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Inscrita en el Departament de Justícia de la Generalitat de Catalunya amb el núm. 31681 - NIF: G64126311

BARCELONA, DRASSANES PER ÀFRICA®

Memòria d'activitats 2026

31 de desembre de 2026

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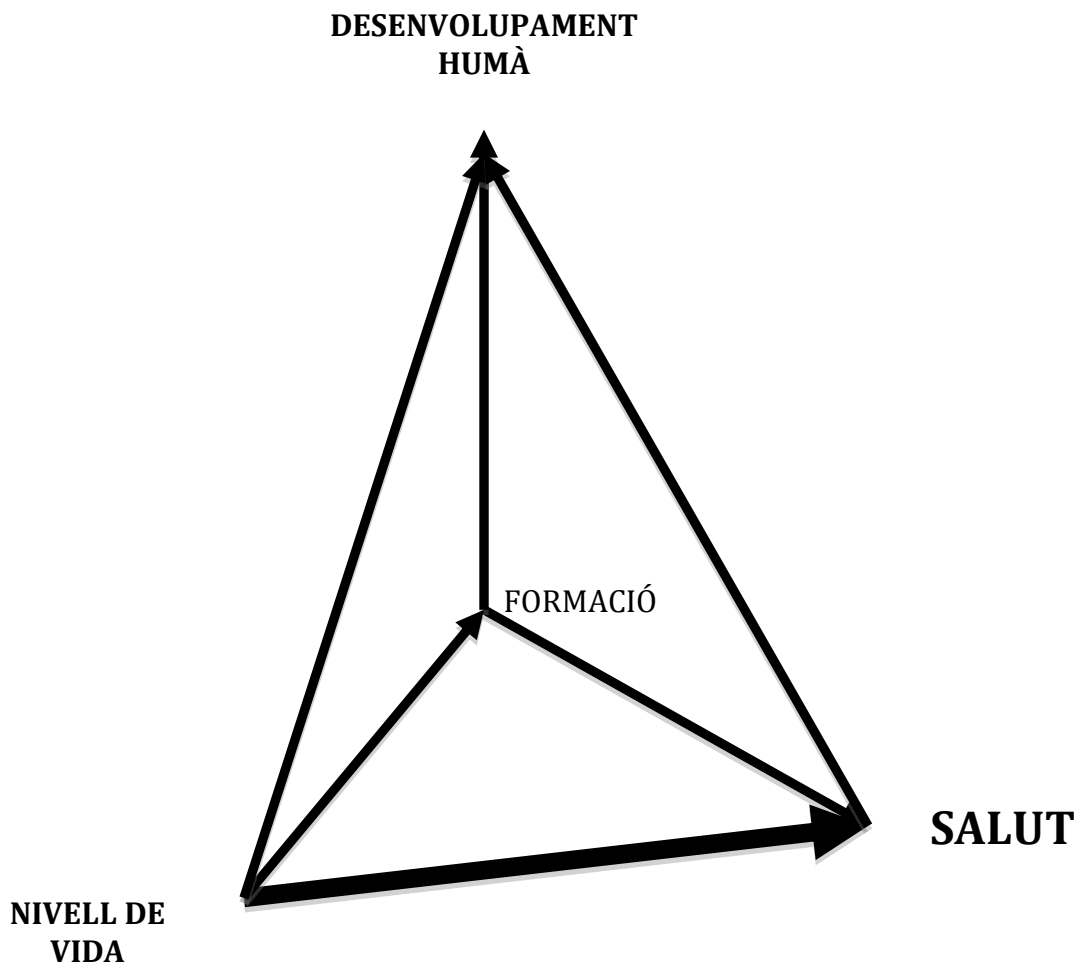
MODEL SDRCA EN COOPERACIÓ INTERNACIONAL



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La nostra missió

Difondre les realitats culturals africanes, crear intercanvis i sinèrgies enriquidores cap a una societat intercultural, i allotjar nuclis de riquesa en països africans.

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Properties of Brewers' Spent Grains

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Journal of Biotechnology and ots Applications
Submited, March 27, 2026

INTRODUCTION [1-5]

The beer making process includes the following stages: malting of barley, maceration of malt, and fermentation of the wort.

Brewers' Spent Grains (BSG) are the set of residual insoluble materials that, during the brewing process, are separated from the wort by filtration at the end of the malt maceration stage.

For the manufacture of beer, varieties of Hordeum vulgare, L. and Hordeum distichon are cultivated, in the corresponding grains of barley the following parts can be distinguished: husk (7-13%), pericarp and testa (<1%), aleurone layer (1-3%), endosperma (80-85%), and embryo (3-5%).

Brewers' Spent Grains (BSG) can be considered, within certain limits, as a by-product of relatively constant composition over time and largely invariable with the geographical location of the breweries.

Gross chemical composition described for BSG (ETSEIB, Barcelona, 1986):

188 g/kg cellulose
246 g/kg hemicelluoses (only arabinoxylans)
68 g/kg starch
11 g/kg soluble sugars
83 g/kg fatty substances
250 g/kg nitrogen-containing substances (proteins)
39 g/kg mineral substances

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123 g/kg other substances without nitrogen (lignins)

BSG carbohydrates (Biotechnology & Bioengineering, 1987)

155 g/kg anhidroxylose
75 g/kg anhidroarabinose
12 g/kg anhidrogalactose
5 g/kg anhidromannose
0 g/kg anhydroramnose
0 g/kg anhidrofucpse
188 g/kg anhydroglucose

BSG proteins (S.A. DAMM, Barcelona, 1983):

16.7% arginine
11.1% lisine
6.6% cistine
4.4% metionine
3.7% triptofane

Summarizing: 50% carbohydrates, 25% proteins (very rich contents of nutrients for livestock, for instance)

BSG as livestock nutrients (metabolizable energy)

10.9 kJ/kg for ruminants
9.2 kJ/kg for poultry
7.0 kJ/kg for pigs

BSG conservation processes:

- Dehydration from 71.2% moisture to 11.2% (ETSEIB, 1983) [dry]
- Ensilage with SILODOSE™ (Cayla, Toulouse, 1985) [wet as received]
- Ensilage with H_3PO_4 for human feed, to pH 3.5 (SDRCA, 2019) [wet as received]

EXPERIMENTAL [1, 4, 5]

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A sample of BSG is separated once the malt maceration is finished. just at the moment of unloading one of the press filters of the brewery that S.A. DAMM had at Carrer del Rosselló 515 in Barcelona. The BSG is a water-containing material that comes out at 74ºC.

in the ETSEIB, using plastic bags, they are packaged, frozen and preserved at -20ºC. This prevents the fermentative decomposition of the moisture-containing BSG otherwise inevitable at ambient temperature.

It is obtained by its subsequent drying. A material with a minimum moisrure content is obtained thar facilitates its use and conservation.

To avoid possible thermal degradation of the material, a very low temperature is chosen, but high enough to avoid microbiological degradation.

Moisture determination

A quantity of wet BSG from the sampling is dried at 95ºC under 100 Hg mm (vacuum oven) until constant mass is achieved. The quantity of wet BSG are equivalent to about 2 g of dry matter. Capsules of at least 50 mm diameter and 40 mm depth are used. The mass loss of the wet BSG represents 71.2% of the moisture content.

Using capsules like the previous ones, about 2 g of BSG dried at 60ºC (substrate) are weighed, after having cooled in the laboratory environment. The sample is dried at 135ºC (simple oven) for 2 h.

The mass loss of the dried BSG represents 11.2% of moisture

Neutralizing character

Dried hay, followed by washing and drying, shows a relatively high neutralizing power.

Although this neutralizing property can be attributed to the surface hydroxyl groups, the fact that purified celluloses absorb materials that present this

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property to a lesser extent, suggests that other components with basic characteristics contribute in hay.

This neutralizing character is detected by adding BSG in dilute sulfuric acid solutions. Following the experimental results obtained in acid hydrolysis, it is postulated that the capture of protons takes place in a reversible way.

The addition of dried BSG to buffered solutions of citric acid and sodium citrate of different pH again demonstrates the neutralizing property of natural lignocellulosic materials.

pH citric/citrate	pH 98,5 g BSG / L citric/citrate
2.0	3.3
3.0	3.7
4.0	4.1
5.0	4.9
6.0	6.0
6.5	6.5

Since the pectines, a group of amorphous, water-soluble polymers that constitute the intercellular cement, contain galacturonic acids, the cells are said to have a polyanionic envelope.

On the other hand, D-glucuronic acid, as well as 4-O-methyl-D-glucuronic acid, is a common constituent of xylan chains. The presence of this large number of carboxylic groups confers the property of exchanging ions with liquids, explaining the retention of mineral elements.

As a consequence, the neutralizing property of natural lignocellulosic materials, in addition to the hydroxyl groups that tend to protonate in acidic environments, could be largely explained by the presence of these carboxylates, which are essentially basic.

The fact that washed hay has a lower neutralizing power than unwashed hay would be interpreted by the elimination of pecines. The difference between a

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solely cellulosic material and another containing hemicelluloses would be explained by the uronic acids incorporated into the xylan chains.

Granulometric analysis

Dried BSG (substrate) were sieved with a Retsch sieve equipped with a series of standardized sieves of 0.090, 0.120, 0.170, 9.250, 0.400, 0.500, 0.750, 0.999, 1.200. 2.000, and 2.800 mm mesh light

Based on the experimental data obtained, the average diameters based on mass (0.975 mm), specific surface area (0.697 mm), and volume (0.436 mm) are evaluated, which are calculated from the corresponding cumulative diagram.

Contribution of BSG particles of different grain sizes to enzymatic hydrolysis

Slurries preparation

The high amount of water that accompanies the BSG separated from the brewing process makes it necessary to consider drying it. In this way, a substrate with a minimum and stable moisture content is available, which facilitates its handling in the usual laboratory conditions.

BSG mixtures with enzyme complex preparations are handled as follows. A given mass of substrate is introduced into the reactor. Separately, a 0.05M citric/citrate pH buffer solution is prepared and poured into the reactor containing dry BSG.

The initial concentration of BSG B_0 is expressed according to

$$B_0 = 1{,}000\ b\ (1-h) / (b \cdot h + V)$$

where b is the mass of substrate in g, h its humidity per unit, and V is the volume of pH buffer solution in mL

The slurry is stirred for 1 h to obtain a homogeneous mixture, during which the pH is monitored. The neutralizing nature of BSG causes the pH to increase with

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respect to that of the prepared buffer solution and, therefore, is adjusted using a few drops of concentrated HCl.

The hydrolysis process begins after the addition of powdered enzyme complex, while maintaining the agitation of the reaction system.

The initial concentration of ebzimaric complex E_0 is also expressed in g/L according to

$E_0 = 1{,}000\ e\ /\ (b \cdot h + V)$

where e is the complex mass in g

Enzymatic hydrolysis of different grain sizes

Considering the BSG particles as spheres, one can define the surface concentration by $c_S = 6\ /\ d_i$

For the same concentration of 22.1 g/L of BSG particles, the corresponding Eadie representation can be done

$v_{0i} = k_3' \cdot E_0 - K_M' \cdot v_{0i} \cdot d_i$

where v_{0i} is the inicial velocity as a function of the initial mean diameter d_i.

The Eadie plot demonstrates that enzymatic hydrolysis of BSG takes place on the particles surface.

Slurry sterility

The use of chemicals to prevent microbial growth during enzymatic hydrolysis of lignocelluloses has been widely considered. In this way, an energetically expensive step is avoided, while being able to work with conventional reactors without additional precautions of sampling and enzyme addition.

However, the use of these chemicals (such as sodium azide) prevents the subsequent acetobutylic fermentation of the hydrolysates.

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Based on the data obtained, it should be noted that, during the incubation period at 50ºC, the enzymes experience at least 2 types of deactivation as a consequence of (1) temperature and (2) formol.

These facts are expressed by

$-dE/dr = k_F \cdot E \cdot F$

where E is the enzyme concentrarion and F represents also the formol concentration. It was therefore evaluate k_F as 0.0085/h based on our own experimental data.

Hydrolysates fermentation to ABE

Acetobutylic fermentation has been extensively studied considering its microbiology, growth requirements, and product effects. The production of ABE has been considered in batch, semi-continuous, and continuous processes. At the same time, the production of solvents by *Clostridium acetobutylicum* has been analyzed from a biochemical and genetic point of view.

The strain *Clostridium acetobutylicum* ATCC824 is maintained in 38 g/L solutions of Oxoid Reinforced Clostridial Medium (RCM). The cultures are grown for one week at 37°C and once sporulated they are stored at 5°C. Enzymatic hydrolysates are prepared to verify that the effects of formol, added as a biocide in the enzymatic hydrolysis step, can be eliminated.

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DISCUSSION

The initial velocities relative to the mean diameters of 0.149 and 0.214 mm are kept out of the regression analysis due to their differential behavior with respect to the others. These differences are attributed to different relative compressions of both fractions, probably with a higher starch content. However, this discrepancy is not considered too important when considering their low relative weight.

In an industrial environment, such as BSG slurries, it must be understood that the effective concentration of formol with respect to enzymes is lower than the analytical one because formol interacts with other proteins, inhibiting microbial growth. Bacteria, yeasts and fungi were initially detected.

The hydrolysates are obtained from 100 g/L of BSG after 48 h with 8 g/L of *Penicillium occitanis* complex at 50ºC and pH 4.8

These hydrolysates (8.8 g/L GLU, 24.7 g/L XYL, 12.0 g/L ARA) yield 1.3 g/L ABE (72.1% BUT, 10.5% ACE, 8.4% ETH) by acetobutylic fermentation.

CONCLUSIONS

By introducing the average diameter in specific surface area (d_S), evaluated for BSG particles (0.697 mm) in the determined rate equation, a theoretical initial rate of reducing sugar release of 1.06 g/L/h is obtained, which is in agreement with the experimentally observed (1.13 g/L/h).

$$V_{0i}^{\#} = 2.0770 / (1+ 1.382 \cdot d_i)$$

The analysis of yields after 24 h as a function of the average diameter of the BSG particles suggests a logarithmic relationship between coordinates

$$\mathbf{Ln}\ y_{24i}^{\#} = 1.9857 - 0.1856\ \mathrm{Ln}\ d_i$$

Therefore, if grinding the BSG particles to 1/2 d_S were considered as a pretreatment, the expected initial sugar release rate would be 1.32 times higher and the yield 1.14 times higher.

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In other words, if d_S were 0.325 mm, the performance achieved in 24 h with d_S = 0.697 mm would be achieved in 9 h, which is a 62.5% less time.

As a result, within the technical possibilities, milling is presented as a solution to reduce the costs of obtaining reducing sugars both in terms of reducing treatment time (which is equivalent to reducing capital investment and labor costs and increasing enzyme recovery) and in terms of increasing the yield and concentration of sugars (which corresponds to an increase in the effective substrate concentration and the possibility of reducing enzyme consumption).

In this sense and according to the deactivation rate constants, it can be considered that the addition of formalin contemplated (4.78 mL/L) has an effect comparable to the action of heat.

It is concluded that the enzymatic hydrolysates of BSG are fermentable after removing the formol by autoclaving.

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ACKNOWLEDGEMENTS

Facultat de Química (Universitat de Barcelona), ETSEIB (Universitat Politècnica de Catalunya), Institut d'Estudis Catalans (Barcelona), S.A. DAMM (Barcelona), La Seda de Barcelona, S.A. (Barcelona), Société CAYLA (Toulouse), AKZO Research Laboratories (Arnhem)

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