Use of the Genetic Algorithm in the Simulation and Optimization of the Hydrolysis Process of Cassava Starch to Obtain the Bioethanol

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Abstract

The Genetic Algorithm consists in a meta-heuristic optimization technique inspired on biological natural selection process, that has been used for solving optimization problems in several areas over the last decades, mainly because its efficiency in irregular search spaces. In this context, this work aimed to simulate and optimize the hydrolysis of cassava starch by α -amylase from Aspergillus niger, using genetic algorithm and to produce the bioethanol from this syrup. The hydrolysis occurred at temperatures ranging from 30 to 60 °C, with starch concentrations from 8 to 22 g/L. After ten trials it was obtained the optimal value for the efficiency of hydrolysis and 84.08% and, for concentration, temperature and time, optimal values equal to 23.4 g/L, 61.9°C and 111.0 min, respectively. In this condition, an ethanol yield of 88% was found, after fermentation process. Results pointed that this kind of metaheuristic optimization could be applied in the industrial bioethanol production.

Keywords: genetic algorithm; optimization; starch hydrolysis.

1 Introduction

1.1 Second generation alcohol

Ethanol and biodiesel are considered important energy options as a substitute for fossil fuels, because they are renewable resources and endless and can be produced from biomass in general, a culture of farming. The current belief is that replacing fossil fuels with ethanol and biodiesel can reduce emissions of greenhouse gases (Giraçol et al., 2011; Leão et al., 2011; Ometto and Roma, 2010; Pereira and Ortega, 2010).

As Shi et al. (2011), who showed the correct way to obtain the glucose syrup by hydrolysis of Dacotah switchgrass. This raw biomass was pretreated with sulfuric acid solutions of 0.5-01.0 wt.% concentrations and 3 wt.% sulfur dioxide at 140-180°C a range of times, following of enzymatic hydrolysis with 48.6 mg enzyme/g. For sulfur dioxide pretreatment, the highest total sugar yield of about 87% of the initial glucan in raw biomass occurred, in that conditions. And Shen et al. (2011), who made the steam-pretreated sweet sorghum bagasse (SSB) and Douglas-fir (DF) for SHF and SSF, to evaluate the effects of xylanase supplementation and delignification on ethanol production. Results indicated that final ethanol concentration in SHF could reach 28.4 g/L (SSB) and 20.4 g/L (DF) by xylanase supplementation with the increase of 46% and 61% in comparison with controls.

Telebnia et al. (2010) showed an attractive alternative form to utilization of wheat straw in bioethanol production Based on the type of pretreatment method applied, a sugar yield of 74–99.6% of maximum theoretical was achieved after enzymatic hydrolysis of wheat straw. Various bacteria, yeasts and fungi have been investigated with the ethanol yield ranging from 65% to 99% of theoretical value.

However, according to Dias et al. (2011), much of the controversy surrounding second generation ethanol production arises from the assumed competition with first generation ethanol production; however, in Brazil, where bioethanol is produced from sugarcane, sugarcane bagasse and trash will be used as feedstock for second generation ethanol production. Thus, second generation ethanol production may be primarily in competition with electricity production from the lignocellulosic fraction of sugarcane.

1.2 Starch as a saccharide source

Starch, the main reserve carbohydrate of several crops, is highly abundant in nature and can be easily extracted with high purity and at low cost (Cereda *et al*., 2001). The food industry is using starch for a long time as ingredient because of its functional properties. Depending on the source, the starches have different applications, improving consistency, stability, and other properties (Smith, 1982). The most common plant sources for food starches used all over the world are maize, wheat, potato, and manioc. However, there is a constraint because of the desire of the industries in bringing new products to the market and improving others (Takizawa *et al*., 2004). All starches have structural and compositional differences and a great number of studies are being done to show each particularity. These differences directly affect the yield of starch hydrolysis (Cereda *et al*., 2001).

Starchy crops like cassava are candidates for commercial bioethanol. Starch hydrolysis at 30–80°C could reduce energy costs and improve fermentation yields (Krishnan et al., 2000; Yingling et al., 2011).

An example of these differences is the presence of amylose in potato, sweet potato, corn, and cassava starches, which are 25.4% (w/w), 19.1% (w/w), 24.5% (w/w), and 18.6% (w/w), respectively (Cereda *et al*., 2001; Curvelo-Santana et al., 2008; Ferreira *et al*., 2005; Takizawa *et al*., 2004).

The hydrolysis of starch to products with low molecular weight, catalyzed by amylases, is the most important commercial enzyme process. The hydrolyzed products are widely applied in food, paper, and textile industries (Konzula and Liakopoulon-Kyriakides, 2004).

An alternative to replace this raw material is the use of syrups obtained by the hydrolysis of starch.

especially corn (*Zea mays*), rice (*Oriza sativa*), cassava (*Manihot spp*) and others (Biazus et al., 2009; Curvelo-Santana et al., 2008 and 2009; Ferreira *et al*., 2005; Leonel and Cereda, 2000). According to Alves (2005), one ton of sugar-cane produces 85 liters of bioethanol and, one ton of manioc produces 104 liters of bioethanol.

Starch hydrolysis has been achieved to ethanol production from corn (Krishnan et al., 2000; Lamsal et al., 2008; Matsumoto et al., 2011; Naidu et al., 2007), cassava (Curvelo Santana et al., 2008; Yingling et al., 2011), potato (Liu and Xu, 2008; Mathew, and Adlercreutz, 2009; Matsumoto et al., 2011) and wheat starches (Matsumoto et al., 2011).

1.3 Genetic Algorithm

The Genetic Algorithm (GA) consists in a meta-heuristic technique of optimization inspired on biological natural selection process that has been used for solving optimization problems in several areas over the last decades, mainly because its efficiency in irregular search spaces (Goldenberg, 1989; Santana et al., 2010).

The GA operates over a population of individuals (usually binary strings) also called chromosomes that represent possible solutions to the investigated problem. At the beginning, a population of individuals is randomly generated and evolved to one solution by means of applying of GA operators (selection, crossover and mutation). In each generation, the chromosomes are evaluated and the most able ones among them are selected and crossed each other, generating new chromosomes better than those ones of the previous population. It makes to increase the probability of one or more individuals be a solution of the problem. Different from other search methods, the GA operates over the population in parallel, yielding various solutions at a time. Hence, this method has been used to solve many problems involving complex combinatorial optimization (Pham and Pham, 1999; Santana et al., 2010).

In the evaluation process, a fitness function is used to measure the aptitude of the individuals of the population, providing information such as the number of new individuals each one can generate according with its aptitude. Usually the fitness function is based on objective function to be minimized or maximized depending on the problem (Pham and Pham, 1999; Santana et al., 2010).
A GA approach for solving a cell formation problem in cellular manufacturing was developed by Mahdavi et al. (2009). In their work, the authors proposed a model to solve the real size problems, which involve nonlinear terms and integer variables. A GA technique was applied in mechanic field to investigate cutting process parameters influence on workpiece price formation using the concepts of contribution margin. The obtained results from GA application presented in this work are in good agreement with others already reported and could be properly used in cutting process optimization parameters (Librantz et al., 2011).
Santana et al. (2010) studied the kinetic simulation and drying process optimization of corn malt by GA for estimation of temperature and time parameters, such that it maintains the maximum activity of α and β-amylases enzymes into the obtained product. Besides, drying temperature and time showed a square influence on the enzymatic activity. Optimizations by GA showed that at 54° C and between 5.6-6.4 h of drying process, values of specific activity of 5.26±0.06 SKB/mg and 15.69±0.10% of remaining moisture into corn malt were found.
However, the use of this technique in the simulation of hydrolysis is not cited by literature, until the current moment. Thus, this work has been proposed simulation and optimization of the hydrolysis of manioc starch by α-amylase from *Aspergillus niger*, a metaheuristic technique using genetic algorithms from a mathematical model presented in Curvelo-Santana et al. (2009). The hydrolysis occurred at temperatures ranging from 30 to 60 °C, with starch concentrations from 8 to 22 g/L.

2 Material and Methods

2.1 Raw matter preparation

Manioc roots were washed with water at 2 ppm of chlorine concentration, their rinds were removed, and roots were cut in pieces of about 2 cm^3. They were dried at 100°C every 8 h in a drier with air circulation. Drying roots were crushed in a Willye TE 650 flour-mill (Tecnal, São Paulo, Brazil) to obtain manioc flour (Curvelo-Santana et al., 2008; Takizawa *et al.*, 2004). The percent composition of manioc flour was determined by methodologies showed by Ascar (1985), Bradford (1976), Ferreira *et al.* (2005), Leonel and Cereda (2000) and Takizawa *et al.* (2004).

2.2 Kinetic study methodology

Bioreactors with thermal heating, containing starch concentrations from 8 to 22g / L at temperatures of 30 to 60 °C at pH 4.8 in acetate buffer 0.1 M. For the study of the hydrolysis bioreactor contained 25 ml of cassava starch solution and these were added 500µL of enzyme solution. Concentrations of reducing sugars (RS, given glucose) were measured from time to time, to obtain the kinetic curve for the appropriate temperatures and concentrations of starch used. Operation temperature and cassava starch contents were varied. The glucose concentration ($C_{Glucose}$, g/L) released into bioreactor was measured by DNS method (Miller, 1959). Protein content was determined according to the dye binding method of Bradford (1976) with bovine serum albumin as protein standard. The yield of the hydrolysis process ($\%Y_{Hydrolysis}$) was obtained by the concentration of the hydrolyzed starch ($\boldsymbol{C_{Hydrolyzed\ Starch}}$) and the initial concentration of starch ($C_{\boldsymbol{Starch}}$) or the relationship between glucose generated ($\boldsymbol{C_{Glucose}}$) and the theoretical concentration of glucose($\boldsymbol{C_{Theoretical\ Glucose}}$ = 1,111*$\boldsymbol{C_{Initial\ Starch}}$), according to Equation 1 (Curvelo-Santana et al., 2008; Ferreira et al., 2005; Sleiman & Venturi Neto, 2004).

$$\%Y_{Hydrolysis} = \left(\frac{C_{HidrolyzedStarch}}{C_{Starch}} \right) 100 = \left(\frac{C_{Glucose}}{C_{TheoreticalGlucose}} \right) 100$$

(1)

2.3 Scale-up of hydrolysis and fermentation

The experiment of starch degradation was assayed in a bioreactor with 2 L of total volume, with mechanic agitator for mixing, and a jacket for heating at operation temperatures. Bioreactors were operated in boat-load, contained 1 L of 2 g/L cassava starch solution, and 20 mL of amylase solution (FORILASE NTL®), at constant agitation of 70 rpm and pH 5 (Ferreira et al., 2005; Konzula and Liakopoulon-Kyriakides, 2004; Marshal *et al*., 1999).

After hydrolysis process, it was added and mixed 1 g of $(NH_4)H_2PO_4$, 0.1 g of $MgSO_4$ and sugar mass until obtaining of 50 g/L of total reduce sugar content. The pH was adjusted at 4.5. Fractions of approximately 10 mL, 100 mL and 1 L from the principal vats were distributed among different flasks, referred as vessels. They were heated in an autoclave and cooled rapidly in running water with the sole objective of sterilization of the medium. *Saccharomyces cerevisae* yeast (Flashmman, Jundiaí -SP, Brazil) at 1 g/L was added into the first vessel (10 mL) and the fermentation process occurred for one day. The total volume of fermented was transferred to the second vessel (500 mL) and the fermentation process occurred for one day. After the end of fermentation in the second vessel, the fermented was transferred to the principal vessel (1 L) until full fermentation of must. All fermentation occurred at 27°C and samples were collected daily for alcohol, total and reducer sugar determinations (Cadogan and Hanks, 1995; Ferreira et al., 2005). Equation 2 shows to obtain the fermentation yield ($Y_{Fermentation}$, %), as follows:

$$Y_{Fermentation} = \left(\frac{C_{Ethanol\ \ measured}(mL/100mL)}{C_{Theoretical\ \ Ethanol}(mL/100mL)} \right) 100$$

(2)

2.4 Modeling of the hydrolysis

New models for starch hydrolyses have been achieved for Curvelo-Santana et al. (2009). It can be proposed with base in Michaelis-Menten model for first order kinetics. It uses some considerations to M-M model, showing into Equations 3 and 4, and they were fitted by GA technique. Following model had been adjusted to simulate the dependence of substratum concentration ([S], or starch concentration) to hydrolysis time (t):

$$[S] = [S]_{eq} + ([S]_0 - [S]_{eq})e^{-kt}$$

(3)

Where: $[S]_{eq}$ and $[S]_0$ are the substratum concentrations at the equilibrium and initial of the reaction; and *k* is the kinetic constant of product formation.

To simulate the dependence of the conversion rate of starch to the reduced sugar (d[S]/dt), the following model was used (Curvelo-Santana et al., 2008; Reguly, 2000; Wiseman, 1987):

$$\frac{d[S]}{dt} = e^{\frac{V_{\max}}{K_M}[S]}$$

(4)

Where: $\boldsymbol{K_M}$ is Michaelis-Menten constant and $\boldsymbol{V_{max}}$ is the maximum speed of starch hydrolysis by enzyme.

The V_{max} was obtained by the following Equation (Curvelo Santana et al., 2008; Reguly, 2000; Wiseman, 1987):

$$V_{\max} = k * [E]_0$$

(5)

Where: $[E]_0$ is the initial concentration of enzyme, which is considered as the total protein concentration inoculated, obtained by the method of Bradford (1976).

2.5 Genetic Algorithm Procedure

The Genetic Algorithms (GA), consists of an optimization technique based on the natural biological selection process and has been used to solve optimization problems in some areas in recent decades (Librantz et al., 2011; Santana et al., 2010a). The GA is an optimization method inspired by non-deterministic process of natural evolution. This procedure typically uses binary strings called chromosomes or individuals to represent the solutions of the problem. In fact, the chromosome can be encoded as binary, integer or real valued string. Through the competition, the chromosomes of the population most able are selected and crossed together to generate new chromosomes better than those of the former population. Thus, in each generation, the probability of one or more individuals to be a solution to the problem is greater (Librantz et al, 2011).

2.5.1 Selection

The selection operation consists of the choice of the best individuals for reproduction. Hence, the individuals with better aptitudes are selected while the other ones are discarded, that is, each individual has a probability to be selected according to its aptitude. There are many selection operators mentioned in the literature. The most common are: proportional selection, elitist selection and tournament selection. In the first one, the probability of an individual to be selected is proportional to its aptitude. In the second, among the better individuals selected in each generation, a few group remains intact to the next generation. In the tournament selection, the group of individuals that is responsible to compose the next generation is randomly chosen.

2.5.2 Crossover

The crossover operator is the main GA operator. It mixes the genetic material of the best chromosomes generating the individuals of the next population. The underlying idea is to generate a new population better than the previous one. To this end, a number of crossover points needs to be determined. Usually is used one or two points, fix or random. In order, to illustrate the crossover operation, it is showed by Figure 1.

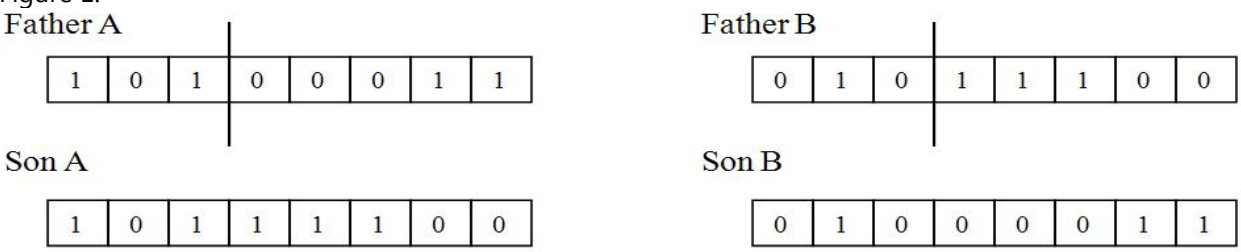


Figure 1. Crossover scheme with one cutting point.

2.5.3 Mutation

Finally, the mutation operation consists in changing a small amount of bits randomly, with some small probability (less than 10% according to the literature). It is performed in order to preventing the population of chromosomes becoming too similar to each other, maintaining the diversity in the population. This operation is essential to avoid the premature convergence (Librantz et al., 2011; Linhares et al., 2011; Santana et al., 2010a).

It is important to remember that the suitable convergence of the GA depends on its configuration, which is made by determining some control parameters such as: size of chromosome, size of population, crossover points and mutation rate. Unfortunately, the determination of these parameters is a notoriously difficult task and, in the majority cases, they are empirically defined (Myers, R., 2001; Santana et al., 2010a). Nevertheless, since the initial population is randomly generated, the performance and the results of GA vary at each execution. Therefore, GA is considered a stochastic-search adaptive method and a stochastic approach is, by nature, non-deterministic. So, the global solution can not be guarantied. Besides, to

demonstrate the performance of GA, the average over several executions of GA is more suitable than just one execution (Librantz et al., 2011; Linhares et al., 2011; Santana et al., 2010a). However, non-deterministic methods as GA have been widely applied to achieve almost optimal solutions mainly in problems where the total number of possible solutions grows exponentially, in which deterministic methods can be very time-consuming.
Figure 2 shows the main steps of the process of Genetic Algorithm. This process was performed in the optimization of parameters (concentration, temperature and time) hydrolysis of cassava starch.

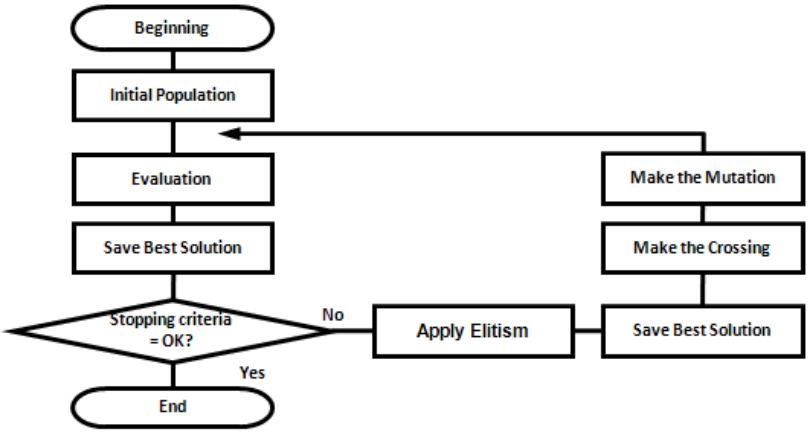


Figure 2. Process flow diagram of the GA.

Thus, in each generation the probabilities of one or more individuals become a solution to the problem is increased. Unlike other search methods, GA works on the population in parallel, yielding several solutions at a time.

2.5.4 Objective function

In the proposed problem, the technique of GA was applied to determine the values of the three variables involved in the hydrolysis of cassava starch in order to optimize the yield of hydrolysis (Equation 6), of according to Benvenga et al. (2011). They are the following variables: ***C*** ***($_{Starch}$ g/L)*** (starch concentration), ***T (°C)*** (Reaction temperature) and ***t (min)*** (Reaction time).

$$\%Y_{Hydrolysis} = 44.07 - 1.25C + 8.23T + 11.50t - 0.51C^2 + 3.67T^2 - 5.34t^2 - 0.36CT + 2.98Ct + 2.61Tt$$

2.5.5 Parameters of GA

The areas of restrictions on the values of variables C (concentration), T (temperature) e t (time) are respectively the following: $C \in$ [6.59 to 23.375], $T \in$ [28.18 to 61.82] and $t \in$ [9 to 111].

To process the simulations the algorithm was calibrated with the following parameters:

Initial population of 400 randomly generated individuals, 100 individuals per generation, elitism rate of 40% and mutation rate of 4%. The stopping criterion of the algorithm was processed for 10 generations.

3 Results and Discussion

3.1 Calibration of GA

Figures 3-7 present the results of experiments for performance analysis of the AG with the variation of its parameters in order to get the best performance of the algorithm in optimizing the hydrolysis of cassava.

The parameters were:

a. Number of generations,

b. Population size,

c. Mutation rate,

d. Elitism rate and,

e. Crossing scheme.

The number of generations or iterations of the GA is a parameter that determines the amount of repetitions or cycles that the algorithm will be processed. There is no pre-established rule that determines the best value for this parameter; this depends on a little bit of experience and intuition of the process operator.

Figure 3 shows the curves of the performance of GA for different numbers of generations keeping the other parameters fixed. Performances were tested with 5, 10, 15 and 20 generations, and the best performance occurred with 10 generations. Population parameter determines the number of individuals that will be evaluated according to the objective function in the process of finding the best solution to the problem proposed. In this work, the initial population was determined randomly, ie, 400 individuals were randomly selected. These 400 individuals passed the first evaluation and then formed the population follows. After the initial population, other populations are formed by means of evaluation and selection of the best individuals. The number of individuals who made these populations was also tested in the performance of GA.

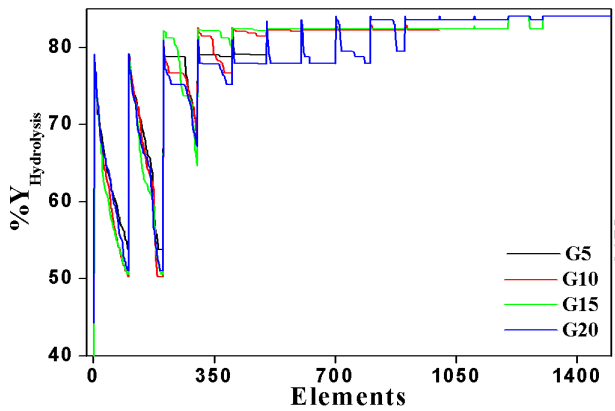


Figure 3. Number or Generations

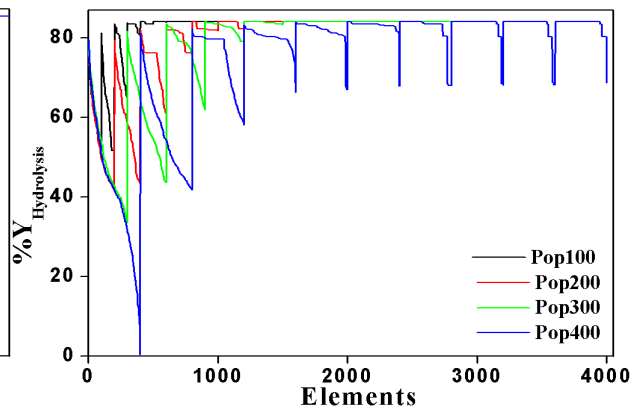


Figure 4. Population Size

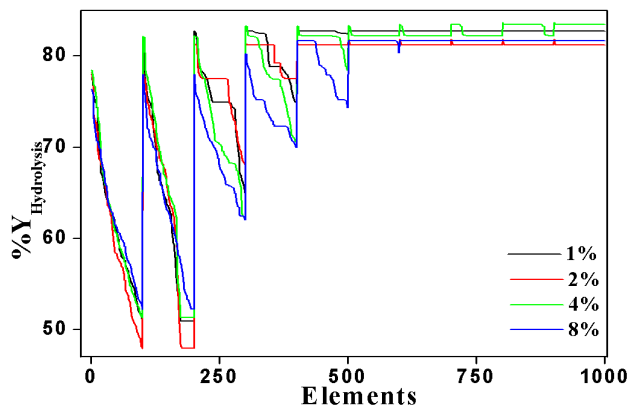


Figure 5. Mutation Rates

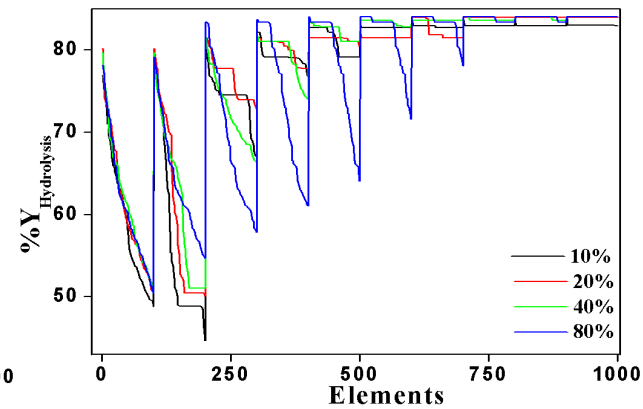


Figure 6. Elitism Rate

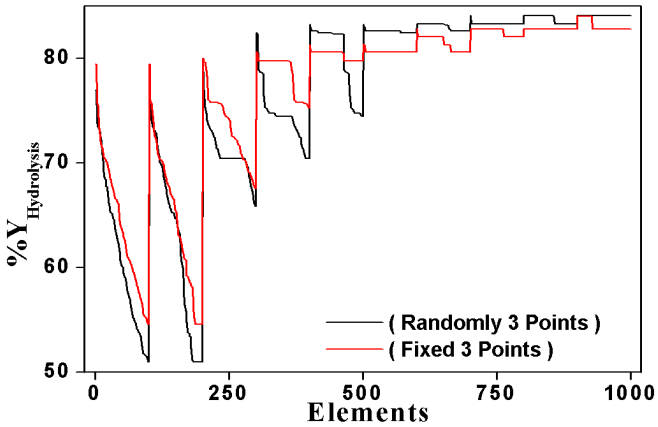


Figure 7. Crossing Schemes

Table 1 shows the best results of the experiments carried out with the query parameters of the GA, extracted from GA performance. The values presented in Table 1 for the parameters of GA were applied to optimize the hydrolysis process of cassava. Table 2 presents the results obtained after the execution of ten trials.

Table 1. Best results of experiments on the performance of GA

Parâmeter	Melhor Desempenho
Number of generations	10
Population size	100
Mutation rate	4%
Elitism rate	40%
Crossover scheme	Three (3) points chosen randomly

3.2 Hydrolysis optimization

The data optimized for the hydrolysis process were obtained from the tests generated by the GA and shown in Table 2. After ten trials, we obtained the best yield of hydrolysis at a rate of 84.08% (test 7), the variables concentration, temperature and time with the following values, respectively: 23.4 g/L of stach concentration, 61.9°C and 111.0 min of hydrolysis time.

Table 2. Optimal values of AG obtained after 10 trials.

Trials	C_{Starch}*(g/L)*	*T(°C)*	*t(min)*	$\%Y_{Hidrolysis}$	*Generation*
1	21.9	61.9	109.0	83.40	5
2	21.0	61.8	97.2	81.23	2
3	21.9	61.9	101.1	82.35	3
4	22.2	61.9	101.2	82.42	3
5	17.7	61.5	109.8	80.70	0
6	21.3	61.7	97.2	81.03	2
7	23.4	61.9	111.0	84.08	4
8	15.5	61.9	111.0	80.39	1
9	23.4	61.8	111.0	83.80	4
10	23.0	61.9	101.4	82.57	1

In the Roulette Addict, according to Librantz et al (2010), we add all the evaluations for each individual and allocate equal space to the evaluation of individual divided by the sum of the assessments of all individuals. In the process of Addicted to Roulette, individuals with better assessment have a greater

chance of being selected for the next generation. Once you obtain the best set of parameters, they were applied in the simulation. The results are shown in section 3.4., Simulation of the Hydrolysis.

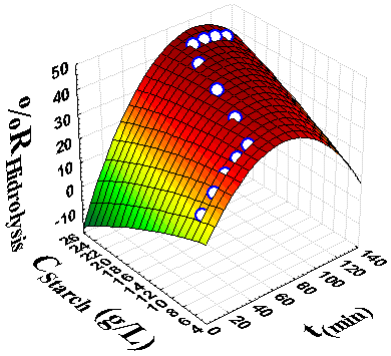
These results are similar to obtain for Matsumoto et al. (2011), who were the saccharification of starch using microwave irradiation with addition of activated carbon at 190-200°C and, more than saccarification of starch for similar method at 10-20°C.

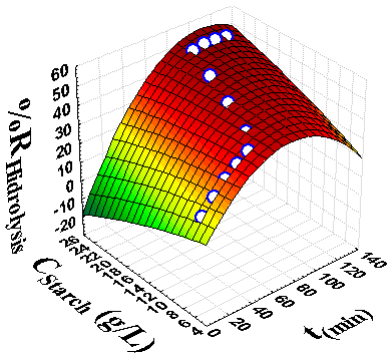
3.3 Hydrolysis Simulation

Hydrolysis of cassava was evaluated by means of computer simulation Figures 8, 9 and 10 showed the result of the simulations for temperatures of 35°, 45° and 55° respectively.

Figure 8 shows the optimal points obtained by the GA in the reaction of the enzyme to ten minutes. The best value for the hydrolysis yield of 35° C was 45.64% with 100 minutes of reaction and 23.4% concentration.

Figure 9 shows the optimal points obtained by the GA in the reaction of the enzyme to ten minutes. The best value for the yield of the hydrolysis temperature of 45° C was 53.23% with 110 minutes of reaction and 23.4% percent concentration.





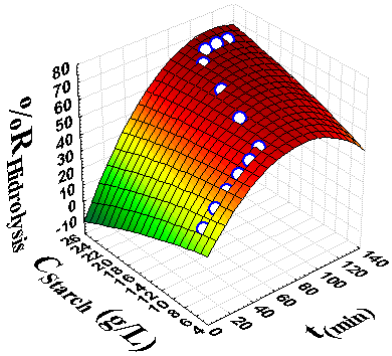


Figure 8. Temperature 35°C. Figure 9. Temperature 45°C. Figure 10. Temperature 45°C.

On the other hand, Figure 10 shows the optimal points obtained by the GA in the reaction of the enzyme to ten minutes. The best value for the yield of the hydrolysis temperature of 55° C was 68.87% with 110 minutes of reaction and 23.4% percent concentration.

3.4 Hydrolysis scale-up and fermentation

Figure 11 shows the alcohol and sugar reducing varying the fermentation time, for the syrup must fermentation by *S. cerevisae*. After the end of fermentation process, an alcohol content of 3.4°GL and alcohol yield of 45% is obtained.

According to Ferreira et al. (2005), Lima et al. (2001) and Reguly (2000). According to Lima et al. (2001) and Reguly (2000) and Wiseman (1987) the sugars released after starch hydrolysis are amylase inhibitors. Thus, when these sugars are consumed, the active sites from enzymes are reactivated. In this work there

was starch hydrolysis by amylase during the fermentation process, for the sugars produced of starch hydrolyses had been consumed during fermentation time. Therefore, hydrolysis yield of about 99% was found, for the simultaneous processes of hydrolysis and fermentation of cassava starch.

Ferreira et al (2005) and Curvelo-Santana (2008) produced ethanol by fermentation of the syrup obtained from the cassava starch. The *S. cerevisae* yeast was used for ethanol fermentation of the sugars from manioc syrup. An ethanol yield of 39% was found in the best fermentation condition. Neves et al (2006) had obtained alcohol by simultaneous saccharification and fermentation of low-grade wheat flour was done in an agitated bioreactor. For fermentation of the wheat syrup *S. cerevisae* yeast was used. After total fermentation, an alcohol yield of 27% was found.

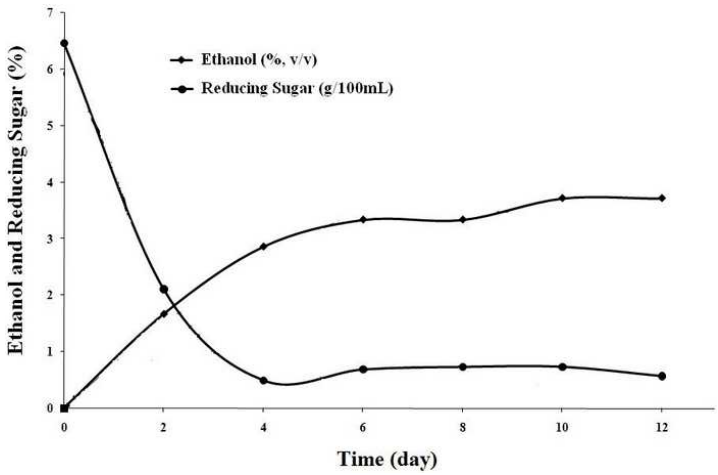


Figure 11. Curves of fermentation of cassava must

However, either common the use of *S. cerevisae* yeast in the fermentation processes, a culture of *Kluyveromyces marxianus* for ethanol fermentation was described in recent scientific research. The lactose from cheese was used as a substratum in a packed column bioreactor. The highest ethanol yield coefficient (35%) was obtained with a feed sugar content of 50 g/L (Ozmihci and Kargi, 2009 and 2008). Naidu et al. (2007) reported ethanol yields increasing from 10.4% to 12.6% v/v for corn starch. Thus, this work had obtained an ethanol yield more than Curvelo-Santana et al. (2008), Ferreira et al (2005 and 2006), Naidu et al. (2007), Neves et al. (2006) and Ozmihci and Kargi (2009 and 2008).

However, Yingling et al. (2011) studied the enzymatic hydrolysis of cassava starch and their conditions for bioethanol production by response surface methodology and on the best conditions, at 79°C and 117 min, a hydrolysis yield amongst 92.50–94.64% were obtained and 60°C and 85 min for ethanol concentration, while an ethanol concentration between 15.96 and 18.25 wt.% were obtained. Lamsal et al. (2011) have been evaluated the efficient processing of corn into ethanol by granular starch hydrolysis and simultaneous fermentation by *S. cerevisiae* yeast. The hydrolysis yields were amongst 91 and 96%, while ethanol concentrations were amongst 18-19%. These hydrolysis and ethanol yields are similar to archived in this work.

4 Conclusion

The amylase-type FORILASE NTL® showed a good activity for hydrolysis of soluble starch, achieving a total conversion between 20-100 minutes and the yielding that came to reach 80% conversion of starch in the reducing sugar (RA, in terms of glucose), Once highest value was found at 45° C for a starch concentration of 22 g/L.

Results showed that the models that best fit for both cases were formed by exponential equations. For the dependence of substrate [S], the starch, the hydrolysis time (t), a type models $[S] = e^{-kt}$, where *k* is the

kinetic constant of product formation and to simulate the dependence of reaction rate (*V*) it was found that a type model $V = e^{\frac{V_{\max}}{K_M}[S]}$ showed the Best fit, where V_{max} and K_M are the maximum and constant Michaelis-Menten. The equations obtained allow predicting the concentration of substratum and product within a bioreactor on the investigated conditions, and thus allows to simulate this process.

The optimized conditions were shown to be feasible and reliable through verification tests. This kind of meta-heuristic optimization is of potential importance in industrial bioethanol production.

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