indicates that a poorer substrate was used in the industrial scale. The product inhibition power (n) obtained under industrial scale was lower than
FIGURE 8. Characteristic curve of the kinetic model for continuous fermentation.

That one obtained under laboratory scale which indicates that yeast strains under industrial conditions exhibit higher tolerance for the ethanol. Volumetric productivity for the BIOES procedure was found to be 9.41 g/l.h.

FINAL CONSIDERATIONS

The application of different continuous fermentation processes used in Brazil have led to some observations which can be summarized, as follows.

Cascade vs one-step fermentation: Cascade fermentation is more stable since the end-product inhibition effects are relevant only at later stages. For this reason, productivity (ethanol produced/h. volume of fermenter) is much higher.

Fermentation rate: Residence time is influenced by the process itself and substrate characteristics. It is well known that exhausted molasses tends to produce a slower fermentation rate in comparison to juice fermentation.

Kinetic model: The use of the kinetic model of the fermentation process is very helpful for the determination of the design parameters of the fermenter. In the
cascade procedure the fermentation performance indicates, however, that it is necessary to increase the capacity of the first stage when compared with data obtained from the kinetic model, to reduce the fermentation rate, and so, the evolution of carbon dioxide to overcome stron foaming characteristics in this stage; the resulting higher ethanol concentration in this case controls the bacteria proliferation in this stage as well.

Agitation: In spite of all turbulence due to release of carbon dioxide, mechanical agitation is very helpful to provide backmixing and to prevent sedimentation of yeast cells or other suspended matter. The industrial operation has shown that better fermentation performance is obtained when agitation is applied, due to even work of the yeast separators, resulting higher concentration of the yeast cream, consequently lessening inhibitors.

Air injection: It is well known that oxygen is essential for the synthesis of lipid components of the membranes, i.e., unsaturated fatty acids and sterol (ergosterol), without which growth and reproduction of the cells cease. An insufficiency of these substances leads to the degeneration of the yeast. However, to increase the fermentative activity of the cells, prior to their introduction into the fermentation medium they should be transferred to anaerobic conditions Lisyuk and Permyakova. The observations carried out to verify the influence exerted by aeration on the fermentation rate, cell viability and yield indicate that only small amount of oxygen is necessary when a yeast recycling procedure is used. Thus, it is experienced that, the dissolved oxygen of the dilution fresh water used for the yeast cream dilution is sufficient for the cells requirements and the control of cell propagation is exercised through the dilution ratio of cream.

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FERMENTATION EN CONTINU POUR LA PRODUCTION D’ETHANOL

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RESUME

La production d’alcool au Brazil a augmenté durant les 10 dernières années pour atteindre 12 billions de litres par an. A peu près 25% de ce volume est obtenu par fermentation continue. On présente les technologies employées, les modèles mathématiques de fermentation en cascade et les expériences pratiques au sujet du processus.

PROCESOS DE FERMENTACION CONTINUA EN LA PRODUCCION DE ALCOHOL

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RESUMEN

La producción de etanol en Brasil ha aumentado durante la última década y alcanza en el presente la cifra de 12 billones de litros anuales. Se estima que una cuarta parte de esta producción es obtenida por fermentación continua. En este artículo son revisadas las tecnologías actualmente aplicadas en los procedimientos de la fermentación, un modelo matemático de reactores de fermentación por cascada y también son discutidas las experiencias prácticas adquiridas en distintos procesos.