Journal of Industrial Microbiology & Biotechnology (2002) 29, 264–267

www.nature.com/jim

Bioconversion of acid- and gamma-ray-treated sweet potato residue to microbial protein by mixed cultures

NH Aziz and GI Mohsen

Microbiology Department, National Center for Radiation Research and Technology, Nasr City, Cairo, Egypt

Sweet potato residue, a starchy agricultural waste, was used as a substrate to produce microbial protein by *Fusarium moniliforme* and *Saccharomyces cerevisiae* in submerged fermentation. Acid- and gamma-irradiation-pretreated sweet potato residue enhanced the biomass yield and protein production when the residue was fermented with *F. moniliforme* and *S. cerevisiae.* A mixed culture of *F. moniliforme* and *S. cerevisiae* efficiently and rapidly utilized free sugars; the maximal biomass yield (13.96 g/l) and protein production (65.8%) were obtained after 3 days fermentation. Lower carbon utilization by the two microbial strains occurred in the waste-containing media as compared to control, increasing the economic value of the waste usage.
Journal of Industrial Microbiology & Biotechnology (2002) **29,** 264–267 doi:10.1038/sj.jim.7000297

Keywords: mixed cultures; SCP; irradiation; sweet potatoes; *Fusarium*; *Saccharomyces*

Introduction

Recently, there has been much effort to develop a source of protein independent of agricultural land utilization. Owing to a shortage of protein for animal feed and problems with disposal of agricultural waste products, attempts have been made to produce single-cell protein (SCP) from these wastes [7,10,14,26].

Sweet potato is one of the major tuber crops grown in different countries of the humid tropics and is abundant in Egypt (265,000 tons per year) [13]. A major portion of sweet potato tubers is used for the extraction of starch, which usually results in problems for disposal of the residue [3,24].

Chemical, physical and enzymatic treatments have been investigated to determine their effectiveness in providing protein-rich biomass [1,8,9]. Recently, mixed culturing of microorganisms proved to be the best method for converting carbohydrate wastes into high yields of microbial protein using short fermentation times [8,11,15,19].

In the present study, we investigated the possibility of bioconversion of sweet potato residue into SCP by submerged mixed fermentation of two fast-growing organisms, *Fusarium moniliforme* and *Saccharomyces cerevisiae.* The experiments included the determination of sugar and nitrogen utilization and the crude protein content produced by the mono and mixed cultures of *F. moniliforme* and *S. cerevisiae* during fermentation of this waste pretreated with acid and gamma irradiation.

Materials and methods

Microorganisms

S. cerevisiae was isolated from commercial baker's yeast, while the fungus *F. moniliforme* (EN-1 96) was isolated from local wheat grains. The medium consisting of (g/l distilled water) D-glucose 10.0, $(NH_4)_2S0_4$ 5.0, KH_2P0_4 1.0, $MgS0_4{\cdot}7H_20$ 0.5, NaCl 1.0, $CaCl_2$ 0.1, pH 5.5 [20] was used as the control medium as well as for the maintenance of yeast and fungus cultures.

The waste used

Sweet potato residue was purchased from local markets in Cairo, Egypt, washed with distilled water to remove dust, peeled and screened with a 14–16 mesh to remove large aggregates. The substrate was hydrolyzed with 0.5 N HCl at a liquid: solid ratio of 6:1. Hydrolysis was carried out at 121°C for 20 min and the material was allowed to cool. The pH of the substrate was adjusted to 7.0 with NaOH. The homogenized hydrolysate samples were then irradiated with 1, 3 and 5 kGy using Egypt's Industrial Mega-Gamma Irradiator (Cobalt-60) located at the National Center for Radiation Research and Technology, Cairo, Egypt. The dose rate at the time of the experiment was 64 Gy/min. These pretreated substrates were fermented with *F. moniliforme* and *S. cerevisiae.*

Fermentation media

F. moniliforme and *S. cerevisiae* were inoculated into media containing pretreated sweet potato residue as a carbon source. The medium contained $(NH_4)_2S0_4$ 4.0 g, KH_2P0_4 2.0, NaCl 0.2 g, $CaCl_2{\cdot}2H_2O$ 0.2 g, $MgS0_4{\cdot}7H_2O$ 0.2, yeast extract 0.5 g and the pretreated homogenate sweet potato residues 10.0 g. These ingredients were brought to 1 l in volume with distilled water, adjusted to pH 5.5.and autoclaved at 121°C for 15 min.

Media (200-ml aliquots) were inoculated with 1.0 ml of *F. moniliforme* (10^6 conidia/ml) or *S. cerevisiae* (10^5 cells/ml) based upon total plate count. The inoculated medium was shaken in a rotary water bath (New Brunswick Model G76, New Brunswick Scientific, New Brunswick, NJ) at 200 rpm and at 28°C for 7 days. After shaking, flasks were allowed to stand for 30 min after which the supernatant was withdrawn. For the mixed culture 1.0 ml each of *F. moniliforme* and *S. cerevisiae* were added to the fermentation medium and samples were taken daily for 7 days. Fungal and yeast biomass were separated by centrifugation at 10,000×*g* for 20 min,

Correspondence: Dr Nagy H Aziz, National Center for Radiation Research and Technology, 3 Ahmed El-Zumer Street, PO Box 29, Nasr City, 113701, Cairo, Egypt
Received 25 October 2001; accepted 22 June 2002

Table 1 Growth of *F. moniliforme* and utilization of carbohydrates and nitrogen in HCl- and gamma-irradiation-treated sweet potato residue medium[1]

Dose (kGy)	Biomass (mg/100 ml)	Reducing sugars			Total nitrogen		
		Initial amount (g/l)	Final amount (g/l)	Sugar utilization[2] (%)	Initial amount (g/l)	Final amount (g/l)	Nitrogen utilization[3] (%)
0	316[a]	6.47	3.39	47.60[a]	61.08	18.78	69.25[a]
1	489[b]	7.87	3.82	51.46[b]	62.92	10.93	82.63[b]
3	594[c]	10.44	4.73	54.69[b]	46.18	8.93	86.09[b]
5	677[c]	12.29	4.84	60.62[c]	46.02	8.54	87.06[b]

[1]Values are means of four replications. Means with different superscripts differ from each other ($P<0.05$) within pairs within rows. Data were collected after 7 days at 30°C.
[2]Sugar utilization=[(amount of sugar utilized)/(initial sugar concentration)]×100.
[3]Nitrogen utilization=[(amount of nitrogen utilized)/(initial nitrogen concentration)]×100.

washed with double-distilled water, dried at 80°C to a constant weight and kept in a desiccator.

Analysis of media and microbial biomass

The clear solution obtained after centrifugation of inoculated samples (to remove microbial cells) was used to determine sugar content. Reducing sugar, expressed as glucose, was measured by the dinitrosalicylic acid (DNSA) method of Noelting and Bernfeld [17] using glucose as the standard. Total protein of fungal and yeast cells were calculated by multiplying percentage nitrogen (N) by 6.25 as determined by the micro-Kjeldahl method [2].

Statistical analysis

The data (four replicates) were subjected to analysis of variance (ANOVA) [21]. Multiple range and multiple F tests [6] were applied to the data when significant ($P<0.05$) F values were obtained by ANOVA.

Results and discussion

The basal medium was supplied with 10 g/l of sweet potato residue pretreated with HCl and gamma irradiation. It contained 14% moisture, 3.1% crude protein, 3.6% ash, 18% crude fiber and 70% carbohydrate. Tables 1 and 2 show that assimilation of reducing sugars that took place by the monocultures of *S. cerevisiae* and *F. moniliforme* was significantly ($P<0.05$) more active using pretreated sweet potato residue compared with the raw sweet potato residue. Also, the assimilation of reducing sugars by the mixed culture using pretreated sweet potato residue was significantly higher ($P<0.05$) (Table 3). In addition, these data (Table 3) showed that biomass production after 3 days of fermentation with pretreated sweet potato residue using the mixed culture was significantly higher ($P<0.05$) compared to monocultures of *F. moniliforme* and *S. cerevisiae.* From Tables 1–3 it is clear that the rate of sugar assimilation was significantly ($P<0.05$) increased by increasing gamma-irradiation dose levels; the highest growth was attained in 5-kGy-irradiated sweet potato residue.

Concerning nitrogen assimilation (Tables 1–3) the rate of nitrogen assimilation was significantly ($P<0.05$) higher in pretreated sweet potato residue compared with untreated ones and gradually increased by increasing the irradiation dose levels of pretreated sweet potato residue. Maximum nitrogen utilization occurred in the presence of 5-kGy-irradiated sweet potato residue by mono and mixed cultures. There were no significant differences ($P<0.05$) in biomass production, sugar utilization and nitrogen assimilation when sweet potato residue was pretreated with HCl or gamma irradiation alone and then fermented with single or mixed cultures (data are approximately equivalent to controls). Fermentation carried out on the sweet potato residue obtained after an acid and gamma-irradiation treatment was much higher than that on raw potato residue due to degradation of monocrystalline polymers (starch) to a dilute solution of simple sugars [23]. Our data are in agreement with the report of Steinboch *et al* [22] who showed that starch was hydrolysed to low molecular weight dextrin and glucose using several chemical, physical and enzymatic treatments on different agricultural wastes. Chemical, physical and enzymatic treatments were evaluated on rice straw, wheat straw, sugar cane bagasse, sugar beet pulp, orange peel, dried corn cobs and stalks, groundnut pod shells and carob pods. All

Table 2 Growth of *S. cerevisiae* and utilization of carbohydrates and nitrogen in HCl- and gamma-irradiation-treated sweet potato residue medium[1]

Dose (kGy)	Biomass (mg/100 ml)	Reducing sugars			Total nitrogen		
		Initial amount (g/l)	Final amount (g/l)	Sugar utilization[2] (%)	Initial amount (g/l)	Final amount (g/l)	Nitrogen utilization[3] (%)
0	232[a]	5.41	2.44	54.90[a]	61.08	17.31	71.66[a]
1	273[a]	6.92	2.69	61.13[b]	62.92	11.82	61.21[b]
3	362[b]	8.53	2.53	70.00[b]	64.18	8.23	87.18[b]
5	449[b]	9.02	2.31	74.39[b]	66.02	7.39	88.81[b]

[1]Values are means of four replications. Means with different superscripts differ from each other ($P<0.05$) within pairs within rows. Data were collected after 7 days at 30°C.
[2]Sugar utilization=[(amount of sugar utilized)/(initial sugar concentration)]×100.
[3]Nitrogen utilization=[(amount of nitrogen utilized)/(initial nitrogen concentration)]×100.

Table 3 Profile of mixed culturing of *S. cerevisiae* and *F. moniliforme* in HCl- and gamma-irradiation-treated sweet potato medium[1]

Dose (kGy)	Biomass (mg/100 ml)	Reducing sugars			Total nitrogen		
		Initial amount (g/l)	Final amount (g/l)	Sugar utilization[2] (%)	Initial amount (g/l)	Final amount (g/l)	Nitrogen utilization[3] (%)
0	442[a]	6.70	2.24	66.60[a]	61.80	13.47	78.20[a]
1	561[b]	8.10	2.28	71.90[b]	62.90	8.30	86.80[b]
3	870[c]	14.30	2.06	85.60[c]	64.10	4.50	92.98[c]
5	1396[d]	16.80	0.59	95.90[d]	66.30	2.78	95.81[c]

[1]Values are means of four replications. Means with different superscripts differ from each other ($P<0.05$) within pairs within rows. Data were collected after 7 days at 30°C.
[2]Sugar utilization=[(amount of sugar utilized)/(initial sugar concentration)]×100.
[3]Nitrogen utilization=[(amount of nitrogen utilized)/(initial nitrogen concentration)]×100.

treatments were effective in increasing the microbial digestibility as well as in increasing the yield of a protein rich-biomass [1,8,9,12,14,16,18,25].

Previous studies conducted in our institute on protein levels and nutritional qualities of certain fungi suggested the cultivation of *F. moniliforme* and *S. cerevisiae* on agricultural by-products consisting of the potato starch factory effluent as a source of microbial protein [3]. In these studies, *F. moniliforme* and *S. cerevisiae* were cultivated in batch and semicontinuous cultures. The same fungus and yeast had already been reported to grow on agricultural wastes for microbial protein production [1,14]. Thus, studies were performed to assess their suitability for producing microbial protein by continuous fermentation of the sweet potato residue. Results given in Table 4 show the production of' biomass protein by the monocultures and the mixed culture in HCl- and gamma-irradiation-pretreated sweet potato residue. Degradation of starch and utilization of reducing sugars were faster with mixed cultures of *F. moniliforme* and *S. cerevisiae* than with either organism alone. There were significant differences ($P<0.05$) in biomass production with various pretreated gamma-irradiation dose levels of sweet potato when monocultures of *F. moniliforme* were compared with that of *S. cerevisiae.* Highest biomass yield of 677 mg/100 ml, equivalent to 2.51 g/l of crude protein, was obtained by fermentation of 5-kGy-treated sweet potato with *F. moniliforme* while biomass production was 449 mg/100 ml, equivalent to 1.21 g/l of crude protein, when 5-kGy-pretreated sweet potato was fermented with *S. cerevisiae*. As supported by the rapid growth of mixed cultures, the maximal biomass yield obtained by fermentation of 5-kGy-pretreated sweet potato residue after 3 days incubation was 1396 mg/100 ml, equivalent to 9.18 g/l of crude protein. As shown in Table 4, the total crude protein percentage of the mixed culture biomass was 41%, 63.2% and 65.8% in 1-, 3- and 5-kGy-pretreated sweet potato residue, respectively, which was significantly higher ($P<0.05$) than for *F. moniliforme* (23.0% to 37%) and *S. cerevisiae* (14.2% to 27.0%). In addition, the values of the protein conversion coefficient (protein yield; grams protein per gram potato sugar consumed) was significantly higher ($P<0.05$) in the mixed culture fermentation than that occurring with a monoculture fermentation. The maximum level of protein conversion coefficient obtained by fermentation of 5-kGy-pretreated sweet potato residue with a mixture of *F. moniliforme* and *S. cerevisiae* was 56.6% after 3 days of fermentation. Protein and total amino acid contents of *F. moniliforme*, *F. oxysporum*, *Aspergillus niger* and *Candida tropicalis* were investigated by Christias *et al* [4]. *F. moniliforme* and *F. oxysporum* contained approximately 30% more protein and total amino acids than *A. niger* and the authors concluded that *Fusarium* spp. may be suitable for commercial production of microbial protein, especially when low-cost agricultural or industrial waste products are readily available as energy source. Ghanem [11] found that a combination of *Trichoderma reesi* and *Kluyveromyces marxianus* gave high SCP yields (5 1%) and

Table 4 Biomass characteristics in the tested media formed by the monoculture and the mixed cultures of *F. moniliforme* and *S. cerevisiae*

Biomass characters	Organisms	Irradiation doses (kGy)			
		0	1	3	5
Biomass yield (mg/100 ml)	*F. moniliforme*	316[a]	489[c]	594[c]	677[d]
	S. cerevisiae	232[b]	273[b]	362[a]	449[c]
	F. moniliforme+*S. cerevisiae*	422[d]	561[d]	870[c]	1396[f]
Protein (mg/ml)	*F. moniliforme*	0.72[a]	1.12[a]	1.38[a]	2.51[b]
	S. cerevisiae	0.33[a]	0.48[a]	0.69[a]	1.21[a]
	F. moniliforme+*S. cerevisiae*	1.68[a]	2.32[b]	5.50[c]	9.18[d]
Protein (% biomass)	*F. moniliforme*	22.88[a]	22.69[a]	23.22[a]	37.08[c]
	S. cerevisiae	14.22[b]	14.63[b]	19.06[b]	26.95[b]
	F. moniliforme+*S. cerevisiae*	39.71[c]	41.39[c]	63.22[d]	65.81[d]
PCC (%)	*F. moniliforme*	23.38[a]	27.65[a]	23.22[a]	33.69[c]
	S. cerevisiae	11.11[b]	11.35[b]	11.5[b]	18.03[b]
	F. moniliforme+*S. cerevisiae*	37.67[c]	39.86[c]	63.22[d]	56.63[c]

Values are means of four replications. Means with different superscripts differ from each other ($P<0.05$) within pairs within rows. Data were collected after 7 days at 30°C for monocultures and after 3 days for mixed cultures.
PCC=protein conversion coefficient=[(weight of protein produced)/(weight of sugar utilized)]×100.

efficiently converted beet pulp into proteins (39.4%). Yeast extract in the basal medium could be substituted. Using mixed cultures of *T. reesi* and *K. marxianus* the beet pulp level increased from 2% to 4%; the highest efficiency of beet pulp conversion into protein was achieved (41.8%) and protein yields reached a maximum value of 54%.

Based on the data obtained in the present study, it may be concluded that sweet potato residue substrates can be fermented with *F. moniliforme* or *S. cerevisiae* to produce microbial biomass. Mixed culture fermentation with *F. moniliforme* and *S. cerevisiae* improve the production of biomass and protein content. Microbial proteins have applications as food ingredients in contrast to their use as protein supplements. Desirable properties of proteins for food applications include water and fat binding, emulsion stability, whippability and foam stability, dispensability, gel formation and thickening [5].

The results of this study combined with the previously reported good appearance and nutritional quality of the mixed culture of *F. moniliforme* and *S. cerevisiae* when fermented in HCl- and 5 kGy gamma-ray-irradiated sweet potato residue make this combination potentially useful for consideration in the production of microbial protein from agricultural by-products.

References

1 Abou-Hamed NAA. 1993. Bioconversion of wheat straw yeasts into single-cell protein. *Egypt J Microbiol* 28: 1–9.
2 A0AC. 1960. In: Horwitz WR (Ed), Official Methods of Analysis, Vol. 9. Association of Official Analytical Chemists, Washington, DC.
3 Aziz NH. 2000. Single-cell protein from acid-treated potato starch effluent by *F. moniliforme* and *S. cerevisiae*. *The 10th International Conference on Environmental Protection Is a Must*. Alexandria University, Alexandria, Egypt, pp. 225–232 (9–11 May).
4 Christias C, C Couvaraki, SG Gerorgopoulos, B Macris and V Vomvoyanni. 1975. Protein content and amino acid composition of certain fungi evaluate for microbial protein production. *Appl Microbiol* 29: 250–254.
5 Cooney GL, C Rha and SR Tannenbaum. 1980. Single cell protein: engineering economics and utilization in foods. In: Chichester CO (Ed), Advances in Food Research, Vol. 26. Academic Press, New York, pp. 1–52.
6 Duncan DB. 1955. New multiple range and multiple *F* tests. *Biometries* 11: 3–42.
7 El-Refai AH, KM Ghanem and AH El-Sabaeny. 1990. Single-cell protein production from orange waste by *Sporotrichum thermophile* cultured under optimal conditions. *Microbios* 63: 254–262.
8 Fields ML, S Tantratian and RE Baldwin. 1991. Production of bacterial and yeast biomass in ground corn cob and ground corn stalk media. *J Food Prot* 54: 117–120.
9 Garg SK and S Neelakantan. 1981. Effect of cultural factors on cellulase activity and protein production by *Aspergillus terreus*. *Biotechnol Bioeng* XXIII: 1653–1659.
10 Ghaly AE and RM Ben-Hassan. 1995. Kinetics of batch production of single cell protein from cheese whey. *Appl Biochem Biotechnol* 50: 79–94.
11 Ghanem KM. 1992. Single-cell protein production from beet pulp by mixed culture. *Microbiologia* 8: 39–43.
12 Han YW and DC Cullihan. 1974. Cellulose fermentation: effect of substrate pretreated on microbial growth. *Appl Microbiol* 27: 159–165.
13 Khalaf MA. 1998. Effect of irradiation and environmental conditions on the bioconversion of potato processing waste by certain microorganisms. PhD thesis. Botany Department, Faculty of Science, Zagazig University, Egypt.
14 Macris BJ and R Kokke. 1978. Continuous fermentation to produce fungal protein. Effect of growth rate on the biomass yield and chemical composition of *Fusarium moniliforme*. *Biotechnol Bioeng* XX: 1027–1035.
15 Manilal VB, CS Narayanan and C Balagopalan. 1991. Cassava starch effluent treatment with concomitant SCP production. *World J Microbiol Biotechnol* 7: 185–190.
16 Neeraj K. 1995. Single cell-protein from chemically pretreated groundnut pod shells using *Pleurouts* sp. *J Food Sci Technol* 32: 141–143.
17 Noelting G and P Bernfeld. 1948. Sur les enzymes amylolytiques: III. La beta amylase: dosage d'activite control de l'absence d'alpha-amylase. *Helv Chim Acta* 31: 286–287.
18 Pandey A and KP Tiwari. 1980. Effects of ultraviolet radiation on amino acid composition of single-cell protein. *J Indian Chem Soc* 59: 686–687.
19 Pasari AB, RA Korus and RC Heimsch. 1989. A model continuous fermentations with amylolytic yeasts. *Biotechnol Bioeng* 32: 338–343.
20 Phaff HJ, MW Miller and EM Mark. 1966. The life of yeasts. Harvard University Press, Cambridge, MA, p. 186.
21 Snedecor GW and WG Cochran. 1982. Statistical Methods. Iowa State University Press, Ames.
22 Steinbock FA, I Hedle, S Choojun and CP Kubicek. 1991. Regulatory aspects of carbohydrate metabolism in relation to citric acid accumulation by *A. niger*. *Acta Biotechnol* II: 511–581.
23 Tsao GT, MR Ladish and HR Bungay. 1987. Biomass refining. In: Bungay HR and G Belfurt (Eds), Advanced Biochemical Engineering. Wiley, New York.
24 Thanh NC and JS Wu. 1975. Treatment of tapioca starch waste waters by torula yeast. *Can Inst Food Sci Technol J* 8: 202.
25 Vaccorino C, R Lo Curto, MM Tripodo, R Patane, G Lagaiia and A Ragno. 1989. SCP from orange peel by fermentation with fungi acid treated peel. *Biol Wastes* 30: 1–10.
26 Yousri RM. 1982. Single cell protein, its potential use for animal and human nutrition. *World Rev Anim Prod* 8(2):49–67, (3): 5–6.