ISSN: 2755-0168

Journal of Biotechnology & Bioinformatics Research

SCIENTIFIC Research and Community

Review Article

Open Access

Enzymatic Hydrolysis of Brewers' Spent Grains

Josep Juanbaro

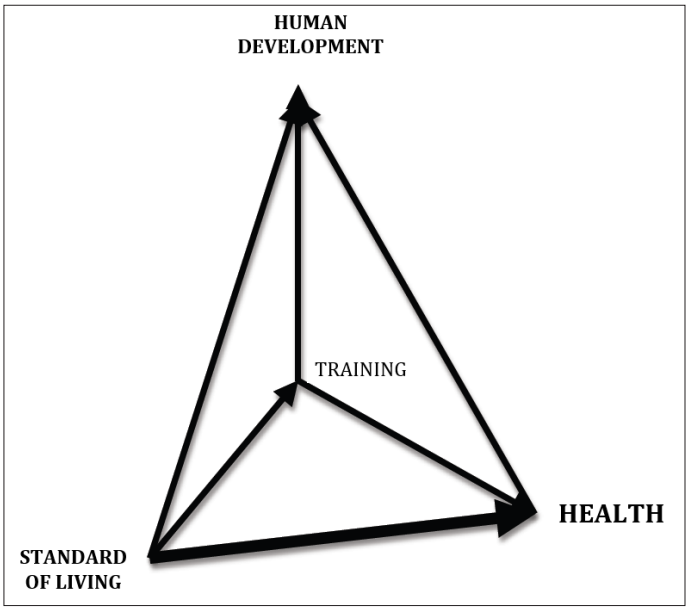
SDRCA.es, Barcelona, Spain

***Corresponding author**
Josep Juanbaro, SDRCA.es, Barcelona, Spain.

Received: April 02, 2026; **Accepted:** April 10, 2026; **Published:** April 16, 2026

BARCELONA, SHIPYARDS FOR AFRICA
Activity Report 2026
December 31, 2026

SDRCA MODEL IN INTERNATIONAL COOPERATION



Our mission
Disseminate African cultural realities, create enriching exchanges and synergies towards an intercultural society, and host centers of wealth in African countries.

Introduction
The conditions that must be used in industrial applications of enzyme systems are generally quite different from those in which they are analyzed through basic biochemical studies.

However, although a series of compromises must be reached for technical and economic reasons and in order for these processes to be controlled by factors other than those found with pure enzyme substrates in dilute solutions, it is necessary to have a precise biochemical knowledge of the polysaccharolytic systems involved [1-5].

The most important parameters that affect the speed and extent of enzymatic hydrolysis reactions are

- The chemical structure of the polysaccharide, including the nature of the monomeric units, the types of linkages, the branching of the chains, the molecular size
- The physical structure of the polysaccharide, such as crystallinity and surface of attack
- The other polymers adjacent to the substrate
- The characteristics of the enzyme
- The substrate concentration
- The type and concentration of products
- The chemical and physical operating conditions, such as pH, temperature and agitation
- The other products present (inhibitors, activators, stabilizers)

In general, industrial hydrolysis processes require a first stage of breaking down the physical structure of the substrate. In technical applications, high concentrations of substrate and products are desired, in order to achieve low product recovery costs and small reactor volumes.

If the final products are not removed by physical means (ultrafiltration) or biochemical means (fermentation), high substrate levels imply high concentrations of products that hinder the complete performance of their hydrolysis, giving rise to increased inhibition per product.

In addition, high concentrations of sugars favor transglycosylation. On the other hand, highly concentrated substrate slurries pose problems for their handling and reactor design.

The characteristics of the enzymes affect the design details of the process. The enzymes can be applied sequentially or they must be applied simultaneously.

In a series of consecutive reactions, the optimal conditions (pH, temperature) for each step can be determined. On the other hand, a compromise must be found when several enzymes are used simultaneously, where the optimum is not necessarily the most suitable for each particular activity.

The production of fructose syrups from starch is an industrial example of the sequential application of enzymes (a-amylase,

glucoamylase and immobilized glucose isomerase). For the hydrolysis of cellulose, the use of the cellulase complex is required, as well as hemicellulases for arabinoxylans.

However, considering that more than 50 years ago diastases were also considered an enzyme complex, it remains unproven today whether the application under appropriate conditions of the enzymes that make up the cellulase complex could give a better performance.

Polysaccharolytic Enzymatic Complexes

Diastases: Glucosylhydrolases and glycosyltransferases are the 2 families of enzymes related to the enzymatic modification of starch.

Glucosylhydrolases are classified into 3 groups of glucosidases:

- Amylases or enzymes specific for a-1,4 linkages (a-amylase, b-amylase)
- Debranching enzymes or enzymes specific for a-1,6 linkages (R-enzyme, pullulanase, isoamylase)
- Enzymes specific for a-1,4 or a-1,6 linkages (glucoamylase)

The physical structure of starch determines the enzymatic degradation activities. Thus, the hydrolysis of granular starch in aqueous suspension is limited by its surface area. Once gelatinization is achieved, the substrate becomes much more accessible to enzymes.

Cellulases: In the production of chemicals and fuels, cellulose hydrolysis has been one of the most widely considered approaches for the transformation of pulp into low molecular weight compounds that can serve as fermentation substrates.

The biological degradation of cellulose consists of a hydrolysis catalyzed by cellulases, enzyme complexes produced by fungi or bacteria (myxobacterial, actinomycetal and eubacterial).

Although the commercial application of these enzymes is still insignificant and it is difficult to predict when they will be economically profitable, the study of these systems is one of the most intensive research disciplines in microbial enzymology.

Unlike acid hydrolysis, the attack on cellulose is localized, given the large molecular size of the enzymes which, therefore, cannot diffuse as quickly into it.

This contributes to the lower loss of mass (4% versus 25%) and degree of polymerization (4,200 versus 225) compared to acid hydrolysis [cotton pulp with an average degree of polymerization of 4,760 despite the decrease in mechanical resistance of the cellulose.

The enzymatic hydrolysis of cellulose is significantly affected by its structural characteristics. Thus, viscose rayon is less attacked the greater the strain applied in the manufacturing process.

It is therefore necessary to bear in mind the multiplicity of the substrate, where some areas are more susceptible to attack. Among them are the amorphous regions and specific layers of crystalline cellulose, such as ends, folds and dislocations. Each region is differently sensitive to enzymatic attack in progressive periods of degradation.

Hemicellulases: The level of knowledge of hemicellulases and their mode of action is less than that of the cellulase system.

Experimental

The Penicillium occitanis complex allows obtaining the highest concentrations of reducing sugars, glucose, xylose and arabinose from BSG after 24 h of BSG hydrolysis when compared with the action, under the same conditions, of the Trichoderma reesei CL 847 complexes and of the complex of a strain of the thermophilic fungus Talaromyces emersonii [1,4,5]. It is also the one that gives the lowest concentration of oligosaccharides (Table 1).

Table 1: Concentrations of Sugars Achieved After 24 h Hydrolysis of 314 g/L of BSG at pH 4.8 and 50ºC by means 2 g/L Enzymaric Complexes from Different Sources

Sugars	Trichoderma Reesei CL847	Talaromyces Emersonii	Penicillium Occitanis
	Conc. g/L	Conc. g/L	Conc. g/L
Reducing	43.5	31.0	57.5
Oligosac.	15.1	12.3	10.0
Glucose	9.8	4.3	15.2
Xylose	9.2	8.2	13.0
Atabinose	4.4	2.0	10.4

Table 2: Specific Yields of Sugars Achieved Alter 24 h of hydrolysis of 98.5 g/L BSG at pH 4.8 and 50ºC by means of 2.0 g/L of Enzymaric Complexes from Different Trichoderma Reesei Strains

Sugars	Caylase	*b6* S6	*CL* 847
	Yield / %	Yield / %	Yield / %
Reducing	16.4	15.5	13.2
Oligosac.	6.1	4.1	4.4
Glucose	22.4	21.8	16.6
Xylose	16.4	18.8	15.3
Atabinose	14.7	19.2	14.4

As a consequence, it must be concluded that the genetic route is especially promising when it comes to the techno-economic optimization of the hydrolysis of BSG, although enzymes specifically improved due to the physicochemical characteristics of the substrate under consideration have not been used.

Discussion

The results obtained using the Penicillium occitanis complex suggest that starch hydrolysation using preparations with diastase activity is the last strategy to obtain maximum conversion of BSG into soluble sugars, the starch hydrolysis using preparations with diastase activity

The starch that forms part of BSG may come partly from that of the malt swollen during the gelatinization process and from the raw gelatinized grain. In both cases there could be a fraction with modular organization, but it is plausible to consider a rather amorphous starch with a lower degree of polymerization and a higher proportion of amylose than at the beginning of the maceration.

a-Amylase solubilizes granular starch by randomly hydrolyzing the a-1,4 linkages of amylose and amylopectin to yield glucose, maltose, and a-dextrins containing a-1,6 linkages.

These branched products can be hydrolyzed by glucoamylase which catalyzes the production of glucose from the non-reducing ends of the a-glucan chains. Its action also extends to the a-1,6

linkages of a-amylopectin, hydrolyzing them more slowly than the a-1,4 ones and slowing down as the degree of polymerization decreases. Glucoamylase is capable of partially degrading the intact starch grain.

The addition of bacterial α-amylase to glucoamylase preparations greatly increases the degree of hydrolysis of granular starch. As a result, the use of Aspergillus niger glucoamylase and thermostable α-amylase of bacterial origin for the hydrolysis of BSG starch are considered.

Conclusions

Hydrolysis is carried out at pH 4.8 and 50ºC with 300 mL of 0.05M citric/citrate buffer solution containing 4.7 mL/L formol, considering 98.5 g/L of BSG and different enzyme preparations and shaking at 250 rpm using a New Brunswick incubator.

The release of reducing sugars using 8.0 mL/L of thermostable a-amylase of bacterial origin and 8.0 mL/L of glucoamylase from Aspergillus niger gives yields of reducing sugars and oligosaccharides of 15.0% and 4.9% respectively after 24 hours.

The same concentrations of diastases are added together with 2 g/L of Trichoderma reesei CL847 enzyme complex so that the yields of reducing sugars and oligosaccharides are at 24 h 35.2% and 10.2%, with respect to the total polysaccharide.

However, the best results in the production of soluble sugars and glucose are obtained in a two-stage enzymatic hydrolysis in which 2 g/L of Trichoderma reesei CL847 complex are first added, and then 8 mL/L of Aspergillus niger glucoamylase and 8 mL/L of thermostable a-amylase of bacterial origin, where the presence of amylase accelerates the hydrolysis of starch, confirming the fragmenting action of a-amylase that favors the attack with glucoamylase.

The comparative analysis of glucose yields with respect to the total glucans present in BSG indicates that, despite the production of this monosaccharide if b-glucanases and a-glucanases are used together, the glucose yield is lower than the sum of the yields given separately by the actions of the cellulase and diastase complexes, although it is equivalent when oligosaccharides are considered.

Therefore, the release of glucose through the joint action of the Trichoderma complex and Aspergillus niger glucoamylase is hindered by an analogous cause that produces similar effects to the use of the Penicillium occitanis complex. As a result, it is concluded that the b-glucanase activities are inhibited when they have to act together with the a-glucanases.

Thus, in order to obtain the best glucose yields, a 2-stage process in which the Trichoderma complex acts first and then the Aspergillus glucoamylase seems to be the most suitable.

On the other hand, while maintaining the yield of reducing sugars of this double process, the use of the maximum yield of monosaccharides, giving a reasonably high conversion to glucose and very high concentrations of xylose and, above all, arabinose and reducing the ratio between oligosaccharides and monosaccharides in the use of a Trichoderma complex with Aspergillus glucoamylase.

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.

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.

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

Acknowledgements

Faculty of Chemistry (University of Barcelona), ETSEIB (Polytechnic University of Catalonia), Institute of Catalan Studies (Barcelona), S.A. DAMM (Barcelona), La Seda de Barcelona, S.A. (Barcelona), CAYLA Society (Toulouse), AKZO Research Laboratories (Arnhem)

References

1. Josep Juanbaro (1990) Hydrolysis of the polysaccharides of the beer hops for the production of ABE. University of Barcelona: 557.
2. Wikipedia (2014) Bagàs (Rests) https://ca.wikipedia.org/wiki/Bag%C3%A0s_(restes).
3. Wikipedia (2019) Bagazo de cerveza (Beer feces). https://es.wikipedia.org/wiki/Bagazo_de_cerveza.
4. Josep Juanbaro (2019) Brewers' Spent Grains for Cattle Feeding. Journal of Animal Husbandry and Dairy Science 3: 25-26.
5. Josep Juanbaro (2026) Properties of Brewers' Spent Grains. Biotechnology and its Applications Journal.