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[EN]A device for the production of ethanol from lignocellulosic biomass

[EN]Device (100) for obtaining ethanol from lignocellulosic biomass, the device (100) comprising:a closed container (102) for collecting and storing the lignocellulosic biomass such as rice hulls in the form of powder, the powder being obtained by mixing with a mixer, wherein the collected powder is stored at a temperature below room temperature;a pre-treatment chamber (104) in connection with a closed container (102) for performing a variety of pre-treatment processes on the collected rice hull powder, wherein a variety of reagents/chemicals are added to the added to collected rice husk powder to conduct delignification and de-toxification;a filter crucible (106) in communication with the pre-treatment chamber (104) for collecting and filtering the pre-treated rice husk after washing with distilled water under suction and drying at 30-40°C; an enzyme extraction flask (108) in connection ung with the pre-treatment chamber (104) for the extraction of crude cellulase enzyme from Trichoderma reesei, the extraction being carried out by adding (per liter) 40-50 g wheat bran, 10-20 g yeast extract, 5-15 g glucose, 1. 5-3 g NH4Cl, 0.5 g thiamine hydrochloride, 2.0 g K2HPO4, 0.5 g MgSO4.7H2O, 0.1 g CaCl2 and 0.5 g KCl, wherein Trichoderma reesei is incubated and centrifuged to produce a supernatant which is a source of enzyme;a test tube (110) in Connection to the flask (108), mixing 0.5 to 2 ml of 0.05 M sodium citrate pH 4.8 with 0.5 ml of the enzyme to prepare a second solution, placing a strip of filter paper in the test tube and leaving it in for 60 minutes a water bath at 50°C, after 60 minutes the test tube is taken out and the amount of sugars released by the cellulase is determined using the dinitrosalicylic acid (DNA) method;a vessel (112) in connection with the enzyme extraction co lben (108) and the test tube (110) for the enzymatic hydrolysis of 200-400 g of native and delignified rice husk (ie 2% nitric acid (HNO3), 10% HNO3, 2% sodium hydroxide (NaOH) and 10% NaOH), the rice hulls are soaked in a citrate buffer for 1-5 hours prior to addition of the prepared enzymes, with sodium azide being added at a concentration of 0.005% to limit microbial growth during the course of enzyme hydrolysis; andan Erlenmeyer flask (114) in connection with the vessel (112) for preparing ethanol by mixing 20 g/l of pre-treated rice hulls from hydrolysis with 200 ml of citrate buffer (pH 5.0 ± 0.2, 50 mM), followed by sterilization for 15 minutes at 15 psi (121°C) with rice hulls soaked in citrate buffer and supplemented with cellulase at a substrate to enzyme ratio of 1:5 (20 g pretreated substrate: 100 ml crude cellulase), inoculating immobilized yeast cells at various concentrations , fermented and centrifuged to analyze the ethanol concentration.

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FIELD OF THE INVENTION

[0001] The present invention relates to the field of ethanol production. In particular, the present invention relates to an apparatus for the production of ethanol from lignocellulosic biomass such as rice hulls, followed by optimization of the simultaneous saccharification and fermentation parameters by Saccharomyces cerevisiae NCIM 3455.

BACKGROUND OF THE INVENTION

[0002] In recent decades, the various problems related to the global environment and economy have been seriously considered. Therefore, the prevailing scientific research has focused on exploring various manufacturing processes and technologies from renewable resources or raw materials. Rising fossil fuel prices and daunting greenhouse gas emissions have pushed research into alternative and sustainable energy sources to the fore. The production of ethanol and other biofuels from lignocellulosic biomass has gained great importance due to the increasing worldwide energy demand, the depletion of fossil fuels and the dangers to the environment.

US10047299B2 discloses the production of fuel from FCC products. It comprises a hydrotreated catalytic slurry Oil composition having a density at ~15°C from about 0.92 g/cc to about 1.02 g/cc, a T50 distillation point from about 340°C to about 390°C, and a T90 distillation point from about 450°C to about 525° C., wherein the hydrogenated catalytic slurry oil composition has about 1.0% by weight or less of n-heptane insolubles, about 50% by weight to about 70% by weight aromatics, a sulfur content of about 1000 wppm or less and a Hydrogen content from about 10.0% to 12.0% by weight, with a ~700°F (~371°C) portion of the hydrotreated catalytic slurry oil composition containing less than about 5.0% by weight paraffins.

In this day and age it is necessary to new biomass such. B. less used agricultural waste, in the portfolio of biomass raw materials to be incorporated, leading to diversification and ensuring the sustainability of bioethanol production in the future. Due to its overabundance in nature, lignocellulose as a source of biomass has received tremendous attention.

US10723621B2 describes the conversion of organic material into biogas by anaerobic digestion, wherein the biogas is cleaned to obtain a combustible liquid feedstock with methane. A fuel manufacturing plant uses a combustible liquid feedstock to produce renewable hydrogen, which is used to hydrogenate hydrocarbons derived from crude oil in a process to produce transportation or heating fuel. The renewable hydrogen is combined with hydrocarbons from crude oil that have been desulfurized under conditions to hydrogenate the liquid hydrocarbon with the renewable hydrogen,

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or alternatively, the renewable hydrogen can be fed to a reactor operated to simultaneously desulfurize and hydrogenate the hydrocarbons.

However, the **prior** art mentioned above discloses the conversion of organic matter into biogas and then into a combustible liquid containing methane. However, it does not contain any information on the production of fuel for transport.

[0007] Lignocellulosic biomass is today an excellent source for the production of liquid fuels. One possibility in this direction is the physical-chemical and enzymatic degradation of rice husks into fermentable sugars and the subsequent conversion into bioethanol. However, none of the prior art mentioned above describes the production of ethanol from rice hulls.

[0008] Therefore, there is a need for a cost effective and environmentally friendly bio-progressive system for the production of cellulosic ethanol develop from rice husks.

The technical advance **disclosed** by the present invention overcomes the limitations and disadvantages of existing ones and conventional systems and methods.

SUMMARY OF THE INVENTION

The present invention **relates** generally to an apparatus for recovering ethanol from rice hulls. aim of the present Invention is to develop an apparatus for recovering ethanol from hulls at risk. Another object of the present invention is to develop an inexpensive and environmentally friendly device, and Another object of the present invention is to achieve maximum ethanol concentration.

[0011]

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In one embodiment, a device for obtaining ethanol from lignocellulosic biomass, the device comprising: a closed container for collecting and storing the lignocellulosic biomass such as rice hulls in the form of powder, the powder being obtained by mixing with a mixer, the collected powder is stored at a temperature below room temperature; a pre-treatment chamber in connection with a closed container for performing a variety of pre-treatment processes on the collected rice husk powder, adding a variety of reagents/chemicals to the collected rice husk powder to perform delignification and de-toxification; a filter crucible in connection with the pre-treatment chamber for collection and filtering the pretreated rice hulls after washing with distilled water under suction and drying at 30-40°C; an enzyme extraction flask in connection with the pre-treatment chamber for the extraction of crude cellulase enzyme from Trichoderma reesei, the extraction being carried out by adding (per liter) 40-50 g wheat bran, 10-20 g yeast extract, 5-15 g glucose, 1.5-3 g NH4Cl, 0.5 g thiamine hydrochloride, 2.0 g K2HPO4, 0.5 g MgSO4.7H2O, 0.1 g CaCl2 and 0.5 g KCl, wherein the Trichoderma reesei is incubated and centrifuged to produce a supernatant which is an enzyme source; a test tube in connection with the flask, in which 0. 5-2 ml of 0.05 M sodium citrate pH 4.8 is mixed with 0.5 ml of the enzyme to prepare a second solution, placing a strip of filter paper in the test tube and 60 minutes in a water bath at 50°C, after 60 minutes the tubes are removed and the amount of sugars released from the cellulase is determined using the dinitrosalicylic acid (DNSA) method; and a vessel in connection with the enzyme extraction flask for the enzymatic hydrolysis of 200-400 g of native and deligned rice hulls (i.e. 2% nitric acid (HNO3), 10% HNO3, 2% sodium hydroxide (NaOH) and 10% NaOH), the rice hulls before the addition of the prepared enzymes are soaked in a citrate buffer for 1-5 hours, with the addition of sodium azide at a concentration of 0.005% to limit microbial growth during the course of the enzymatic hydrolysis; and an Erlenmeyer flask in connection with the flask for ethanol production by mixing 20 g/L of pretreated rice hulls derived from hydrolysis with 200 mL of citrate buffer (pH 5.0 ± 0.2.50 mM), followed by sterilization for 15 minutes at 15 psi (121°C) with rice hulls soaked in citrate buffer and supplemented with cellulase at a substrate:enzyme ratio of 1:5 (20 g pretreated substrate: 100 ml crude cellulase), inoculating immobilized yeast cells at various concentrations , fermented and centrifuged to analyze the ethanol concentration.

In one **embodiment ,** the collected rice husk is processed into 100 mesh (0.15 mm) fine powder with the mixer at 3000 rpm, keeping the pulverized rice husk in the closed container at 3-5°C to avoid any probable degradation or deterioration.

In one embodiment, the variety of pretreatment processes performed in the pretreatment chamber include steam blast, overliming, neutralization, and charcoal treatment, using the standard methods of overliming (calcium oxide), neutralization (HCl), and charcoal treatment for the detoxification process .

In one embodiment, a vacuum filtration chamber in communication with the pretreatment chamber filters the precipitate and the **post** salts formed during neutralization, leaving the neutralized mixture under normal mixing for 20-40 minutes to obtain a filtered solution.

In one **embodiment ,** an orbital shaker is connected to the pretreatment chamber to form a first mixture after adding 2.5% activated carbon to the filtered solution and continuously mixing for 20-40 minutes, the first mixture being refiltered twice to removing the activated carbon and adjusting the pH to 6.0-6.5.

In one embodiment, the plurality of reagents/chemicals for performing the plurality of pretreatment methods comprises : dilute nitric acid (1-5% v/v), concentrated nitric acid (5-15% v/v), sodium hydroxide (0.1-5% w/v), sodium hydroxide (5-15% w/v) with vapor explosion at 120- 200°C for about 15-30 minutes.

In one embodiment, T. **reesei** is grown separately and inoculated with actively growing and isolated fungi, the masks are incubated for 10 days on a rotary shaker, after 8-12 days of incubation, the culture broth is incubated at 10,000 rpm for 10-30 minutes /min to remove mycelia and spores, collecting the supernatant and using it as an enzyme source, which is stored at 1-10°C.

In one **embodiment ,** the hydrolysis is carried out in a vessel fitted with an agitator for agitation and an outer jacket for water circulation to maintain the required temperature and 3 L of citrate

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buffer (pH 5.0 ± 0.2, 50 mM, 50 ± 0.5°C) at 100 rpm.

In one **embodiment ,** the substrate soaked in the citrate buffer is supplemented with cellulase 5 FPU/g at a substrate to enzyme ratio of 1:5, the samples removed after 48 h, centrifuged and the supernatant for the total reducing sugars released is analyzed estimating the amount of reducing sugars by the DNSA method.

In one **embodiment ,** the immobilized yeast cells are used as an inoculum in various concentrations, namely 2, 4 or 6%. MgSO4, 0.5 g/l; KCl, 0.5 g/l, and FeSO4 0.01 g/l are used as common nutrients in all fermentations, whereby the fermentation

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is carried out for 72 hours, after which the samples are removed and centrifuged in a laboratory centrifuge at 1200 rpm and the supernatants analyzed for ethanol concentration.

In order to further clarify the advantages and features of the present invention, a more detailed description of the invention will be made by reference to specific embodiments thereof illustrated in the attached figures. It is understood that these figures show only typical embodiments of the invention and therefore should not be considered as limiting its scope. The invention will be described and illustrated with additional specificity and detail with the accompanying figures.

LIST **OF**

FIGURES These and other features, aspects and advantages of the present invention will be better understood when the following detailed description is read with reference to the accompanying figures, in which like characters represent like parts throughout the figures, wherein : **FIG** Block diagram of an apparatus for the production of ethanol from lignocellulosic biomass, **Fig. 2** shows a tabular representation of the nutrient components used in various nutrient parameters, **Fig. 3** shows a tabular representation of the sugars released after the steam explosion pretreatment of rice husks, **Fig. 4** Figure 5 tabulates the three-level CCD and experimental responses to the dependent variable Y, and **Figure 5** tabulates the quadratic polynomial model for ethanol production based on ANOVA.

[0023] Those skilled in the art will understand that the elements in the figures are shown for simplicity and are not necessarily to scale were drawn. For example, the flow charts illustrate the method of key steps to enhance understanding of aspects of the present disclosure. Furthermore, one or more components of the device may be represented in the figures by conventional symbols and the figures show only the specific details relevant to an understanding of the embodiments of the present disclosure, in order not to obscure the figures with details overload that are readily apparent to those skilled in the art familiar with the present specification.

DETAILED DESCRIPTION

To promote understanding of the invention, reference will now be made to the embodiment shown in the figures and described in specific words . It should be understood, however, that no limitation on the scope of the invention is intended, and such alterations and further modifications to the illustrated system and such further applications of the principles of the invention set forth therein are contemplated as would occur to those skilled in the art invention would normally come to mind.

It **should** be understood by those skilled in the art that the foregoing general description and the following detailed description are exemplary and illustrative of the invention and are not intended to limit it.

Whenever in this description of "an aspect", "another aspect" or the like, it means that a particular feature, structure, or characteristic described in connection with the embodiment is included in at least one embodiment of the present invention. Thus, the phrases "in one embodiment", "in another embodiment" may be different. and similar expressions in this specification all refer to the same embodiment, but need not do so.

The terms "comprising", "including&quot ; or other variations thereof are intended to be non-exclusive cover, so that a process or method that includes a list of steps does not include only those steps,

but may include other steps not expressly listed or pertaining to such a process or method. Likewise, one or more devices or subsystems or elements or structures or components identified by "comprises...a" are initiated does not exclude, without further limitation, the existence of other devices, or other subsystems, or other elements, or other structures, or other components, or additional devices, or additional subsystems, or additional elements, or additional structures, or additional components.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one skilled in the art to which this invention pertains. The system, methods, and examples provided herein are for purposes of illustration only and are not intended to be limiting.

Embodiments of the present invention are described in detail below with reference to the attached figures.

According to one aspect of the present invention, a sample of rice hulls from rice mills of Bilaspur District (22°05'N 82#09'E/22#09'N 82°15'E), Chhattisgarh, India.

According to another aspect of the present invention, the wild-type strain of Saccharomyces cerevisiae NCIM 3455 and T. **reesei** NCIM 1052 obtained from National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory, Pune, India.

The **yeast** culture is kept on malt-yeast agar medium with the following composition (g/l): malt extract, 3; yeast extract, 3; peptone, 5; glucose, 10; Agar, 20, pH 7.0 ± 0.2. The strain of T. reesei NCIM 1052 is maintained on PDA layers consisting of (g/l): potato, 200; dextrose, 20; Agar, 25, pH 4.8 ± 0.2. Stock cultures are stored at 4°C. The liquid medium for growth of the inoculum for yeast is YEPD medium consisting of (g/l): yeast extract, 10; peptone, 20; Dextrose, 20, pH 5.00 ± 0.2 for 48 hours at 28 ± 0.5°C. The inocula are grown aerobically in 250 ml Erlenmeyer flasks with the above medium at 28°C in a shaker (Remi Scientific) at 200 rpm for 24 hours. The active cells are spun in a clinical centrifuge (1200 rpm), washed with sterile water and used as inoculum.

Fig. 1 shows a block diagram of a device (100) for the production of ethanol from lignocellulosic biomass, wherein the Apparatus (100) comprising: a closed container (102), a pretreatment chamber (104), a filter crucible (106), an enzyme extraction flask (108), a vacuum filtration chamber (104a), an orbital shaker (104b), a test tube (110) and a Vessel (112) and an Erlenmeyer flask (114).

Closed container (102) for collecting and storing the lignocellulosic biomass such as rice hulls in the form of powder, wherein the Powder is obtained by mixing with a mixer, storing the collected powder at a temperature below room temperature. The collected rice hulls are crushed into a powder of 100 mesh (0.15 mm) with the mixer at 3000 rpm, and the pulverized rice hulls are kept in a closed container at 3-5°C to avoid any probable degradation or decay to avoid.

The **pretreatment** chamber (104) in conjunction with a closed vessel (102) for performing a variety of pretreatment processes on the collected rice husk powder, adding a variety of reagents/chemicals to the collected rice husk powder to perform delignification and detoxification. The variety of pre-treatment processes performed in the pre-treatment chamber (104) include the steam explosion, overliming processes, neutralization and activated carbon treatment, using the standard processes of overliming (calcium oxide), neutralization (HCl) and activated carbon treatment for the detoxification process.The variety of reagents /Chemicals to perform the variety of pre-treatment processes includes: dilute nitric acid

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(1-5% v/v), concentrated nitric acid (5-15% v/v), sodium hydroxide (0.1-5% w/v), sodium hydroxide (5-15% w/v) with vapor explosion at 120-200° C for about 15-30 min.

The multiple **pretreatment** chambers (104) consist of: a vacuum filtration chamber (104a) and an orbital shaker (104b).

The vacuum filtration chamber (104a) filters out the precipitate and the salts formed after neutralization, the neutralized mixture is left under normal mixing for 20-40 minutes to obtain a filtered solution.

The orbital shaker (104b) is connected to the pre-treatment chamber (104) to form a first mixture after adding 2.5% activated carbon to the filtered solution and continuously mixing for 20-40 minutes, the

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the first mixture is refiltered twice to remove the activated carbon and the pH is adjusted to 6.0-6.5.

The **filter** crucible (106) in connection with the pre-treatment chamber (104) for collecting and filtering the pre-treated rice hulls after washing with distilled water under suction and drying at 30-40°C. Enzyme extraction flask (108) in connection with the pre-treatment chamber (104) for extracting crude cellulase enzyme from Trichoderma reesei, the extraction being carried out by adding (per litre) 40-50 g wheat bran, 10-20 g yeast extract, 5-15 g glucose , 1. 5-3 g NH4Cl, 0.5 g thiamine hydrochloride, 2.0 g K2HPO4, 0.5 g MgSO4.7H2O, 0.1 g CaCl2 and 0.5 g KCl, wherein Trichoderma reesei is incubated and centrifuged to generate a supernatant, which is a source of enzyme. T reesei is grown separately and inoculated with actively growing and isolated fungi, the masks are incubated for 10 days on a rotary shaker, after 8-12 days of incubation the culture broth is centrifuged at 10,000 rpm for 10-30 minutes to Remove mycelia and spores, collecting the supernatant and using it as an enzyme source, which is stored at 1-10°C.

The test tube (110) in conjunction with the flask (108), **mixing** 0.5 to 2 ml of 0.05 M sodium citrate pH 4.8 with 0.5 ml of the enzyme to prepare a second solution, using a strip of filter paper placed in the test tube and kept in a water bath at 50°C for 60 minutes, after 60 minutes the test tube is removed and the amount of sugars released by the cellulase is determined using the dinitrosalicylic acid (DNA) method.

The **vessel** (112) in connection with the enzyme extraction flask (108) and the test tube (110) for the enzymatic hydrolysis of 200-400 g of native and delignified rice hull (ie 2% nitric acid (HNO3), 10% HNO3, 2% Sodium hydroxide (NaOH) and 10% NaOH), the rice hulls are soaked in a citrate buffer for 1-5 hours prior to the addition of the prepared enzymes, with sodium azide being added at a concentration of 0.005% to inhibit microbial growth during the course of the enzymatic limit hydrolysis.

The **hydrolysis** is carried out in a vessel equipped with an agitator for agitation and an outer jacket for water circulation to maintain the required temperature and containing 3-1 citrate buffer (pH 5.0 ± 0.2, 50 mM, 50 ± 0.5 °C) at 100 rpm.
The substrate soaked in the citrate buffer is treated with 5 FPU/g cellulase in a ratio of substrate to enzyme of 1:5, the samples are taken after 48 h, centrifuged and the supernatant analyzed for the total reducing sugars released, the amount of reducing Sugar is estimated by the DNSA method.

Erlenmeyer flask (114) in connection with the vessel (112) for the production of ethanol pretreated by mixing 20 g / l, by Hydrolysis of recovered rice hulls with 200 mL of citrate buffer (pH 5.0 ± 0.2, 50 mM) followed by sterilization at 15 psi (121°C) for 15 minutes, the rice hulls being soaked in citrate buffer and treated with cellulase in a substrate-enzyme ratio of 1:5 (20 g pretreated substrate: 100 ml crude cellulase), inoculating immobilized yeast cells at various concentrations, fermenting and centrifuging for analysis of ethanol concentration.

The **yeast** is immobilized by the sodium alginate method. The batch attempt is composed according to the central Experimental plan for ethanol production performed in a 500mL Erlenmeyer flask.

Figure 2 shows a tabular representation of the nutrient components used for various nutrient parameters. The parameters such as temperature, inoculum concentration and nutrient factor were selected as the most important. The process is performed with an initial substrate concentration of 20 g/L pretreated substrate (ie rice hulls) and 200 mL citrate buffer (pH 5.0 ± 0.2, 50 mM), followed by sterilization at 15 psi (121°C) for 15 minutes. Cellulase is added to the substrate soaked in citrate buffer at a ratio of 1:5 (20 g pretreated substrate: 100 ml crude cellulase) or 5 FPU cellulase per gram substrate is used for hydrolysis. Saccharification is carried out at 50°C for 24 hours . Then, in the same vessel, simultaneous fermentation is carried out by adding 50 ml of sterilized detoxified hydrolyzate (after pre-treatment) and various nutrients at lower temperatures (30/32/34°C).

[0046] Immobilized yeast cells are used as inoculum in various concentrations, namely 2, 4 or 6%. MgSO4, 0.5 g/l; KCl, 0.5 g/l, and FeSO4 0.01 g/l are used as general nutrients in all fermentation experiments, except for the nutrient parameters above. The fermentation lasts 72 hours. Thereafter, the samples are removed and centrifuged in a laboratory centrifuge at 1200 rpm and the supernatants are analyzed for their ethanol concentration.

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Total reducing sugars are determined using the dinitrosalicylic acid method. The quantitative and qualitative analysis of the sugars in the hydrolysates after the pre-treatment is carried out using high-performance liquid chromatography (HPLC) with a C18 column and an SPD 20A UV detector at 284 nm and a data processor with a register. Before injection, the samples are filtered through a 0.45 ÿm membrane filter (Millipore). The temperature of the column is kept at 35 °C, the injection valve is set to 20 µl. The UV detector is operated at 40 °C. Water (90%) and methanol (10%) as the mobile phase are used as solvents at a flow rate of 1 mL/min. All experiments were performed in duplicate and all results reported are means. The average standard deviation of the results obtained is less than 4%.

In the central composite design (CCD), the total number of experimental combinations is 2K + 2K + **n0 ,** where K is the number of independent variables and n0 is the number of repetitions of the experiments at the central point, which means that for this procedure requires 20 attempts. The dependent variable chosen for this study is ethanol concentration, Y (g/L). The incubation temperature (30, 32 and 34°C) X1, the inoculum content (2, 4 and 6%) X2 and the nutrients (1/2/3) were used as independent variables.
X3 chosen. A mathematical model is developed that describes the relationships between the process dependent variable and the independent variables in a second-order equation. Experimental data are fitted according to the following second order polynomial equation (1). Y=bo+i=1kbixi+i=1kbijx2i+ii<jk i<jkbijxixj+e where i and j are linear and quadratic coefficients, respectively, while "b" is the regression coefficient, k is the number of examined and optimized factors in the experiment, and "e" is the random error. The quality of fit of the second-order equation is expressed by the R2 coefficient of determination, and its statistical significance is determined by the F-test. The significance of each coefficient is determined using Student's t-test. Student's t-test is used to determine the significance of the regression coefficients of the parameters. The P-values (probability value) are used as a tool for verification

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of the significance of the interaction effects, which in turn can provide clues to the patterns of interactions between the variables. In general, larger values of t and smaller values of P indicate that the corresponding coefficient is significant.

Figure 3 shows a tabular representation of the sugars released after steam explosion pretreatment of rice hulls. From all Chemicals, steam explosion pretreatment of bagasse with HNO3 (6% v/v) resulted in maximum hydrolysis.

The **hydrolyzate** obtained after this pretreatment consisted of 11.58% xylose, 6.35% glucose and 4.52% fructose. During the pre-treatment of lignocellulose, there is a significant reduction in the lignin content and decrystallization of the cellulose. Part of the hemicellulose is also reduced during the pretreatment, which affects the micro and macro accessibility of the cellulases to the cellulose.
In addition, the pre-treatment provides an improved surface area for the catalytic reactions that can take place.

The **acidic** reaction causes the conversion of cellulose to glucose and hemicellulose to xylose components. Xylose, a hemicellulose sugar, is the largest sugar released in the filtrate. The amorphous nature of hemicellulose is responsible for its easy degradation. However, the crystalline nature of cellulose does not allow it to be readily degraded by mineral acids, especially when their concentration is low. The steam explosion pretreatment with 6% HNO3 has shown that the easily hydrolyzable fraction of the xylan is degraded. The sugar remained more concentrated at an average hydrolyzate volume of 10.0 ml. On a large scale, where the volume of hydrolyzate is larger, the same condition would be reversed.

This **pretreated** biomass can consist of up to 90% cellulose and thus support the enzymatic hydrolysis considerably. Cellulosic microfibrils appear to be more amenable to enzymatic attack in cell walls with little or no lignification. The conversion of cellulose into glucose by enzymatic hydrolysis is one of the most important steps in the conversion of cellulosic biomass to produce biofuel. In this study, the enzymatic hydrolysis of rice hulls pretreated with 6% HNO3 is performed to depolymerize the carbohydrate fraction of the cell wall into fermentable sugars. The enzyme activity (U/ml) of the crude cellulase obtained from T.reesei NCIM 1052 is 311.1 imole/ml/min and is loaded at 5 FPU/g substrate. The enzymatic hydrolysis is then carried out with physical parameters (50±0.5° C., 100 rpm). As expected, cellulose turnover increased with increasing incubation time. Throughout the course of the enzymatic hydrolysis, there is a regular increase in the released sugars up to 60 hours, after which it remains constant. The enzymatic saccharification of mit

Rice hulls pretreated with 6% HNO3 yielded a maximum of 150.10 ± 0.38 mg/g (1.5 ± 0.40 g/l) glucose with a degree of hydrolysis of 60 ± 0.34% after 60 hours of treatment.

An **enzyme** concentration of 5 FPU/g substrate has proven to be suitable for breaking down the cellulose present in the pretreated rice husk.

[0054] Saccharomyces species are being explored for bioethanol production from various substrates. Key process variables for ethanol production from pretreated substrates are optimized using the Response Surface Method (RSM) based on CCD (Central Composite Design) experiments.

Figure 4 shows a tabular representation of the three-level CCD and the experimental responses of the dependent variable Y. The three-level matrix of the central composite design and the experimental responses of the dependent variable (ethanol concentration) are shown in **Figure 4 .**

Figure 5 shows a tabular representation of the quadratic polynomial model for ethanol production based on ANOVA.
In addition to the linear effect of the ethanol concentration Y g/l, the response surface method also gives insight into the quadratic and combined effects of the parameters. The analyzes are performed using the statistical instruments Fisher's F-test and Student's t-test. The regression coefficients, t and P values for all linear, quadratic and combined effects at the 95% level of significance are presented in **Figure 5 .**

In this case, A, B, C, AB, AC, BC, A2, B2, C2 are significant model terms. Values >0.1000 indicate that the model terms are not significant. ANOVA of the regression model for ethanol yield showed that the model is significant due to a very high F value and a very low probability value. The regression equation obtained from the ANOVA shows that the R2 (measure of certainty) is 0.9202 (a value of >0.75 indicates the significance of the model). This is an estimate of the proportion of the total variation in the data that is explained by the model and thus the model is able to explain 99.9% of the variation in the response. The "adjusted R2" is 0.8484, indicating that the model is good (for a good statistical model, the R2 value should be in the range 0-1.0, and the closer the value is to 1.0, the more fit the model is than good). The ´´Predicted R2” of 0.8034 is in reasonable agreement with the "Adjusted R2" of 0.8484, ie the difference is <0.2. The ´´Reasonable Accuracy” measures the signal-to-noise ratio. A ratio of >4 is desirable. A ratio of 13,427 indicates a reasonable signal. Therefore, this model can be used to navigate the design space.

The **ethanol** concentration rose steadily with increasing inoculum concentration up to 6% and temperature up to 32°C. a higher one Thus, inoculum concentration and higher temperature increase the ethanol yield.

[0059] For ethanol production from rice hulls, a single study was conducted at the modular fermenter level. The reaction analysis showed that the maximum ethanol concentration (6.24 g/l) of S. cerevisiae NCIM 3455 could be reached from rice husk under the optimal process conditions. For fermentation, the optimal values for the independent variables according to RSM are 32°C incubation temperature, 6% inoculum concentration and the nutrient factor 2. Based on the device, the optimal working conditions are determined to achieve a high ethanol yield. These optimized parameters were set for 72-hour ethanol production in the modular fermentor.
Gas-Liquid Chromatography (GLC) results showed that the fermented broth of the modular fermentor had an ethanol concentration of 6.02 g/L after 72 hours.

It is concluded that rice hulls are a potential, renewable and inexpensive biomass for the production of **ethanol** fermentation are. Rice husks pretreated with 6% HNO3 showed the highest saccharification. From this it can be concluded that cellulosic ethanol is obtained by simultaneous saccharification and fermentation of rice husks with the yeast S. cerevisiae NCIM 3455 under the optimized process conditions in anaerobic batch fermentation. Further research is needed on other combinations and on improving various aspects of this method towards higher cellulosic ethanol yields, as well as on feasibility studies and commercial viability. The ease of availability and cost-effectiveness make rice hulls popular. Their excess availability and renewability symbolize a real advantage over dwindling fossil fuels for bioethanol production.

Using rice hulls could solve the disposal problem and reduce waste treatment costs.

The **figures** and the preceding description give examples of embodiments. Those skilled in the art will understand that one or several of the elements described into one

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functional element can be combined. Alternatively, certain elements can be broken down into multiple functional elements.
Elements from one embodiment may be added to another embodiment. For example, the order of the processes described herein may be changed and is not limited to the manner described herein.

Also, the acts of a flowchart need not be performed in the order presented ; also, not all actions have to be performed. Also, the actions that are not dependent on other actions can be performed in parallel with the other actions. The scope of the embodiments is in no way limited by these specific examples. Numerous variations are possible, regardless of whether they are explicitly mentioned in the description or not, e.g. B. Differences in structure, dimensions and use of materials. The scope of the embodiments is at least as broad as indicated in the following claims.

[0063] Advantages, other advantages, and solutions to problems have been described above with respect to particular embodiments. However, the benefits, advantages, problem solutions, and components that can cause an advantage, benefit, or solution to occur or become more pronounced are not to be construed as a critical, required, or essential feature or component of any or all claims.

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Vessel
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Erlenmeyer flask

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QUOTES INCLUDED IN DESCRIPTION

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Patent Literature Cited

[0000] US 10047299 B2 [0003] US 10723621 B2 [0005]

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[1] Device (100) for obtaining ethanol from lignocellulosic biomass, the device (100) comprising: a closed container (102) for collecting and storing the lignocellulosic biomass such as rice hulls in the form of powder, the powder being mixed with a mixer, the collected powder being stored at a temperature below room temperature; a pre-treatment chamber (104) in communication with a closed vessel (102) for performing a variety of pre-treatment processes on the collected rice husk powder, wherein a variety of reagents/chemicals are added to the collected rice husk powder to perform delignification and de-toxification; a filter pot (106) in communication with the pre-treatment chamber (104) for collecting and filtering the pre-treated rice hulls after washing with distilled water under suction and drying at 30-40°C; an enzyme extraction flask (108) in connection with the pre-treatment chamber (104) for the extraction of crude cellulase enzyme from Trichoderma reesei, the extraction being carried out by adding (per liter) 40-50 g wheat bran, 10-20 g yeast extract, 5-15 g glucose , 1. 5-3 g NH4Cl, 0.5 g thiamine hydrochloride, 2.0 g K2HPO4, 0.5 g MgSO4.7H2O, 0.1 g CaCl2 and 0.5 g KCl, wherein Trichoderma reesei is incubated and centrifuged to produce a supernatant which is an enzyme source; a test tube (110) in connection with the flask (108) wherein 0.5 to 2 ml of 0.05 M sodium citrate pH 4.8 is mixed with 0.5 ml of the enzyme to prepare a second solution with a strip of filter paper in the test tube placed and maintained in a water bath at 50°C for 60 minutes, after 60 minutes the test tube is removed and the amount of sugars liberated by the cellulase is determined using the dinitrosalicylic acid (DNSA) method; a vessel (112) in connection with the enzyme extraction flask (108) and the test tube (110) for the enzymatic hydrolysis of 200-400 g of native and delignified rice hull (i.e. 2% nitric acid (HNO3), 10% HNO3, 2% sodium hydroxide (NaOH) and 10% NaOH), the rice hulls being soaked in a citrate buffer for 1-5 hours prior to the addition of the prepared enzymes, with sodium azide being added at a concentration of 0.005% to limit microbial growth during the course of enzyme hydrolysis; and an Erlenmeyer flask (114) in connection with the vessel (112) for ethanol production by mixing 20 g/l of pre-treated rice hulls from hydrolysis with 200 ml of citrate buffer (pH 5.0 ± 0.2, 50 mM), followed by 15 minutes Sterilization at 15 psi (121°C) with rice hulls soaked in citrate buffer and supplemented with cellulase at a substrate to enzyme ratio of 1:5 (20 g pretreated substrate: 100 ml crude cellulase) using immobilized yeast cells at various concentrations inoculated, fermented and centrifuged for analysis of ethanol concentration.

[2] The apparatus according to claim 1, wherein the collected rice hulls are made into 100 mesh (0.15 mm) thick powder by the mixer at 3000 rpm be processed, keeping the powdered rice husk in the closed container at 3-5°C to avoid any probable degradation or decay.

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The apparatus of claim **1 ,** wherein the plurality of pretreatment processes performed in the pretreatment chamber (104) include steam explosion, overliming, neutralization and charcoal treatment, using the standard methods of overliming (calcium oxide), neutralization (HCl) and charcoal treatment for the detoxification process .

[4] Apparatus according to claim 3, wherein a vacuum filtration chamber (104a) in communication with the pre-treatment chamber (104) filters out the precipitate and salts formed after neutralization, leaving the neutralized mixture under normal mixing for 20-40 minutes to to obtain a filtered solution.

[5] Apparatus according to claim 3, wherein the pre-treatment chamber (104) is associated with an orbital shaker (104b) to form a first mixture after adding 2.5% activated carbon to the filtered solution and to mix continuously for 20-40 minutes, wherein the first mixture is refiltered twice to remove activated charcoal and pH is adjusted to 6.0-6.5.

[6] The apparatus of claim 1, wherein the plurality of reagents/chemicals for performing the plurality of pretreatment processes comprises: dilute nitric acid (1-5% v/v), concentrated nitric acid (5-15% v/v), sodium hydroxide (0.1 -5% w/v), sodium hydroxide (5-15% w/v) with steam explosion at 120-200°C for about 15-30 min.

[7] The device of claim 1, wherein T. reesei is cultured separately and inoculated with actively growing and isolated fungi, the masks being incubated for 10 days on a rotary shaker, after 8-12 days of incubation the culture broth for 10-30 minutes at 10000 rpm cent

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is rifuged to remove mycelia and spores, the supernatant being collected and used as an enzyme source, which is stored at 1-10°C.

[8] The apparatus of claim 1, wherein the hydrolysis is carried out in a vessel equipped with an agitator for agitation and an outer jacket for water circulation to maintain the required temperature, and containing 3-1 citrate buffer (pH 5.0 ± 0.2, 50 mM, 50 ± 0.5°C) at 100 rpm.

[9] The device of claim 1, wherein the substrate soaked in the citrate buffer contains cellulase 5 FPU/g at a substrate to enzyme ratio of 1:5, samples are taken after 48 h, centrifuged and the supernatant analyzed for the total reducing sugars released, estimating the amount of reducing sugars by the DNSA method.

[10] The device according to claim 1, wherein the immobilized yeast cells as an inoculum in different concentrations, namely 2, 4 or 6%, be used. MgSO4, 0.5 g/l; KCl, 0.5 g/l, and FeSO4 0.01 g/l are used as usual nutrients in all fermentations, the fermentation being carried out for 72 hours, after which the samples are taken and centrifuged in a laboratory centrifuge at 1200 rpm and the supernatants are discarded the ethanol concentration can be analyzed.

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