Manuscript Number: CEJ-D-13-02942R1

Title: Optimization of furfural production by acid hydrolysis of Eucalyptus globulus in two stages

Article Type: Research Paper

Keywords: Furfural, acid hydrolysis, Eucalyptus globulus

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Abstract: The aim of this study was to optimize the production of furfural by hydrolysis with dilute acid of Eucalyptus globulus wood in two stages, using a factorial experimental design and multiple regression models to maximize furfural yields. The two-stage process could provide a solid phase with less degraded cellulose and older applications that the solid phase of a conventional acid hydrolysis. The selectivity for furfural of the process was increased by effect of the glucan and lignin fractions being efficiently removed after the first acid hydrolysis stage.

The operational ranges are especially suitable for the extraction of xylose, which was virtually quantitative at 170 ºC and virtually independent of the operation time. It is therefore advisable to use non-isothermal conditions or short times. This is quite important if one considers the low acid concentration used. The liquid phase obtained after autohydrolysis of the raw material under the optimum conditions contained 2.3% glucose, 9.8% xylose, 2.5% xylo-oligomers, 0.6% arabinose, 1.1% furfural and 0.1% 5-OH methyl furfural.

After the residual solid from the first hydrolysis stage is removed, the acid concentration is the most influential variable on the conversion of xylo-oligomers or xylose into furfural. Conversions to furfural were quite substantial (greater than 10 g/L). Hemicelluloses in the raw material were extracted by 32-57.7% and xylan by 40.5-84%. The highest conversions to furfural were obtained by using a medium operation time (15 min) and low temperature (170 ºC) and pH=2.

The process additionally provides hemicellulose derivatives that are scarcely contaminated with cellulose derivatives. The concentrations of gluco-oligomers were very low and those of glucose in the region of 3% of the raw material.

Response to Reviewers: Optimization of furfural production by acid hydrolysis of Eucalyptus globulus in two stages. Ms. Ref. No.: CEJ-D-13-02942

LIST OF CHANGES

Reviewers' comments:

REVIEWER 1: The manuscript dealed with the furfural production from woody biomass. If the manuscript just focused on the optimization of two stage of furfural production, the novelty seems not enough. The authors were suggested to provide some evidences for advantages of two stage process as compared with single stage. In many parts, the writing hard to be understood.

RESPONSE TO REVIEWER COMMENT:

(*)
The use of lignocellulosic biomass as a sustainable, renewable raw material for the production of chemicals and energy is steadily gaining interest. Despite the difficulties, especially as regards the need to fractionate its components in order to make the process cost-effective, there is increasing experimental evidence of its viability. For example, research has shown the possibility of obtaining ethanol as main product, but also that other structural components such as hemicelluloses and polyphenols (lignin) can be useful to obtain foods, pharmaceuticals, plastics, paper and basic chemicals, among others, by using a series of process known as "Biorefining".
An industrial process capable of using all structural components in lignocellulosic materials to obtain chemicals would also minimize the production of waste. In our case, the residual solid fraction is not waste proper, but rather an effective raw material for obtaining bioethanol, cellulose pulp and paper, among other products.
Closely related to the integral exploitation of raw materials is the widely accepted assumption that the biorefining industry must necessarily develop at the local level in order to facilitate the use of diverse raw materials available in different regions to obtain a wide range of chemicals and energy forms.
This was the standpoint for our conduct of the present work. Optimizing the production of furfural, a hemicellulose derivative, by using a two-step process improving on the traditional method involving acid hydrolysis in a single step is important. So, is, however, preserving the physico–chemical properties of the cellulose and lignin fractions in order to facilitate their subsequent use. Therefore, the process was optimized for a twofold purpose, namely: to maximize furfural yield while minimizing cellulose degradation. Also, a two-step process including separation of the solid phase after the first hydrolysis step would facilitate the obtainment of fractions containing less degradation products (e.g. polyphenols or furfural derivatives acting as microbial inhibitors and hindering potential uses in fermentation processes).
Developing a scheme for the integral exploitation of all fractions in a lignocellulosic material would involve very extensive work obviously beyond the scope of a research study such us this. Also, no accurate economic conclusions can be drawn from a study on a specific aspect of the process; rather, this would require a comprehensive study of all biorefining fractions at a purposely set industrial exploitation biorefinery when in fact the aim of this paper was to improve existing knowledge on the topic with a view to facilitating future industrial developments. Therefore, although our work does not address the general problem (viz. developing a complete industrial biorefining line), it is, to our minds, interesting and innovative enough for publication in this journal.
Judging by the Reviewers' comments, the original manuscript may have failed to clarify our aims and contribution, and this has led us to make the following changes:

The next paragraphs were added in introduction section:

-"Actually, in the field of exploitation of the forest resources (pulp and paper industry), it accepts the idea of prior extraction of hemicelluloses, makes that the mechanical properties of handsheets prepared with previous autohydrolysis/hot water extraction or exploded chips were slightly lower (Mendes et al., 2010; Martín-Sampedro et al., 2010). However, recently much interest has developed on the concept of the forest product biorefinery, where wood is hydrolyzed prior to conventional pulping and papermaking processes and the hydrolyzate consisting of hemicellulose sugars are to be used as a feedstock for biofuels, bioplastics or other uses (Duarte et al. 2010)".

The next sentence was added in abstract section and objective of article respectively:
-Abstract: "The two-stage process could provide a solid phase with less degraded cellulose and older applications that the solid phase of a conventional acid hydrolysis".
-Objective: "The two-stage process could provide a solid phase with less degraded cellulose and older applications that the solid phase of a conventional acid hydrolysis. Therefore, the process was optimized for a twofold purpose, namely: to maximize furfural yield while minimizing cellulose degradation. Also, a two-step process including separation of the solid phase after the first hydrolysis step would facilitate the obtainment of fractions containing less degradation products (e.g. polyphenols or furfural derivatives acting as microbial inhibitors and hindering potential uses in fermentation processes)".

REVIEWER 1: The following issues should be addressed before further considering:
1.Since the optimum conditions for both stages were almost same, why not carry out the single-stage process as control to clarify the advantages of two-stage process? Otherwise, the research might be meaningless.

RESPONSE TO REVIEWER COMMENT:

See response (*) in reviewer 1.
The conditions for both stages were not almost same. Little diferences are important in optimization process. The first stage was optimized with two objectives:
-Obtain the highest concentrations of furfural and xylose and…
-Minimize the extraction of gluco-oligomers to obtain a usable solid phase.
Also, our initial hypothesis was that the selectivity for furfural was increased by effect of the glucan and lignin fractions remotion after the first acid hydrolysis stage.

In the second stage, furfural production exceed those obtained by others authors from Eucalyptus in a single step.

REVIEWER 1. 2.The range of variables seems to be narrow. The optimum conditions might be out of the range.

RESPONSE TO REVIEWER COMMENT:

(**)
It´s difficult to select an appropriate range of variables for process optimization, but the authors think that the range of variables is suitable. In previous works, others temperatures and operation time were assayed to rule out inappropriate ranges (for example: García, M.T., García Domínguez, J.C., Fería, M.J., Gómez-Lozano, D.M., López, F., Díaz, M.J. Furfural production from Eucaliptus globulus: Optimizing by using neural fuzzy models. Chemical Engineering Journal, 221, 185-192. 2013). Basically and briefly:

-If temperature, operation time or sulphuric acid concentration are too low, the hemicelluloses extraction was not significative…
-If temperature, operation time or sulphuric acid concentration are too high, the cellulose degradation increase.

REVIEWER 1. 3.The first section of results and discussion "3.1. Raw material characteristics" just want to say they used right material not others. It is unnecessary! Just describe the data in material preparation section.

RESPONSE TO REVIEWER COMMENT:

-The first section of results and discussion was deleted and included in section 2.1
-The sections of "Results and Discussion" were renumbered.

REVIEWER 1. 4.The filtration through 0.45 mm membranes before HPLC analysis is correct? The pore size seems too big for sample pretreatment.

RESPONSE TO REVIEWER COMMENT:

-This is a standard procedure. We can see works of Garrote et al., for example, or others authors.

REVIEWER 1. 5.The authors often mentioned " data not shown"," results not shown", some data were important data. Some data were present in the final manuscript as supplementary materials.

RESPONSE TO REVIEWER COMMENT:

-(***) A mistake was detected: Section 3.2. "figure 2" was changed by "Fig. 3"
-Some figures were added as supplementary materials 1 and 2:

Supplementary materials 1: Variations of glucose, arabinose, xylooligomers and 5-OH methyl furfural contents as a function of independent variables of acid hydrolysis process.
Supplementary materials 2. Variations of arabinose, glucooligomers and 5-OH methyl furfural contents as a function of independent variables of acid hydrolysis process.

REVIEWER 1. 6.When citing the specific figure or table, the format of "Fig. 1 or Fig. 2 ..." or "Table 1, Table 2 .." should be followed.

RESPONSE TO REVIEWER COMMENT:

-"table" was changed by "Table", "fig." was changed by "Fig, "figure" was changed by "Fig." and "Figure" was changed by "Fig."along the text.

REVIEWER 1. 7."Xylose concentrations were relatively high (figure 2) but conversions to furfural were greater than they would have with a high temperature (180 or 190 ºC)." what's your mean? Why use the "but"?

RESPONSE TO REVIEWER COMMENT:

-A mistake was detected: "Fig. 2" was changed by "Fig. 3"

-(****) The sentence: "Xylose concentrations were relatively high (Fig. 3) but conversions to furfural were greater than they would have with a high temperature (180 or 190 ºC)" was changed by: "Xylose concentrations were relatively high (Fig. 3) and his conversion is not complete but conversions to furfural were greater than they would have with a high temperature (180 or 190 ºC)".

REVIEWER 1. 8.P 9, L49, "The time in not isothermal temperature....."?

RESPONSE TO REVIEWER COMMENT:

-Page 9, line 49. The sentence: "The time in not isothermal temperature…" was changeg by: "The time to reach the operating temperature in not isothermal process…"

REVIEWER 1. 9.P10, L31, full stop and % were lost. There are too many these kinds of errors!!!! The authors should check the manuscripts carefully.

RESPONSE TO REVIEWER COMMENT:

-Page 10, line 31, "%." was added.
-Page 10, line 1, "%" was added.
-Page 10, line 9, "%" was added.
-Page 13, line 14, "%" was added.
-Page 13, line 26, "wt%" was added.
-Page 14, line 29, "%" was added.

REVIEWER 1. 10.P17, L5, What's the meaning of "Conversions to furfural were quite."?

RESPONSE TO REVIEWER COMMENT:

-I'm sorry. It's a mistake. The sentence: "Conversions to furfural were quite" was changed by: "Conversions to furfural were quite substantial (greater than 10 g/L, which is equivalent to 40% conversion of all xylan initially present in the raw material to furfural)".

REVIEWER 1. 11.P11, L7, finish the sentence!

RESPONSE TO REVIEWER COMMENT:

-"…in all cases has been" was changed by: "…in all cases has been obtained".

REVIEWER #2: The manuscript number CEJ-D-13-02942 reports an investigation regarding the optimization of furfural production by hydrolysis with dilute acid of Eucalyptus globulus wood in two stages. In general, the manuscript is well written, the experimental approach is correct and the results are well discussed and analyzed using proper statistics.

RESPONSE TO REVIEWER COMMENT:

-O.K.

REVIEWER 2. However, the authors had a very unfortunate choice of how materials to carry on their studies. Furfural is largely produced in China, about 60% of world's production which was half a million tons per year in 2012. The raw materials used are cheap biomass rich in xylans like grasses, certain grains (oats), and agricultural and agro-industrial residues in general. In other words, cheap raw materials are used to make furfural. The authors have chosen Iberian E. globulus to make furfural. This wood costs a lot of money in the Iberian countries because it has many other important applications. The content on xylans in this wood is very low if compared with many grasses. On the other hand, the milling of the wood to make it into small particles is highly energy intensive. Therefore, E. globulus may not be cost-competitive with currently used raw materials. Furthermore, the authors do not give any hint on how they are going to handle the solid waste derived from the furfural production, which will be large and highly acidic material.

RESPONSE TO REVIEWER COMMENT:

-See response (*) in reviewer 1.

REVIEWER 2. Regarding innovation, the process chosen (acid hydrolysis) is the standard technology used worldwide. The only novel twist presented in the work is the two stage approach associated with fancy statistical (central point experimental) analyses.

RESPONSE TO REVIEWER COMMENT:

-See response (*) in reviewer 1.

REVIEWER 2. In order to be published, two things must be done: (1) a general economic evaluation of the process proposed and (2) some explanations on how the large amount of acidic solid residues will be handled, and how that affects the economics.

RESPONSE TO REVIEWER COMMENT:

-See response (*) in reviewer 1.

REVIEWER 2. The aim of this study was optimizing the production of furfural by hydrolysis with dilute acid of Eucalyptus globulus wood in two stages, using a factorial experimental design and multiple regression models to maximize furfural yields. A lot of the information presented is not novel. Therefore, it is paramount that authors add one or two paragraphs in the end of the introduction showing what the novelty of the work was. Include economical evaluation of the process and comment on the solid wastes generated. What to do with the acidic solid waste?

RESPONSE TO REVIEWER COMMENT:

-See response (*) in reviewer 1.

REVIEWER 2. Some minor comments are shown below:
- I would suggest author to confirm the punctuation in lines 30-33, page 6.

RESPONSE TO REVIEWER COMMENT:

-Page 6, line 33. A "." was added.

REVIEWER 2.
- Please, explain your choice of the specific parameters and their levels (time, temperature, concentration of acid, etc) in of the acid hydrolysis experiments in relation to other similar papers.

RESPONSE TO REVIEWER COMMENT:

-See response (**) in reviewer 1.

REVIEWER 2.
- I would suggest author to present more clearly the sentence "with low concentration or glucose or derivates of glucan." in line 25 page 7.

RESPONSE TO REVIEWER COMMENT:

-The sentence: “A second acid hydrolysis stage were performed at the first stage liquor with the aim of achieving a higher concentration of furfural with low concentration or glucose or derivates of glucan” was chagend by: “A second acid hydrolysis stage were performed at the first stage liquor with the aim of achieving a higher concentration of furfural and limit degradation of glucan (low concentration or glucose or derivates of glucan were obtained)”.

REVIEWER 2.

- What was done with solid residues generated in the first stage? And what composition of this solid?

RESPONSE TO REVIEWER COMMENT:

-See response (*) in reviewer 1.
-The composition of all solids was done. A resume of results was showed in page 10, lines 17 to 24.

REVIEWER 2.

- In line 39 page 15 explain the "highest conversions to furfural were obtained by using high pH".

RESPONSE TO REVIEWER COMMENT:

-Page 15, line 39. The sentence: “….obtained by using high pH (2)” was changed by “…obtained by using high pH (pH = 2 according the range of variation of pH).

REVIEWER 2.

- In page 16 add a reference to confirm the sentence "Xylose concentrations were relatively high (figure 2) but conversions to furfural were greater than they would have with a high temperature (180 or 190 ºC)" and explain why high temperature decrease conversions to furfural.

RESPONSE TO REVIEWER COMMENT:

-I’m sorry. There is a mistake in “figure 2”. It’s Fig.3.
-It’s the same question in reviewer 1, point 7. See response (****) in reviewer 1.

REVIEWER 2.

- Figure 1 should be prepared with higher quality.

RESPONSE TO REVIEWER COMMENT:

-Fig.1 has been changed.

REVIEWER 2.

- Please, add in the legend of Figure 2 the operating conditions correspondence of normalized values in experimental design (-1, 0, 1).

RESPONSE TO REVIEWER COMMENT:

-Figure 2, Supplementary materials 1, Supplementary materials 2 and figure 3. The operating conditions correspondence of normalized values in experimental design (-1, 0, 1) were added.

REVIEWER 2.

- Figure 3 is not in the manuscript, but it is in the captions of figures. Where is Figure 3?? Or do you refer to Figure 2?

RESPONSE TO REVIEWER COMMENT:

I'm sorry. This is a mistake. See response (***) in reviewer 1.

REVIEWER 2.

- Finally, many references cited along the reference list are not properly cited:

Line 27 in page 18: [5] Lange, J., ignocellulose conversion: an introduction to, [6] Kazi FK, Fortman JA, Anex RP, Hsu DD, Aden A, Dutta A, Kothandaraman And many others references: [8], [9], [34], [36], [37].

RESPONSE TO REVIEWER COMMENT:

-References 5, 6, 8, 9, 34, 36 and 37 (now 37, 39 and 40) were corrected.

REVIEWER #3: The paper works with an interesting topic. The literature is good and also the statistical method used and the experiments.

RESPONSE TO REVIEWER COMMENT:

-O.K.

REVIEWER 3. However the main problem of the paper is that it is written in a very bad way that makes very difficult for the reader to follow it.
There are paragraphs and sentences without meaning (for example in page 17 the sentence was "Conversion of furfural were quite."
I strongly recommend the paper to be written again from scratch. This is the most weak point of this paper.
I propose major modifications and I would like to see it again but completely different.

RESPONSE TO REVIEWER COMMENT:

-Some mistakes were corrected. See comments in previous reviewers.

REVIEWER 3. Other points for modifications:

1) 2nd paragraph of 2.1 is very confusing

RESPONSE TO REVIEWER COMMENT:

The second paragraph, in 2.1 section, explain some international standards (TAPPI) and characteristics determination. Also, according with reviewer 1, a third paragraph was added.

REVIEWER 3.

2) post hydrolysis vs second stage. What is the difference? Why thet are doing post hydrolysis

RESPONSE TO REVIEWER COMMENT:

After the first stage, the solid residue is removed. The two-stage process could provide a solid phase with less degraded cellulose and older applications that the solid phase of a conventional acid hydrolysis. In our opinion, this could be a very interesting advantage of two stage process as compared with single stage process. See response (*) in reviewer 1.

REVIEWER 3.
3) mass balances should be given for the tests

RESPONSE TO REVIEWER COMMENT:

The mass balances can be obtained by difference between the composition of the raw material and the results of table 2.

The next sentence was added at the end of second paragraph in section 3.1: "The error in mass balance was less than 5% in all components".

REVIEWER 3.
4) Table 2. It gives only normalized values of indepented variables. Rael number should be given.

RESPONSE TO REVIEWER COMMENT:

-Table 2. A foot note was added in the table.

Also:

-The bibliographic references were renumbered.
-The references were renumbered in the text.

-The next references were added:

[29] Mendes, C.V.T., Rocha, J.M.S., Sousa, G.D.A., Carvalho, G.V.S., Extraction of hemicelluloses prior to kraft cooking: a step for an integrated biorefinery in the pulp mill, XXI TECNICELPA Conference and Exhibition / VI CIADICYP 2010. Book of abstracts (2010) 178-179. Ed. TECNICELPA, Lisbon, Portugal. ISBN: 978-989-20-1926-0.
[30] Martín-Sampedro, R., Revilla, E., Martín, J.A, Eugenio, M.E., Villar, J.C., Evaluation of steam explosion as a pretreatment prior to kraft pulping of eucalyptus globulus wood. XXI TECNICELPA Conference and Exhibition / VI CIADICYP 2010. Book of abstracts (2010) 63-64. Ed. TECNICELPA, Lisbon, Portugal. ISBN: 978-989-20-1926-0.
[31] Duarte, G.V., Ramarao, B.V., Amidon, T.E., Ferreira, P.T., Properties of Eucaliptus globulus fiber after hot water extraction. XXI TECNICELPA Conference and Exhibition / VI CIADICYP 2010. Book of abstracts (2010) 61-61. Ed. TECNICELPA, Lisbon, Portugal. ISBN: 978-989-20-1926-0.

CHEMICAL ENGINEERING JOURNAL

Dr. Francisco López Baldovín
Chemical Engineering Dept.
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Avda. 3 de marzo s/n
21071 HUELVA (SPAIN)

Huelva, 5 november 2013

Dear Mr. Editor

I am enclosing the revised version of our article “Optimization of furfural production by acid hydrolysis of *Eucalyptus globulus* in two stages”**, which we would like to be considered for review and publication in CHEMICAL ENGINEERING JOURNAL.**

Also, I am enclosing an itemized letter (list of changes) answering all comments made and describing all changes made in response, or the reason why no change should be made.

We confirm that:

The author has consulted the Guide for Authors in preparing the submitted manuscript. The authors must also confirm that they have prepared the manuscript incompliance with the Ethics in Publishing Policy as described in the Guide for Authors.Manuscript is not a re-submission.

Sincerely
Dr. Francisco López Baldovín
Corresponding author

Optimization of furfural production by acid hydrolysis of *Eucalyptus globulus* in two stages. Ms. Ref. No.: CEJ-D-13-02942

<u>List of changes</u>

<u>Reviewers' comments:</u>

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An industrial process capable of using all structural components in lignocellulosic materials to obtain chemicals would also minimize the production of waste. In our case, the residual solid fraction is not waste proper, but rather an effective raw material for obtaining bioethanol, cellulose pulp and paper, among other products.

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This was the standpoint for our conduct of the present work. Optimizing the production of furfural, a hemicellulose derivative, by using a two-step process improving on the traditional method involving acid hydrolysis in a single step is important. So, is, however, preserving the physico–chemical properties of the cellulose and lignin fractions in order to facilitate their subsequent use. Therefore, the process was optimized for a twofold purpose, namely: to maximize furfural yield while minimizing cellulose degradation. Also, a two-step process including separation of the solid phase after the first hydrolysis step would facilitate the obtainment of fractions containing less degradation products (e.g. polyphenols or furfural derivatives acting as microbial inhibitors and hindering potential uses in fermentation processes).

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The next sentence was added in abstract section and objective of article respectively:

-Abstract: "The two-stage process could provide a solid phase with less degraded cellulose and older applications that the solid phase of a conventional acid hydrolysis".

-Objective: "The two-stage process could provide a solid phase with less degraded cellulose and older applications that the solid phase of a conventional acid hydrolysis. Therefore, the process was optimized for a twofold purpose, namely: to maximize furfural yield while minimizing cellulose degradation. Also, a two-step process including separation of the solid phase after the first hydrolysis step would facilitate the obtainment of fractions containing less degradation products (e.g. polyphenols or furfural derivatives acting as microbial inhibitors and hindering potential uses in fermentation processes)".

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6.When citing the specific figure or table, the format of "Fig. 1 or Fig. 2 ..." or "Table 1, Table 2 .." should be followed.

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7."Xylose concentrations were relatively high (figure 2) but conversions to furfural were greater than they would have with a high temperature (180 or 190 ºC)." what's your mean? Why use the "but"?

-A mistake was detected: "Fig. 2" was changed by "Fig. 3"

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11.P11, L7, finish the sentence!

-"…in all cases has been" was changed by: "…in all cases has been obtained".

<u>Reviewer #2:</u> *The manuscript number CEJ-D-13-02942 reports an investigation regarding the optimization of furfural production by hydrolysis with dilute acid of Eucalyptus globulus wood in two stages. In general, the manuscript is well written,*

the experimental approach is correct and the results are well discussed and analyzed using proper statistics.

-O.K.

However, the authors had a very unfortunate choice of how materials to carry on their studies. Furfural is largely produced in China, about 60% of world's production which was half a million tons per year in 2012. The raw materials used are cheap biomass rich in xylans like grasses, certain grains (oats), and agricultural and agro-industrial residues in general. In other words, cheap raw materials are used to make furfural. The authors have chosen Iberian E. globulus to make furfural. This wood costs a lot of money in the Iberian countries because it has many other important applications. The content on xylans in this wood is very low if compared with many grasses. On the other hand, the milling of the wood to make it into small particles is highly energy intensive. Therefore, E. globulus may not be cost-competitive with currently used raw materials. Furthermore, the authors do not give any hint on how they are going to handle the solid waste derived from the furfural production, which will be large and highly acidic material.

-See response (*) in reviewer 1.

Regarding innovation, the process chosen (acid hydrolysis) is the standard technology used worldwide. The only novel twist presented in the work is the two stage approach associated with fancy statistical (central point experimental) analyses.

-See response (*) in reviewer 1.

In order to be published, two things must be done: (1) a general economic evaluation of the process proposed and (2) some explanations on how the large amount of acidic solid residues will be handled, and how that affects the economics.

-See response (*) in reviewer 1.

The aim of this study was optimizing the production of furfural by hydrolysis with dilute acid of Eucalyptus globulus wood in two stages, using a factorial experimental design and multiple regression models to maximize furfural yields. A lot of the information presented is not novel. Therefore, it is paramount that authors add one or two paragraphs in the end of the introduction showing what the *novelty of the work was. Include economical evaluation of the process and comment on the solid wastes generated. What to do with the acidic solid waste?*

-See response (*) in reviewer 1.

Some minor comments are shown below:
- *I would suggest author to confirm the punctuation in lines 30-33, page 6.*

-Page 6, line 33. A "." was added.

- *Please, explain your choice of the specific parameters and their levels (time, temperature, concentration of acid, etc) in of the acid hydrolysis experiments in relation to other similar papers.*

-See response (**) in reviewer 1.

- *I would suggest author to present more clearly the sentence "with low concentration or glucose or derivates of glucan." in line 25 page 7.*

-The sentence: "A second acid hydrolysis stage were performed at the first stage liquor with the aim of achieving a higher concentration of furfural with low concentration or glucose or derivates of glucan" was chagend by: "A second acid hydrolysis stage were performed at the first stage liquor with the aim of achieving a higher concentration of furfural and limit degradation of glucan (low concentration or glucose or derivates of glucan were obtained)".

- *What was done with solid residues generated in the first stage? And what composition of this solid?*

-See response (*) in reviewer 1.
-The composition of all solids was done. A resume of results was showed in page 10, lines 17 to 24.

- *In line 39 page 15 explain the "highest conversions to furfural were obtained by using high pH".*

-Page 15, line 39. The sentence: "….obtained by using high pH (2)" was changed by "…obtained by using high pH (pH = 2 according the range of variation of pH).

- *In page 16 add a reference to confirm the sentence "Xylose concentrations were relatively high (figure 2) but conversions to furfural were greater than they would have with a high temperature (180 or 190 ºC)" and explain why high temperature decrease conversions to furfural.*

-I'm sorry. There is a mistake in "figure 2". It's Fig.3.
-It's the same question in reviewer 1, point 7. See response (****) in reviewer 1.

- *Figure 1 should be prepared with higher quality.*

-Fig.1 has been changed.

- *Please, add in the legend of Figure 2 the operating conditions correspondence of normalized values in experimental design (-1, 0, 1).*

-Figure 2, Supplementary materials 1, Supplementary materials 2 and figure 3. The operating conditions correspondence of normalized values in experimental design (-1, 0, 1) were added.

- *Figure 3 is not in the manuscript, but it is in the captions of figures. Where is Figure 3?? Or do you refer to Figure 2?*

I'm sorry. This is a mistake. See response (***) in reviewer 1.

- *Finally, many references cited along the reference list are not properly cited:*
Line 27 in page 18: [5] Lange, J., ignocellulose conversion: an introduction to, [6] Kazi FK, Fortman JA, Anex RP, Hsu DD, Aden A, Dutta A, Kothandaraman And many others references: [8], [9], [34], [36], [37].

-References 5, 6, 8, 9, 34, 36 and 37 (now 37, 39 and 40) were corrected.

Reviewer #3: The paper works with an interesting topic. The literature is good and also the statistical method used and the experiments.

-O.K.

However the main problem of the paper is that it is written in a very bad way that makes very difficult for the reader to follow it.
There are paragraphs and sentences without meaning (for example in page 17 the sentence was "Conversion of furfural were quite."
I strongly recommend the paper to be written again from scratch. This is the most weak point of this paper.
I propose major modifications and I would like to see it again but completely different.

-Some mistakes were corrected. See comments in previous reviewers.

Other points for modifications:

1) *2nd paragraph of 2.1 is very confusing*

The second paragraph, in 2.1 section, explain some international standards (TAPPI) and characteristics determination. Also, according with reviewer 1, a third paragraph was added.

2) *post hydrolysis vs second stage. What is the difference? Why thet are doing post hydrolysis*

After the first stage, the solid residue is removed. The two-stage process could provide a solid phase with less degraded cellulose and older applications that the solid phase of a conventional acid hydrolysis. In our opinion, this could be a very interesting advantage of two stage process as compared with single stage process. See response (*) in reviewer 1.

3) *mass balances should be given for the tests*

The mass balances can be obtained by difference between the composition of the raw material and the results of table 2.

The next sentence was added at the end of second paragraph in section 3.1: "The error in mass balance was less than 5% in all components".

4) *Table 2. It gives only normalized values of indepented variables. Rael number should be given.*

-Table 2. A foot note was added in the table.

Also:

-The bibliographic references were renumbered.
-The references were renumbered in the text.

-The next references were added:

[29] Mendes, C.V.T., Rocha, J.M.S., Sousa, G.D.A., Carvalho, G.V.S., Extraction of hemicelluloses prior to kraft cooking: a step for an integrated biorefinery in the pulp mill, XXI TECNICELPA Conference and Exhibition / VI CIADICYP 2010. Book of abstracts (2010) 178-179. Ed. TECNICELPA, Lisbon, Portugal. ISBN: 978-989-20-1926-0.
[30] Martín-Sampedro, R., Revilla, E., Martín, J.A, Eugenio, M.E., Villar, J.C., Evaluation of steam explosion as a pretreatment prior to kraft pulping of eucalyptus globulus wood. XXI

TECNICELPA Conference and Exhibition / VI CIADICYP 2010. Book of abstracts (2010) 63-64. Ed. TECNICELPA, Lisbon, Portugal. ISBN: 978-989-20-1926-0.

[31] Duarte, G.V., Ramarao, B.V., Amidon, T.E., Ferreira, P.T., Properties of Eucaliptus globulus fiber after hot water extraction. XXI TECNICELPA Conference and Exhibition / VI CIADICYP 2010. Book of abstracts (2010) 61-61. Ed. TECNICELPA, Lisbon, Portugal. ISBN: 978-989-20-1926-0.

Optimization of furfural production by acid hydrolysis of Eucalyptus globulus in two stages. Ms. Ref. No.: CEJ-D-13-02942

LIST OF CHANGES

Reviewers' comments:

REVIEWER 1: The manuscript dealed with the furfural production from woody biomass. If the manuscript just focused on the optimization of two stage of furfural production, the novelty seems not enough. The authors were suggested to provide some evidences for advantages of two stage process as compared with single stage. In many parts, the writing hard to be understood.

RESPONSE TO REVIEWER COMMENT:

(*)

The use of lignocellulosic biomass as a sustainable, renewable raw material for the production of chemicals and energy is steadily gaining interest. Despite the difficulties, especially as regards the need to fractionate its components in order to make the process cost-effective, there is increasing experimental evidence of its viability. For example, research has shown the possibility of obtaining ethanol as main product, but also that other structural components such as hemicelluloses and polyphenols (lignin) can be useful to obtain foods, pharmaceuticals, plastics, paper and basic chemicals, among others, by using a series of process known as “Biorefining”. An industrial process capable of using all structural components in lignocellulosic materials to obtain chemicals would also minimize the production of waste. In our case, the residual solid fraction is not waste proper, but rather an effective raw material for obtaining bioethanol, cellulose pulp and paper, among other products.

Closely related to the integral exploitation of raw materials is the widely accepted assumption that the biorefining industry must necessarily develop at the local level in order to facilitate the use of diverse raw materials available in different regions to obtain a wide range of chemicals and energy forms.

This was the standpoint for our conduct of the present work. Optimizing the production of furfural, a hemicellulose derivative, by using a two-step process improving on the traditional method involving acid hydrolysis in a single step is important. So, is, however, preserving the physico–chemical properties of the cellulose and lignin fractions in order to facilitate their subsequent use. Therefore, the process was optimized for a twofold purpose, namely: to maximize furfural yield while minimizing cellulose degradation. Also, a two-step process including separation of the solid phase after the first hydrolysis step would facilitate the obtainment of fractions containing less degradation products (e.g. polyphenols or furfural derivatives acting as microbial inhibitors and hindering potential uses in fermentation processes). Developing a scheme for the integral exploitation of all fractions in a lignocellulosic material would involve very extensive work obviously beyond the scope of a research study such us this. Also, no accurate economic conclusions can be drawn from a study on a specific aspect of the process; rather, this would require a comprehensive study of all biorefining fractions at a purposely set industrial exploitation biorefinery when in fact the aim of this paper was to improve existing knowledge on the topic with a view to facilitating future industrial

developments. Therefore, although our work does not address the general problem (viz. developing a complete industrial biorefining line), it is, to our minds, interesting and innovative enough for publication in this journal.
Judging by the Reviewers' comments, the original manuscript may have failed to clarify our aims and contribution, and this has led us to make the following changes:

The next paragraphs were added in introduction section:

-"Actually, in the field of exploitation of the forest resources (pulp and paper industry), it accepts the idea of prior extraction of hemicelluloses, makes that the mechanical properties of handsheets prepared with previous autohydrolysis/hot water extraction or exploded chips were slightly lower (Mendes et al., 2010; Martín-Sampedro et al., 2010). However, recently much interest has developed on the concept of the forest product biorefinery, where wood is hydrolyzed prior to conventional pulping and papermaking processes and the hydrolyzate consisting of hemicellulose sugars are to be used as a feedstock for biofuels, bioplastics or other uses (Duarte et al. 2010)".

The next sentence was added in abstract section and objective of article respectively:
-Abstract: "The two-stage process could provide a solid phase with less degraded cellulose and older applications that the solid phase of a conventional acid hydrolysis".
-Objective: "The two-stage process could provide a solid phase with less degraded cellulose and older applications that the solid phase of a conventional acid hydrolysis. Therefore, the process was optimized for a twofold purpose, namely: to maximize furfural yield while minimizing cellulose degradation. Also, a two-step process including separation of the solid phase after the first hydrolysis step would facilitate the obtainment of fractions containing less degradation products (e.g. polyphenols or furfural derivatives acting as microbial inhibitors and hindering potential uses in fermentation processes)".

REVIEWER 1: The following issues should be addressed before further considering:
1.Since the optimum conditions for both stages were almost same, why not carry out the single-stage process as control to clarify the advantages of two-stage process? Otherwise, the research might be meaningless.

RESPONSE TO REVIEWER COMMENT:

See response (*) in reviewer 1.
The conditions for both stages were not almost same. Little diferences are important in optimization process. The first stage was optimized with two objectives:
-Obtain the highest concentrations of furfural and xylose and…
-Minimize the extraction of gluco-oligomers to obtain a usable solid phase.
Also, our initial hypothesis was that the selectivity for furfural was increased by effect of the glucan and lignin fractions remotion after the first acid hydrolysis stage.

In the second stage, furfural production exceed those obtained by others authors from Eucalyptus in a single step.

REVIEWER 1. 2.The range of variables seems to be narrow. The optimum conditions might be out of the range.

RESPONSE TO REVIEWER COMMENT:

(**)
It´s difficult to select an appropriate range of variables for process optimization, but the authors think that the range of variables is suitable. In previous works, others temperatures and operation time were assayed to rule out inappropriate ranges (for example: García, M.T., García Domínguez, J.C., Fería, M.J., Gómez-Lozano, D.M., López, F., Díaz, M.J. Furfural production from Eucaliptus globulus: Optimizing by using neural fuzzy models. Chemical Engineering Journal, 221, 185-192. 2013). Basically and briefly:

-If temperature, operation time or sulphuric acid concentration are too low, the hemicelluloses extraction was not significative…
-If temperature, operation time or sulphuric acid concentration are too high, the cellulose degradation increase.

REVIEWER 1. 3.The first section of results and discussion "3.1. Raw material characteristics" just want to say they used right material not others. It is unnecessary! Just describe the data in material preparation section.

RESPONSE TO REVIEWER COMMENT:

-The first section of results and discussion was deleted and included in section 2.1
-The sections of "Results and Discussion" were renumbered.

REVIEWER 1. 4.The filtration through 0.45 mm membranes before HPLC analysis is correct? The pore size seems too big for sample pretreatment.

RESPONSE TO REVIEWER COMMENT:

-This is a standard procedure. We can see works of Garrote et al., for example, or others authors.

REVIEWER 1. 5.The authors often mentioned " data not shown"," results not shown", some data were important data. Some data were present in the final manuscript as supplementary materials.

RESPONSE TO REVIEWER COMMENT:

-(***) A mistake was detected: Section 3.2. "figure 2" was changed by "Fig. 3"
-Some figures were added as supplementary materials 1 and 2:

Supplementary materials 1: Variations of glucose, arabinose, xylooligomers and 5-OH methyl furfural contents as a function of independent variables of acid hydrolysis process.
Supplementary materials 2. Variations of arabinose, glucooligomers and 5-OH methyl furfural contents as a function of independent variables of acid hydrolysis process.

REVIEWER 1. 6.When citing the specific figure or table, the format of "Fig. 1 or Fig. 2 ..." or "Table 1, Table 2 .." should be followed.

RESPONSE TO REVIEWER COMMENT:

-"table" was changed by "Table", "fig." was changed by "Fig, "figure" was changed by "Fig." and "Figure" was changed by "Fig."along the text.

REVIEWER 1. 7."Xylose concentrations were relatively high (figure 2) but conversions to furfural were greater than they would have with a high temperature (180 or 190 ºC)." what's your mean? Why use the "but"?

RESPONSE TO REVIEWER COMMENT:

-A mistake was detected: "Fig. 2" was changed by "Fig. 3"

-(****) The sentence: "Xylose concentrations were relatively high (Fig. 3) but conversions to furfural were greater than they would have with a high temperature (180 or 190 ºC)" was changed by: "Xylose concentrations were relatively high (Fig. 3) and his conversion is not complete but conversions to furfural were greater than they would have with a high temperature (180 or 190 ºC)".

REVIEWER 1. 8.P 9, L49, "The time in not isothermal temperature....."?

RESPONSE TO REVIEWER COMMENT:

-Page 9, line 49. The sentence: "The time in not isothermal temperature…" was changeg by: "The time to reach the operating temperature in not isothermal process…"

REVIEWER 1. 9.P10, L31, full stop and % were lost. There are too many these kinds of errors!!!! The authors should check the manuscripts carefully.

RESPONSE TO REVIEWER COMMENT:

-Page 10, line 31, "%." was added.
-Page 10, line 1, "%" was added.
-Page 10, line 9, "%" was added.
-Page 13, line 14, "%" was added.
-Page 13, line 26, "wt%" was added.
-Page 14, line 29, "%" was added.

REVIEWER 1. 10.P17, L5, What's the meaning of "Conversions to furfural were quite."?

RESPONSE TO REVIEWER COMMENT:

-I’m sorry. It’s a mistake. The sentence: “Conversions to furfural were quite“ was changed by: “Conversions to furfural were quite substantial (greater than 10 g/L, which is equivalent to 40% conversion of all xylan initially present in the raw material to furfural)”.

REVIEWER 1. 11.P11, L7, finish the sentence!

RESPONSE TO REVIEWER COMMENT:

-“…in all cases has been” was changed by: “…in all cases has been obtained”.

REVIEWER #2: The manuscript number CEJ-D-13-02942 reports an investigation regarding the optimization of furfural production by hydrolysis with dilute acid of Eucalyptus globulus wood in two stages. In general, the manuscript is well written, the experimental approach is correct and the results are well discussed and analyzed using proper statistics.

RESPONSE TO REVIEWER COMMENT:

-O.K.

REVIEWER 2. However, the authors had a very unfortunate choice of how materials to carry on their studies. Furfural is largely produced in China, about 60% of world's production which was half a million tons per year in 2012. The raw materials used are cheap biomass rich in xylans like grasses, certain grains (oats), and agricultural and agro-industrial residues in general. In other words, cheap raw materials are used to make furfural. The authors have chosen Iberian E. globulus to make furfural. This wood costs a lot of money in the Iberian countries because it has many other important applications. The content on xylans in this wood is very low if compared with many grasses. On the other hand, the milling of the wood to make it into small particles is highly energy intensive. Therefore, E. globulus may not be cost-competitive with currently used raw materials. Furthermore, the authors do not give any hint on how they are going to handle the solid waste derived from the furfural production, which will be large and highly acidic material.

RESPONSE TO REVIEWER COMMENT:

-See response (*) in reviewer 1.

REVIEWER 2. Regarding innovation, the process chosen (acid hydrolysis) is the standard technology used worldwide. The only novel twist presented in the work is the two stage approach associated with fancy statistical (central point experimental) analyses.

RESPONSE TO REVIEWER COMMENT:

-See response (*) in reviewer 1.

REVIEWER 2. In order to be published, two things must be done: (1) a general economic evaluation of the process proposed and (2) some explanations on how the large amount of acidic solid residues will be handled, and how that affects the economics.

RESPONSE TO REVIEWER COMMENT:

-See response (*) in reviewer 1.

REVIEWER 2. The aim of this study was optimizing the production of furfural by hydrolysis with dilute acid of Eucalyptus globulus wood in two stages, using a factorial experimental design and multiple regression models to maximize furfural yields. A lot of the information presented is not novel. Therefore, it is paramount that authors add one or two paragraphs in the end of the introduction showing what the novelty of the work was. Include economical evaluation of the process and comment on the solid wastes generated. What to do with the acidic solid waste?

RESPONSE TO REVIEWER COMMENT:

-See response (*) in reviewer 1.

REVIEWER 2. Some minor comments are shown below:

- I would suggest author to confirm the punctuation in lines 30-33, page 6.

RESPONSE TO REVIEWER COMMENT:

-Page 6, line 33. A "." was added.

REVIEWER 2.

- Please, explain your choice of the specific parameters and their levels (time, temperature, concentration of acid, etc) in of the acid hydrolysis experiments in relation to other similar papers.

RESPONSE TO REVIEWER COMMENT:

-See response (**) in reviewer 1.

REVIEWER 2.

- I would suggest author to present more clearly the sentence "with low concentration or glucose or derivates of glucan." in line 25 page 7.

RESPONSE TO REVIEWER COMMENT:

-The sentence: "A second acid hydrolysis stage were performed at the first stage liquor with the aim of achieving a higher concentration of furfural with low concentration or glucose or derivates of glucan" was chagend by: "A second acid hydrolysis stage were performed at the first stage liquor with the aim of achieving a higher concentration of furfural and limit degradation of glucan (low concentration or glucose or derivates of glucan were obtained)".

REVIEWER 2.

- What was done with solid residues generated in the first stage? And what composition of this solid?

RESPONSE TO REVIEWER COMMENT:

-See response (*) in reviewer 1.
-The composition of all solids was done. A resume of results was showed in page 10, lines 17 to 24.

REVIEWER 2.
- In line 39 page 15 explain the "highest conversions to furfural were obtained by using high pH".

RESPONSE TO REVIEWER COMMENT:

-Page 15, line 39. The sentence: "….obtained by using high pH (2)" was changed by "…obtained by using high pH (pH = 2 according the range of variation of pH).

REVIEWER 2.
- In page 16 add a reference to confirm the sentence "Xylose concentrations were relatively high (figure 2) but conversions to furfural were greater than they would have with a high temperature (180 or 190 ºC)" and explain why high temperature decrease conversions to furfural.

RESPONSE TO REVIEWER COMMENT:

-I'm sorry. There is a mistake in "figure 2". It's Fig.3.
-It's the same question in reviewer 1, point 7. See response (****) in reviewer 1.

REVIEWER 2.

- Figure 1 should be prepared with higher quality.

RESPONSE TO REVIEWER COMMENT:

-Fig.1 has been changed.

REVIEWER 2.

- Please, add in the legend of Figure 2 the operating conditions correspondence of normalized values in experimental design (-1, 0, 1).

RESPONSE TO REVIEWER COMMENT:

-Figure 2, Supplementary materials 1, Supplementary materials 2 and figure 3. The operating conditions correspondence of normalized values in experimental design (-1, 0, 1) were added.

REVIEWER 2.

- Figure 3 is not in the manuscript, but it is in the captions of figures. Where is Figure 3?? Or do you refer to Figure 2?

RESPONSE TO REVIEWER COMMENT:

I'm sorry. This is a mistake. See response (***) in reviewer 1.

REVIEWER 2.

- Finally, many references cited along the reference list are not properly cited:

Line 27 in page 18: [5] Lange, J., ignocellulose conversion: an introduction to, [6] Kazi FK, Fortman JA, Anex RP, Hsu DD, Aden A, Dutta A, Kothandaraman And many others references: [8], [9], [34], [36], [37].

RESPONSE TO REVIEWER COMMENT:

-References 5, 6, 8, 9, 34, 36 and 37 (now 37, 39 and 40) were corrected.

REVIEWER #3: The paper works with an interesting topic. The literature is good and also the statistical method used and the experiments.

RESPONSE TO REVIEWER COMMENT:

-O.K.

REVIEWER 3. However the main problem of the paper is that it is written in a very bad way that makes very difficult for the reader to follow it.

There are paragraphs and sentences without meaning (for example in page 17 the sentence was "Conversion of furfural were quite."

I strongly recommend the paper to be written again from scratch. This is the most weak point of this paper.

I propose major modifications and I would like to see it again but completely different.

RESPONSE TO REVIEWER COMMENT:

-Some mistakes were corrected. See comments in previous reviewers.

REVIEWER 3. Other points for modifications:

1) 2nd paragraph of 2.1 is very confusing

RESPONSE TO REVIEWER COMMENT:

The second paragraph, in 2.1 section, explain some international standards (TAPPI) and characteristics determination. Also, according with reviewer 1, a third paragraph was added.

REVIEWER 3.

2) post hydrolysis vs second stage. What is the difference? Why thet are doing post hydrolysis

RESPONSE TO REVIEWER COMMENT:

After the first stage, the solid residue is removed. The two-stage process could provide a solid phase with less degraded cellulose and older applications that the solid phase of a conventional acid hydrolysis. In our opinion, this could be a very interesting advantage of two stage process as compared with single stage process. See response (*) in reviewer 1.

REVIEWER 3.
3) mass balances should be given for the tests

RESPONSE TO REVIEWER COMMENT:

The mass balances can be obtained by difference between the composition of the raw material and the results of table 2.

The next sentence was added at the end of second paragraph in section 3.1: “The error in mass balance was less than 5% in all components”.

REVIEWER 3.
4) Table 2. It gives only normalized values of indepented variables. Rael number should be given.

RESPONSE TO REVIEWER COMMENT:

-Table 2. A foot note was added in the table.

Also:

-The bibliographic references were renumbered.
-The references were renumbered in the text.

-The next references were added:

[29] Mendes, C.V.T., Rocha, J.M.S., Sousa, G.D.A., Carvalho, G.V.S., Extraction of hemicelluloses prior to kraft cooking: a step for an integrated biorefinery in the pulp mill, XXI TECNICELPA Conference and Exhibition / VI CIADICYP 2010. Book of abstracts (2010) 178-179. Ed. TECNICELPA, Lisbon, Portugal. ISBN: 978-989-20-1926-0.
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CIADICYP 2010. Book of abstracts (2010) 61-61. Ed. TECNICELPA, Lisbon, Portugal. ISBN: 978-989-20-1926-0.

FurfuralCEJ-Hihglights.docx

- Production of furfural from eucalyptus in two stages was optimized.
- Conversions to furfural were quite substantial, greater than 10 g/L.
- The process provides furfural that are scarcely contaminated with cellulose derivatives.

Optimization of furfural production by acid hydrolysis of *Eucalyptus globulus* in two stages

López, F. (1)*, García, M.T. (2), Feria, M.J. (1), García, J.C. (1), de Diego, C.M.(3), Zamudio, M.A.M.(4), Díaz, M.J. (1)

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Abstract

The aim of this study was to optimize the production of furfural by hydrolysis with dilute acid of Eucalyptus globulus wood in two stages, using a factorial experimental design and multiple regression models to maximize furfural yields. The two-stage process could provide a solid phase with less degraded cellulose and older applications that the solid phase of a conventional acid hydrolysis. The selectivity for furfural of the process was increased by effect of the glucan and lignin fractions being efficiently removed after the first acid hydrolysis stage.

The operational ranges are especially suitable for the extraction of xylose, which was virtually quantitative at 170 ºC and virtually independent of the operation time. It is therefore advisable to use non-isothermal conditions or short times. This is quite important if one considers the low acid concentration used. The liquid phase obtained after autohydrolysis of the raw material under the optimum conditions contained 2.3% glucose, 9.8% xylose, 2.5% xylo-oligomers, 0.6% arabinose, 1.1% furfural and 0.1% 5-OH methyl furfural.

After the residual solid from the first hydrolysis stage is removed, the acid concentration is the most influential variable on the conversion of xylo-oligomers or xylose into furfural. Conversions to furfural were quite substantial (greater than 10 g/L). Hemicelluloses in the raw material were extracted by 32–57.7% and xylan by 40.5–84%. The highest conversions to furfural were obtained by using a medium operation time (15 min) and low temperature (170 ºC) and pH=2.

The process additionally provides hemicellulose derivatives that are scarcely contaminated with cellulose derivatives. The concentrations of gluco-oligomers were very low and those of glucose in the region of 3% of the raw material.

Keywords

Furfural, acid hydrolysis*, Eucalyptus globulus,*

1. INTRODUCTION

To date fossil sources have been the main resources to obtain energy or chemicals but these resources produce negative effects due to environmental pollutions [1]. To remedy this situation, among the potential industrial biorefineries, the lignocellulose biorefinery will most probably be pushed through with the greatest success, because the raw material situation is optimal and conversion products have a good position in the traditional petrochemical as well as in the future bio-based product market [2-4]. In these biorefinery schemes, the hemicelluloses fraction, are not being isolated for industrial use yet, but they have a high potential and they could be used in many applications, not only for liquid biofuels production, notably bioethanol [5, 6], and butanol[7, 8]. An important derivate from hemicelluloses fraction is furfural [9]. Furfural, an important derived from hemicelluloses, is used for the production of a wide range of important non-petroleum derived chemicals. such as furan, tetrahydrofuran, and furfuryl alcohol[10]. It is also used as an extractive, fungicide, nematocide[11], in oil refineries, as well as in the plastics, food, pharmaceutical and agricultural industries [12, 13]. Resins with excellent thermosetting properties and extreme physical strength can also be produced by condensation of furfural with formaldehyde, phenol, acetone, or urea [11]. There is no synthetic route available for furfural production in chemical industry. Furfural is exclusively produced from hemicelluloses of lignocellulosic biomass [14]. About 250.000 tons of furfural, probably the only unsaturated, large volume, organic chemical, are prepared from carbohydrate sources annually [15].

A lot of literature is written about different pretreatment methods to enhance the extraction of carbohydrates in the process from lignocellulosic materials. Hot water or weak acid hydrolysis at an elevated temperature is the first step that is typically used to hydrolyze carbohydrates from lignocellulosic materials[16-18]. Steam explosion and dilute acid pretreatment are the two most commonly used methods because of their effectiveness and inexpensiveness [19]. In research, acid prehydrolysis has already found a broad area of applications, however, most of them are not commercially applied yet. For example, acid prehydrolysis is applied to remove hemicelluloses for (dissolving) pulp production [20]. Furfural production is carried out by hydrolysis of hemicelluloses pentosans to monomeric pentoses, and their subsequent acid-catalyzed dehydration into furfural [21]. This reaction would occur with or without mineral acids addition due to the liberation of acetic acid from the acetyl groups of the hemicelluloses. Industrial production of furfural requires a minimum content of 15–20% of pentosans and only a third of this amount can be converted into furfural [22]. Currently, furfural is obtained mainly from agricultural wastes as a source of hemicellulose or pentosan and by using acid catalyzed hydrolysis [22, 23] or steam pressure [24]. The application of various catalysts in the furfural conversion has been well documented in the literature, including mineral acids, organic acids and solid acids [25]. In one-stage technology, depolymerization of xylans in xylose and dehydration into furfural occur simultaneously. The solid residue can be used as a source of fuel [26]. In two-stage technology, a dissolution and partial depolymerization of hemicelluloses occur, followed by dehydration in furfural. In this manner, the residual lignocelluloses is almost unaltered and can be used for conversion to ethanol, glucose [14] or pulp and paper [3, 4, 27, 28] and the process has a higher furfural yield [26].

Actually, in the field of exploitation of the forest resources (pulp and paper industry), it accepts the idea of prior extraction of hemicelluloses, makes that the mechanical properties of handsheets prepared with previous autohydrolysis/hot water extraction or exploded chips were slightly lower [29, 30] (Mendes et al., 2010; Martín-Sampedro et al., 2010). However, recently much interest has developed on the concept of the forest product biorefinery, where wood is hydrolyzed prior to conventional pulping and papermaking processes and the hydrolyzate consisting of hemicellulose sugars are to be used as a feedstock for biofuels, bioplastics or other uses [31] (Duarte et al. 2010).

The aim of this study was to optimize the production of furfural by hydrolysis with dilute acid of *Eucalyptus globulus* wood in two stages, using a factorial experimental design and multiple regression models to maximize furfural yields. The two-stage process could provide a solid phase with less degraded cellulose and older applications that the solid phase of a conventional acid hydrolysis. Therefore, the process was optimized for a twofold purpose, namely: to maximize furfural yield while minimizing cellulose degradation. Also, a two-step process including separation of the solid phase after the first hydrolysis step would facilitate the obtainment of fractions containing less degradation products (e.g. polyphenols or furfural derivatives acting as microbial inhibitors and hindering potential uses in fermentation processes). The selectivity for furfural of the process was increased by effect of the glucan and lignin fractions being efficiently removed after the first acid hydrolysis stage. The material balances and compositions of the solid and liquid phases were assessed, especially as regards the concentration of furfural and the composition of the liquid phase. Fig. 1 depicts the operational scheme used to optimize the two stages of the process.

2. MATERIALS AND METHODS

2.1. Characterization and storage of Raw material.

E. globulus samples from local plantations (Huelva, Spain) were milled to pass an 8 mm screen, since in preliminary studies no diffusional limitations were observed for this particle, air-dried, homogenized in a single lot to avoid differences in compositions among aliquots, and stored.

Aliquots of raw material were milled to particle size <0.5 mm and subjected to moisture and determination of extractable compounds (TAPPI T-264-om-88 and Tappi 204 cm-07), 1% NaOH soluble (Tappi 212 om-98) and to quantitative acid hydrolysis with 72% H_2SO_4 following standard methods (TAPPI T-249-em-85). The solid residue after hydrolysis was recovered by filtration and considered as Klason lignin. The compounds (glucose, xylose and arabinose, furfural and 5-hydroxymethylfurfural –HMF-) and acetic acid contained in the hydrolysates were determined by HPLC, so the HPLC features are below mentioned. Then, glucan, xylan, arabinan and acetyl groups in raw material can be calculated. Ashes were determined by calcination (TAPPI 211 om-07).

The chemical characterization of *E. globulus* used in this study and the characterizacion by various authors are shown in Table 1. The major fraction is cellulose (analyzed as glucan), to 42.8% or 41.0% (at TAPPI T 203-om-93), followed by the Klason lignin to 21.2 % (after quantitative acid hydrolysis) and hemicelluloses fraction (calculated as the sum of xylan, araban, acetyl groups and others) to 28.5%.

This composition is similar to that found by other authors [32, 35]. In this sense, *Eucaliptus globulus* could be used as suitable raw material according with criteria of a industrial production of furfural that requires a minimum content of 15–20% of pentosans [22].

2.2. Acid hydrolysis processing of wood samples. First stage

Raw material and water were mixed in the desired proportions and treated in a 2 dm^3 stainless steel reactor (Parr Instruments Company, Moline, Illinois, USA) using a liquid/solid ratio (LSR) of 8 kg water/kg raw material, on dry basis (the moisture content of material was considered as water). The liquid/solid ratio had been recognized as an important factor in furfural yield at low acid concentration and its optimal value (8–10) has been found in previous studies [21]. In our study, liquid-solid rate was 8:1 in all experiments. The reactor was fitted with four-blade turbine impellers, heated by an external fabric mantle and cooled by cool water circulating through an internal loop. The reaction media stirred at 150 rpm and heated to reach the desired temperature. Time zero was considered to be the beginning of the isothermal stage. The operating conditions were 130 ºC, 150 ºC and 170 ºC temperature; 30, 45 and 60 min operating time and 0.5%, 1.25% and 2% H_2SO_4 content and a liquid/ solid ratio of 8 kg water by kg raw material, on a dry basis.When the pretreatment was finished, the reactor was immediately cooled down by cooling water and then removed from heating jacket.

After treatment, solid residues were recovered by filtration, washed with water, air-dried, weighted for yield determination. Aliquots of the solid phase were analyzed for moisture and composition (duplicate) using the same methods as for raw material analysis. An aliquot of the liquors was filtered through 0.45 mm membranes and used for direct HPLC determination of monosaccharides, furfural, HMF, oligomers and acetic acid. A second aliquot was subjected to quantitative post-hydrolysis with 4% H_2SO_4 at 121 ºC for 45 min, before 0.45 mm membranes filtration an HPLC analysis. The increase in monosaccharide and acetic acid concentration caused by posthydrolysis provided a measure of the oligomer concentration. HPLC analyses were performed using a BioRadAminex HPX-87H column at 30 ºC eluted with 0.01 M H_2SO_4 at a flow rate of 0.6 mL min^{-1} using a refractive index detector to quantify glucose, xylose, arabinose, acetic acid, HMF and furfural.

2.3. Acid hydrolysis processing of liquor. Second stage

A second acid hydrolysis stage were performed at the first stage liquor with the aim of achieving a higher concentration of furfural and limit degradation of glucan (low concentration or glucose or derivates of glucan were obtained). In this second process, the operating conditions were 170 ºC, 180 ºC and 190 ºC temperature; 0, 15 and 30 min operating time and 2, 1.5 and 1 pH (-1, 0 and 1 normalized values in experimental design).

In the same way as in point 2.2, when the process was finished, the reactor was immediately cooled down by cooling water and then removed from heating jacket.

After treatment, the concentration of different components in the sample (glucose, xylose, arabinose, acetic acid, oligomers, furfural and HMF) were determined by HPLC.

2.4. Experimental design for acid hydrolysis processes. Multiple regresion model

In order to relate the dependent (yield, glucose, xylose, arabinose, acetic acid, oligomers, furfural and HMF) and independent variables (temperature, time and acid concentration or pH of process) in process with the minimum possible number of experiment, a 2^n central composite factor experimental design was used, making it possible to construct a second-order polynomial in the independent variables and the identification of statistical significance in the variables was used. Independent variables were normalized by using the following equation.

$$X_n = \frac{X - \overline{X}}{(X_{\max} - X_{\min})/2}$$

Where *X*is the absolute value of the independent variable concern $\overline{X}$ is the average value of the variable, and X_{max} and X_{min} are its maximum and minimum values respectively. Temperature and operation time have the highest influence. The range of variation of independent variables was determinated according previous work (data not show).

The number of tests required was calculated as $N = 2^n + 2 \cdot n + n_c$, 2^n being the number of points constituting the factor design, 2n that of axial points, and n_c that of central points. Under our conditions, N = 16. The experimental results were fitted to the following second-order polynomial:

$$Y = a_o + \sum_{i=1}^{n} b_i X_{ni} + \sum_{i=1}^{n} c_i X_{ni}^2 + \sum_{i=1; j=1}^{n} d_i X_{ni} X_{nj} \quad (i < j)$$

The independent variables used in the equations relating to both types of variables were those having a statistical significant coefficient (viz. those not exceeding a significance level of 0.05 in the student´s-test and having a 90% confidence interval excluding zero).

3. RESULTS AND DISCUSSION

3.1. First acid hydrolysis stage

Table 2 shows the normalized values of independent variables (temperature, acid concentration and operation time), the solid yield and glucose, xylose, arabinose, oligomers, furfural, 5-OH methylfurfural (HMF) and acetyl groups contents in the liquid phase relative to the initial raw material. The time to reach the operating temperature in not isothermal process between 130 and 170 ºC has not been taken into consideration due to the short time heating the reactor, between both temperatures, elapsed compared to both heating from ambient temperature. The influence of differences in non-isothermal operation time can be quite accurately approximated by that of temperature. Differences in liquid phase yield (100 – solid yield) were quite substantial (9.3% to 29.5%), which is suggestive of little solid degradation.

Table 2 show percentage composition of liquid fraction respect to initial raw material and solid yield. The next results were the variations ranges in liquid phase: 60.5%-90.7% solid yield; total-glucose (glucose + glucooligomers + 5-OH-methyl furfural) between 1.4% and 5.3%; total xylose (xylose + xylooligomers + furfural), between 1.9% and 17.0%. Also, the solid phase from hydrolysis was characterized: glucan between 32.1% and 45.1%, xylan between 9% and 15.5%, acetyl groups between

0.1% and 3%, Klason lignin between 10.3% and 29.4% and araban was extracted completely in each sample. The error in mass balance was less than 5% in all components.

These results imply that glucose was extracted between 3.3% and 12.4% concerning the initial content of glucan in the raw material and xylose between 11.1% and 99.0%. It is assumed to be a considerable difference because the working hypothesis was to maximize the extraction of hemicellulose derivatives while minimizing that of cellulose derivatives in order to obtain as pure as possible a furfural solution.

According with section 2.4, to assess the relative influence of the selected independent variables (time, temperature and acid concentration) on each dependent variable (solid yield, glucose content, xylose content, arabinose content, glucooligomers, xylooligomers and degradation products: furfural and 5-OH-methyl furfural), the polynomial mathematical models were obtained (Table 3) by substituting the values of the measured independent variables for each dependent variable, and applying a polynomial model analysis. Each value used to obtain the equations is the average of three measurements. The differences between the experimental values and those that were estimated using the previous equations never exceeded 10% for glucose, solid yield, glucooligomers, xylooligomers and 5% in the rest. Very good fits with values of r^2 greater than 0.94 in all cases has been obtained.

In order to better envisage the influence of operational variables on extraction liquor of wood, and to compare different conditions, the surface responses in Fig. 2 were plotted. The space among three response surfaces represents the whole range of values for each dependent variable that was used at three extreme values, +1, 0, -1, of the most influential variable (operation temperature in the case of glucose and xylose contents). It can be seen that solid yield decreases with increasing any conditions (figure not shows).The higher the temperature and lower acid concentration the xylose (Fig. 2), xyloligomers(figure not shows) and arabinose (figure not shows) more were produced. Only the xylose content, at high temperature, shown a little increase. Xylose degradation was noticed in all reactions providing good potential for furfural to be produced. This is probably due to the speed of the reaction and the relatively high temperature, which increased the rate of xylose formation but also xylose degradation. Xylose formation occurred quickly, due to the heterogeneous structure of the hemicelluloses [37]. The results in this work were higher than reported by Weigi et al. [38] (xylose between 0.62% and 4.13%, with a máximum temperature of 200ºC and operation time 20 min) and similars to results to that found for Orozco et al. and Gütsch et al. [37, 39] (with a extraction of 13.5 g/100 g with conditions of 2.5 wt% H_3PO_4 at 175 ºC in 10 min reaction time and 13.1% over dry wood with conditions of 0.1 mol/L H_2SO_4 at 140 ºC in 35 min reaction time, respectively). The utilization of sulfuric acid reduces treatment temperatures at a given wood yield as compared to autohydrolysis[39].

For glucose, glucooligomers (Fig. 2), furfural (Fig. 2) and 5-OH-methyl furfural, it can be seen that extraction rate increase with increasing acid concentration or temperature. Similar values were obtained or lower than other authors [37, 39]. In these works the maximum yield for glucose production was an average of 5 g/100 g dry mass with conditions of 5 wt% H_3PO_4 at 175 ºC and 30 min reaction time and 1.0% over dry wood with conditions of 0.1 mol/L H_2SO_4 at 140 ºC in 50 min reaction time, respectively. Due to its crystalline structure, it was not possible at the reaction conditions to obtain a higher yield of glucose. Glucose degradation to HMF was observed at the highest temperature. Finally, there was a decline of glucoligomers for increased concentration of acid and temperature, and conversely an increase in glucoligomers with increasing time is observed.

According with previous discussion, operating conditions of 170 ºC, 30 min, 2% H_2SO_4 were selected as optimum conditions. Using a temperature of 170 ºC (T +1) provided the highest concentrations of furfural and xylose (Fig. 2), and the lowest concentrations of oligomers (results not shown). The operation time and acid concentration used had little effect on the furfural and xylose concentrations; however, using high acid concentrations reduced the contents in xylo-oligomers (results not shown) and minimized the extraction of gluco-oligomers (Fig. 2). The predictions under these optimum conditions differed by 5–10% from the experimental values for the liquid phase after autohydrolysis, namely: 2.3% glucose, 9.8% xylose, 2.5% xylo-oligomers, 0.6% arabinose, 1.1% furfural and 0.1% HMF. If we combine the amounts of xylose, xylo-oligomers and furfural, and consider the potential errors in the models of Table 3 and that in the determinations of the raw material in Table 1, then xylan in the raw material was extracted by 68–90%; similarly, if we add the amount of arabinose and assume hemicelluloses to comprise xylan, araban, acetyl groups and the “others” faction in Table 1, then hemicelluloses were extracted by 43–56%. If the “others” fraction of the raw material is excluded, hemicelluloses were extracted by el 57%–76%. These results are very similar to that obtained by Feria et al. [28] in *Eucaliptusglobulus*and high extraction values have been achieved.

In one-stage technology, depolymerization of xylans in xylose and dehydration into furfural occur simultaneously. The typical concentration of furfural in the product stream from the reactor is low, around (0.7 wt% – 3.3 wt%) and the solid residue can be used as a source of fuel [15, 26].

3.2. Second acid hydrolysis stage

Table 4 shows the normalized values of independent variables (temperature, acid concentration and pH), and dependent variables: glucose, xylose, arabinose, oligomers, furfural, 5-OH methylfurfural (HMF) and acetyl groups contents in the liquid phase in g/L, and relative to the initial raw material, after two steps of diluted acid hydrolysis. The process is similar to that described in the previous section 3.2.

As can be seen, the concentrations of cellulose and hemicellulose oligomers after the second hydrolysis stage were very low. Conversions to furfural were substantial: above 10 g/L, equivalent to more than 40% of all xylan initially present in the raw material, in some cases. Based on the relatively substantial amounts of xylose in the material, one can expect even greater concentrations of furfural to be obtained by using a different temperature, operation time or acid concentration than those falling in the ranges spanned by the experimental design. Probably, however, the alternative conditions would cause greater degradation of polysaccharides.

The furfural production values of Table 4 exceed those obtained by Riansa-ngawong and Prasertsan [26] by delignifying palm with a similar two-stage process using an identical solid/liquid ratio and 5% sulphuric acid at 125 ºC (8.67 g/L); and also those obtained by Canettieri [40] from *Eucalyptus grandis* subjected to acid hydrolysis in a single step, solid/liquid ratios of 3.4/1 to 8.6/1 and 0.6% sulphuric acid (1.23 g/L).

Based on the results of Table 4, hemicelluloses in the raw material ("others" fraction included) were extracted by 32%–57.7% and xylan was converted to an extent of 40.5%–84%.

The procedure used also allowed the obtainment of hemicellulose derivatives scarcely contaminated with cellulose derivatives. The amounts of gluco-oligomers were in fact very small and that of glucose never exceeded 3% of the raw material. Therefore, only about 7% of all glucan initially present in the material was degraded. The concentrations of 5-OH-methyl furfural were also very low (conversion of the starting glucan never exceeded 0.65%).

In the same way as in the previous section, to assess the relative influence of the selected independent variables (time, temperature and pH) on each dependent variable (glucose content, xylose content, arabinose content, glucooligomers, xylooligomers and degradation products: furfural and 5-OH-methyl furfural), the polynomial mathematical models were obtained (Table 5) by substituting the values of the measured independent variables for each dependent variable, and applying a polynomial model analysis. Each value used to obtain the equations is the average of three measurements. The differences between the experimental values and those that were estimated using the previous equations never exceeded 15% for glucooligomersand 10% in the rest of variables.

Also, In order to better envisage the influence of operational variables on extraction liquor of wood, and to compare different conditions, the surface responses in Fig. 3 were plotted. The space between three response surfaces represents the whole range of values for each dependent variable that was used at three extreme values, +1, 0, -1, of the most influential variable (operation time in the case of furfural contents and pH in the case of xylose, glucose and xylooligomers contents).

As can be seen from Fig. 3, the highest conversions to furfural were obtained by using a medium operation time (15 min), a low temperature (170 ºC) and a high pH (pH = 2 according the range of variation of pH). These conditions corresponded to point (T + 1, operation time 0, pH + 1) in the experimental design, consistent with previous results of Zhang [41]. Zhang [41] reported that the conversión of xylose to furfural can be divided into three stages based on the reaction temperature: between 120 and 140 ºC, only trace amounts of xylose were converted to furfural; between 140 and 160 ºC, the rate of xylose conversion was increased and between 160 and 200 ºC, a much higher conversion rate, and a linear relationship was observed between the xylose conversion and furfural yield. These conditions resulted in concentrations of xylo-oligomers falling in the low end of the experimental range that suggest complete degradation of xylan extracted in the first hydrolysis step to xylose and furfural (results not shown). Xylose concentrations were relatively high (Fig. 3) and his conversion is not complete but conversions to furfural were greater than they would have with a high temperature (180 or 190 ºC). The arabinose concentration fell in the high-value end of the experimental range. Interestingly, the concentrations of glucose (Fig. 3) and 5-OH-methyl furfural in the liquid phase from the second acid hydrolysis stage fell in the low end of the range (results not shown). This indicates a high selectivity in the conversion of hemicellulose derivatives to furfural in relation to the simultaneous conversion of glucan derivatives to 5-OH methylfurfural.

4. CONCLUSIONS

The proposed two-stage acid hydrolysis process allows increased amounts of furfural to be obtained from Eucalyptus globulus wood under more selective extraction conditions.

The operational ranges are especially suitable for the extraction of xylose, which was virtually quantitative at 170 ºC and virtually independent of the operation time. It is therefore advisable to use non-isothermal conditions or short times. This is quite important if one considers the low acid concentration used. The liquid phase obtained after autohydrolysis of the raw material under the optimum conditions contained 2.3% glucose, 9.8% xylose, 2.5% xylo-oligomers, 0.6% arabinose, 1.1% furfural and 0.1% 5-OH methyl furfural.

After the residual solid from the first hydrolysis stage is removed, the acid concentration (pH) is the most influential variable on the conversion of xylo-oligomers or xylose into furfural. Conversions to furfural were quite substantial (greater than 10 g/L, which is equivalent to 40% conversion of all xylan initially present in the raw material to furfural). Hemicelluloses in the raw material were extracted by 32–57.7% and xylan by 40.5–84%. The highest conversions to furfural were obtained by using a medium operation time (15 min) and low temperature (170 ºC) and a high pH (2).

The process additionally provides hemicellulose derivatives that are scarcely contaminated with cellulose derivatives. The concentrations of gluco-oligomers were very low and those of glucose in the region of 3% of the raw material.

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Optimization of furfural production by acid hydrolysis of *Eucalyptus globulus* in two stages

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Abstract

The aim of this study was to optimize the production of furfural by hydrolysis with dilute acid of Eucalyptus globulus wood in two stages, using a factorial experimental design and multiple regression models to maximize furfural yields. The two-stage process could provide a solid phase with less degraded cellulose and older applications that the solid phase of a conventional acid hydrolysis. The selectivity for furfural of the process was increased by effect of the glucan and lignin fractions being efficiently removed after the first acid hydrolysis stage.

The operational ranges are especially suitable for the extraction of xylose, which was virtually quantitative at 170 ºC and virtually independent of the operation time. It is therefore advisable to use non-isothermal conditions or short times. This is quite important if one considers the low acid concentration used. The liquid phase obtained after autohydrolysis of the raw material under the optimum conditions contained 2.3% glucose, 9.8% xylose, 2.5% xylo-oligomers, 0.6% arabinose, 1.1% furfural and 0.1% 5-OH methyl furfural.

After the residual solid from the first hydrolysis stage is removed, the acid concentration is the most influential variable on the conversion of xylo-oligomers or xylose into furfural. Conversions to furfural were quite substantial (greater than 10 g/L). Hemicelluloses in the raw material were extracted by 32–57.7% and xylan by 40.5–84%. The highest conversions to furfural were obtained by using a medium operation time (15 min) and low temperature (170 ºC) and pH=2.

The process additionally provides hemicellulose derivatives that are scarcely contaminated with cellulose derivatives. The concentrations of gluco-oligomers were very low and those of glucose in the region of 3% of the raw material.

Keywords

Furfural, acid hydrolysis*, Eucalyptus globulus,*

1. INTRODUCTION

To date fossil sources have been the main resources to obtain energy or chemicals but these resources produce negative effects due to environmental pollutions [1]. To remedy this situation, among the potential industrial biorefineries, the lignocellulose biorefinery will most probably be pushed through with the greatest success, because the raw material situation is optimal and conversion products have a good position in the traditional petrochemical as well as in the future bio-based product market [2-4]. In these biorefinery schemes, the hemicelluloses fraction, are not being isolated for industrial use yet, but they have a high potential and they could be used in many applications, not only for liquid biofuels production, notably bioethanol [5, 6], and butanol[7, 8]. An important derivate from hemicelluloses fraction is furfural [9]. Furfural, an important derived from hemicelluloses, is used for the production of a wide range of important non-petroleum derived chemicals. such as furan, tetrahydrofuran, and furfuryl alcohol[10]. It is also used as an extractive, fungicide, nematocide[11], in oil refineries, as well as in the plastics, food, pharmaceutical and agricultural industries [12, 13]. Resins with excellent thermosetting properties and extreme physical strength can also be produced by condensation of furfural with formaldehyde, phenol, acetone, or urea [11]. There is no synthetic route available for furfural production in chemical industry. Furfural is exclusively produced from hemicelluloses of lignocellulosic biomass [14]. About 250.000 tons of furfural, probably the only unsaturated, large volume, organic chemical, are prepared from carbohydrate sources annually [15].

A lot of literature is written about different pretreatment methods to enhance the extraction of carbohydrates in the process from lignocellulosic materials. Hot water or weak acid hydrolysis at an elevated temperature is the first step that is typically used to hydrolyze carbohydrates from lignocellulosic materials[16-18]. Steam explosion and dilute acid pretreatment are the two most commonly used methods because of their effectiveness and inexpensiveness [19]. In research, acid prehydrolysis has already found a broad area of applications, however, most of them are not commercially applied yet. For example, acid prehydrolysis is applied to remove hemicelluloses for (dissolving) pulp production [20]. Furfural production is carried out by hydrolysis of hemicelluloses pentosans to monomeric pentoses, and their subsequent acid-catalyzed dehydration into furfural [21]. This reaction would occur with or without mineral acids addition due to the liberation of acetic acid from the acetyl groups of the hemicelluloses. Industrial production of furfural requires a minimum content of 15–20% of pentosans and only a third of this amount can be converted into furfural [22]. Currently, furfural is obtained mainly from agricultural wastes as a source of hemicellulose or pentosan and by using acid catalyzed hydrolysis [22, 23] or steam pressure [24]. The application of various catalysts in the furfural conversion has been well documented in the literature, including mineral acids, organic acids and solid acids [25]. In one-stage technology, depolymerization of xylans in xylose and dehydration into furfural occur simultaneously. The solid residue can be used as a source of fuel [26]. In two-stage technology, a dissolution and partial depolymerization of hemicelluloses occur, followed by dehydration in furfural. In this manner, the residual lignocelluloses is almost unaltered and can be used for conversion to ethanol, glucose [14] or pulp and paper [3, 4, 27, 28] and the process has a higher furfural yield [26].

Actually, in the field of exploitation of the forest resources (pulp and paper industry), it accepts the idea of prior extraction of hemicelluloses, makes that the mechanical properties of handsheets prepared with previous autohydrolysis/hot water extraction or exploded chips were slightly lower [29, 30] (Mendes et al., 2010; Martín-Sampedro et al., 2010). However, recently much interest has developed on the concept of the forest product biorefinery, where wood is hydrolyzed prior to conventional pulping and papermaking processes and the hydrolyzate consisting of hemicellulose sugars are to be used as a feedstock for biofuels, bioplastics or other uses [31] (Duarte et al. 2010).

The aim of this study was to optimize the production of furfural by hydrolysis with dilute acid of *Eucalyptus globulus* wood in two stages, using a factorial experimental design and multiple regression models to maximize furfural yields. The two-stage process could provide a solid phase with less degraded cellulose and older applications that the solid phase of a conventional acid hydrolysis. Therefore, the process was optimized for a twofold purpose, namely: to maximize furfural yield while minimizing cellulose degradation. Also, a two-step process including separation of the solid phase after the first hydrolysis step would facilitate the obtainment of fractions containing less degradation products (e.g. polyphenols or furfural derivatives acting as microbial inhibitors and hindering potential uses in fermentation processes). The selectivity for furfural of the process was increased by effect of the glucan and lignin fractions being efficiently removed after the first acid hydrolysis stage. The material balances and compositions of the solid and liquid phases were assessed, especially as regards the concentration of furfural and the composition of the liquid phase. Fig. 1 depicts the operational scheme used to optimize the two stages of the process.

2. MATERIALS AND METHODS

2.1. Characterization and storage of Raw material.

E. globulus samples from local plantations (Huelva, Spain) were milled to pass an 8 mm screen, since in preliminary studies no diffusional limitations were observed for this particle, air-dried, homogenized in a single lot to avoid differences in compositions among aliquots, and stored.

Aliquots of raw material were milled to particle size <0.5 mm and subjected to moisture and determination of extractable compounds (TAPPI T-264-om-88 and Tappi 204 cm-07), 1% NaOH soluble (Tappi 212 om-98) and to quantitative acid hydrolysis with 72% H_2SO_4 following standard methods (TAPPI T-249-em-85). The solid residue after hydrolysis was recovered by filtration and considered as Klason lignin. The compounds (glucose, xylose and arabinose, furfural and 5-hydroxymethylfurfural –HMF-) and acetic acid contained in the hydrolysates were determined by HPLC, so the HPLC features are below mentioned. Then, glucan, xylan, arabinan and acetyl groups in raw material can be calculated. Ashes were determined by calcination (TAPPI 211 om-07).

The chemical characterization of *E. globulus* used in this study and the characterizacion by various authors are shown in Table 1. The major fraction is cellulose (analyzed as glucan), to 42.8% or 41.0% (at TAPPI T 203-om-93), followed by the Klason lignin to 21.2 % (after quantitative acid hydrolysis) and hemicelluloses fraction (calculated as the sum of xylan, araban, acetyl groups and others) to 28.5%.

This composition is similar to that found by other authors [32, 35]. In this sense, *Eucaliptus globulus* could be used as suitable raw material according with criteria of a industrial production of furfural that requires a minimum content of 15–20% of pentosans [22].

2.2. Acid hydrolysis processing of wood samples. First stage

Raw material and water were mixed in the desired proportions and treated in a 2 dm^3 stainless steel reactor (Parr Instruments Company, Moline, Illinois, USA) using a liquid/solid ratio (LSR) of 8 kg water/kg raw material, on dry basis (the moisture content of material was considered as water). The liquid/solid ratio had been recognized as an important factor in furfural yield at low acid concentration and its optimal value (8–10) has been found in previous studies [21]. In our study, liquid-solid rate was 8:1 in all experiments. The reactor was fitted with four-blade turbine impellers, heated by an external fabric mantle and cooled by cool water circulating through an internal loop. The reaction media stirred at 150 rpm and heated to reach the desired temperature. Time zero was considered to be the beginning of the isothermal stage. The operating conditions were 130 ºC, 150 ºC and 170 ºC temperature; 30, 45 and 60 min operating time and 0.5%, 1.25% and 2% H_2SO_4 content and a liquid/ solid ratio of 8 kg water by kg raw material, on a dry basis.When the pretreatment was finished, the reactor was immediately cooled down by cooling water and then removed from heating jacket.

After treatment, solid residues were recovered by filtration, washed with water, air-dried, weighted for yield determination. Aliquots of the solid phase were analyzed for moisture and composition (duplicate) using the same methods as for raw material analysis. An aliquot of the liquors was filtered through 0.45 mm membranes and used for direct HPLC determination of monosaccharides, furfural, HMF, oligomers and acetic acid. A second aliquot was subjected to quantitative post-hydrolysis with 4% H_2SO_4 at 121 ºC for 45 min, before 0.45 mm membranes filtration an HPLC analysis. The increase in monosaccharide and acetic acid concentration caused by posthydrolysis provided a measure of the oligomer concentration. HPLC analyses were performed using a BioRadAminex HPX-87H column at 30 ºC eluted with 0.01 M H_2SO_4 at a flow rate of 0.6 mL min^{-1} using a refractive index detector to quantify glucose, xylose, arabinose, acetic acid, HMF and furfural.

2.3. Acid hydrolysis processing of liquor. Second stage

A second acid hydrolysis stage were performed at the first stage liquor with the aim of achieving a higher concentration of furfural and limit degradation of glucan (low concentration or glucose or derivates of glucan were obtained). In this second process, the operating conditions were 170 ºC, 180 ºC and 190 ºC temperature; 0, 15 and 30 min operating time and 2, 1.5 and 1 pH (-1, 0 and 1 normalized values in experimental design).

In the same way as in point 2.2, when the process was finished, the reactor was immediately cooled down by cooling water and then removed from heating jacket.

After treatment, the concentration of different components in the sample (glucose, xylose, arabinose, acetic acid, oligomers, furfural and HMF) were determined by HPLC.

2.4. Experimental design for acid hydrolysis processes. Multiple regresion model

In order to relate the dependent (yield, glucose, xylose, arabinose, acetic acid, oligomers, furfural and HMF) and independent variables (temperature, time and acid concentration or pH of process) in process with the minimum possible number of experiment, a 2^n central composite factor experimental design was used, making it possible to construct a second-order polynomial in the independent variables and the identification of statistical significance in the variables was used. Independent variables were normalized by using the following equation.

$$X_n = \frac{X - \overline{X}}{(X_{\max} - X_{\min})/2}$$

Where $X$is the absolute value of the independent variable concern $\overline{X}$ is the average value of the variable, and X_{max} and X_{min} are its maximum and minimum values respectively. Temperature and operation time have the highest influence. The range of variation of independent variables was determinated according previous work (data not show).

The number of tests required was calculated as $N = 2^n + 2\cdot n + n_c$, 2^n being the number of points constituting the factor design, 2n that of axial points, and n_c that of central points. Under our conditions, N = 16. The experimental results were fitted to the following second-order polynomial:

$$Y = a_o + \sum_{i=1}^{n} b_i X_{ni} + \sum_{i=1}^{n} c_i X_{ni}^2 + \sum_{i=1;j=1}^{n} d_i X_{ni} X_{nj} \quad (i < j)$$

The independent variables used in the equations relating to both types of variables were those having a statistical significant coefficient (viz. those not exceeding a significance level of 0.05 in the student´s-test and having a 90% confidence interval excluding zero).

3. RESULTS AND DISCUSSION

3.1. First acid hydrolysis stage

Table 2 shows the normalized values of independent variables (temperature, acid concentration and operation time), the solid yield and glucose, xylose, arabinose, oligomers, furfural, 5-OH methylfurfural (HMF) and acetyl groups contents in the liquid phase relative to the initial raw material. The time to reach the operating temperature in not isothermal process between 130 and 170 ºC has not been taken into consideration due to the short time heating the reactor, between both temperatures, elapsed compared to both heating from ambient temperature. The influence of differences in non-isothermal operation time can be quite accurately approximated by that of temperature. Differences in liquid phase yield (100 – solid yield) were quite substantial (9.3% to 29.5%), which is suggestive of little solid degradation.

Table 2 show percentage composition of liquid fraction respect to initial raw material and solid yield. The next results were the variations ranges in liquid phase: 60.5%-90.7% solid yield; total-glucose (glucose + glucooligomers + 5-OH-methyl furfural) between 1.4% and 5.3%; total xylose (xylose + xylooligomers + furfural), between 1.9% and 17.0%. Also, the solid phase from hydrolysis was characterized: glucan between 32.1% and 45.1%, xylan between 9% and 15.5%, acetyl groups between

0.1% and 3%, Klason lignin between 10.3% and 29.4% and araban was extracted completely in each sample. The error in mass balance was less than 5% in all components.

These results imply that glucose was extracted between 3.3% and 12.4% concerning the initial content of glucan in the raw material and xylose between 11.1% and 99.0%. It is assumed to be a considerable difference because the working hypothesis was to maximize the extraction of hemicellulose derivatives while minimizing that of cellulose derivatives in order to obtain as pure as possible a furfural solution.

According with section 2.4, to assess the relative influence of the selected independent variables (time, temperature and acid concentration) on each dependent variable (solid yield, glucose content, xylose content, arabinose content, glucooligomers, xylooligomers and degradation products: furfural and 5-OH-methyl furfural), the polynomial mathematical models were obtained (Table 3) by substituting the values of the measured independent variables for each dependent variable, and applying a polynomial model analysis. Each value used to obtain the equations is the average of three measurements. The differences between the experimental values and those that were estimated using the previous equations never exceeded 10% for glucose, solid yield, glucooligomers, xylooligomers and 5% in the rest. Very good fits with values of r^2 greater than 0.94 in all cases has been obtained.

In order to better envisage the influence of operational variables on extraction liquor of wood, and to compare different conditions, the surface responses in Fig. 2 were plotted. The space among three response surfaces represents the whole range of values for each dependent variable that was used at three extreme values, +1, 0, -1, of the most influential variable (operation temperature in the case of glucose and xylose contents). It can be seen that solid yield decreases with increasing any conditions (figure not shows).The higher the temperature and lower acid concentration the xylose (Fig. 2), xyloligomers(figure not shows) and arabinose (figure not shows) more were produced. Only the xylose content, at high temperature, shown a little increase. Xylose degradation was noticed in all reactions providing good potential for furfural to be produced. This is probably due to the speed of the reaction and the relatively high temperature, which increased the rate of xylose formation but also xylose degradation. Xylose formation occurred quickly, due to the heterogeneous structure of the hemicelluloses [37]. The results in this work were higher than reported by Weigi et al. [38] (xylose between 0.62% and 4.13%, with a máximum temperature of 200ºC and operation time 20 min) and similars to results to that found for Orozco et al. and Gütsch et al. [37, 39] (with a extraction of 13.5 g/100 g with conditions of 2.5 wt% H_3PO_4 at 175 ºC in 10 min reaction time and 13.1% over dry wood with conditions of 0.1 mol/L H_2SO_4 at 140 ºC in 35 min reaction time, respectively). The utilization of sulfuric acid reduces treatment temperatures at a given wood yield as compared to autohydrolysis[39].

For glucose, glucooligomers (Fig. 2), furfural (Fig. 2) and 5-OH-methyl furfural, it can be seen that extraction rate increase with increasing acid concentration or temperature. Similar values were obtained or lower than other authors [37, 39]. In these works the maximum yield for glucose production was an average of 5 g/100 g dry mass with conditions of 5 wt% H_3PO_4 at 175 ºC and 30 min reaction time and 1.0% over dry wood with conditions of 0.1 mol/L H_2SO_4 at 140 ºC in 50 min reaction time, respectively. Due to its crystalline structure, it was not possible at the reaction conditions to obtain a higher yield of glucose. Glucose degradation to HMF was observed at the highest temperature. Finally, there was a decline of glucoligomers for increased concentration of acid and temperature, and conversely an increase in glucoligomers with increasing time is observed.

According with previous discussion, operating conditions of 170 ºC, 30 min, 2% H_2SO_4 were selected as optimum conditions. Using a temperature of 170 ºC (T +1) provided the highest concentrations of furfural and xylose (Fig. 2), and the lowest concentrations of oligomers (results not shown). The operation time and acid concentration used had little effect on the furfural and xylose concentrations; however, using high acid concentrations reduced the contents in xylo-oligomers (results not shown) and minimized the extraction of gluco-oligomers (Fig. 2). The predictions under these optimum conditions differed by 5–10% from the experimental values for the liquid phase after autohydrolysis, namely: 2.3% glucose, 9.8% xylose, 2.5% xylo-oligomers, 0.6% arabinose, 1.1% furfural and 0.1% HMF. If we combine the amounts of xylose, xylo-oligomers and furfural, and consider the potential errors in the models of Table 3 and that in the determinations of the raw material in Table 1, then xylan in the raw material was extracted by 68–90%; similarly, if we add the amount of arabinose and assume hemicelluloses to comprise xylan, araban, acetyl groups and the “others” faction in Table 1, then hemicelluloses were extracted by 43–56%. If the “others” fraction of the raw material is excluded, hemicelluloses were extracted by el 57%–76%. These results are very similar to that obtained by Feria et al. [28] in *Eucaliptusglobulus*and high extraction values have been achieved.

In one-stage technology, depolymerization of xylans in xylose and dehydration into furfural occur simultaneously. The typical concentration of furfural in the product stream from the reactor is low, around (0.7 wt% – 3.3 wt%) and the solid residue can be used as a source of fuel [15, 26].

3.2. Second acid hydrolysis stage

Table 4 shows the normalized values of independent variables (temperature, acid concentration and pH), and dependent variables: glucose, xylose, arabinose, oligomers, furfural, 5-OH methylfurfural (HMF) and acetyl groups contents in the liquid phase in g/L, and relative to the initial raw material, after two steps of diluted acid hydrolysis. The process is similar to that described in the previous section 3.2.

As can be seen, the concentrations of cellulose and hemicellulose oligomers after the second hydrolysis stage were very low. Conversions to furfural were substantial: above 10 g/L, equivalent to more than 40% of all xylan initially present in the raw material, in some cases. Based on the relatively substantial amounts of xylose in the material, one can expect even greater concentrations of furfural to be obtained by using a different temperature, operation time or acid concentration than those falling in the ranges spanned by the experimental design. Probably, however, the alternative conditions would cause greater degradation of polysaccharides.

The furfural production values of Table 4 exceed those obtained by Riansa-ngawong and Prasertsan [26] by delignifying palm with a similar two-stage process using an identical solid/liquid ratio and 5% sulphuric acid at 125 ºC (8.67 g/L); and also those obtained by Canettieri [40] from *Eucalyptus grandis* subjected to acid hydrolysis in a single step, solid/liquid ratios of 3.4/1 to 8.6/1 and 0.6% sulphuric acid (1.23 g/L).

Based on the results of Table 4, hemicelluloses in the raw material (“others” fraction included) were extracted by 32%–57.7% and xylan was converted to an extent of 40.5%–84%.

The procedure used also allowed the obtainment of hemicellulose derivatives scarcely contaminated with cellulose derivatives. The amounts of gluco-oligomers were in fact very small and that of glucose never exceeded 3% of the raw material. Therefore, only about 7% of all glucan initially present in the material was degraded. The concentrations of 5-OH-methyl furfural were also very low (conversion of the starting glucan never exceeded 0.65%).

In the same way as in the previous section, to assess the relative influence of the selected independent variables (time, temperature and pH) on each dependent variable (glucose content, xylose content, arabinose content, glucooligomers, xylooligomers and degradation products: furfural and 5-OH-methyl furfural), the polynomial mathematical models were obtained (Table 5) by substituting the values of the measured independent variables for each dependent variable, and applying a polynomial model analysis. Each value used to obtain the equations is the average of three measurements. The differences between the experimental values and those that were estimated using the previous equations never exceeded 15% for glucooligomersand 10% in the rest of variables.

Also, In order to better envisage the influence of operational variables on extraction liquor of wood, and to compare different conditions, the surface responses in Fig. 3 were plotted. The space between three response surfaces represents the whole range of values for each dependent variable that was used at three extreme values, +1, 0, -1, of the most influential variable (operation time in the case of furfural contents and pH in the case of xylose, glucose and xylooligomers contents).

As can be seen from Fig. 3, the highest conversions to furfural were obtained by using a medium operation time (15 min), a low temperature (170 ºC) and a high pH (pH = 2 according the range of variation of pH). These conditions corresponded to point (T + 1, operation time 0, pH + 1) in the experimental design, consistent with previous results of Zhang [41]. Zhang [41] reported that the conversión of xylose to furfural can be divided into three stages based on the reaction temperature: between 120 and 140 ºC, only trace amounts of xylose were converted to furfural; between 140 and 160 ºC, the rate of xylose conversion was increased and between 160 and 200 ºC, a much higher conversion rate, and a linear relationship was observed between the xylose conversion and furfural yield. These conditions resulted in concentrations of xylo-oligomers falling in the low end of the experimental range that suggest complete degradation of xylan extracted in the first hydrolysis step to xylose and furfural (results not shown). Xylose concentrations were relatively high (Fig. 3) and his conversion is not complete but conversions to furfural were greater than they would have with a high temperature (180 or 190 ºC). The arabinose concentration fell in the high-value end of the experimental range. Interestingly, the concentrations of glucose (Fig. 3) and 5-OH-methyl furfural in the liquid phase from the second acid hydrolysis stage fell in the low end of the range (results not shown). This indicates a high selectivity in the conversion of hemicellulose derivatives to furfural in relation to the simultaneous conversion of glucan derivatives to 5-OH methylfurfural.

4. CONCLUSIONS

The proposed two-stage acid hydrolysis process allows increased amounts of furfural to be obtained from Eucalyptus globulus wood under more selective extraction conditions.

The operational ranges are especially suitable for the extraction of xylose, which was virtually quantitative at 170 ºC and virtually independent of the operation time. It is therefore advisable to use non-isothermal conditions or short times. This is quite important if one considers the low acid concentration used. The liquid phase obtained after autohydrolysis of the raw material under the optimum conditions contained 2.3% glucose, 9.8% xylose, 2.5% xylo-oligomers, 0.6% arabinose, 1.1% furfural and 0.1% 5-OH methyl furfural.

After the residual solid from the first hydrolysis stage is removed, the acid concentration (pH) is the most influential variable on the conversion of xylo-oligomers or xylose into furfural. Conversions to furfural were quite substantial (greater than 10 g/L, which is equivalent to 40% conversion of all xylan initially present in the raw material to furfural). Hemicelluloses in the raw material were extracted by 32–57.7% and xylan by 40.5–84%. The highest conversions to furfural were obtained by using a medium operation time (15 min) and low temperature (170 ºC) and a high pH (2).

The process additionally provides hemicellulose derivatives that are scarcely contaminated with cellulose derivatives. The concentrations of gluco-oligomers were very low and those of glucose in the region of 3% of the raw material.

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Table 1: Average chemical composition for Eucalyptus globulus (EG) used and other bibliographic EG composition[1].

		Eucalyptus globulus				
	Present study	[32]	[33]	[34]	[35]	[36]
1% NaOH sol. (%)	13.1 ± 1.6	12.4	nd	nd	nd	6.5
Ethanol extracts (%)	2.7 ± 0.1	1.2	nd	1.7	0.6	1.3
Ash (%)	0.7 ± 0.1	n.d.	0.5	0.4	0.4	12.1
Glucan (%)	42.8 ± 2.4	46.3	46.8	41.7	46.1	68.4
Klason lignin (%)	21.2 ± 1.7	22.9	19.9	22.9	19.8	26.6
Xylan (%)	17.1 ± 1.4	16.6	23.9	15.3	17.1	23.2
Arabinan (%)	0.7 ± 0.1	0.54	0.37	0.4	0.8	2.7
Acetyl groups (%)	3.5 ± 0.2	3.54	4.32	3.3	nd	nd
Others (%)	7.2 ± 0.4	10.1	nd	nd	nd	nd

[1] Raw material percentages (100 kg dry matter).

Table 2: Extraction rate, in liquid phase, of monomeric sugars, oligomers and degradation products (% to respect original raw material) from first step

Normalized values of temperature, time and acid concentration			Yield, %	Glucose, %	Glucoligomers, %	Xylose, %	Xyloligomers, %	Arabinose, %	Acetyl Groups, %	HMF, %	Furfural, %
0	0	0	77.29	1.34	1.93	4.97	4.58	0.57	4.28	0.12	0.57
0	0	0	76.80	1.56	2.27	5.11	4.26	0.67	2.96	0.11	0.56
-1	-1	-1	90.66	0.25	1.62	0.27	1.62	0.25	0.06	0.02	0.04
-1	-1	+1	68.02	2.23	0.63	10.39	5.64	1.03	1.99	0.15	0.93
-1	0	0	81.35	0.91	2.67	3.13	3.59	0.74	0.68	0.09	0.42
-1	+1	-1	89.32	0.22	5.02	0.42	3.32	0.59	0.19	0.04	0.07
-1	+1	+1	65.62	1.81	1.42	10.28	3.24	1.12	2.42	0.26	1.86
0	-1	0	79.84	1.09	1.46	4.59	6.93	0.59	1.48	0.02	0.03
0	0	-1	82.17	0.76	1.68	2.46	1.62	0.39	1.75	0.04	0.27
0	0	+1	64.54	2.27	0.70	10.55	1.52	0.80	4.62	0.26	1.59
0	+1	0	75.83	1.46	3.09	5.63	6.44	0.79	3.72	0.11	0.50
+1	-1	-1	89.04	0.60	0.73	1.24	1.52	0.41	0.55	0.05	0.36
+1	-1	+1	69.86	2.25	0.93	9.06	2.03	0.69	6.29	0.14	0.99
+1	0	0	79.35	1.53	1.38	4.33	1.02	0.46	2.88	0.14	0.62
+1	+1	-1	78.68	1.38	0.26	5.41	1.39	0.70	3.67	0.05	0.43
+1	+1	+1	60.52	3.72	0.29	9.68	0.38	0.59	8.50	0.39	1.99

The operating conditions were 130 ºC, 150 ºC and 170 ºC temperature (normalized values -1, 0 and 1); 30, 45 and 60 min operating time (normalized values -1, 0 and 1) and 0.5%, 1.25% and 2% H_2SO_4 (normalized values -1, 0 and 1).

Table 3. Equation obtained for each dependent variable of autohydrolysis process.

Equation	**R^2**	**F-Snedecor**
$Y_{Yi} = 77,404 – 1,752\ X_{ac} – 2,745\ X_t – 10,131\ X_T + 3,019\ X_{ac}\ X_{ac} – 3,976\ X_T\ X_T – 1,995\ X_{ac}\ X_t + 1,125\ X_{ac}\ X_T$	0,993	166,25
$Y_{Glu} = 1.313 + 0.405\ X_{ac} + 0.217\ X_t + 0.907\ X_T + 0.234\ X_T\ X_T + 0.337\ X_{ac}\ X_t$	0.956	66.91
$Y_{Xyl} = 4.951 + 0.523\ X_{ac} + 0.587\ X_t + 4.016\ X_T – 0.972\ X_{ac}\ X_{ac} + 1.803\ X_T\ X_T + 0.594\ X_{ac}\ X_t – 0.986\ X_{ac}\ X_T – 0.476\ X_t\ X_T$	0.991	95.51
$Y_{Ar} = 0.605 – 0.088\ X_{ac} + 0.082\ X_t + 0.188\ X_T + 0.071\ X_t\ X_t – 0.144\ X_{ac}\ X_T – 0.081\ X_t\ X_T$	0.946	45.53
$Y_{Gluol} = 2,132 – 0,778\ X_{ac} + 0,471\ X_t – 0,533\ X_T – 0,661\ X_{ac}\ X_t + 0,602\ X_{ac}\ X_T – 0,347\ X_t\ X_T$	0,943	18,896
$Y_{Xyol} = 4,234 – 1,105\ X_{ac} – 1,835\ X_{ac}\ X_{ac} + 2,543\ X_t\ X_t – 2,572\ X_T\ X_T – 0,555\ X_{ac}\ X_t – 0,703\ X_t\ X_T$	0,941	23,97
$Y_{HMF} = 0.108 + 0.025\ X_{ac} + 0.044\ X_t + 0.104\ X_T – 0.029\ Xt\ Xt + 0.052\ X_T\ X_T + 0.019\ X_{ac}\ X_t + 0.051\ X_t\ X_T$	0.968	65.41
$Y_{Fur} = 0.509 + 0.107\ X_{ac} + 0.248\ X_t + 0.618\ X_T – 0.177\ X_t\ X_t + 0.484\ X_T\ X_T + 0.229\ X_t\ X_T$	0.982	138.83

$Y_{Xyl} = 4.951 + 0.523\ X_{ac} + 0.587\ X_t + 4.016\ X_T – 0.972\ X_{ac}\ X_{ac} + 1.803\ X_T\ X_T + 0.594\ X_{ac}\ X_t – 0.986\ X_{ac}\ X_T – 0.476\ X_t\ X_T$

Where: Y_{Yl} denotes solid yield (%); Y_{Xyl}: xylose content ; $Y_{Glu,}$ glucose content; $Y_{Ar,}$ arabinose content; $Y_{Gluol,}$ glucoligomers content; Y_{Xyol}: xyloligomers content; Y_{HMF}, Hydroxymethylfurfural content; Y_{Fur}, Furfural content. X_T, X_t and X_{ac} denote normalized temperature, time and acid concentration, respectively.

Table 4: Extraction rate, in liquid phase, of monomeric sugars, oligomers and degradation products (g/L and % to respect original raw material) from second step

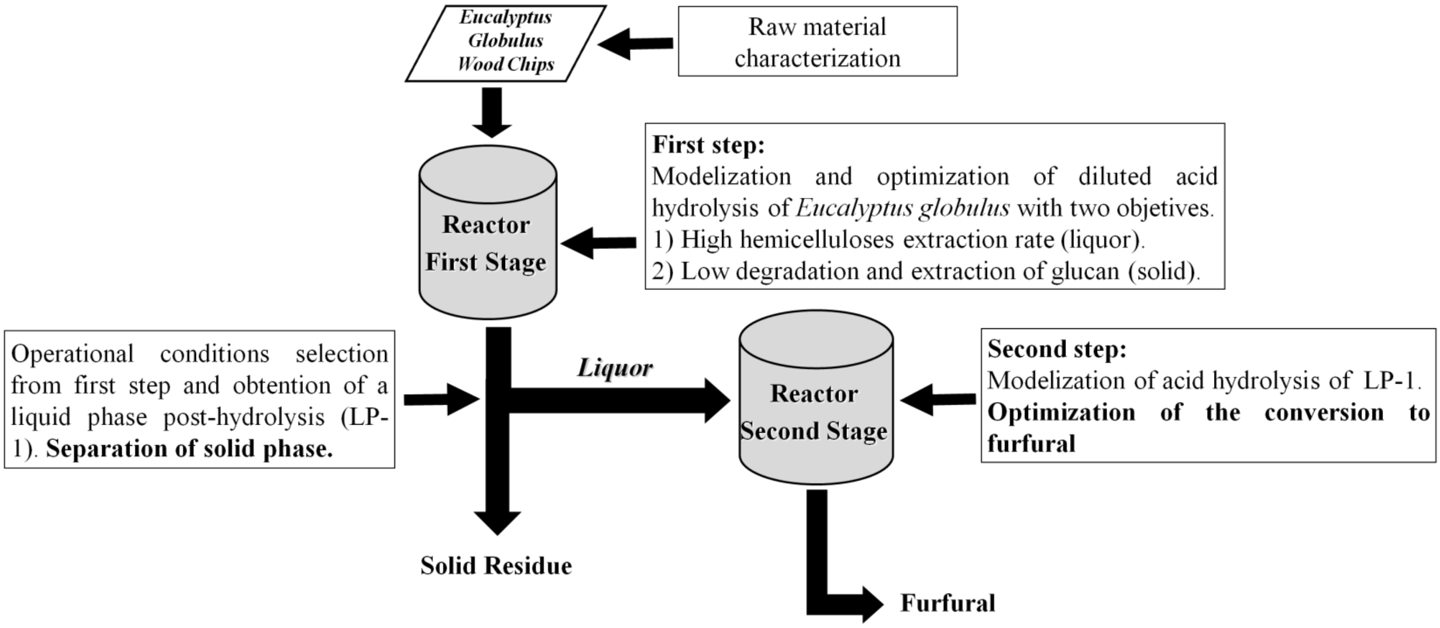
Normalized values of temperature, time and pH			**Glucose, g/L, %**	**Glucoligomers, g/L, %**	**Xylose, g/L, %**	**Xyloligomers, g/L, %**	**Arabinose, g/L, %**	**Acetyl groups, g/L, %**	**Furfural, g/L, %**	**HMF**
1	1	1	2.97/2.22	0.50/0.37	9.56/6.98	0.38/0.28	0.54/0.39	2.40/1.75	9.46/6.91	0.22/0.16
1	1	-1	1.21/0.90	0.34/0.25	3.96/2.89	0.67/0.49	0.59/0.43	2.48/1.81	8.20/5.99	0.13/0.10
1	-1	1	2.87/2.14	0.45/0.34	10.87/7.94	0.35/0.26	0.60/0.44	2.39/1.75	6.70/4.89	0.17/0.13
1	-1	-1	2.99/2.23	0.38/0.28	7.62/5.57	0.07/0.05	0.47/0.34	2.30/1.68	9.19/6.71	0.22/0.16
-1	1	1	3.63/2.71	0.43/0.32	12.22/8.93	0.00/0.00	0.59/0.43	2.36/1.72	7.33/5.35	0.27/0.20
-1	-1	1	4.04/3.02	0.24/0.18	12.52/9.15	0.72/0.53	0.68/0.50	2.48/1.81	5.93/4.33	0.23/0.17
-1	1	-1	1.61/1.20	0.10/0.07	5.36/3.92	0.09/0.07	0.53/0.39	2.48/1.81	4.03/2.94	0.1/0.07
-1	-1	-1	2.12/1.58	0.35/0.26	5.86/4.28	0.22/0.16	0.37/0.27	2.33/1.70	4.17/3.05	0.15/0.11
1	0	0	3.30/2.47	0.08/0.06	7.06/5.16	0.05/0.04	0,46/0.34	2.28/1.67	9.39/6.86	0.37/0.28
-1	0	0	3.98/2.97	0.01/0.01	8.08/5.90	0.00/0.00	0.44/0.32	2.28/1.67	6.34/4.63	0.33/0.25
0	1	0	1.52/1.14	0.20/0.15	7.63/5.57	0.01/0.01	0.51/0.37	2.70/1.97	9.67/7.06	0.36/0.27
0	-1	0	2.04/1.52	0.29/0.22	8.89/6.49	0.13/0.09	0.52/0.38	2.56/1.87	10.04/7.33	0.34/0.25
0	0	1	3.50/2.61	0.33/0.25	10.07/7.36	0.62/0.45	0.61/0.45	2.04/1.49	8.98/6.56	0.21/0.16
0	0	-1	2.22/1.66	0.04/0.03	4.57/3.34	0.53/0.39	0.49/0.36	2.11/1.54	8.23/6.01	0.11/0.08
0	0	0	2.72/2.03	0.11/0.08	8.05/5.88	0.21/0.19	0.44/0.32	2.31/1.69	10.20/7.50	0.35/0.26
0	0	0	2.76/2.07	0.13/0.10	8.11/5.92	0.17/0.15	0.52/0.38	2.27/1.65	10.43/7.60	0.37/0.28

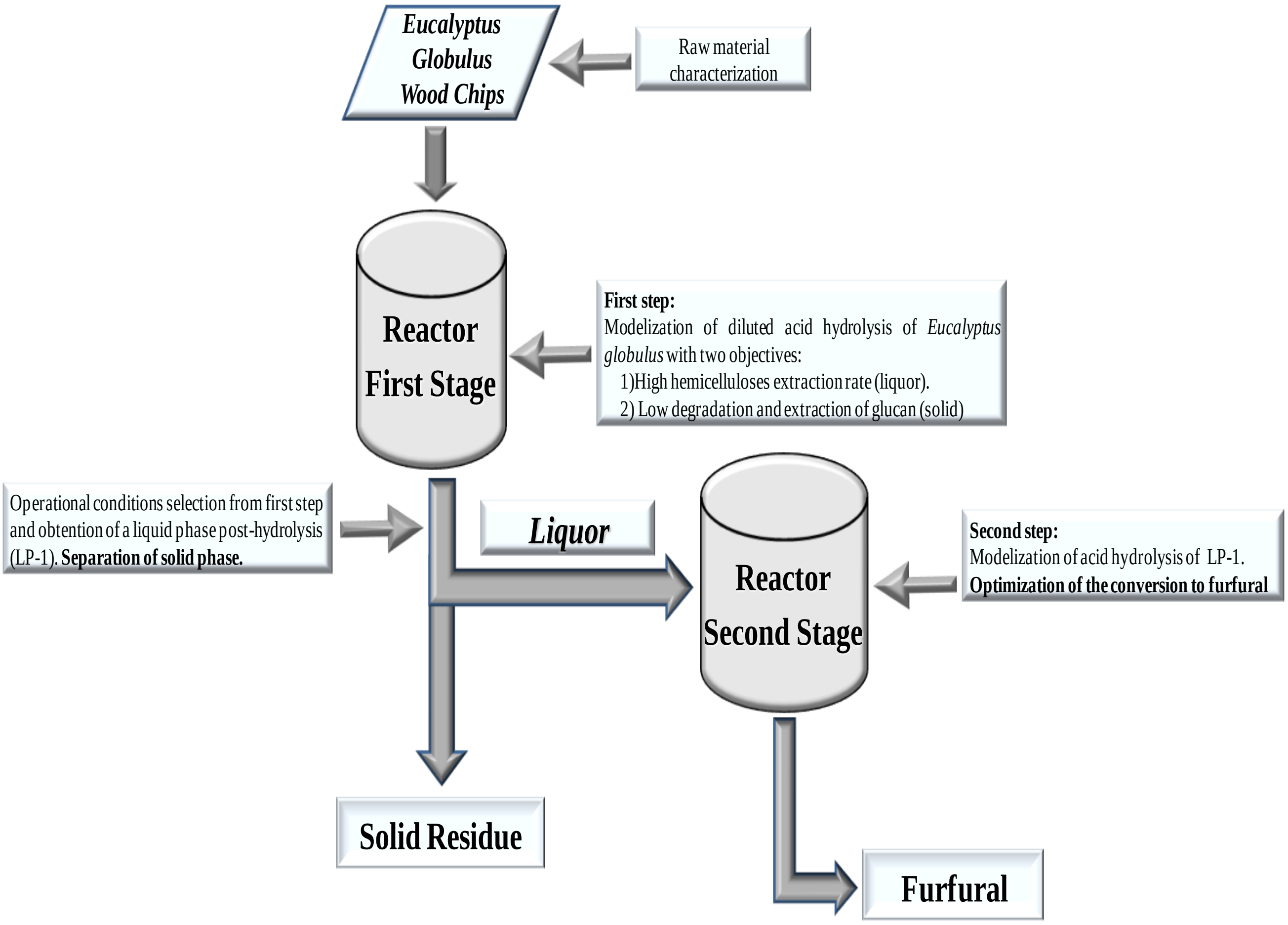
Table 5. Equation obtained for each dependent variable of autohydrolysis process.

Equation	R^2	F-Snedecor
$Y_{Glu} = 2.824 - 0,127\ X_t - 0.3899\ X_T + 0.763\ X_{pH} + 0.811\ X_tX_t - 1.049\ X_TX_T - 0.386\ X_t\ X_{pH} + 0.344\ X_TX_{pH}$	0.94	33.2
$Y_{Xyl} = 7.572 - 0.359\ X_t - 0.881\ X_T + 2.965\ X_{pH} + 0.739\ X_TX_T - 0.298\ X_TX_t - 0.791\ X_t\ X_{pH} + 0.036\ X_TX_{pH}$	0.99	119.6
$Y_{Ar} = 0.487 + 0.014\ X_t + 0.066\ X_{pH} - 0.039\ X_tX_t + 0.026\ X_TX_T + 0.061\ X_{pH}X_{pH} + 0.011\ X_TX_t - 0.047\ X_tX_{pH} - 0.042\ X_TX_{pH}$	0.99	126.5
$Y_{GrA} = 2.289 + 0.042\ X_T + 0.331\ X_TX_T - 0.224\ X_{pH}X_{pH} - 0.048\ X_T\ X_{pH}$	0.98	138.1
$Y_{Gluol} = 0.070 + 0.063\ X_t + 0.074\ X_{pH} + 0.170\ X_TX_T + 0.110\ X_{pH}X_{pH} + 0.066\ X_TX_{pH}$	0.89	14.7
$Y_{Xyol} = 0.187 + 0.056\ X_t - 0.042\ X_T + 0.067\ X_{pH} - 0.157\ X_tX_t - 0.117\ X_TX_T + 0.388\ X_{pH}X_{pH} + 0.195\ X_TX_t - 0.065\ X_tX_{pH} - 0.122\ X_TX_{pH}$	0.99	53.6
$Y_{HMF} = 0.352 + 0.044\ X_{pH} - 0.176\ X_{pH}X_{pH} - 0.027\ X_tX_{pH} + 0.036\ X_TX_{pH}$	0.97	83.5
$Y_{Fur} = 9.978 + 1.725\ X_t + 0.669\ X_{pH} - 2.059\ X_tX_t - 1.319\ X_{pH}X_{pH} + 0.328\ X_tX_T - 1.056\ X_TX_{pH} + 0.042\ X_TX_{pH}$	0.99	87.4

Where: Y_{Glu}, glucose content (g/L); Y_{Xyl}: xylose content (g/L); Y_{Ar}, arabinose content (g/L); Y_{GrA}, groups acetyl content (g/L); Y_{Gluol}, glucoligomers content (g/L); Y_{Xyol}: xyloligomers content (g/L); Y_{HMF}, Hydroxymethylfurfural content (g/L); Y_{Fur}, Furfural content (g/L). X_T, X_t and X_{ac} denote normalized temperature, time and acid concentration, respectively. The differences between the experimental values and those estimated by using the previous equations never exceeded 10%.

Figure


Eucalyptus Globulus Wood Chips
Raw material characterization
Reactor First Stage
First step:
Modelization and optimization of diluted acid hydrolysis of Eucalyptus globulus with two objetives.
1) High hemicelluloses extraction rate (liquor).
2) Low degradation and extraction of glucan (solid).
Operational conditions selection from first step and obtention of a liquid phase post-hydrolysis (LP-1). Separation of solid phase.
Liquor
Reactor Second Stage
Second step:
Modelization of acid hydrolysis of LP-1.
Optimization of the conversion to furfural
Solid Residue
Furfural



First step:
Modelization of diluted acid hydrolysis of *Eucalyptus globulus* with two objectives:
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Operational conditions selection from first step and obtention of a liquid phase post-hydrolysis (LP-1). **Separation of solid phase.**

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Modelization of acid hydrolysis of LP-1.
Optimization of the conversion to furfural