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## UPGRADING BREWER'S SPENT GRAINS BY TREATMENT WITH ASPERGILLUS SPECIES

*Hydrolysis of brewer's spent grains (BSG) starch was examined using Aspergillus oryzae and A. awamori, at various conditions (pH, spore concentrations). Both fungi performed well, although A. oryzae proved more efficient in terms of process times and enzyme stability. BSG slurries were either treated directly with spore suspensions at 30°C, or with crude enzyme solutions at 45°C. In the first case, simultaneous biomass production and starch hydrolysis occurred, but in the second, transformation of sugars to biomass was avoided. In both cases, fermentable sugar production was not efficient (0.24–0.95 g/l and 0.47–1.83 g/l respectively) to support the use of the BSG hydrolyzates (BSGHs) as substrates e.g. for yeast propagation within the brewery. Alternatively, BSG treated directly with fungi could be proposed as protein enriched animal feeds. BSGHs were also evaluated as nutritious supplements in yeast growth media containing mixtures of molasses and orange pulp (as carbon sources), resulting in significantly improved biomass yields.*

*Key words: Brewer's spent grains, Aspergillus oryzae, Aspergillus awamori, starch, hydrolysis, yeast production, animal feed.*

Agro-industrial wastes rich in dietary fibre, starch and fermentable carbohydrates, such as molasses, cereals and their spent grains, citrus and potato residues are often used as animal feed or incorporated in feed formulations. They may also be treated with bacteria and fungi to obtain protein enriched feeds, leading at the same time to creation of added value and reduction of the pollution caused by their disposal [1–5]. Many microorganisms have been used for starch hydrolysis as well as the production of starch degrading enzymes, mainly bacteria such as *Bacillus* sp. and filamentous fungi. Especially *Aspergillus* sp. (*A. oryzae*, *A. niger* and *A. awamori*), are extensively used for industrial enzyme production (mainly  $\alpha$ -amylases and glucoamylases) due to their superior ability to secrete enzymes [5–6]. Some of these fungi are traditionally used in fermented food production such as *sake*, *miso*, *koji* and *shochu* [7–9]. Microbial amylases, their properties and biotechnological applications have been extensively reviewed [10–15]. *A. oryzae* is widely used for commercial  $\alpha$ -amylase production, which is typically used in baking, brewing, in dietary supplements, in alcohol production and other food grade applications [7]. It has also been evaluated as probiotic in animal feed production [16]. At research level, the depolymerization of starch contained in various substrates using *A. oryzae* and *A. awamori* strains for biomass or enzyme production, waste organic load reduction, animal feed production, etc., in submerged (SmF) and solid state fermentation (SSF) processes, has been widely studied [5,17–22]. Immobilization techniques have also been proposed for ethanol

production from starch, e.g. involving co-immobilized systems of starch degrading *A. awamori* with alcohol forming *Saccharomyces* or *Zymomonas* sp. [23–28]. In SmF the growth rate and product formation of filamentous fungi may be affected by the morphology of the culture, the medium composition and culture conditions (oxygen, pH, temperature, agitation, shear forces), the inoculum concentration, etc. [9,20,29].

Brewer's spent grains (BSG), produced after the mashing stage in beer production, are the main by-product of the brewing industry and are available at low or no cost from large factories as well as local breweries. They consist of about (% w/w on dry matter) 16–25 cellulose, 12–28 lignin, 11–26 apparent starch (glucose, maltodextrins and residual starch), 15–25 crude protein, 15–20 crude fibre, 6–10 digestible fibre, 6–10 lipids and 3–5 total ash [1,4,30–32]. Their composition depends on the barley variety and on the malting and mashing programme. Due to their high fibre and protein content, including all essential amino acids, BSG are widely used as animal feed. Also, their high moisture (>70%), protein and lignocellulosic content, have made them good substrates for the cultivation of mushrooms leading to increase of their nutritional value [32–34]. BSG residual starch has been evaluated as sole carbon source for the synthesis of enzymes by filamentous fungi such as *A. oryzae* and *A. awamori* [1,35].

This study explores ways to upgrade BSG, and other by-products of the food industry such as molasses, citrus and potato wastes, through treatment with *A. awamori* and *A. oryzae* for fermentable sugar or microbial biomass production. The treated materials were evaluated as potential substrates (carbon sources or nutritional supplements) for yeast biomass production in the brewery, or alternatively, as potential protein enriched animal feeds.

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## MATERIALS

### Microorganisms and inocula standardization

The *Aspergillus* sp. used in this study were the strains *A. oryzae* IMI283876 (CABI-International Mycological Institute, Oxfordshire, UK) and *A. awamori* Nakazawa DSM63272 (DSMZ, Braunschweig, Germany). They were grown on 100 ml potato dextrose agar (PDA) (20 g/l glucose) in 500 ml conical flasks at 25 °C. After growth and sporulation, the spores were collected by scrapping the culture surface twice with 100ml of sterile water containing 0.1% Tween-80 under aseptic conditions. The spore suspensions produced this way contained  $8 \cdot 10^7$  spores/ml of *A. oryzae* and  $10^8$  spores/ml of *A. awamori*, respectively. Baker's yeast was a commercial *S. cerevisiae* strain commonly used in the Greek baking industry, in the form of pressed blocks (70% w/w moisture).

### Media

Brewer's spent grains (BSG) were obtained from the Athenian Brewery S.A. They contained 79.2% moisture, 2.5oP remaining extract (washable), 25.6% crude protein (on dry matter), and had pH 6.0. Soluble, extra pure starch was supplied by MERCK. Gelatinization of starch solutions was carried out at 70°C for 20 min. Molasses, orange and potato pulp, and their mixtures were used after dilution with water at suitable densities (2–5 °Be). Crude enzyme solutions were prepared with and without addition of CaCl<sub>2</sub> (0.5 g/l). All media were sterilized at 120 °C for 15 min.

### Direct treatment of BSG with *Aspergillus* spores

In the first case, hydrolysis of BSG residual starch (including dextrans and fermentable sugars) with simultaneous growth of *A. oryzae* or *A. awamori* was carried out on BSG as sole carbon source (Figure 1, Table 1). Mixtures containing 75 g BSG and 150 ml water, and having pH 4, 6 or 8, after heating for starch gelatinization, were inoculated with various amounts of the stock spore suspensions ( $9 \cdot 10^5$ ,  $10^6$  and  $2 \cdot 10^6$  spores/ml). The systems were incubated at 30°C with agitation and starch hydrolysis was monitored at various time intervals by measurement of the decrease in iodine staining power. The hydrolyzed slurries (BSGH1) were subjected to sonication, assayed for produced fermentable sugar (glucose and maltose) and crude protein (total nitrogen), and tested as substrates for yeast biomass production. In the case that the produced fermentable sugars were totally converted to fungal biomass, the treated material could be alternatively evaluated as potential protein enriched animal feed.

### Treatment of BSG with crude enzyme solutions

In a second set of experiments (Figure 1, Table 2), crude enzyme solutions were prepared by growth of *A.*

*oryzae* or *A. awamori* at 30°C in various media containing potato pulp or orange pulp or molasses, as well as their mixtures. All media, except potato pulp, contained approx. 4 g/l initial fermentable sugar. The produced crude enzyme solutions were treated by sonication, and were used for the hydrolysis of BSG residual starch at 45°C, at which hydrolysis was favoured but fungal growth was inhibited. Specifically, 50 ml of crude enzyme solution were mixed with 150 g of BSG in a 1 l glass cylinder, and the volume was fixed to 300 ml with water. The system was allowed to ferment and experiments were carried out with and without addition of 0.5 g/l CaCl<sub>2</sub>, which is considered to increase amylase stability [36]. Starch hydrolysis was monitored at various time intervals and at the end of each experiment the hydrolyzed slurries (BSGH2) were assayed for produced fermentable sugar and crude protein, and tested as substrates for yeast biomass production.

### Yeast production using BSG hydrolysates

Yeast biomass production experiments were carried out in 1 l glass cylinders, at 30°C with air supply (3 l/min). The substrates used were a) the BSGH1 (Table 1) and BSGH2 slurries (Table 2), produced as described above without addition of other carbon sources or nutrients, and b) solutions of molasses or orange pulp or their mixtures, with and without the addition of BSGH1 as nutritious supplement (potential source of minerals and other nutritional substances such as amino acids and peptides) (Table 3). An initial amount of 1 g of yeast was used to pitch each system. Specifically, media containing 300 ml of molasses or orange pulp or mixture of molasses and orange pulp, diluted to 5°Be density, were used for yeast biomass production with or without addition of 130 ml of BSGH1. When the fermentations were completed (about 24 h), the produced yeast was harvested by centrifugation at 5000 rpm for 10 min (~82% moisture), and the biomass yield was assessed as the g of yeast produced (dry weight) per g of sugar utilized (g/g). The supernatants were analyzed for residual sugar by HPLC. All experiments were carried out in triplicate and the results are given as average values.

### Assays

Residual starch (decrease of iodine staining power) was determined according to the method described by Apar and Ozbek [36]. Starch hydrolysis was monitored at 620 nm, using a solution of 200 µl water and 5 ml of iodine solution as blank. For the determination of residual starch concentration, 300 µl samples, removed at various time intervals, were mixed with 5 ml iodine solution (0.5% KI and 0.15% I<sub>2</sub>), and the volume was fixed to 15 ml with distilled water. The absorbance was measured at 620 nm, using a solution of 5 ml iodine solution and 10 ml distilled water as blank.

Residual starch concentrations were determined using a standard curve. Glucose, maltose, sucrose and fructose were determined by HPLC, on a Shimadzu LC-9A Liquid Chromatograph (SCR-101N stainless steel column; LC-9A pump; CTO-10A oven at 60°C; RID-6A refractive index detector). Three times distilled and filtered water was used as mobile phase (0.8 ml/min) and butanol-1 was used as internal standard. The sugar concentration was calculated using standard curves. Protein was determined according to the AOAC Official Method 991.20 (Kjeldahl method) used for determination of total nitrogen in milk [37].

## RESULTS AND DISCUSSION

In this study, ways to upgrade BSG and various by-products of the food industry (molasses, wasted oranges and potato residues), through treatment with *A. awamori* and *A. oryzae*, were evaluated. The work focused on residual BSG starch hydrolysis for fermentable sugar or fungal biomass production, and the hydrolysed BSG slurries (BSGHs) were evaluated as potential substrates (sole carbon sources or nutritional supplements) for yeast biomass production, e.g. in the brewery, or alternatively, as potential protein enriched animal feeds.

### Yeast production using BSGHs

BSG slurries were treated directly with spore suspensions at 30°C, at which simultaneous starch hydrolysis and fungal growth occurred (Figure 1, left path). Therefore, if the treated BSG were to be used as carbon sources for yeast production, they had to be treated with crude enzyme solutions at a higher temperature (45°C) to favour hydrolysis and avoid consumption of the produced sugars to fungal biomass,

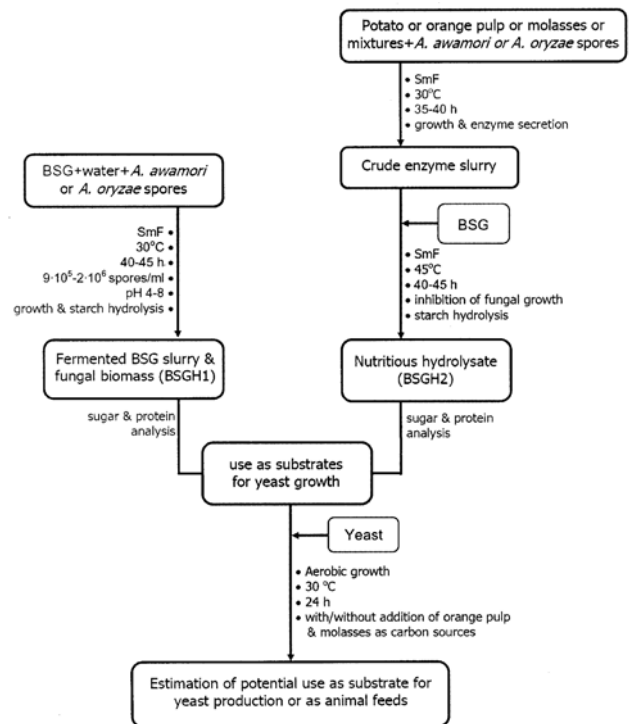


Figure 1. Treatment of BSG and food industry by-products with *Aspergillus* species for fermentable sugar, microbial biomass or animal feed production: experimental outline

which would be desirable only in the case of protein enriched animal feed production. Consequently, crude enzyme solutions were produced by growth of the fungi at 30°C on molasses, orange pulp, potato pulp and their combinations, which are low cost and abundant food industry by-products (Figure 1, right path). In both cases the produced BSGHs were treated by sonication, for cell lysis and nutrient liberation, and were used as substrates for aerobic yeast production.

Table 1. Parameters of the SmF of BSG slurries using *A. oryzae* or *A. awamori* spores at 30°C, and yeast production using the fermented slurries (BSGH1) as substrates

SmF of BSG (40–45 h process)			Characteristics of BSGH1					Yeast production using BSGH1 (24 h)		
Biocatalyst	Inoculum (spores/ml)	pH	produced sugar			% protein (dry) (g/100g)	% protein increase (dry)	total residual sugar (g/l)	total bio-mass (dry) (g)	biomass yield (dry) (g/g sugar)
			glucose (g/l)	maltose (g/l)	total (g/l)					
No treatment	–	–	–	–	–	25.6	–	–	–	–
<i>A. awamori</i>	10 <sup>6</sup>	4	0.32±0.04	0.14±0.06	0.46±0.10	33.5±2.3	30.9±9.0	0.08±0.03	0.33±0.10	0.86±0.65
	10 <sup>6</sup>	6	0.52±0.06	0.18±0.06	0.70±0.12	34.8±3.3	35.9±12.5	0.06±0.02	0.60±0.05	0.93±0.14
	10 <sup>6</sup>	8	0.12±0.06	0.12±0.04	0.24±0.10	32.5±2.4	27.0±9.4	0.03±0.03	0.21±0.15	0.89±0.43
	9·10 <sup>5</sup>	6	0.41±0.08	0.20±0.03	0.61±0.05	30.8±2.7	20.3±10.5	0.05±0.03	0.48±0.03	0.85±0.16
	2·10 <sup>6</sup>	6	0.36±0.06	0.18±0.06	0.54±0.00	34.9±2.7	36.3±10.5	0.08±0.01	0.44±0.12	0.95±0.24
<i>A. oryzae</i>	10 <sup>6</sup>	4	0.18±0.02	0.15±0.05	0.33±0.03	29.8±3.6	16.4±14.1	0.07±0.02	0.25±0.12	0.96±0.43
	10 <sup>6</sup>	6	0.61±0.12	0.22±0.05	0.83±0.17	30.3±0.5	18.4±2.0	0.08±0.02	0.70±0.11	0.93±0.41
	10 <sup>6</sup>	8	0.54±0.02	0.28±0.12	0.82±0.14	29.6±3.0	15.6±11.7	0.06±0.01	0.68±0.10	0.89±0.32
	9·10 <sup>5</sup>	6	0.49±0.11	0.23±0.06	0.72±0.14	28.9±0.71	2.9±2.7	0.06±0.03	0.61±0.15	0.92±0.03
	2·10 <sup>6</sup>	6	0.68±0.08	0.27±0.14	0.95±0.06	31.2±1.83	0.9±8.8	0.10±0.04	0.83±0.05	0.98±0.04

a) *Direct treatment with spores.* Specifically, in a first set of experiments (Figure 1, left path), BSG slurries of different pH (4, 6 and 8) were inoculated with various amounts of fungi spore suspensions ( $9 \cdot 10^5$ ,  $10^6$  and  $2 \cdot 10^6$  spores/ml) and hydrolysis was carried out at 30°C with simultaneous mycelia production. The results are shown in Table 1 and Figures 2–3. No significant effect of pH and initial spore concentration on starch hydrolysis kinetics was observed. The systems were monitored for starch conversion (reduction of iodine staining power at 550 nm) for 40–45 h but process times required for growth, enzyme secretion and starch hydrolysis, were only 10–25 h. As shown in Figure 2 the best starch conversion with *A. oryzae*, was achieved at pH 8 and 6. At pH 4 on the other hand, the hydrolysis of starch was not efficient and did not proceed further after a period of 25 h. This was not observed in the case of *A. awamori* (Figure 2). For both fungi, no significant differences on the hydrolysis kinetics were observed using different initial concentrations of spores (ISC) (Figure 3). The highest produced fermentable sugar concentration for *A. oryzae* was 0.95 g/l, achieved at pH 6 and ISC  $2 \cdot 10^6$  spores/ml, and for *A. awamori* it was 0.70 g/l, achieved at pH 6 and ISC  $10^6$  spores/ml. The highest fungal biomass production (34.9% w/w crude protein on dry weight basis) was obtained for *A. awamori* at pH 6 and ISC  $2 \cdot 10^6$  spores/ml. The produced hydrolysed BSG slurries from both microorganisms (BSGH1) were used as substrates for aerobic yeast production, with no addition of extra nutrients (Table 1). Yeast production was low (0.21–0.60 g and 0.25–0.83 g dry yeast for *A. awamori* and for *A. oryzae*, respectively) since the available fermentable sugars were low, but

yeast biomass yields were high (0.85–0.98 g dry yeast/g utilized sugar), showing that addition of extra nutrients was not necessary.

Experiments carried out with pure gelatinised aqueous starch solutions (data not shown) yielded similar results to those obtained in the presence of BSG. These results demonstrate that the two microorganisms presented similar behaviours in hydrolyzing BSG starch under the given conditions. Process times were very low, although amylases seemed to be inactivated after a time period and starch conversion was not total. Also, the produced fermentable sugars were not adequate to support use of BSGH1 as raw materials for yeast production. Therefore, the BSGH1 containing higher amounts of fermentable sugar and fungal protein might be used as a more nutritious animal feed, compared to the conventional non-treated BSG. Towards this scope, analysis of essential amino acid and nucleic acid content as well as tests with animals, such as fish and poultry, are necessary.

b) *Treatment with crude enzyme solutions.* To avoid transformation of sugars to *Aspergillus* biomass, crude amylolytic enzyme solutions were prepared by growth of the fungi in various substrates containing potato pulp, orange pulp, molasses and their combinations (Figure 1, right path). The produced crude enzyme solutions were used to hydrolyze BSG residual starch at 45°C and pH 6, and the results are shown in Table 2. After experiments for fungal growth and enzyme secretion in various combinations of the above media, the one containing blended potato pulp was found to be the most efficient, leading to fast growth and higher amylase activity within 25–30 h for both strains. The activities of

Table 2. Parameters of the SmF of BSG slurries using *A. oryzae* or *A. awamori* crude enzyme solutions at 45°C and pH 6, and yeast production using the fermented slurries (BSGH2) as substrates

Crude enzyme production (30–35 h process)		SmF of BSG (40–45 h process)			Yeast production using BSGH2 (24 h process)		
Biocatalyst	Substrates	Characteristics of BSGH2			Total residual sugar (g/l)	Total bio- mass (dry) (g)	Biomass yield (dry) (g/g sugar)
		Glucose (g/l)	Maltose (g/l)	Total (g/l)			
<i>A. awamori</i>	Potato	0.89±0.12	0.64±0.19	1.53±0.07	0.16±0.05	1.30±0.27	0.95±0.12
	Orange	0.21±0.14	0.35±0.12	0.56±0.26	0.08±0.02	0.43±0.34	0.89±0.07
	Molasses	0.38±0.08	0.41±0.18	0.62±0.14	0.09±0.02	0.51±0.08	0.96±0.13
	Orange/molasses	0.29±0.09	0.46±0.18	0.75±0.09	0.12±0.03	0.59±0.25	0.93±0.20
	Orange/potato	0.56±0.08	0.62±0.17	1.18±0.09	0.14±0.07	0.98±0.13	0.94±0.07
	Molasses/potato	0.69±0.20	0.71±0.35	1.40±0.15	0.19±0.06	1.14±0.10	0.94±0.01
	Potato/CaCl <sub>2</sub>	1.12±0.31	0.71±0.10	1.83±0.41	0.16±0.02	1.50±0.40	0.90±0.04
<i>A. oryzae</i>	Potato	0.73±0.25	0.36±0.10	1.09±0.15	0.09±0.01	0.95±0.06	0.95±0.12
	Orange	0.18±0.10	0.29±0.08	0.47±0.18	0.07±0.02	0.39±0.10	0.98±0.26
	Molasses	0.26±0.05	0.39±0.13	0.65±0.18	0.08±0.05	0.55±0.18	0.96±0.01
	Orange/molasses	0.25±0.13	0.33±0.06	0.58±0.19	0.08±0.04	0.44±0.24	0.88±0.08
	Orange/potato	0.31±0.11	0.49±0.20	0.80±0.09	0.09±0.03	0.66±0.14	0.93±0.06
	Molasses/potato	0.36±0.20	0.58±0.17	0.94±0.03	0.10±0.04	0.75±0.13	0.89±0.03
	Potato/CaCl <sub>2</sub>	0.54±0.24	0.42±0.17	0.96±0.07	0.13±0.05	0.76±0.23	0.92±0.06

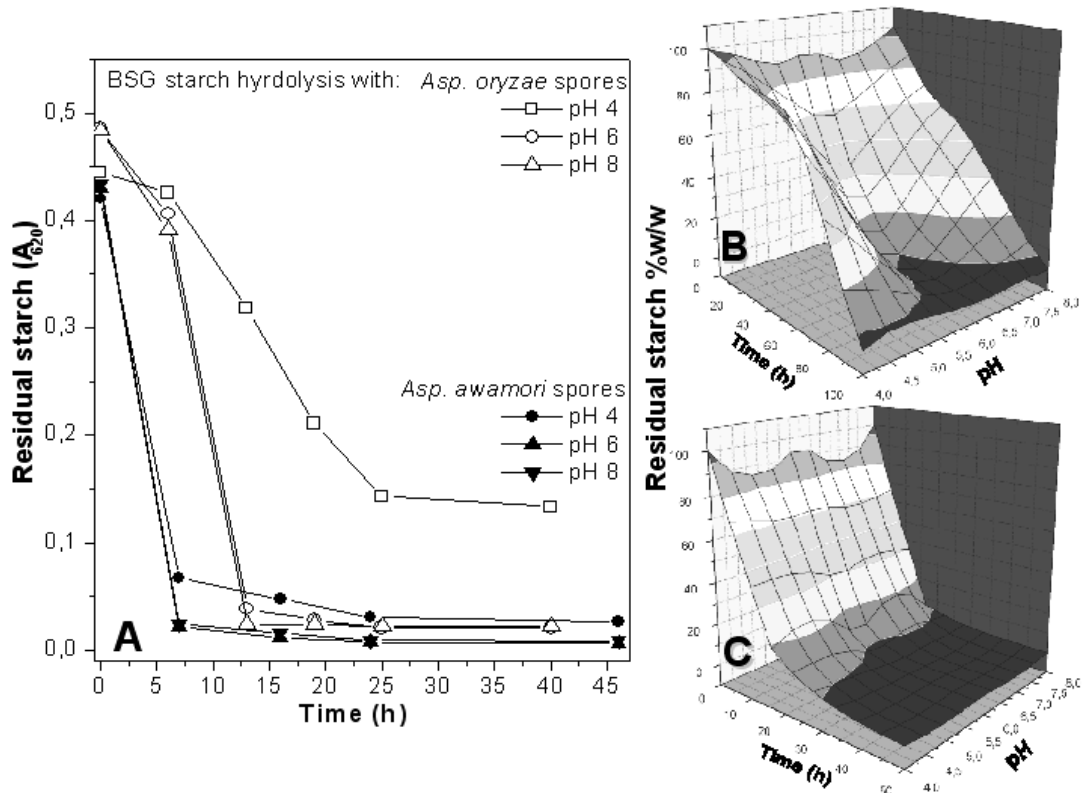


Figure 2. Starch hydrolysis kinetics ( $A_{620}$ ) at pH 4, 6 or 8 (A), and surface response graphs of BSG residual starch (%w/w) as functions of pH and process times, during the SmFs of BSG using *A. oryzae* (B) and *A. awamori* (C) spore suspensions

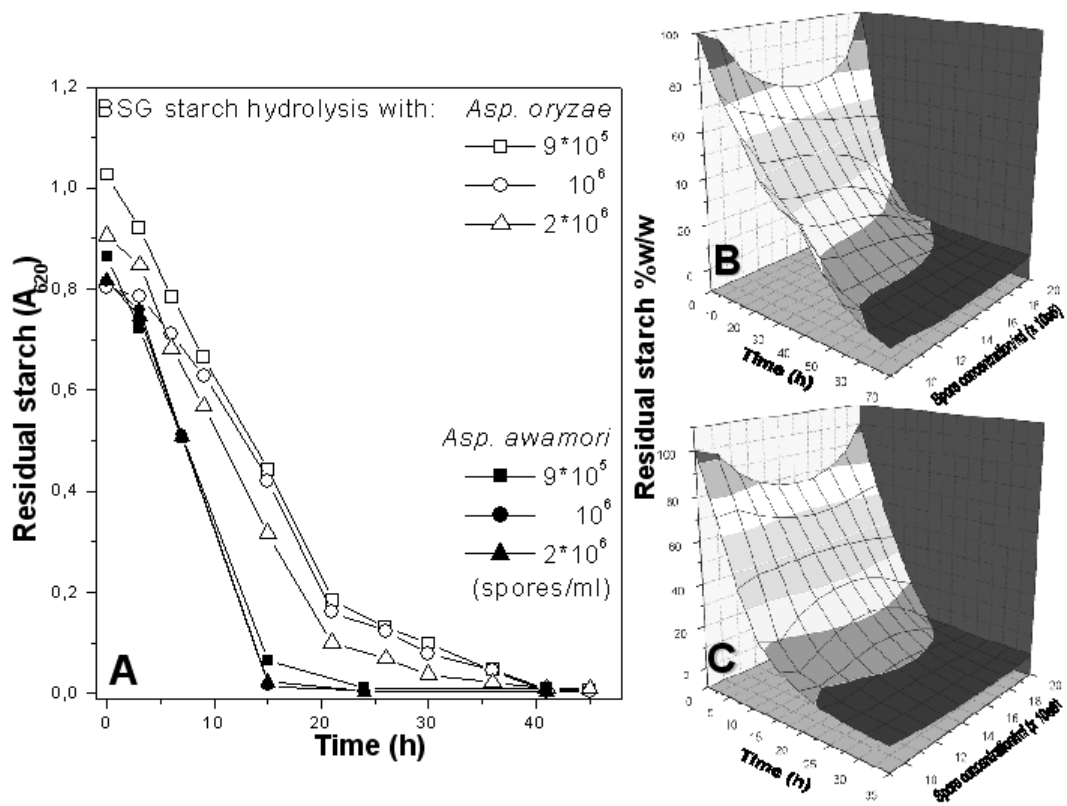


Figure 3. Starch hydrolysis kinetics ( $A_{620}$ ) using inocula of  $9 \times 10^5$ ,  $10^6$ , or  $2 \times 10^6$  spores/ml (A), and surface response graphs of BSG residual starch (%w/w) as functions of inocula concentrations and process times, during the SmFs of BSG using *A. oryzae* (B) and *A. awamori* (C) spore suspensions

the crude enzyme solutions were higher when  $\text{CaCl}_2$  was added to the growth medium. The highest amount of fermentable sugar (1.83 g/l) was produced in the case of *A. awamori* crude enzyme solution prepared in medium containing potato pulp enriched with  $\text{CaCl}_2$ . The produced hydrolysed BSG slurries from both microorganisms (BSGH2) were used as substrates for aerobic yeast production, with no addition of extra nutrients (Table 2). Yeast production was low (0.39–1.50 g), but higher than in the case of the direct treatment of BSG with spores (Table 1) since the produced fermentable sugars were not converted to fungal biomass, and were totally available for yeast production. Yeast biomass yields on the other hand were very high (0.88–0.98 g dry yeast/g utilized sugar) as reported in the previous case.

### Yeast production using BSGH1 and food industry by-products

In the case of BSG containing low amounts of residual starch and dextrins, the hydrolysis process is not expected to yield high amounts of fermentable sugars, as shown above, therefore their hydrolysed slurries cannot be used as sole carbon sources for yeast production within the brewery. Francis et al. [35] reported the use of BSG residual starch as sole carbon source for the synthesis of  $\alpha$ -amylase in SSF using *Aspergillus oryzae*, with good results, while the supplementation with external carbon sources was found to repress the enzyme synthesis. For yeast production on the other hand, supplementation is necessary to yield high amounts of biomass. Therefore, it was thought that BSG treated with filamentous fungi, apart from being used as protein enriched animal feeds, they could also be evaluated as sources of minerals and nitrogen compounds in media containing other materials as carbon sources. To evaluate this possibility, experiments of yeast biomass production were carried

out using media containing molasses, orange pulp and their mixtures, with and without the addition of BSGH1, prepared by BSG treatment with *A. oryzae* and *A. awamori* and subsequent treatment with ultrasonic waves, to obtain a nutritious extract. The results are shown in Table 3. It is obvious that biomass yields (g dry yeast per g sugar utilized) were improved when BSGH1 was added to the growth medium, for both fungi and for all the tested media.

### CONCLUSIONS

The above results demonstrate that both strains had similar behaviour in hydrolysing BSG starch as far as process times and enzyme stability were concerned. Also, the hydrolysis using crude enzyme solutions at 45°C compared with the direct treatment with fungi spores at 30°C, was faster, leading to higher amounts of fermentable sugars whose transformation to fungal biomass was avoided. The hydrolysed BSG slurries (BSGH1 & BSGH2) produced by both methods contained low amounts of fermentable sugars to be utilized as sole carbon sources e.g. for yeast growth within the brewery plant. On the other hand, it was found that BSG treated directly with spores (BSGH1), could be used as nutritious additives in yeast propagation media containing other cheap, food grade materials like molasses or orange pulp as carbon sources. In all cases BSG treated with *A. oryzae* and *A. awamori* in combination with other cheap and easily available food industry by-products (molasses, citrus and potato residues) could be evaluated as protein enriched animal feeds. Tables 1 shows that direct treatment of BSG slurries either with *A. oryzae* or *A. awamori*, increased their protein content by 20–36%. Work concerning the production of protein enriched animal feeds based on BSG treated with *A. oryzae* and *A. awamori*, and their suitability for fish or poultry, is in progress. The above efforts aim in utilizing BSG to create added value and avoid environmental problems caused by their disposal.

Table 3. Yeast production (24 h process) using media containing molasses (M), orange pulp (P) and their mixtures (MP), with and without the addition of BSGH1 produced after treatment of BSG with *A. oryzae* or *A. awamori*.

	Substrate	Glucose (g/l)		Fructose (g/l)		Sucrose (g/l)		Total fermentable sugar (g/l)		Produced biomass (82% moist) (g)	Produced biomass (dry weight) (g)	Biomass yield (dry weight) (g/g sugar)
		in	res	in	res	in	res	in	res			
Without addition of BSGH1	M	1.39±0.03	1.06±0.06	1.92±0.09	2.08±0.08	2.97±0.07	0.60±0.47	6.28±0.17	3.74±0.49	5.8±0.5	1.0±0.1	0.41±0.02
	P	1.89±0.11	0.00±0.00	1.97±0.12	0.63±0.06	1.29±0.14	0.06±0.02	5.15±0.33	0.69±0.08	18.5±2.6	3.3±0.5	0.75±0.17
	MP	1.37±0.12	0.08±0.05	1.63±0.13	0.67±0.06	1.63±0.11	0.09±0.03	4.63±0.07	0.84±0.14	16.1±3.7	2.9±0.7	0.76±0.22
With addition of Asp. Awamori BSGH	M/BSGH1	1.01±0.15	0.51±0.11	1.42±0.04	1.21±0.23	2.07±0.08	0.07±0.04	4.50±0.13	1.79±0.16	12.9±2.8	2.3±0.5	0.86±0.28
	P/BSGH1	1.36±0.08	0.44±0.10	1.46±0.11	1.20±0.10	0.9±0.35	0.06±0.02	3.72±0.28	1.70±0.22	11.4±2.0	2.1±0.4	1.02±0.08
	MP/BSGH1	1.0±0.06	0.08±0.01	1.22±0.13	0.71±0.11	1.14±0.20	0.01±0.00	3.36±0.33	0.80±0.10	13.9±1.2	2.5±0.2	0.98±0.26
With addition of Asp. oryzae BSGH	M/BSGH1	0.98±0.05	0.29±0.08	1.34±0.20	1.08±0.13	2.10±0.23	0.11±0.03	4.42±0.41	1.48±0.18	14.1±2.0	2.5±0.4	0.86±0.06
	P/BSGH1	1.34±0.13	0.13±0.06	1.37±0.37	0.73±0.03	1.02±0.14	0.06±0.02	3.73±0.69	0.92±0.05	15.3±1.5	2.8±0.3	0.98±0.14
	MP/BSGH1	0.97±0.14	0.00±0.00	1.14±0.12	0.52±0.47	1.09±0.16	0.00±0.00	3.20±0.15	0.52±0.47	14.7±3.6	2.6±0.6	0.99±0.37
in: initial (before aerobic fermentation for yeast production)												
res: residual (after aerobic fermentation for yeast production)												

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