SIMULATION, ANALYSIS AND EVALUATION OF FERMENTATION TEMPERATURE IN AN *IN SITU* GAS STRIPPING FERMENTATION PROCESS

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RESUMO- Fermentation is the main step in bioethanol production. The major fermentation problems are the yeast inhibition when high ethanol concentrations are reached, and the high amounts of water added to the process. One way to overcome these problems is to couple fermentation to a continuous process removal, or known as *in situ* removal techniques. Such techniques allow the use of high sugar concentrations in the broth, keeping ethanol concentration in acceptable ranges to microorganism metabolism. Gas stripping *in situ* removal presents itself as a simple technique that requires no membrane or expensive chemicals and easy to integrate with fermentation process. In this work, the simulation of a gas stripping fermentation process was carried out using ASPEN PLUS® V.7.3, in order to evaluate the ethanol separation process behavior related to different fermentation temperatures. Initially a case study was performed, where a concentrate sugar stream at 30 % wt was fed to a reactor operating at a temperature of 34 °C with 7 L/min of CO_2 gas flow rate. In this simulation, 67 % of ethanol was stripped which allowed ethanol concentration to be kept below threshold of toxicity. The results shown that high ethanol recoveries were reached as high as the temperature employed. Nonetheless, the temperature range studied was limited by the *S. cerevisiae* yeast behavior, so, in practice, only narrow temperature ranges could be applied for gas stripping fermentation process.

1. INTRODUCTION

Biofuels have been presented as an important option for energy supply, notably as renewable substitutes for fossil fuels. They are considered a renewable and endless resource, since they are produced from biomass, usually from agricultural crop. Among all Biomass fuels currently in production, ethanol from sugar cane is the most commercially successful, has a positive energy balance and has been benefited from the support of government policies (Pereira and Ortega, 2010; Goldberg, 2009). Driven by government support, Brazil and U.S. are the dominant industrial players, accounting for 87% of global biofuel production. Brazil is now the biggest exporter and the second biggest fuel ethanol world producer with around 30 bilion liters/annum (2008) (Bjapai, 2013).

The ethanol production on a large scale in Brazil takes place via the fermentation step, in this step the sugars presents in the fermentation broth are converted into ethanol due specific metabolic paths performed by *Sacchamoryces cerevisiae* yeast. Such microorganism usually cannot tolerate more than about 10-12% by volume of ethanol in the broth, thus, it is necessary to start the fermentation with a relatively dilute sugar solution, usually not more than about 16% by weight, in order to achieve complete conversion in a reasonable time. In this way, sugarcane molasses (about 52% by weight of sugar) which is the main sugar raw material of fermentation, have to be diluted (conditioned). Costs associated with distillation, centrifugation and evaporation can be minimized by reducing this amount of water that is added to the process, besides, large process equipment would not be necessary (Taylor *et al.*, 1995; 2010).

Aiming to solve such problems, several techniques were proposed for simultaneous fermentation and product recovery from fermentation broth. Since volatile products could be removed, the toxicity effects of solvents are eliminated, enhancing sugar utilization concentration, which in turns, results in lesser water added in the fermentation step. The gas stripping *in situ* removal process is a simple technique that requires no membrane and expensive chemicals, is free of emulsion formation, has low energy requirement and easy to integrate with fermentation process (Vrije *et al.*, 2013; Ezeji *et al.*, 2012; Yang *et al.*, 2013).

Gas stripping technique is based on passing a carrier gas (such as CO_2 or N_2*)* through a sparger, resulting in the formation of gas bubbles in the bioreactor. Gas sparging induces ethanol vapourization, which after passing through a condenser is recovered by condensation. The carrier gas is then purged or recycled back to the reactor for be used to recover more ethanol from the fermentation broth (Durre *et al.*, 2013).

Several variables can play a significant role in gas stripping technique such as: gas flow rate, condensation temperature, system agitation, bubble size, broth viscosity, fermentation temperature and different kinds of gases utilized. The fermentation temperature represents a critical step in ethanol production, it directly influences in ethanol recovery by gas stripping and metabolic activity of microorganisms. Higher temperatures make many process parameters, including the stripping factor more favorable for fermentation. The ratio of ethanol to inert gas is a strong function of the stripping temperature because the partial pressure of a volatile compound (ethanol or water) in the gas phase is determined, among other factors, by saturated vapor pressure, which raises as temperature increases (Vane, 2008). Besides, higher temperatures can decrease density, surface tension and viscosity on fermentation broth, increasing the solubility of most substrates (Liu and Hsu, 1990). In the other hand, higher temperatures can negatively impact in biomass production and yeast behavior, diminishing the ethanol content in the final wine (Dias *et al.*, 2009).

The objective of this work was to investigate how different fermentation temperatures may influence the gas stripping fermentation process performance, searching for high percentual of ethanol recovery, keeping the ethanol concentration lower than threshold of toxicity. For this reason, process simulations were carried out employing ASPEN PLUS® V.7.3 software, in laboratory scale ranges. Sensibility analysis were performed being a significant tool for recognize variable impacts in the final results, helping the outline laboratory scale trials in order to find the best applicable temperatures ranges.

2. SIMULATION DEVELOPMENT OF *IN SITU* GAS STRIPPING

The fermentation process simulation with *in situ* gas stripping was carried out employing the ASPEN PLUS® V.7.3. A conversion reactor (fermentor) was used in the simulation to convert the broth sugars, continuously. During the fermentation reactions, sucrose (from sugarcane) is hydrolyzed into fructose and glucose (by microorganisms), which are converted into carbon dioxin (CO_2) and ethanol (Reaction 1). The raw material used industrially and in laboratory scale is the sugarcane molasses, however a simulation approach was made to molasses being only glucose and water. Some by-products are also formed in fermentation step, as a result of parallel fermentation reactions, cell growth and impurities in the sugar juice, among other factors. In addition, around 4% of the glucose is not consumed by the yeast (Dias *et al.*, 2009).

The extend of fermentation conversion is set according to industrial data of large scale units. Glucose utilization is 94.63 %, with 1.37 % glucose converted to yeast cells. The ethanol, glycerol, succinic acid, and isoamylic alcohol yields are 0.9048, 0.0267, 0.0029, 0.0119 and 3.1×10^{-6} g/(g glucose), respectively (Dias *et al.*, 2009). Major reaction involved in the glucose fermentation include following:

$$C_6H_{12}O_6 \longrightarrow 2 \cdot CO_2 + 2 \cdot C_2H_5OH \quad \text{(Ethanol)} \quad (1)$$

$$C_6H_{12}O_6 + 4H^+ \longrightarrow 2 \cdot C_3H_8O_3 \quad \text{(Glycerol)} \quad (2)$$

$$C_6H_{12}O_6 + 1.143 \cdot NH_3 \longrightarrow 5.714 \cdot Yeast + 0.286 \cdot CO_2 + 2.57 \cdot H_2O \quad (3)$$

The yeast compound was created as a solid type in ASPEN PLUS®, in order to represent cell growth reaction (Reaction 3). The gas stripping process is simulated by a flash drum at the same fermentor conditions once stripping is not available at conversion reactor block. Thus, the thermodynamic equilibrium in the conversion reactor is carried out using a flash drum (flash separator type). The fermentation with *in situ* gas stripping process flow sheet is presented in the Figure 1.

Sequentially, a concentrate sugars stream is fed in reactor (I1), after conversion of sugars this stream follows to the flash drum, for separation step. The bottoms flash drum stream (B1) contains, basically, all glucose components converted and not converted. This stream is then centrifuged, in order to separate biomass from the main broth. Almost all biomass stream (R5) returns to the conversion reactor and the surplus biomass is purged out of the system, likewise happens with the broth stream (R1) where a small part of its flow leaves the process and the most part of it is recycled for keep the system mass balance.

The top flash drum product contains, in its majority, stripped gas and ethanol/water vapour. This stream is condensed and the vapour stream (partially exhausted in volatile compounds) is recycled back to the flash drum. The condenser is simulated as a heat exchanger followed by a flash drum (FB2) at the same heat exchanger conditions. With flash drum insertion, it was employed the same thermodynamic approach done before (fermentor), in order to become easier the phase separation.

A small amount of the gas flow rate (T3) is purged out from the system (about 5%). The main top flash stream (T3) is recycled by a peristaltic pump which acts raising the pressure of gas stream (R4). The amount of carbon dioxin produced during fermentation is not the same amount of gas bled out of the process (P1), thus, small amounts of additional stripping gas are required in this case.

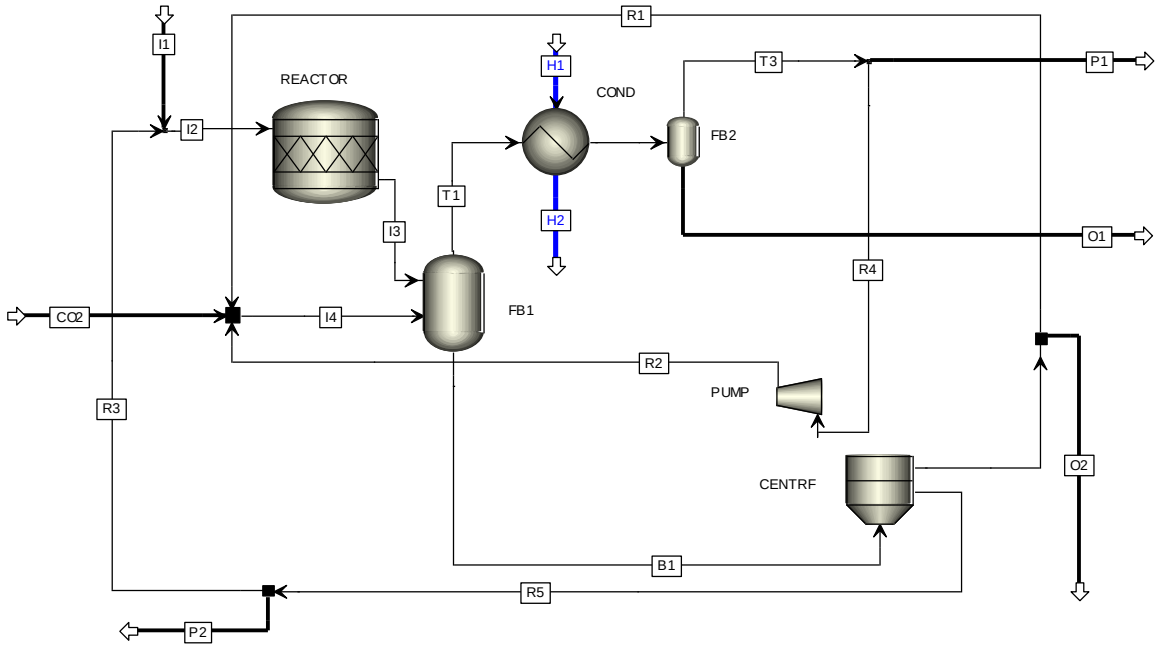

Figure 1- Flowsheet of continuous fermentation process with *in situ* gas stripping carried out in ASPEN PLUS®. Wider Lines indicate the process streams boundaries.

2.1 Thermodynamic Model

To calculation of the activity coefficient on the liquid phase, the NRTL (non-random two-liquid) model was used. In the same way, to calculate non idealities of the vapour phase, the Hayden-O'Connell model was used (NRTL-HOC). According to Dias *et al.* (2009), NRTL model provides the best estimations for the boiling temperature of sucrose solutions, when compared either UNIQUAC or equation of state Peng-Robinson. The Hayden-O'Connell equation was used because it can predict dimerization in the vapour phase on mixtures containing carboxylic acids (acetic acid).

3. RESULTS AND DISCUSSION

Initially, a simulation case study was carried out according conventional values of process variables, respecting the laboratory applicable ranges. Thereby, the gas stripping fermentation process was conducted continuously with 7 L/min of CO_2 gas flow rate at -2 ºC of cooling temperature and 30 wt % of glucose concentration in feed. The CO_2 was used as a carrier gas, mostly because it is already produced in fermentation, besides no inhibitory effect is attributable to its use. For this case of study, the fermentation temperature was set as 34ºC. The main streams and components results are shown in Table 1, as follows:

Table 1: Stream values of the case study

Stream ID Stream	I1 Main Feed	I3 Reactor output	I4 Flash Feed	T1 Stripped Gas	B1 Stripped Broth	01 Cond.	T3 Main Output	R2 Gas Recycled
Temperature (K)	307.2	307.2	301.3	307.2	307.1	271.1	271.1	272.0
Vapor Fraction	0.000	0.073	0.133	1.000	0.000	0.000	1.000	1.000
Mass Flow (Kg/hr)	0.099	0.100	2.991	0.780	0.060	0.026	0.032	0.723
Volume (L/min)	0.001	0.001	7.252	7.658	0.001	0.000	0.266	6.031
Mass Fraction								
Glucose	0.300	0.012	0.015	0.000	0.020	0.000	0.000	0.000
Water	0.690	0.695	0.665	0.022	0.884	0.603	0.002	0.002
Ethanol	0.000	0.139	0.056	0.017	0.073	0.360	0.005	0.005
Glycerol	0.000	0.008	0.010	0.000	0.014	0.000	0.000	0.000
Acetic Acid	0.000	0.002	0.003	0.000	0.004	0.000	0.000	0.000
CO_2	0.000	0.134	0.247	0.959	0.001	0.011	0.992	0.992

Analysing the results presented in Table 1 was possible to notice a high ethanol concentration leaving the fermentor (I3 stream), with about 21 ºGL in ethanol (166 g/L or 13,9 % wt). Due to gas stripping technique, the broth final ethanol concentration may reach 7,3 % wt (about 9 ºGL). As it could be seen, this ethanol decrease in the broth didn't reach the microorganism (*Saccharomyces cerevisiae*) threshold of toxicity, which is about 10-12% by volume of ethanol (Taylor *et al.*, 1995).

Specifically using 34ºC of fermentation temperature and -2 ºC of condensation temperature (FB2 temperature), was also noted that 67 % of produced ethanol (on stream I3) was recovered in the condensate stream (O1 stream), being the condenser efficiency for ethanol compound about 70%. The selectivity of the process was calculated as well (defined as $S= (y_{ethanol}/y_{water})/(x_{ethanol}/x_{water})$), it was reached a value of 7,2 (ethanol over water).

Performing the fermentation temperature sensitivity analysis (considering theoretical conversions independent of the temperatures effects in the microorganisms), keeping the other specifications as assigned before (30% wt of sugars concentration in feed, 7 L/min of CO_2 permanent gas flow rate and -2 ºC of cooling temperature), a profile of overall ethanol recovered from the process (in percentual) and its concentration in the condensate (in mass fraction) was provided varying the fermentation temperature. The results of this analysis are shown in Figure 2.

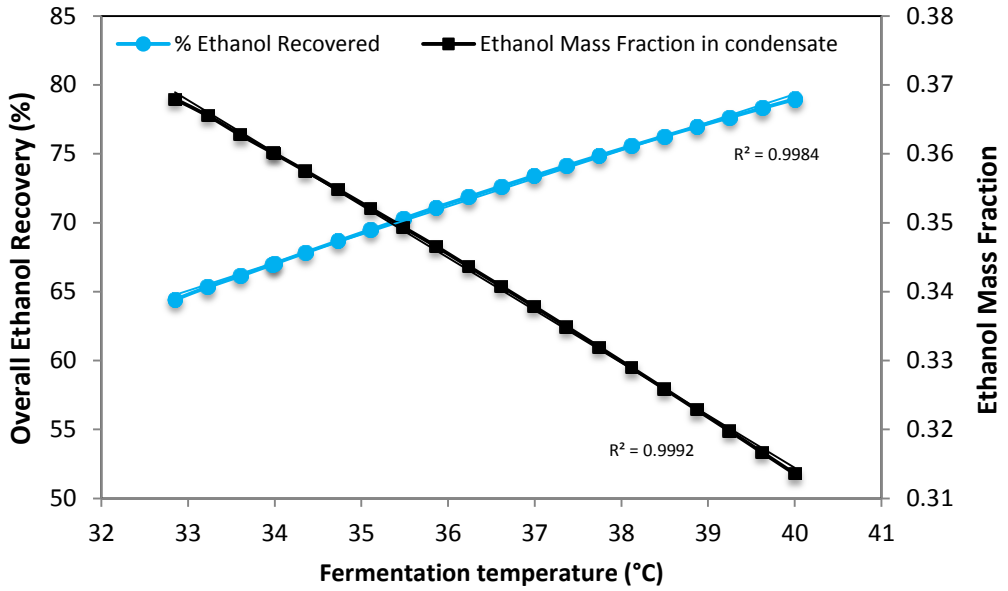

Figure 2- Relationship between the fermentation temperature towards percentage of recovered ethanol and its concentration in the condensate.

From the chart, it became clear that the variable effects follow opposite linear trends as fermentation temperature ranging. As the fermentation temperatures increases, the amount of ethanol stripped (recovered) raises. On the other hand, the condensate concentration decreases as reactor temperature increases. This occur mainly because more water is stripped as reactor temperature is raised and the heat of condensation of water per unit mass is 2.7 times that of ethanol, thus much more water is condensed over ethanol (Vane, 2008). The ethanol concentration in the condensate portrays the energy consumption in the overall ethanol production process. Low concentrations in condensate raises the energy required in the end-of-pipe process (distillation process), considering to produce ethanol in commercials concentrations (Hydrated Ethanol).

Again, higher temperatures result in higher ethanol stripped amount from the broth, but usually temperatures above 35 ºC are not used for experimental and industrial purposes. According to Philapasong *et al.* (2005), higher fermentation temperatures cause inhibition effect on cell growth of *Saccharomyces cerevisiae*, changing saturation level of soluble compounds and solvents in the cells, which might increase the accumulation of toxic concentration including ethanol inside cells. The same authors claim that a maximal level of biomass and ethanol production are seen among 30 ºC e 33 ºC, these levels decrease considerably at temperatures exceeding 35 ºC**.**

As mentioned before, from 35 ºC the yeast presents a huge ethanol production decay. The authors's experience and industrial applications suggest non use of temperatures beyond 35ºC. Alternatively, thermophilic microorganisms (other yeasts, filamentous fungi and bacteria) can be used to enable higher fermentation temperatures. Currently in Brazil, many attempts in this area are being performed, mainly to enable ethanol production of second generation (SSF process). However more studies in thermophilic microorganisms area are still necessary once several drawbacks still not allowed substitution of conventional *S. cerevisiae*.

Since ethanol concentration must be maintained lower than threshold of toxicity, it is essentially important evaluate how fermentation temperature affect the decrease of ethanol concentration in the broth. The Figure 3 presents this analysis carried out considering: 30% wt of sugars concentration in feed, 7 L/ min of CO_2 gas flow rate, -2 ºC of cooling temperature and 90,48% of sugars conversion into ethanol on the reactor.

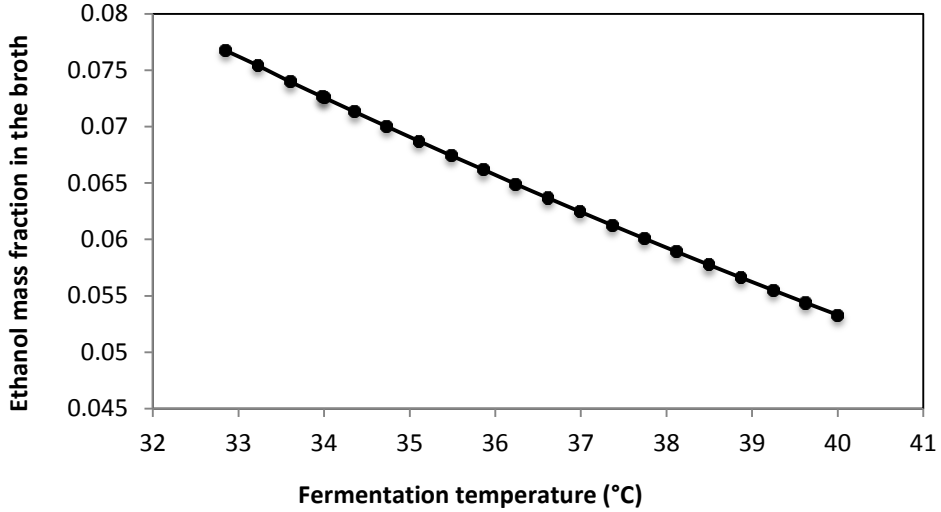

Figure 3- Ethanol concentration in the broth decreasing with the raise of fermentation temperature.

In Figure 3 it is shown that the raise of the fermentation temperature reduces the mass fraction of ethanol in the broth. Higher temperatures were more propitious in order to keep low ethanol concentrations. Nonetheless, within the concentrations ranges studied, the applicable microorganism fermentation temperatures (33-35 ºC) are enough to decrease the ethanol concentration below of inhibition limits (10-12 º GL ethanol).

4. CONCLUSIONS

An *in situ* gas stripping fermentation process was performed through a case study considering high concentrate sugars in the feed. The results showed the efectiveness of the technique in stripping ethanol from the main broth, allowing maintain acceptable ethanol concentrations in it. Through sensitivity analysis, it was could be seen that the fermentation temperature variable causes two ethanol production tradeoffs. The first one is the ethanol condensate concentration and the second one is the percentual of ethanol recovered (stripped ethanol) from the process, both, are inversely proportional to the raise of fermentation temperature.

Such impass was solved when microorganism behaviour was analized, once literature results shown that temperatures above 35 °C greatly diminishes ethanol and biomass production, this temperature value should be considered the limit of work. Taking into account the feed sugars concentration studied and all other the parameters of the process, the best temperature ranges that may be applied in the fermentation process of *in situ* gas stripping would be around 33-35 ºC.

5. REFERENCES

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