Raw stillage as carbon source and inducer for the production of cellulase / hemicellulase complexes

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Introduction:

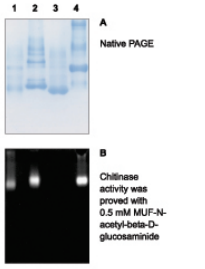
In the past sixty years, various procedures both for the production and the application of cellulolytic enzymes have been developed. However, the application of cellulolytic enzymes for the material and energetic utilization of lignocellulosic substances could not yet be realised economically. One example is the enzymatic modification of lignocellulosic fibre materials for the production of glue-free timber products, esp. Medium Density Fibre Boards (MDF). A few years ago we developed a process for the production of glue-free MDF using cellulase complexes for the enzymatic modification of the fibre materials [1]. This process is still economically limited due to the high costs of commercial enzymes and the lack of knowledge concerning the optimum composition of suitable enzyme complexes. Nevertheless, we could prove a correlation between endoglucanase activity and in some cases xylanase activity as well, with properties of the MDF, esp. the bending strength [2].

Table 1: Fermentation of cellulase- / xylanase-complexes using stillage as substrate / inducer:

Medium / process control	Work. volume [L]	Ferm. time [h]	Extrac. Protein g/L	Enzyme activities Xylanase IU/ml	Endogluc. U/ml	Cellulase IU/ml
Thin stillage 50%, MKC (1.5%), glucose (1.0%), fed-batch (thin stillage), pH 5.0	6	92	-	616	30.0	1.9
Thin stillage plus Linters cellulose (1%), fed-batch at pH 5.0	100	120	24.0	1,140	17.0	6.4
Raw stillage plus MKC (1%), batch at pH 5.5	2.5	120	23.2	2,020	21.1	5.1
Raw stillage plus MKC (1%), batch at pH 5.0	2.5	120	21.7	1,670	22.6	5.1
Raw stillage plus Linters cellulose (1%), fed-batch at pH 5.0	15	115	17.4	701	18.1	7.9
Raw stillage plus Linters cellulose (1%), batch at pH 5.0	400	80	14.0	1,190	14.6	6.2

Table 2: Comparison between three commercial enzymes (No. 1, 2, 3) with the T. reesei-enzyme concentrate SIAB-03 produced on raw stillage

Activities of the enzymes		Enzyme samples 1	2	3	SIAB-03
Cellulase	[FPU/ml]	n.n.	19	14	121
Xylanase	[IU/ml]	1,850	10,545	1,300	13,400
Proof of side-activities with chromogenic substrates					
MUF-α-D-Galactopyranoside		+	+	-	+++
MUF-α-D-Glucopyranoside		-	-	-	-
MUF-α-D-Mannopyranoside		-	-	-	-
MUF-α-L-Arabinopyranoside		-	+	+	+
MUF-ß-D-Cellobioside		+++	+++	+++	+++
MUF-ß-D-Glucoside		+	++	+	+
MUF-ß-D-Fucoside		-	-	+	++
MUF-ß-D-Xylopyranoside		+++	++	-	+++
MUF-N-acetyl--D-glucosaminide		+	++	-	+++



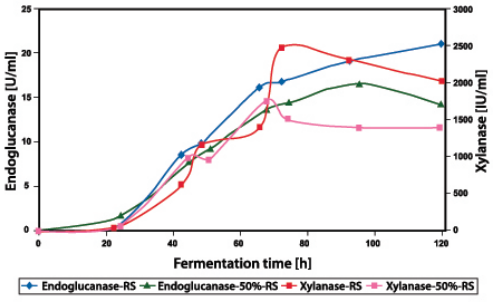


Fig. 1: Comparison of original raw stillage with 50% raw stillage diluted with water, batch, add. of 1% MKC, 5-L-bioreactor

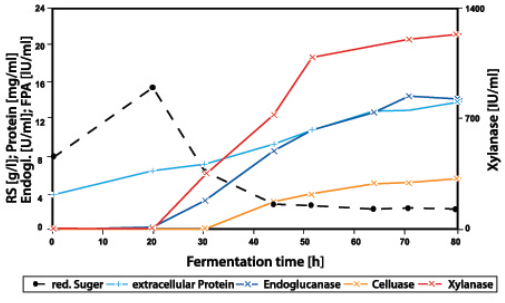


Fig. 2: Fermentation with raw stillage plus 1% cotton linters as additional inducer in pilot scale, 600-L-bioreactor, batch

Results:

We have developed a process for the production of cellulase/hemicellulase complexes on basis of original distillery stillage as fermentation substrate and inducer [3]. By using thin stillage and raw stillage, both from wheat and rye, we investigated the fermentation process up to pilot scale. The composition of the resulting enzyme complexes was assayed depending both on the fermentation medium on basis of stillage and the process control (e.g. Tab.1, Fig.1). The strain used for the enzyme production was the high yielding mutant strain *Trichoderma reesei*-M18-2 which shows high excretion rates of cellulase and xylanase due to switched-off carbon catabolite repression and modified morphology. After optimal induction, the maximum enzyme excretion rate is about 15 mg enzyme protein per hour per gram mycelium.

In comparison to thin stillage, raw stillage as fermentation medium leads to a higher xylanase activity in the enzyme complex. The composition of the excreted enzyme complex can be varied by changing the process conditions. For instance, at higher pH values an increased amount of xylanase and ß-glucosidase is produced in the enzyme complex. Lower pH values and the addition of cellulose to the basic stillage medium, e.g. cotton linters, led to the production of an increased amount of endoglucanase. Raw stillage as well as thin stillage are good inducers not only for cellulase and xylanase, but also for other enzymes (Tab. 2). Apparently, the inducing effect is caused by the non-utilizable di- and oligosaccharides of the stillage. When using raw stillage as substrate, the "boiled" yeast cells of the stillage are utilized during 40 to 60 h of the fermentation time (Fig. 3). During this period the effective viscosity (non-Newtonian flow behaviour) of the fermentation medium increases to a maximum level. After the utilization of the yeast cells the viscosity drops down. When cellulose, e.g. cotton linters, is added to the raw stillage medium as additional inducer, problems with the oxygen transfer may arise, caused by the increased viscosity. Such problems could be solved by a controlled increase of the fermentation pressure during this period.

The enzyme complexes fermented on basis of stillage have been tested successfully for the enzymatic modification of lignocellulosic fibres for the production of glue-free timber products (Fig. 4). The production of glue-free MDF for door blades was tested up to industrial scale [4].

The engineering partner EDL worked out the basic design and dimensioning of the equipment and plant units necessary for the integration of the process into a bioethanol plant. Compared with current commercial products, the mass and energy balances indicate a reduction of the estimated enzyme costs. A pilot plant was designed for a capacity of 4 m³/batch in order to ensure the scale-up from the laboratory.

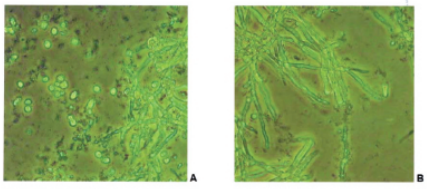


Fig. 3: Microscopic pattern of *T. reesei*-Fermentation on raw stillage

A: after about 40 h fermentation time: mycelium plus dead yeast cells
B: after about 60 h fermentation time: yeast cells are largely utilized (after about 70 h the hard-boiled yeast cells from stillage are utilized)

Fig. 4: A – Industrial fibre material
B – Medium Density Fibre Board

Conclusions:

One of the advantages of this technique is the potential economic exploitation of stillage as a by-product of bio-ethanol production. Further advantages are gained by the possible reduction of time and effort for the sterilisation of the fermentation medium, provided that the original stillage with a temperature of about 100°C is being integrated in the enzyme production process after distilling off the ethanol (Fig. 5). Another important economic factor is the extraction of valuable products, e.g. chitosan from chitin of the fungal cell walls, provided that economic techniques for the separation of chitin and deacetylation are developed.

The application of culture filtrates with at least 30 U/ml endoglucanase and without further downstream processing could improve the economy of the enzymatic modification of lignocellulosic fibres for MDF production. Beside this application, the enzyme complexes produced on basis of raw stillage can be used for conventional applications, as well as in the process of simultaneous saccharification and fermentation (SSF) for the production of bioethanol on basis of the carbohydrate content in pretreated lignocellulosic materials.

The results have shown that there is still a potential for increasing the enzyme production on basis of stillage, mainly by optimizing the induction, e.g. by adding other inducing substances to the stillage medium or by special fed-batch techniques. This technique for the production of enzymes on basis of stillage can be gradually scaled up to industrial production levels, e.g. within an industrial plant.

References:

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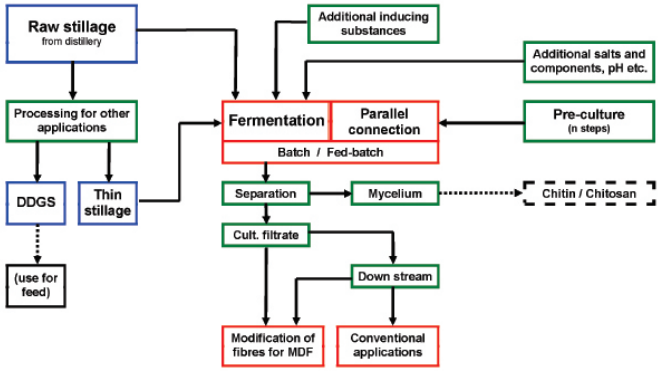

Fig. 5: Reduced flow chart for the production of cellulase- / hemicellulase-complexes on basis of raw stillage