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High Reducing Sugars Concentrations from Brewers' Spent Grains

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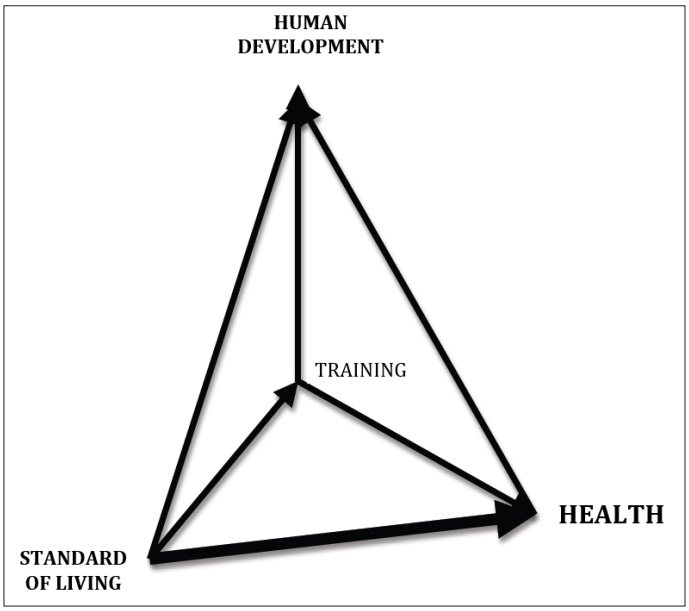

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Introduction

The yield of reducing sugars tends to be constant from a given concentration of BSG so that the increase of a concentration of reducing sugars to a concentration of enzyme complex occurs through an increase in the concentration of BSG slurries.

However, this approach is restricted by the agitation conditions and, in the limit, by the water absorption capacity of BSG [1].

Therefore, the goal of achieving solutions of 60 g/L of reducing sugars, suitable for the subsequent obtaining of ABE (Acetone-Butanol-Ethanol), is tested with a fixed bed reactor with total recirculation (equivalent to a stirred tank).

The hydrolysis juices are collected in a container equipped with magnetic stirring by means of the action of a peristaltic pump and from this container they are sent back to the column field at a speed of 12 mL/min. Higher speeds do not give a significantly different kinetics of sugar reléase [2].

Experimental

Enzymatic hydrolysis are carried out at pH 4.8 and 50ºC using 280 g of BSG with 11.2% moisture (Figure 1) with different amounts of citric/citrate buffer solution 0.05 M, containing 4.7 mL/L of formol and considering various concentrations of Trichoderma reesei CL847 enzyme complex once a maximum BSG concentration is reached.

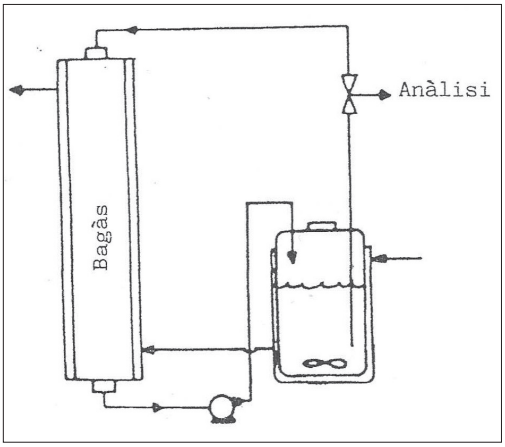

Figure 1: Fixed Bed Reactor with Global Recirculation

Results

When comparing the kinetics of reducing sugar release from increasing concentrations of BSG, it is observed that the same trend achieved up to 200 g/L of BSG is recovered with regard to the initial hydrolysis rate and the concentrations of reducing sugars after the same prolonged action of the Enzymes [3].

Table 1: Reducing sugars concentration achieved after 24 h of hydrolysis of BSG slurries of different concentrations using 2 g/L of Trichoderma reesei CL847 enzyme complex at pH 4.8 and 50ºC using a fixed bed reactor with a recirculation of 12 mL/min

Initial BSG concentration	Reducing sugars concentration after 24 h	
	observed	calculated
BSG_0	c_{red}^{24}	$c_{red}^{24\#}$
g/L	g/L	g/L
197.33	27.0	27,2
245.85	34.5	33.8
295.52	39.5	40.7
314.70	44.0	43.3

$c_{red}^{24\#} = 0.1377\ BSG_0$

The results collected in Table 1 validate the prediction in the sense that from a sufficiently high substrate concentration the yield remains constant, that is, increasing concentrations of reducing sugars, as long as the transfer of matter from the interface to the solution is favored by adequate agitation.

However, the slurry with a maximum BSG concentration at a practical level -with a minimum amount of free liquid that allows agitation and subsequent recovery of the released soluble sugars- only allows for 44 g/L of reducing sugars to be achieved after 24 h [4].

Table 2: Concentrations of reducing sugars from 24 h hydrolysis of 314 g/L BSG slurries at pH 4.8 and 50ºC

Initial *Trichoderma reesei* CL847 enzymatic complex concentration	Reducing sugars concentration after 24 h	
	observed	calculated
E_0	c_{red}^{24}	$c_{red}^{24\#}$
g/L	g/L	g/L
0.50	36.8	36.9
0,98	38.0	39.0
1.94	44.0	41.2
3.98	42.0	43.4
7.97	46.0	45.6

$c_{red}^{24\#} = 39.08 + 3.1564 \ln E_0$

Discussion

High concentrations of enzyme complex do not allow a significant increase in the presence of reducing sugars after 24 h of hydrolysis. Therefore, moderate concentrations of enzymes are considered adequate if the relative cost of their consumption is taken into account.

However, higher enzyme concentrations allow for faster sugar release. In this way, a new strategy can be observed based on obtaining medium-concentration sugar solutions (30 g/L) with minimum hydrolysis times, as long as the recovery of enzymes retained by the substrate and those dissolved in the hydrolysates is considered, since minimum residence times imply minimum denaturation [5].

Table 3: Time required to reach a concentration of 30 g/L of reducing sugars from a slurry of 314 g/L of BSG as a function of the initial concentration of enzyme complex at pH 4.8 and 50ºC

Initial *Trichoderma reesei* CL847 enzymatic complex concentration	Time needed to reach 30 g/L of reducing sugars	
	observed	calculated
E_0	t_{30}	$t_{30}^{\#}$
g/L	h	h
0.50	9.8	13.1
0.98	8.5	8.4
1.94	3.0	3.1
3.98	2.0	1.4
7.97	0.5	0.7

$\ln t_{30}^{\#} = 1.831 - 1.067 \ln E_0$

The enzyme/substrate ratio sometimes considered as a technical-economic parameter is considered inadequate because the release of reducing sugars depends on the concentration of BSG and the enzyme complex.

Conclusions

The impossibility of achieving reducing sugar solutions of 60 g/L using the Trichoderma reesei enzyme complex in an economically viable hydrolysis time leads to the consideration of three possible causes: (1) enzyme denaturation, (2) substrate inaccessibility and (3) competitive inhibition due to the reducing sugars themselves.

The deactivation is analyzed from a practical point of view by preparing a slurry of 314 g/L of BSG using as enzymatic preparation the hydrolysates obtained after 50 h of hydrolysis of 197.3 g/L of BSG at pH 4.8 and 50ºC using 2.0 g/L of Trichoderma reesei enzyme complex.

The observation of the kinetics of release of reducing sugars and the yields of the different monosaccharides up to 26 h allows us to state that there are still sufficient polysaccharolytic activities to allow the hydrolysis of fresh BSG, although the yield is lower when compared to those achieved using 1.9 g/L of virgin complex starting from the same BSG concentration (Figure 2). The markedly lower xylanase activity stands out.

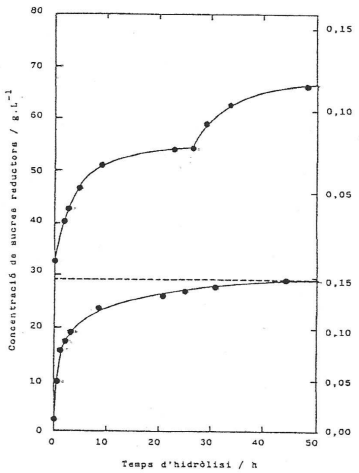

Figure 2: Hydrolysis of 197.3 g/L BSG at 50ºC and pH 4.8 using 2 g/L of Trichoderma reesei CL847 enzyme complex, use

of the hydrolysates for the preparation of a slurry of 314.2 g/L concentration and addition after 26 h of hydrolysis of 1.8 g/L of Trichoderma reesei CL847 enzyme complex

To analyze whether the bottleneck in yields may result from the inhibitory effect of the reducing sugars present, a quantity of powdered enzyme complex is added after 26 h of hydrolysis, representing an additional concentration of 1.8 g/L. Thus, the target of 60 g/L is exceeded.

Although the yield of reducing sugars after the subsequent 24 h of hydrolysis does not reach the yield obtained with a slurry of the same BSG concentration and 1.8 g/L of starting virgin enzymes -attributable in part to the inhibitory effects of reducing sugars-, these results highlight the significant denaturation that the enzymes experience after a prolonged residence time.

In any case, the comparative analysis of the glucose, xylose and arabinose release yields shows the difficulties of cellulose hydrolysis when compared to the hydrolysis of arabinoxylans, explainable by the lack of crystalline zones in arabinoxylans, confirming that only 15% of natural cellulose -fraction consisting of amorphous phase- is easily hydrolysable.

This fact prevents the obtaining of high yields and having to work with large concentrations of BSG that includes a good part of inert materials, implying difficult agitation and recovery techniques of hydrolysates due to their ability to absorb water, makes it necessary to consider the application of some pretreatment that allows to achieve higher yields and to work with more diluted substrate concentrations that at the same time favor the recovery of the reducing sugars released.

The greater accessibility of glycosidic bonds suggests that their acid hydrolysis may be more effective and, therefore, economically more attractive than enzymatic hydrolysis subject to catalyst deactivation.

Finally, it must be concluded that enzyme denaturation, substrate inaccessibility and inhibition due to reducing sugars play a prominent role in the hydrolysis of polysaccharides contained in BSG.

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