Review Article

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Propagation of selected fungal isolates using media formulated from brewery spent waste.

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Five different fungal isolates, *Rhizopus stolonifer, Rhizopus oligosporus, Mucor racemosus, Mucor rouxii and Aspergillus niger* were selected for growth observation on substrates formulated from brewer' spent grains, while commercial yeast extract agar served as the control. Dry biomasses were determined by diameter measurement. The fungi generally showed best growth in substrate combinations of spent grains and yeast extract. *Aspergillus niger* showed the highest extension growth of 380 mm at 144 hrs in 0.9 % w/v of spent grains and yeast extract (SG.YE) in the ratio 1:1. The biomass production by each organism peaked at the 96th hour.

Key words: Fungi, growth, brewers' spent grain, media formulation.

Significant amount of processing wastes and residues are generated from the food industries. The accumulation of these wastes in the environment can result into pollution (Zvidzai *et al*. 2007). According to Torres *et al*. (2004) the changing of economic, social, political and cultural values of the world has challenged many countries to effectively utilize industrial biotechnology for a friendly way of disposing wastes. The industrial waste contain vast amounts of nutrients which can be harnessed for growth of microorganisms and subsequent production of useful primary and secondary products like enzymes (Aikat and Bhattacharyya, 2000), energy production (Bhumibhamon, 1978), biomass production (Wang *et al.,* 2001) and organic acid like lactic acid, butanol, methanol and ethanol (Bartolome *et al.* 2002). The brewing industry generates relatively large amounts of by-products and wastes; spent grain, spent hops and yeast being the most common. However as most of these are agricultural products, they can be recycled and reused. Thus, compared to other industries, the brewing industry tends to be more environmentally friendly (Ishiwaki *et al*. 2000). Spent grain is the most abundant brewing by-product corresponding to around 85% of total by-products generated (Townsley, 1979). Brewers' spent grain (BSG) is a lignocellulosic material containing about 17% cellulose, 28% non-cellulosic polysaccharides, mainly arabinoxylans, and 28% lignin. BSG is available in large quantities throughout the year, but its main application has been limited to animal feeding (Ozturk *et al.* 2002). Attempts have been made to use BSG in biotechnological processes such as in cultivation of single cell protein, source of value added products, such as ferulic and p-coumaric acids, xylose, arabinose, or as raw material for xylitol and arabitol production (Palmquist and Hahn-Hagerdal, 2000; Fastnaught, 2001; Mussato and Roberto, 2004). BSG is a suitable nitrogen and energy source for production of xylanase by *Aspergillus awamori* (Bhumibhamon, 1978). Acid hydrolysis of BSG with diluted sulphuric acid produce a sugar rich hydrolysate which when neutralized supported high biomass yields when fermented with the yeast *Debaryomyces hansenii* (Carvalheiro *et al*. 2004). BSG has been used as substrate for cultivation of microorganisms due to its protein content (Schildbach *et al*. 1992).

Various species of fungi have very important uses both industrially and medically.

They are useful in biological control, food, fermentation, fungal drugs, food processing and paper making (Wainwright, 1992; Gow and Gad, 1995). This work focuses on the use of BSG as the main ingredient of a medium formulated for the cultivation of selected fungal isolates.

MATERIALS AND METHODS

Brewers spent grains (barley) were obtained from the International Brewery Ilesha, Osun State. The source of the test organisms was a seven day old culture of five different isolates selected after Potato Dextrose agar with dilute preparation of yoghurt. The inoculums were prepared by transferring the seven day old culture of each organism into a test tube that contained sterile distilled water. It was homogenized for 20 seconds. The dried barley grains were blended, sieved and an extract was obtained from 60g of barley that was steeped in sterile distilled water and decanted.

Preparation of basal medium: A basal medium containing the following in g/L was prepared: NH_4NO_3, 4; NH_4Cl, 20; KH_2PO_4, 4; K_2HPO_4, 12; Na_2SO_4, Mg_2SO_4, 0.4. The basal medium was autoclaved at 121^oC for 15 minutes. Three different types of basal media were prepared. Basal medium plus spent grains, basal medium plus commercial yeast extract (Control) and a basal medium, spent grains and yeast powder all in varied concentrations. These medium were sterilized at 121^oC for 15mins. One hundred milliliter (100ml) of each combination was dispensed into a 500ml shake flask and 1ml of each homogenized fungal inoculums was introduced into the flasks using a sterile syringe. These were fermented at 28 ± 2^oC while samples were collected at different intervals for analysis. A solid media was prepared by solidifying the previously prepared substrates combinations with 15% Agar No. 3 (Oxoid). Different concentrations of the spent grains and yeast powder were used. The solid media was inoculated with the fungal inoculums and incubated at 30^oC.

Determination of Biomass Content

The biomass was determined at intervals of 48, 72, 96 and 120 hours. The suspension was filtered through Whatman No. 1 filter paper to remove mycelium. The filter cake paper was washed thrice with deionised water, dried at 105^o C to a constant mass in an oven (Gallenkamp), and weighed as the biomass.

Determination of growth extension

The growth extension was also measured at intervals of 48, 72, 96, 120 and 144 hours using a vernier caliper.

Statistical analysis

Analysis of variance and T-test for data was carried out using SPSS (Statistical Package for Social Science) version 15.0 software.

RESULTS

The dry biomasses cropped from the fungal isolates after cultivation in the liquid media are shown in Tables 1 to 5. The fungal isolates showed maximum growth on yeast extract agar with the exception of *A. niger* which showed highest growth in substrates combinations of spent grains and yeast extract (Table 6). *A. niger* had the highest biomass crop in a medium combination of 0.6 % w/v brewers spent grain and yeast extract. *Mucor racemosus* however was at its best in yeast extract broth medium. Generally, rate of increase in biomass production peaked at the 96th hr as shown in Tables 1 to 5.

Five different fungal isolates were cultured and observed for growth at varying intervals on substrates containing differing concentrations of spent brewer grains and yeast extract powder in formulated media. Their extension growth measurement shown on Tables 7 to 11 revealed that *Aspergillus niger* had the highest growth rate amongst all the cultivated organisms. *A. niger* had a growth extension of 380 mm after 144hrs of incubation on a yeast extract agar supplemented with 0.9 % w/v of spent brewers grain (Table 10). *Mucor racemosus* (Table 9) and *Mucor rouxii* (Table 11) showed no significant growth on the solid media throughout the period of observation. *Rhizopus stolonifer* showed its best growth on substrate containing only spent grains as compared with the other substrate combinations. *Rhizopus oligosporus* however showed its best growth on yeast extract agar supplemented with brewers spent grain. *R. oligosporus* had a growth extension of 145 mm after 144hrs of incubation (Table 8).

There was no significant difference in the extension growth measurements of *R. stolonifer,* and *A. niger* on the various substrate combination ($p \leq 0.5$). However there was significant difference in the growth

Table 1: Biomass (g/l) cropped from *Rhizopus stolonifer*

Time (h)	Biomass (g/L)		
	YEA	SG	SG.YE
48	21.08±0.05	15.83±0.05	18.58±0.02
72	30.10±0.04	18.43±0.03	22.51±0.02
96	36.18±0.04	20.86±0.05	25.51±0.01
120	41.85±0.03	23.21±0.04	27.93±0.03

Table 2: Biomass (g/l) cropped from *Rhizopus oligosporus*

Time (h)	Biomass (g/L)		
	YEA	SG	SG.YE
48	19.35±0.04	14.15±0.04	14.35±-.04
72	28.58±0.02	23.49±0.03	21.22±0.03
96	33.80±0.04	27.38±0.04	24.88±0.05
120	36.15±0.03	30.63±0.04	28.09±0.02

Table 3: Biomass (g/l) cropped from *Mucor racemosus*

Time (h)	Biomass (g/L)		
	YEA	SG	SG.YE
48	18.08±0.04	17.42±0.01	20.83±0.05
72	27.41±0.03	25.55±0.04	29.78±0.02
96	35.58±0.04	28.72±0.04	33.95±0.01
120	39.16±0.03	30.64±0.03	36.75±0.02

Table 5: Biomass (g/l) cropped from *Mucor rouxii*

Time (h)	Biomass (g/L)		
	YEA	SG	SG.YE
48	19.00±0.02	16.83±0.03	17.58±0.04
72	27.42±0.02	19.31±0.03	22.13±0.04
96	35.00±0.04	21.64±0.03	26.41±0.01
120	39.25±0.01	23.91±0.04	28.55±0.03

Table 6: Biomass (g/l) cropped from *Aspergillus niger*

Time (h)	Biomass (g/L)		
	YEA	SG	SG.YE
48	19.22±0.03	15.32±0.05	17.34±0.02
72	28.06±0.03	27.66±0.01	25.95±0.15
96	33.41±0.04	36.09±0.05	33.55±0.04
120	38.65±0.03	39.50±0.03	39.87±0.03

Table 7: Growth extension measurement of *Rhizopus stolonifer*

Time (h)	Growth extension (mm)		
	YEA	SG	SG.YE
48	65	45	50
72	70	85	70
96	175	150	125
120	215	195	155
144	225	180	180

Table 8: Growth extension measurement of *Rhizopus oligosporus*

Time (h)	Growth extension (mm)		
	YEA	SG	SG.YE
48	5	-	50
72	25	15	70
96	30	35	120
120	35	40	130
144	40	45	145

Table 9: Growth extension measurement of *Mucor racemosus*

Time (h)	Growth extension (mm)		
	YEA	SG	SG.YE
48	-	-	-
72	-	-	-
96	-	-	-
120	-	-	-
144	-	-	-

Table 10: Growth extension measurement of *Aspergillus niger*

Time (h)	Growth extension (mm)		
	YEA	SG	SG.YE
48	25	70	75
72	90	170	155
96	175	255	210
120	260	345	325
144	335	350	380

Table 11: Growth extension measurement of *Mucor rouxii*

Time (h)	Growth extension (mm)		
	YEA	SG	SG.YE
48	-	-	-
72	-	-	-
96	-	-	-
120	-	-	-
144	-	-	-

response of *R. oligosporus* on the different media combination. The growth was enhanced in yeast extract agar supplemented with 0.6 % and 0.9 % w/v of spent grains. There were no significant difference ($p \leq 0.5$) in biomass production for *Mucor racemosus, Aspergillus niger, Rhizopus oligosporus,* and *Mucor rouxii* in the various substrate combination. However there were significant differences in the biomass production by *Rhizopus stolonifer* in the different media formulation.

DISCUSSION

Among the organisms subjected to growth on the different media combination, *A. niger* gave the fastest extension growth measurement and the highest dry biomass production in substrate combination of spent grains and yeast extract (0.6 % w/v SG.YP). *A. niger* has been reported to produce an array of extracellular enzymes required for the degradation of the major components of brewers spent grain. Balagopopal and Maini (1976) reported that *A. niger* was superior to other species of *Aspergillus* and *Rhizopus* in amylase production. The enzymatic activity of this organism and the rapid breakdown of the substrate may be responsible the highest extension growth and biomass produced by *a. niger* compared with other fungi cultured. The mean dry biomass harvested at the end of 48 h cultivation of *A. niger* in brewers spent grain was 15.32± 0.05. Hang *et al.* (1975) reported a dry biomass of 13g/ L of *A. niger* grown in brewery spent grain liquor. Balagopopal and

Maini (1976), however observed a similar range of 15.60g/ L dry biomass of *A. niger* when grown cassava rind basal medium. A lower biomass of 9.82± 0.35g/ L was observed using cassava whey (Ikenebomeh and Chikwendu, 1997). It has been observed that supplementation greatly increased the biomass and protein content of the biomass of *Calvalia gigantean* and *Candida strearolytica* grown in supplemented and unsupplemented brewery wastes (Shannon and Stevenson, 1975). *Rhizopus stolonifer* showed better growth on yeast extract agar than in formulated medium.

REFERENCES

Aikat K, Bhattacharyya BC, 2000. Optimization of some parameters of solid fermentation of wheat bran for protease production by a local strain of *Rhizopus oryzae. Acta Biotechnological 20*: 149-159.

Balagopopal C, Maini SB, 1976. Evaluation of certain species of fungi for single cell protein and amylase production in cassava liquid medium. *Journal of Root Crops 2*: 49-51.

Bartolome B, Santos M, Jimenze JJ, del Nozal MJ, Gomez cordoves C, 2002. Pentoses and hydroxycinnamic acids in brewers spent grain. *Journal of Cereal Science 36*: 51-58.

Bhumibhamon O, 1978. Production of acid protease and carbohydrate degrading

Carvalheiro F, Esteves MP, Parago JC, Pereira H, 2004. Spent grain. *Bioresource Technology 91*: 93-100.

enzyme by *Aspergillus awamori. The Journal of Agricultural Science 11*: 209-222

Fastnaught CE, 2001. Barley flour. In: S Cho, ML Dreher, SS Cho, eds. Handbook of dietary fiber, Marcel Dekker, New York, pp 519-542.

Gow N, Gad GM, 1995. *The growing fungus.* Chapman Hall, London.

Ikenebomeh MJ, Chikwendu AE, 1997. *Aspergillus niger* biomass production in cassava whey medium. *Nigerian Journal of Microbiology 11*: 52-63.

Ishiwaki N, Murayama H, Awayama H, Kanauchi O, Sato T, 2000. Development of high value uses of spent grain by fractionation technology. MBAA technical Quarterly *37: 261-265.*

Mussato SI, Roberto IC, 2004. Alternatives for detoxification of dilute acid lignocellulosic hydrolysate for use in fermentative process: a review. *Bioresource Technology 93:* 1-10.

Ozturk S, Ozboy O, Carvidoglu I, Koksel H, 2002. Effect of brewers spent grain on the quality and dietary fiber content of cookies. *Journal of the Institute of Brewing 108*: 23-27.

Palmquist E, Hahn-Hagerdal B, 2000. Fermentation of lignocellulosic hydrolysates inhibitors and mechanisms of inhibition. *Bioresource Technology 74:* 25-33.

Schildbach R, Ritter W, Schmithais K, Burbridge M, 1992. New developments in the environmentally safe disposal of spent grains and waste kieselguhr from breweries. *Proceedings of the convetion Institute of brewing* (Asia Pacific section) 22: 139-143.

Shannon LJ, Stevenson KE, 1975. Growth of *Calvatia gigantean* and *Candida stearolytica* in brewery wastes for microbial protein production and BOD reduction. *Journal Food Science 40*: 830-832.

Torres JL, Selga A, Cascane M, 2004. Bioactive products from by-product and wastes. *Agric. Food Chem. 6*: 128-129.

Townsley PM, 1979. Preparation of commercial products from brewer's waste grain. MBAA Technical Quarterly 16: 130-134.

Wainwright M, 1992. *An introduction to fungal biotechnology* Wiley, Chichester.

Wang B, Sakoda A, Suzuki M, 2001. Biological efficiency and nutritional value of *Pleurotus ostreatus* cultivated on spent beer grain. *Bioresource Technology 78*: 293-300.

Zvidzai C, Muzhinji N, Chidzvondo F, Mundembe R, Sithole-Niang I, 2007. Potential commercialization of a microbial medium formulated from industrial food waste. *African Journal of Microbiology 1*(6): 79-87.