“Production of bioethanol from carrot discards”

Authors: N.R. Aimaretti, C.V. Ybalo, M.L. Rojas, F.J. Plou, J.C. Yori

Published in:

Bioresource Technology, 123, 727-732 (**2012**)

doi:10.1016/j.biortech.2012.08.035

PRODUCTION OF BIOETHANOL FROM CARROT DISCARDS

Nora R Aimaretti^{a,b}, Carolina V Ybalo^b, María L Rojas^c, Francisco J Plou^d, Juan C Yori^a*

^a Instituto de Investigaciones en Catálisis y Petroquímica. Facultad de Ingeniería Química. Universidad Nacional del Litoral (Santa Fe, Argentina).

^b Laboratorio de Investigaciones Aplicadas. Facultad de Química. Universidad del Centro Educativo Latinoamericano (Rosario, Argentina).

^c Departamento de Química Inorgánica y Química Técnica. Facultad de Ciencias. Universidad Nacional de Educación a Distancia. (Madrid, Spain).

^d Instituto de Catálisis y Petroleoquímica. Consejo Superior de Investigaciones Científicas. (Madrid, Spain).

* Corresponding author: Dra. Nora Aimaretti. Av Pellegrini 1332. 2000 Rosario (Provincia de Santa Fe). Argentina. Tel/Fax: 00 54 341 4400540. E-mail: naimaretti@ucel.edu.ar, naimaretti@fbc.unl.edu.ar

1
2
3
4
5 ABSTRACT:
6
7

8 A revalorization of discarded carrots as substrate for the production of
9
10 second-generation ethanol is proposed. In order to increase the fermentable sugar
11 concentration of the musts two strategies were studied: Strategy 1 consisted in the
12 enzymatic hydrolysis of bagasse must and Strategy 2 by which carrots were milled,
13
14 dropped into distilled water and hydrolyzed with different enzymes prior to
15
16 compressing and filtering to obtain carrot must. By applying Strategy 2 using 0.05
17
18 % (v/v) of the enzyme Optimase CX255 at 70 °C and pH 5.5 during 2.5 h, the
19
20 fermentable sugars extracted increased 3.5 times. In this way, the production of
21
22 77.5 L of ethanol for each ton of discarded carrots was achieved. This process
23
24 yielded bagasse as byproduct, which could be used for animal feed.
25
26
27
28
29
30
31
32
33

34 KEYWORDS: carrot, fermentation, ethanol, enzymatic hydrolysis
35
36
37
38

39 ABBREVIATIONS:
40

41 RS: reducing sugars
42
43

44 TS: total sugars
45
46

47 CM: carrot must
48

49 BM: bagasse must
50

51 DC: discarded carrot
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1. INTRODUCTION

At present, the dependence on fossil fuels is still strong (85–90 %) in spite of their rapid depletion. In this context, the search for alternative energy sources has become a crucial issue that leads to the development of better technologies for second-generation biofuel production (Simsa et al., 2010).

The use of solar energy by means of the photosynthesis which produces biomass (Kreuger et al., 2011) is the most important source of renewable raw materials (Clark, 2005; Sánchez and Cardona, 2008). The acid or enzymatic hydrolysis of polysaccharides such as cellulose, hemicellulose, starch, inulin and chitosan in hexoses and pentoses allows their use in the alcoholic fermentative process. Of both types of hydrolysis, the enzymatic one allows obtaining a pure product with a low energy demand and a minor effluent production (Tomás, 2009; Ovando and Waliszewski, 2005) but the yield differs considerably depending on the applied technology (Sun and Cheng, 2002). In this way, bioethanol can be obtained from energy crops and lignocellulosic residues. The sustainability of the biotransformation processes must be analyzed from an economic and environmental point of view. Besides the high cost of current technologies and enzymes, another economic issue is to select cheap and abundant raw materials. That is why the use of regional agricultural and agro-industrial discards is very attractive to produce second-generation bioethanol (Laufenberg et al., 2003; Sánchez and Cardona, 2008; Aimaretti, 2011).

In Santa Fe (Argentina), a particular case is carrot (*Daucus carota*) cultivation whose average yield is nearly 40 tons (t) ha⁻¹ with a cultivated area of

1
2
3
4
5 approximately 1,500 ha. During the harvest time, 20-100 t of carrots with an
6
7 optimal degree of freshness and maturity are discarded daily due to a sizing
8
9 problem and then directed to animal feed (Aimaretti, 2006). Carrot is one of the
10
11 most efficient crops in biomass accumulation (Diamantopoulou et al., 2011) and it
12
13 is one of the few plants that accumulate free sugars into vacuoles (40-60% of total
14
15 carbohydrates) as reserve carbohydrates. 95% of free sugars are composed of
16
17 sucrose, fructose and glucose, and reducing sugars (RS) (fructose and glucose)
18
19 are present in an equimolecular amount. The ratio sucrose/RS increases while the
20
21 plant reaches maturity. After harvesting and during the cold storage, the ratio
22
23 begins to fall down (Simon, 2000; Suojala, 2000).
24
25
26
27
28
29

30 Taking into account the above considerations, the present study had two
31
32 main objectives: i) to evaluate the production of second-generation bioethanol
33
34 using discarded carrots as raw material, and ii) to enhance the ethanol yield of the
35
36 process by enzymatic hydrolysis in order to increase the fermentable sugar
37
38 concentration of the must.
39
40
41
42
43

44 **2. MATERIALS AND METHODS**

45 **2.1. Raw material, handling and storage**

46
47 Discarded roots of carrot (DC) (*Daucus carota*) were collected in November-
48
49 December 2010 from a packing shed in the Santa Fe area (31°25'S, 60°20'W),
50
51 Argentina. These discards were by-products from carrot packing processes, in
52
53 which plant leaves were cut and whole roots were washed, dried and selected
54
55 before packing. DC were stored in shed under ambient conditions (20-32 °C; 50-60
56
57
58
59
60
61
62
63
64
65

% humidity). During delivery of random representative samples obtained by sampling methods (ANMAT, 2010), carrots were packed in a polypropylene container and moved to the laboratory where they were used immediately.

2.2. Primary carrot processing

For must preparation, DC were processed after discarding those rotten sections, to extract their juice by a continuous milling, compressing and filtering treatment. As a result of the treatment, two fractions were obtained: i) carrot juice, which was called carrot must (CM) and ii) carrot bagasse which was utilized for the preparation of bagasse must (BM). The yield of the process was: 0.54 Kg kg⁻¹ of CM and 0.46 kg kg⁻¹ of bagasse. On the other hand, BM was prepared by dipping bagasse into a water volume in a proportion of 0.35 kg L⁻¹.

2.3. Enzymes

The enzymes used in the hydrolysis reactions and the operational conditions performed are described in Table 1. The pretreatments were performed in a stirred tank reactor manufactured at UNL, Argentina, equipped with a controlled heating system. Agitation speed was regulated at 20 rpm for all the experiments. Each enzyme has an indicated value of pH defined by its producers, which was adjusted with diluted sulfuric acid.

(Table 1, here)

2.4. Enzymatic hydrolysis of Bagasse Must

The seven commercial enzymes (Table 1) were evaluated under the conditions recommended by suppliers using BM as substrate. In each experiment, the enzyme dose was added after adjusting initial pH and temperature. The

1
2
3
4
5 hydrolysis time was 2.5 h and homogeneous samples were taken every 30 min.
6
7 The samples were centrifuged during 10 min at 5,000 x g and the supernatants
8
9 were used for determining the concentration of total sugars (TS) and reducing
10
11 sugars (RS) by using the DNS method (see 2.8.2) and to analyze the carbohydrate
12
13 composition (see 2.8.3) before the fermentation assay. All experiments were
14
15 performed in duplicate and technical repeats were performed during each single
16
17 experiment.
18
19
20
21

22 **2.5. Enzymatic hydrolysis of carrot prior to prepare carrot must**

23
24 Depending on the enzymatic activity and their availability, enzymes 1, 2, 4
25
26 and 5 (Table 1) were tested for enzymatic hydrolysis prior to preparing CM. In this
27
28 way, DC was milled to a particle size minor to 4 mm, dropped into distilled water
29
30 and then enzymatically hydrolyzed. For these assays, batches containing 0.5 Kg of
31
32 milled DC dipped into water in a total volume of 1 L were mixed with the enzymes,
33
34 after adjusting the initial pH and temperature. The hydrolysis was performed during
35
36 2.5 h and homogeneous samples were taken every 30 min of reaction. The
37
38 samples were compressed and filtered to obtain CM. The concentration of RS and
39
40 TS was determined using the DNS method (see 2.8.2). All experiments were
41
42 performed in duplicate and technical repeats were performed during each single
43
44 experiment.
45
46
47
48
49
50

51 **2.6. Microorganism**

52
53 *Saccharomyces cerevisiae* CCUB filtered and discarded by a local brewing
54
55 industry after five operative cycles, was utilized as biocatalyst in the fermentation
56
57 reactions. Whole yeast cells were kept in a sterile container, without nutrient
58
59
60

addition at 4 °C and saturation humidity during four days (Aimaretti and Ybalo, 2012). For the inoculum preparation, cells were suspended in an isotonic solution and counted by a differential direct count method in order to calculate the volume required (Alfenore et al., 2002).

2.7. Fermentation conditions

For alcoholic fermentations, fresh must was used in every case and its pH was adjusted to 4.5. The inoculum was adjusted to a value of 10^8 cell mL⁻¹ (Aimaretti and Ybalo, 2012). Batch fermentations were developed to 28 °C, in a 500 mL stirred tank bio-reactor (self-manufactured), equipped with a controlled heating system. The agitation speed was regulated at 100 rpm in all the experiments. A reactor hold-up of 0.25 was used, according to Laopaiboon et al. (2009). The alcoholic fermentation progress was monitored following CO₂ production, which was collected in a gasometric probe (ANMAT, 2010). Samples at different reaction times were taken and submitted to centrifugation. Supernatants were transferred to Eppendorf tubes (1.5 mL of capacity) for their storage and conservation at – 20°C. All experiments were performed in triplicate.

2.8. Analytical methods

2.8.1. Moisture

Moisture was determined using Approved Method 44-15A (AACC, 2005).

2.8.2. Sugar concentration

The concentration of reducing sugars was measured by the 3.5-dinitrosalicylic acid (DNS) method (Aimaretti and Ybalo, 2012). The total sugar was

assayed by the same method after acid hydrolysis (1.2 mol L⁻¹ HCl, at 65 °C for 15 min), neutralization with 1 mol L⁻¹ NaOH and filtration (Yu et al., 2009).

2.8.3. Carbohydrate composition

The analysis of carbohydrate composition in hydrolyzed musts was performed by high-performance anion exchange chromatography with pulsed amperometric detection HPAEC-PAD using an ICS-300 system (Dionex Corp, Sunnyvale, CA, USA) with a CarboPac™ PA100 column (4 x 250 mm, Dionex Corporation, CA, USA) with a guard column (4 x 50 mm). The eluent and flow rate were 16 mM NaOH and 1 mL min⁻¹ at 25 °C, respectively. Before the HPAEC-PAD analysis, the hydrolyzed samples were heated up to 85 °C for 5 min in order to inactivate the enzymes; then, they were filtered and treated in a separate Sephadex G-25 column (Pharmacia, PD10) to remove high molecular weight molecules that can affect the HPLC column. 5 µL of hydrolyzed sample together with 5 µL internal standard (Lactulose, Anedra > 99.5 %) were injected in the chromatographer (Aimaretti, 2011). Measurements were made in duplicate.

2.8.4. Ethanol concentration

Ethanol concentration was determined by GC (PERKIN-ELMER, Sigma 3B, Dual FID Chromatograph, United States). A FID detector and a packed column of Chromosorb 102 (2.0m length) were employed. The column oven was operated isothermally at 150 °C and the injection and detector ports were kept at 195 °C and 220 °C, respectively. Nitrogen was used as carrier gas with a flow rate of 30 mL min⁻¹ and the combustion gas was a mixture of hydrogen and air. Isopropanol

(Anedra, >99.9%) was used as internal standard (Ratnam et al., 2003). In the chromatograph of every fermented sample, only two peaks appeared, one corresponding to the internal standard, and the other to ethanol.

2.9. Fermentation parameters

The following fermentation parameters were calculated to compare the responses of different assays:

$Y_{p/s}$: ethanol yield per substrate was considered as the ratio of total ethanol produced and the consumed sugars, $[g\ g^{-1}]$ (Colin and Bjorn, 2002).

$Y_{p/c}$: ethanol yield per carrot was considered as the ratio of total ethanol produced and used carrot (dry base), $[g\ g^{-1}]$ (Colin and Bjorn, 2002).

Productivity: it was defined as total alcohol production over the total fermentation time, $[g\ L^{-1}\ h^{-1}]$ (Colin and Bjorn, 2002).

3. RESULTS AND DISCUSSION

3.1. Carrot fermentation

In the first experiment, two different musts were obtained from the primary processing of carrots (CM and BM) and the concentration of sugars was determined. They were fermented separately under conditions indicated in Section 2.7, comparing with un-inoculated CM and BM.

The concentration of reducing sugars of CM was in average $49.8 \pm 13.4\ g\ L^{-1}$, on a total of sugars $94.0 \pm 11.7\ g\ L^{-1}$. After the CM fermentation, the ethanol concentration obtained was $37.1\ g\ L^{-1}$. On the other hand, the average concentration of reducing sugars of BM was $5.8 \pm 0.4\ g\ L^{-1}$, the concentration of

total sugars: $29.6 \pm 4.7 \text{ g L}^{-1}$, and the concentration of ethanol obtained by fermentation: 7.9 g L^{-1} . Meanwhile, in the control experiments in which CM and BM were not inoculated, the ethanol concentration obtained was 0.0 g L^{-1} . These results are indicative of the fact that ethanol is the main product of the metabolic way and its final yield depends on the sugar concentration, and they are in agreement with the results reported by Aimaretti and Ybalo (2012).

Taking into account that the humidity of CM was 85 % and 60 % for BM, then it can be observed that the fermentation allowed obtaining $Y_{p/c}$ values of 0.134 and 0.068 g g^{-1} after the of CM and BM, respectively. So, the total $Y_{p/c}$ of the primary carrot processing was 0.201 g g^{-1} . In brief, 38.8 L of bioethanol were obtained from 1 t of DC.

With the objective of increasing total sugar concentration of musts and the ethanol yield, two different enzymatic hydrolysis strategies were proposed for carrot processing.

3.2. Strategy 1: Enzymatic hydrolysis of bagasse must (BM).

The use of enzymes to hydrolyze the bagasse which resulted from the primary carrot processing could allow its use as a substrate for a fermentation process, thus increasing the overall productivity of DC (see 2.4).

Enzymes must be adsorbed on the particles surface of insoluble cellulose before the hydrolysis reaction begins. Thus, the three-dimensional structures of enzymes, in combination with their size and form, determine if the β -glycosidic bonds may be accessible to enzymatic attack or not (Tomás, 2009). Figure 1 shows the evolution of the reducing (Figure 1-A) and total sugars (Figure 1-B) as a function of

1
2
3
4
5 hydrolysis time for the different enzymes. It can be observed that all the enzymes were
6
7 capable of hydrolyzing the bagasse must increasing the sugar content in different
8
9 proportions. In particular, the major improvements in total sugar content of must were 78.5
10
11 % for enzyme Optimase CX255L and 75.5% for Enzigrex. It is also worth noting that the
12
13 most active enzyme (OptimaseCX255L) is especially recommended by the supplier for
14
15 reducing the viscosity of the components of the plant cell wall. The enzymatic hydrolysis of
16
17 bagasse was also proposed as an interesting way for re-evaluating the large bagasse
18
19 surplus from the juice industry (Yoon et al., 2005).
20
21
22

23 On the other hand, a noticeable effect was observed: an important
24
25 increase of the reducing sugars catalyzed by Fungamyl (enzyme 7). This result is
26
27 contradictory because, according to the supplier Fugamyl only presents α -amylase
28
29 activity (Table 1) and only 1% of the bagasse fiber is composed of starch. This
30
31 could be explained by the fact that Fungamyl presents some invertase or alpha-
32
33 glucosidase residual activity to hydrolyze sucrose (Yoon et al., 2005).
34
35
36

37 (Figure 1, here)
38
39

40 **3.2.1. Carbohydrate composition of hydrolyzed bagasse musts**

41

42 The analysis of the hydrolyzed BM carbohydrate composition was carried
43
44 out in order to determine the extension of the hydrolysis reaction with each
45
46 enzyme. From the analysis many issues can be raised:
47
48

49 (i) during the filtering step of samples on Sephadex G-25 column, no high
50
51 molecular weight polymers were found, which would indicate the absence of
52
53 intermediate hydrolysis compounds.
54
55
56
57
58
59
60
61
62
63
64
65

(ii) during the chromatography analysis both the not-hydrolyzed BM sample and all the hydrolyzed samples presented only three peaks, corresponding to glucose, fructose and sucrose. It represents one of the advantages of the method, since all these sugars are capable of being fermented (Sun and Cheng, 2002).

The absence of degradation compounds such as xylose and/or arabinose indicated that hydrolysis process was not effective to degrade cellulose and hemicellulose. Probably the enzymatic degradation of cellulose and hemicellulose into simple sugars requires longer reaction times (Sánchez and Cardona, 2008). Therefore, the increase of TS in the hydrolyzed musts might be related with the release of the free sugars accumulated in the carrot vacuoles. Enzymatic hydrolysis was an effective way to cause destabilization of the cell wall structure of the storage vacuoles allowing the release of free sugars.

The sugar profiles of hydrolyzed and control BM samples showed that for all hydrolyzed samples, the glucose content was higher than that of fructose whereas in the control BM samples their concentrations were equimolecular. This is indicative that the process produced a partial hydrolysis of some polysaccharides mainly composed of glucose. The result obtained with enzyme 7 in which sucrose and starch were totally degraded into glucose units must be highlighted.

3.2.2. Fermentation of the hydrolyzed bagasse musts

The above results are not enough to conclude about the benefit that the increase of sugars can exercise on ethanol yields. For this reason, the hydrolyzed musts were fermented. The ethanol obtained and its $Y_{p/s}$ values are shown in Table 2 for the seven enzymes at maximum hydrolysis times. In cases in which the

1
2
3
4
5 increase was slow (enzyme 4 and 7) or too fast (enzyme 6) musts with different
6
7 hydrolysis time were fermented. The analysis of the results shows that the higher
8
9 the concentration of sugars in the hydrolyzed must the higher the ethanol yield
10
11 obtained, as expected. This fact rules out the possibility that among the hydrolysis
12
13 products there are substances that could inhibit the biocatalyst or that are non-
14
15 fermentable sugars, like it was mentioned by Sun and Cheng (2002).
16
17
18
19

20 (Table 2 here)
21

22 Summarizing, Figure 2 showed that it is interesting to note that the
23
24 enzymatic hydrolysis of BM with enzymes Enzigrex or OptimaseCX255, during 2 h
25
26 and 2.5 h, respectively, at their optimal conditions (Table1), allowed triplicating the
27
28 ethanol obtained ($Y_{p/c} = 0.133 \text{ g.g}^{-1}$). Therefore, taking into account the ethanol
29
30 yield with CM (see 3.1.), the total $Y_{p/c}$ reached by Strategy 1 in these conditions
31
32 was 0.267 g g^{-1} . Comparing this value with the one corresponding to primary carrot
33
34 processing, the increase of ethanol yield is 100 % and 51.3 L of bioethanol might
35
36 be obtained from each ton of DC.
37
38
39
40

41 (Figure 2, here)
42
43

44 **3.3 Strategy 2: Enzymatic hydrolysis previous to CM preparation**

45

46 The scientific community has spent much effort in studying the application of
47
48 enzymes in the vegetal juice industry in the past two decades, because it has
49
50 proven to be a versatile tool for releasing components of tissue cells and
51
52 intracellular compounds, thus improving juice productivity (Ovando and
53
54 Waliszewski, 2005). In this way, enzymatic hydrolysis of DC previous to CM
55
56 preparation was studied as a strategy to increase the extracted sugar and
57
58
59
60

consequently the ethanol yield. Enzymes 1, 2, 4 and 5 were used according to the supplier's specifications and their availability.

Results showed that as time elapsed all enzymes increased the extracted volume of juice compared with the original CM. In spite of this, the juice volume extracted at different time of hydrolysis, its sugar concentration and the kinetics were different for each enzyme reaching an increase of about 50-60 % in some cases. Though the majority of the enzymes reached the maximum juice extraction at 2.5 h, enzyme 5 allowed obtaining such volume increase in only 1 h. In order to evaluate the efficiency of the different enzymes, the total sugar extracted after 2.5 h of hydrolysis expressed as ($\text{g kg}^{-1}_{\text{carrot}}$) is indicated in Table 3 together with the sugar concentrations of the must ($\text{g L}^{-1}_{\text{must}}$) used for fermentation. These results show that the higher yield in sugars extraction is obtained with the OptimaseCX255L biocatalyst during 1 h at enzyme optimal conditions. In this case, comparing with CM without enzyme treatment (see 3.1.), the extracted sugar was increased 3.5 times.

As in previous experiments, the analysis of carbohydrate composition of each hydrolyzed must showed that the sugars present were sucrose, fructose and glucose, all of them capable of being used by the yeast through alcoholic fermentation.

3.3.1. Fermentation of CM

The results of the fermentation of the CM obtained by different enzymatic hydrolysis are shown in Table 3 in comparison with those corresponding to original CM. In agreement with previous results (see 3.2.2), it can be observed that by

1
2
3
4
5 increasing the concentration of total sugars in the musts, the $Y_{p/s}$ values remain
6
7 almost constant meaning that all substrates would be fermentable, but instead, $Y_{p/c}$
8
9 values vary substantially due to the different sugar extraction during must
10
11 preparation. In fact, the $Y_{p/c}$ could be increased 3 times if DC was hydrolyzed with
12
13 enzyme 5 before extracting the juice.
14
15

16
17 (Table 3 here)
18
19

20 In this way, Strategy 2 using enzyme OptimaseCX255 at its optimal
21
22 conditions (see Table 1) during 1 h reached an $Y_{p/c} = 0.403 \text{ g g}^{-1}$, as shown in
23
24 Figure 2. So it allowed us to obtain 77.5 L of second-generation bioethanol from 1 t
25
26 of DC turning itself into an interesting alternative which allows increasing the
27
28 ethanol yield of discarded carrots. Thus, the remaining bagasse may be used for
29
30 animal feed, as suggested by Castillo and Gallardo (1989).
31
32

33 It can be observed that though the ethanol yield of each ton of carrot may
34
35 be minor to the one obtained with traditional crops, the high yield of biomass per
36
37 each ha of carrot cultivation in this area gives an ethanol yield of $3,100 \text{ L ha}^{-1}$,
38
39 similar to the yields of corn and sorghum, $2,960 \text{ L ha}^{-1}$ and $3,010 \text{ L ha}^{-1}$,
40
41 respectively (Sánchez and Cardona, 2008).
42
43
44
45
46
47

48 49 **4. CONCLUSIONS**

50 Enzymatic hydrolysis was adjusted to increase the ethanol yield and
51
52 improve discarded carrot valorization. The two Strategies were efficient but
53
54 enzymatic hydrolysis prior to CM preparation (Strategy 2) allowed duplicating the
55
56 ethanol yield with respect to the must prepared without hydrolysis. Considering the
57
58
59
60

1
2
3
4
5 daily average of discarded amount, 4,650 L day⁻¹ of second-generation bioethanol
6
7 could be produced in this area. It is important to continue the global analysis of the
8
9 process since after the distillation of the ethanol, a vinasse rich in water arises that
10
11 might be used for animal feed as the fiber-rich bagasse remaining from the
12
13 preparation of CM.
14
15
16
17
18
19

20 **ACKNOWLEDGEMENTS**

21
22 The authors wish to thank Agustín Codevilla and Mercedes Escorcia for
23
24 their valuable cooperation. Thanks are also given to Agencia Santafesina de
25
26 Seguridad Alimentaria for the use of their equipment. This work was performed
27
28 with the financial support of the Latin American Educational Centre University
29
30 (UCEL), Project ALI 121 grant and Project BIO2010-20508-C04-01 from the
31
32 Spanish Ministry of Science and Innovation supported this research.
33
34
35
36
37
38

39 **REFERENCES**

40
41 AACC International Approved methods of analysis, 2005. Method 44-15.02.
42
43 Moisture e Air-Oven methods. 12th ed. St. Paul, MN, U.S.A: American
44
45 Association of Cereal Chemists (AACC) International.
46
47
48 Aimaretti, N., Ybalo, C., 2012. Valorization of carrot and yeast discards for the
49
50 obtention of ethanol. Biomass and Bioenerg. 42, 18-23.
51
52
53 Aimaretti, N., 2011. Desarrollo de un Proceso Sostenible para la Producción de
54
55 Bioetanol a partir de Desechos Agroindustriales. Dr. Thesis. Facultad de
56
57
58
59
60
61
62
63
64
65

Ciencias. Universidad Nacional de Educación a Distancia. Madrid. España.
2011.

Aimaretti, N., 2006. Aprovechamiento de Residuos Agroindustriales para la
Obtención de Nuevos Productos Alimenticios. M.Sc. Thesis. Facultad de
Ingeniería Química. Universidad Nacional del Litoral. Santa Fe. Argentina.

ANMAT: Administración Nacional de Medicamentos, Alimentos y Tecnología
Médica, 2010. Código Alimentario Argentino.. (www.anmat.gov.ar/codigoa).

Alfenore, S., Molina-Jouve, C., Guillouet, S.E., Uribelarrea, J.L., Goma, G.,
Benbadis, L., 2002. Improving ethanol production and viability of
Saccharomyces cerevisiae by a vitamin feeding strategy during fed-batch
process. *Appl Microbiol Biotechnol.* 60, 67–72.

Castillo, A., Gallardo, M., 1989. Alimentos no tradicionales en ganado lechero.
Consideraciones prácticas para su utilización. E.E.A INTA Rafaela.
Información para extensión. 88, 25-32.

Clark, J., 2005. Green chemistry for the second generation biorefinery- sustainable
chemical manufacturing based on biomass. *J Chem Technol Biotechnol.*
82(7), 603-609.

Colin, R., Bjorn, K., 2002. *Basic Biotechnology* 2nd ed. Cambridge: Cambridge
University Press.

Diamantopoulou, L.K., Karaoglanoglou, L.S., Koukios, E.G., 2011. Biomass Cost
Index: Mapping biomass-to-biohydrogen feedstock costs by a new
approach. *Bioresour Technol.* 102 (3), 2641–2650.

- Kreuger, E., Sipos, B., Zacchi, G., Svensson, S., Björnsson, L., 2011. Bioconversion of industrial hemp to ethanol and methane: The benefits of steam pretreatment and co-production. *Bioresour Technol.* 102 (20), 9524-9531.
- Laopaiboon, L., Nuanpeng, S., Srinophakun, P., Klanrit, P. Laopaiboon, P., 2009. Ethanol production from sweet sorghum juice using very high gravity technology: Effects of carbon and nitrogen supplementations. *Bioresour Technol.* 100 (18), 4176-4182.
- Laufenberg, G., Kunz, B., Nystroem, M., 2003. Transformation of vegetable waste into value added products: (A) the upgrading concept; (B) practical implementations. Review. *Bioresour Technol.* 87, 167–198.
- Ovando, S., Waliszewski, K., 2005. Preparativos de celulasas comerciales y aplicaciones en procesos extractivos. *Universidad y Ciencia.* 21(42), 113-122.
- Ratnam, B., Narasimha Rao, M., Damodara Rao, M., Subba Rao, S., Ayyanna, C., 2003. Optimization of fermentation conditions for the production of ethanol from sago starch using response methodology. *World J Microb Biot.* 19, 523-6.
- Sánchez, O., Cardona, C., 2008. Trends in biotechnological production of fuel ethanol from different feedstocks. *Bioresour Technol.* 99(13), 5270–5295.
- Simon, P., 2000. Domestication, historical development, and modern breeding of carrot. *Plant Breeding*, 19, 157–190.

- 1
2
3
4
5 Simsa, R., Mabeeb, W., Saddlerc, J., Taylord, M., 2010. An overview of second
6 generation biofuel technologies. *Bioresour Technol.* 101(6),1570–1580.
7
8
9
- 10 Suojala, T., 2000. Variation in sugar content and composition of carrot storage
11 roots at harvest and during storage. *Sci Hortic Amsterdam.* 85, 1-19.
12
13
14
- 15 Sun, Y., Cheng, J., 2002. Hydrolysis of lignocellulosic materials for ethanol
16 production: a review. *Bioresour Technol.* 83(1), 1–11.
17
18
19
- 20 Tomás, M., 2009. Bioetanol de paja de trigo: estrategias de integración de las
21 etapas del proceso. Dr Thesis. Facultad de Ciencias Biológicas. Universidad
22 Complutense de Madrid. España
23
24
25
- 26 Yoon, K., Cha, M., Shin, S., Kim, K., 2005. Enzymatic production of a soluble-fibre
27 hydrolyzate from carrot pomace and its sugar composition. *Food Chem.* 92,
28 151-157.
29
30
31
32
33
- 34 Yu, J., Zhang, X., Tan, T., 2009. Optimization of media conditions for the
35 production of ethanol from sweet sorghum juice by immobilized
36 *Saccharomyces cerevisiae*. *Biomass and Bioenerg.* 33, 521-526.
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

FIGURE CAPTIONS

Figure 1: Reducing sugar (top graph) and total sugars (low graph) in BM during different enzymatic hydrolysis. (Ref.: 1: Rohament CL; 2: Rohalase OS; 3: Enzigrex; 4: IndiAge MAX L; 5: Optimase CX255L; 6: Spirizyme Fuel; 7: Fungamyl).

Figure 2: Schemes of the two different strategies proposed.

TABLE CAPTIONS

Table 1: Enzymes' specifications.

Table 2: Total sugars and ethanol obtained in hydrolyzed BM.

Table 3: Total sugars extracted from carrot and fermentation parameters of fermentation of CM previously hydrolyzed.

FIGURE 1

FIGURE 2

TWO STRAGIES TO INCREASE ETHANOL YIELD FORM DISCARDED CARROTS

TABLE 1

Ref.	commercial name	enzymatic activity	Origin	Temp.	pH	Doses
1	Rohament CL	endo-1,4- β -glucanase	<i>Trichoderma reesei</i>	50 °C	5.0	0.05% v/v
2	Rohalase OS	cellulase, betaglucanase, xylanase	<i>Trichoderma reesei</i>	55 °C	5.5	0.05% v/v
3	Enzigrex	experimental cellulase	<i>Trichoderma reesei</i>	50 °C	5.5	0.02% w/v
4	IndiAge MAX L	Cellulase	<i>Trichoderma reesei</i>	50 °C	5.0	0.05% v/v
5	OptimaseCX255L	Thermostable Xylanase	<i>Trichoderma reesei</i>	70 °C	5.5	0.05% v/v
6	Spirizyme Fuel	Amyloglucosidase	<i>Aspergillus niger</i>	65 °C	4.5	0.25% v/v
7	Fungamyl	α -amylase	<i>Aspergillus oryzae</i>	55 °C	4.7	0.32% w/v

TABLE 2

ENZYME ^a	Incubation Time (h)	Total sugars (g L ⁻¹)	Ethanol (g L ⁻¹)	Y _{p/s} (g g ⁻¹)
-	-	28.7 ± 0.1	5.9 ± 0.2	0.205
1	1.5	48.9 ± 0.4	11.5 ± 0.5	0.235
2	1.5	38.6 ± 0.3	8.9 ± 0.4	0.230
3	2.0	51.1 ± 0.4	15.4 ± 0.5	0.301
4	2.0	43.7 ± 0.2	8.9 ± 0.4	0.204
4	2.5	46.2 ± 0.4	10.0 ± 0.3	0.216
5	2.5	53.1 ± 0.3	15.4 ± 0.2	0.290
6	2.0	34.1 ± 0.3	8.2 ± 0.2	0.241
6	2.5	44.9 ± 0.2	11.1 ± 0.3	0.247
7	2.0	34.7 ± 0.1	7.1 ± 0.2	0.205
7	2.5	41.1 ± 0.4	9.0 ± 0.2	0.219

^aTable 2

TABLE 3

ENZYME ^a	TS (g kg ⁻¹ _{carrot})	TS (g L ⁻¹ _{must})	Ethanol (g L ⁻¹)	Y _{p/s} (g g ⁻¹)	Y _{p/c} (g g ⁻¹)
-	42.3 ± 0.3	94.3 ± 0.7	37.1 ± 0.3	0.393	0.133
1	126.9 ± 0.7	84.6 ± 0.6	29.0 ± 0.5	0.343	0.290
2	106.5 ± 0.6	71.0 ± 0.3	27.3 ± 0.5	0.385	0.273
4	102.4 ± 0.6	68.2 ± 0.3	27.9 ± 0.5	0.409	0.279
5	150.2 ± 0.6	95.6 ± 0.6	40.3 ± 0.6	0.422	0.403

^a Table 3