Submerged Culture Growth of Edible Mushrooms on Waste Sulphite Liquors

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Various waste sulphite liquors (WSL) i.e. NH_3-, Mg-, Ca-, and mixed-base, were used as substrates for submerged cultivation of four species of edible mushrooms (morel mushroom): *Morchella* spp., *Morchella crassipes, Morchella deliciosa, Morchella esculenta.* Growth was found to be completely inhibited by calcium found in both Ca-WSL and mixed-WSL. (In NH_3-WSL and Mg-WSL growth generally occurs in large pellet form.

The optimal initial pH for the different morel species on various WSL was found to be between 5.0 and 7.0. The highest yield (approximately 83.4% based on utilized carbohydrate) and dry weight (approximately 4.86 g/l) were obtained when the NH_3-WSL was diluted (1:5 v/v) and used for growth of *Morchella crassipes.* Freeze-dried morel mushroom mycelium (MMM) on WSL contains on a dry basis, 25.7 - 48.0% crude protein, 2.45 - 4.38% fat, 13.7 - 39.2% carbohydrate and 5.9 - 18.0% ash. The spectrum of essential amino acids is comparable to the FAO standard, except for the levels of methionine and isoleucine. A strong mushroom flavor (aroma and taste) is also conserved in freeze-dried powdered samples.

On a employé diverses liqueurs usagées au sulfite (LUS), dont les bases étaient NH_3-, Mg-, Ca- et un mélange de ces cations (MC), comme substrats pour la culture sous l'eau de quatre espèces de champignons comestibles (morilles), nommément *Morchella* spp., *Morchella crassipes, Morchella deliciosa* et *Morchella esculenta.* On a trouvé que le calcium présent dans Ca-LUS empêchait complètement la croissance des dits champignons. Dans le cas de NH_3-LUS et Mg-LUS, la croissance s'est faite sous forme de grosses boulettes.

On a trouvé qu'un pH initial de 5.0 à 7.0 était optimal pour les différentes espèces de morilles cultivées sur les types précités de LUS. On a obtenu le meilleur rendement (environ 83.4% basé sur l'hydrate de carbone utilisé) et le poids sec le plus élevé (environ 4.86 grammes par litre) lorsqu'on dilua NH_3-LUS (1.5 v/v) et l'employa ainsi pour la culture de *Morchella crassipes.* Le mycélium de la morille séchée à l'état de congélation sur LUS contient à l'état sec 25.7 à 48.0% de protéine brute, 2.45 à 4.38% de matière grasse, 13.7 à 39.2% d'hydrate de carbone et 5.9 à 18.0% de cendres. Le spectre des acides aminées essentielles se compare au standard de la FAO, excepté en ce qui a trait aux concentrations du méthionine et de l'isoleucine. Une forte de champignon (arôme et goût) persiste également dans les échantillons en poudres séchés à l'état congelé.

Waste sulphite liquors from pulp and paper mills can be used as substrates for growth of various microorganisms[1,2,3] including morel mushrooms[4]. Because of the abundance and availability of this substrate and because of the disposal problems associated with *WSL*, it is of particular interest to convert the organic material from these liquors to a valuable food or feed product.

Submerged cultivation of morel mushrooms on various substrates has been studied by many authors[5-11]. These edible mushrooms were of interest because of their nutrient content[12] and their potential use as flavoring materials[13] when grown on sugars and because many mushrooms grow on decaying wood and organic matters.

In this paper, the optimal growth conditions in terms of pH and substrate concentration on the yield of biomass are investigated using NH_3-, Mg-, Ca- and mixed-base sulphite liquors for the growth of four different species of morel mushrooms (*Morchella* spp., *Morchella crassipes, Morchella deliciosa, Morchella esculenta).* Their protein, essential amino acids, fats and carbohydrate contents are studied in order to select the best culture for an economical production. Advantages of submerged cultivation of morel mushrooms on *WSL* are also mentioned.

Materials and methods

Waste sulphite liquors

The concentrated *WSL* samples were received from various pulp and paper mills. The insoluble solids were removed by filtration and the liquors were stored in closed plastic tanks for further use. Before utilization, the liquors were stripped of SO_2 by boiling for 30 minutes and then diluted with distilled water. The characteristics of the liquors are presented in Table 1. The carbohydrates were determined by anthrone reagent[14] and are expressed as glucose.

Stock cultures

The original cultures of *Morchella crassipes* NRRL-2369, *Morchella deliciosa* NRRL-2601, *Morchella esculenta* NRRL-2603 were received from the U.S. Department of Agriculture and that of *Morchella* ssp. from the Department of Plant Science of The University of Western Ontario, London, Ontario, Canada. These pure cultures were maintained on stock culture agar slants of the following composition: 2.5% glucose, 2.0% agar, 1.0% peptone, and 0.5% yeast extract. Slants were inoculated with a portion of the mycelium, at least 0.5 cm^2, to minimize the probability of selecting variant types. They were incubated at room temperatures for 6 to 8 days depending upon the fungal species and then were stored in the refrigerator (4°C). Fresh transfers of stock cultures were made every 4 months.

Inoculum

The inocula for shake flask fermentations were always prepared from the fresh growth on agar slants, using the same culture conditions as these described

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for stock cultures. The entire growth of 3 slants was transferred aseptically to a sterile Waring-blender jar containing 150 ml of sterile distilled water and blended for 30 seconds. This suspension was used to innoculate the culture media. The inoculum volume was 5% (*v/v*).

Growth in 4 Erlenmeyer flasks (800 ml of total culture medium) was used to seed large carboy-type bottles operated at 16 liter volume. Relatively small pellets (1-3 mm in diameter) developed in the Erlenmeyer flasks so that blending was not necessary.

Fermentation methods

The shake flask fermentation experiments were performed in 500 ml or 1000 ml Erlenmeyer flasks containing 200 ml or 300 ml of culture medium. The medium was prepared by dilution of a stock solution with the composition as outlined in Table 2. The dilution ratios for individual experiments varied and are shown in the results. The initial pH of the medium was adjusted to the desired value by addition of either 4N HCl or 2.5N NaOH. The shake flasks were sterilized in the autoclave at 15 psig and 120°C for 20 minutes and then cooled to room temperature. The flasks were inoculated and were incubated at 200 RPM for 9 days at room temperature (23°C).

Larger laboratory-scale batch fermentations were also performed in a 5-gallon bottle fermentor containing 16 l of culture medium. The culture medium for these experiments was prepared in the same way as for shake flask experiments. Fermentations were conducted at room temperature at an aeration rate of 4 litres of air per minute or 0.25 vvm (volume of air per broth volume per minute) for 9 days. No agitation was utilized other than that obtained from aeration.

Analytical methods

For the determination of dry weight, the total amount of *MMM** in the culture medium was filtered through dehydrated filter papers (by drying in the oven at 105°C for 2 hours). The filter paper with mycelia was dried in an oven at 105°C to constant weight. The initial inoculum and precipitate was subtracted from the total weight to obtain the dry weight of produced mycelia. Mycelia from the 5-gallon fermentor were filtered by gravity through cotton gauze, then washed twice with either 1% NaCl in distilled water or with distilled water, and were then dried in a New Brunswick Freeze-Dryer. The freeze-dried samples were ground into fine powders and stored in closed bottles at room temperature for further analyses.

Total carbohydrates in the culture medium were determined colorimetrically by anthrone reagent according to the method of Morris[14].

The organic nitrogen in the dry mycelium was determined by the Pregl-Parnas-Wagner micro-Kjeldahl method[15]. Mycelial protein was assumed to contain 16% nitrogen and a factor of 6.25 was used to convert the organic nitrogen to crude protein content. No correction for other nitrogen-containing compounds in the cell was made.

For amino acid determination, the dry mycelium was hydrolyzed by refluxing with 500 ml of 5.7 N HCl within 24 hours. The hydrolyzate was dried in the rotary evaporator and then redissolved in an appropriate volume of buffer. Norleucine was added as internal standard and aliquot was applied to sample cartridge for analysis using Technicon TSM-1 Amino Acid Analyzer.

The total lipid was extracted by shaking overnight the dry mycelium powder with glass beads, glass powder and a solvent mixture of chloroform-methanol (2:1) in a tightly stoppered round bottom flask[16]. The extracts were separated from the solid portion by filtration and were evaporated under vacuum to remove the solvent. Dry materials in this flask were redissolved in chloroform and the nonsoluble portion was separated by filtration. The filtrate was finally evaporated to dryness under vacuum and weighed for total fats.

The moisture and ash contents in dried mycelium powder were determined by the method described by Jacobs[17].

Results and discussion

Optimal initial pH range for growth of MMM on WSL

Studies on the optimal initial pH were performed with four species of morel mushrooms, *Morchella* spp., *Morchella crassipes*, *Morchella deliciosa*, and *Morchella esculenta*, on various *WSL* (*NH_3-WSL*, *Ca-WSL*, *Mg-WSL*, and mixed-*WSL*) in 200 ml/500 ml Erlenmeyer flasks within the pH range of 4.0 to 8.0. Early experiments in shake flasks using concentrated *WSL* as a culture medium were unsuccessful. Therefore the *WSL* was diluted in order to reduce the apparent toxicity level of the liquor. The stock cul-

TABLE 1

CHARACTERISTICS OF *WSL* AS RECEIVED

WSL	Supplier	*pH*	Carbohydrate (*g/l.*)
NH_3 – WSL	Canadian International Paper Co., Temiskaming, Que.	2.00	44
Ca – WSL	Domtar Fine Paper Ltd., Cornwall, Ont.	2.55	50
Mixed – *WSL* (*Ca & Mg*)	Spruce Falls Powers & Paper Co., Ltd., Kapuskasing, Ont.	1.80	70
Mg – WSL	The James Maclaren Co., Ltd., Buckingham, Que.	3.85	46

TABLE 2

COMPOSITION OF THE STOCK CULTURE MEDIUM FOR *MMM* ON *WSL*

Constituent	Quantity	
	Flask	Bottle
Concentrated Waste Sulphite Liquor	1*l*	1*l*
Ammonium Phosphate Dibasic $(NH_4)_2 HPO_4$	20*g*	20*g*
Corn Steep Liquor(1)	50*ml*	5*ml*

(1)Supplied by A. D. Staley Mfg. Co., Decatur, Illinois

*Morel mushroom mycelia.

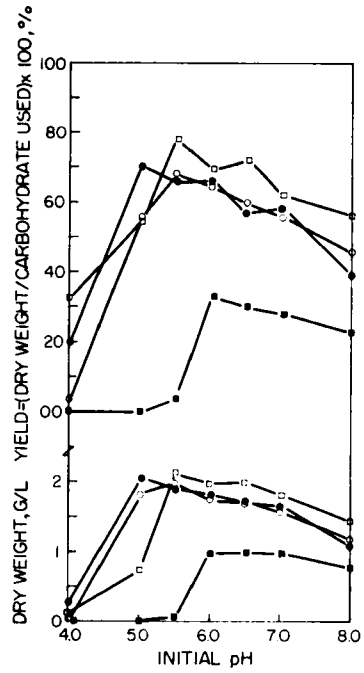

Figure 1 – Effect of initial pH on the growth of MMM on NH_3-WSL.
■ – *Morchella deliciosa*
□ – *Morchella crassipes*
○ – *Morchella esculenta*
● – *Morchella* spp.

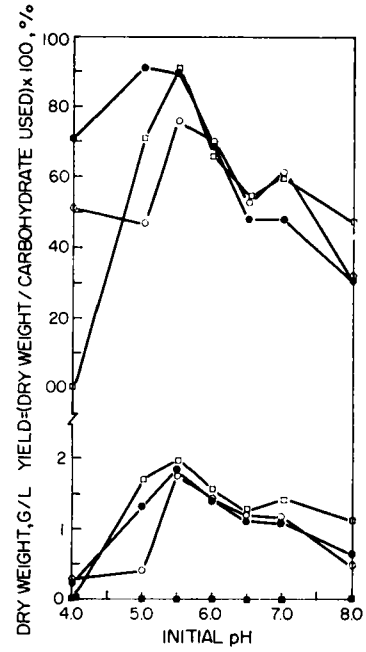

Figure 2 – Effect of initial pH on the growth of MMM on Mg-WSL.
■ – *Morchella deliciosa*
□ – *Morchella crassipes*
○ – *Morchella esculenta*
● – *Morchella* spp.

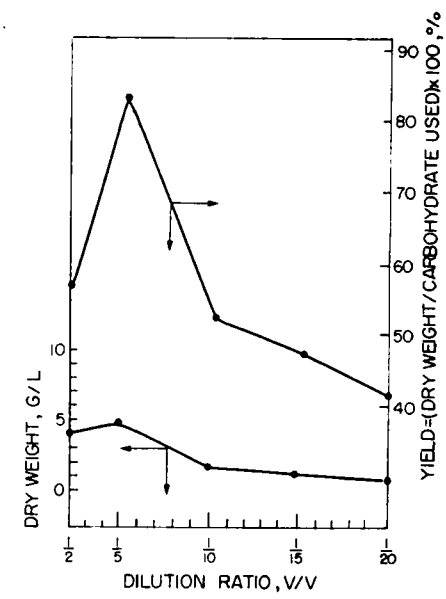

Figure 3 – Effect of the concentration of NH_3-WSL on the growth of *Morchella crasssipes* at optimal initial pH 6.00.

ture medium (Table 2) was diluted to 1:10 (v/v) and used as the culture medium for the studies on the effects of initial pH. The results for *NH_3-* and *Mg-WSL* are shown in Figures 1 and 2.

It was found that none of four species of morel mushrooms grew in *Ca-WSL* nor in the mixed-*WSL* even though high dilution ratios were applied. This fact may be due to the inhibition of growth by calcium compounds which are present in both of these *WSL*. *Morchella crassipes, Morchella esculenta* and *Morchella* spp. grew well on *NH_3-WSL* and *Mg-WSL* and they gave nearly the same yield and dry weight, while *Morchella deliciosa* grew very slowly on *NH_3-WSL* and yielded only a trace of mycelia in the *Mg-WSL*.

The choices of optimal pH for these fermentations were based on the criterion of compromise between the yield (%) and the dry weight (g/l) of *MMM*, because their optimal values are not always at the same initial pH. The optimal initial pH's distribute regularly from 5.0 to 7.0, depending upon the species of morel mushrooms and the *WSL* used. Very little or no effect of compositional differences between the *NH_3-WSL* and the *Mg-WSL* on the optimal initial pH were observed.

However, reports from previous workers confirmed that the optimal initial pH is specific for each species and each culture medium under particular fermentation conditions. Fron[8] reported that the required pH for growth of *Morchella conica, Morchella vulgaris* var. *flava*, and *Morchella esculenta* on Knop and Raulin liquid containing 5% sugar should be neutral or slightly alkaline. The optimum pH range for *Morchella hortensis* growing on glucose medium was found to be 5.5 to 6.5 by Litchfield et al[10]. However, Brock[6] obtained the highest yield of *Morchella esculenta* in glucose-ammonium chloride medium at pH 8.4 which Szuecs[11] reported the best yield of the same species in a glucose synthetic medium at pH 6.5.

It is to be noted that from Figures 1 and 2, two maximum values for dry weight were frequently encountered in the pH range from 4.0 to 8.0. This bimodel phenomenon confirms the results obtained by Brock[6] with *Morchella esculenta* when cultured on glucose-sodium nitrate medium at different initial pH values. Brock attributed the bimodel curve to the effect of pH on the fungus isoelectric point and the minimum point between two maxima was interpreted as the isoelectric point for the cell colloids.

Effects of WSL concentration on growth of MMM

The effects of the WSL concentration on the growth of *MMM* were studied in 300 ml/1000 ml Erlenmeyer flasks. Different dilutions of the stock culture medium (Table 2) were used for the culture medium. The results for *Morchella crassipes* on *NH_3 - WSL* are shown in Figure 3. The dilution ratio 1:5 (v/v) for *NH_3-WSL* was found to be the best for both yields (~83.4%) based on utilized carbohydrate and dry weight (~4.86 g/l) of this species. The yield and dry weight decreased for both higher and lower dilution ratios.

However, due to the relatively low concentration of total carbohydrate in the concentrated *WSL* as shown in Table 1, this optimal dilution ratio (1:5 v/v) is not considered to be ideal for the large scale fermentation. Therefore, because of high volume to be handled, one continuous fermentor system was set up in order to select mutant strains of those morel mushrooms which would grow directly on the concentrated *NH_3-WSL* and *Mg-WSL*, or at least at an improved dilution ratio. This work is still under way.

The low yield of *Morchella hybrida* grown on diluted *WSL* was reported earlier by Reusser et al[2]. Cirillo et al[4] also claimed an unsuccessful growth of *Morchella crassipes* in concentrated *WSL*. The growth in-

hibition might be due to the presence of sulphur dioxide, sulfonic acid radicals, lignosulphonates and polyphenolic components in the medium, which are known to be toxic for many fungi.

Chemical composition of MMM grown on WSL

The chemical composition of the biomass was analyzed in terms of protein, total fats, carbohydrates and ash. The commercial meadow mushrooms (fruiting bodies of *Agaricus campestris*) and the sample of *Morchella* spp. grown on glucose were also analyzed for comparison. The results are shown in Table 3 together with values obtained from other sources.

It was found that freeze-dried *MMM* on *WSL* contain on dry basis 25.7 - 48.0% crude protein. *Morchella* spp on *Mg-WSL* (complex medium) contains 48.0% of protein in comparison with 19.7% protein when grown on a glucose synthetic medium. This phenomenon is commonly found in other fungi and other species of morel mushrooms. Reusser et al[2] reported that *Morchella hybrida* gave only 10.5% of protein in a glucose medium, but 34.8% and even 37.5% of protein on molasses and *WSL* media respectively. The

TABLE 3

COMPOSITION OF *MMM* ON VARIOUS SUBSTRATES

Species	*Protein %* (*Dry Basis*)	*Fat %* (*Dry Basis*)	*Carbohydrate %* (*Dry Basis*)	*Ash %* (*Dry Basis*)	*Substrates*	*References*
M. crassipes	30.6	3.07	—	—	Glucose	(10)
"	30.1	3.35	—	—	Maltose	(10)
"	29.8	3.72	—	—	Lactose	(10)
"	22.8	7.55	51.45*	18.2	Glucose	(12)
"	31.1	—	22.1	14.4	*Mg-WSL*	this work
"	25.7	4.38	39.2	5.9	NH_3-*WSL*	this work
M. esculenta	31.1	1.88	—	—	Glucose	(10)
"	29.6	1.32	—	—	Maltose	(10)
"	30.0	1.93	—	—	Lactose	(10)
"	25.0	3.31	54.39*	17.3	Glucose	(12)
"	36.3	—	13.7	18.0	*Mg-WSL*	this work
M. hortensis	34.8	1.38	—	—	Glucose	(10)
	26.9	3.13	52.37*	17.7	Glucose	(12)
M. hybrida	10.5	6.0	—	—	Glucose	(2)
"	34.8	1.3	—	—	Molasses	(2)
"	37.5	0.8	—	—	*WSL*	(2)
"	48.3 — 54.7	—	—	—	Soybean Whey	(7)
Morchella spp.	19.7	—	30.2	—	Glucose	this work
"	48.0	2.45	22.9	8.0	*Mg-WSL*	this work
Morchella spp. (Commerical Morel Mushroom Flavoring)	51.0	2.18	40.38*	6.44	Glucose	(12)
Agaricus campestris (Fruiting Bodies)	46.3	2.84	19.2	7.8	—	this work

*Value calculated by difference, i.e. carbohydrate and fibrous materials.

TABLE 4

ESSENTIAL AMINO ACIDS OF *MMM* ON VARIOUS SUBSTRATES

(g/16 g nitrogen)

Amino Acids	M. crassipes (12)	M. crassipes on *Mg-WSL**	M. crassipes on *NH₃-WSL**	M. esculenta (12)	M. esculenta on *Mg-WSL**	M. hortensis (12)	Morchella spp. (12)	Morchella spp. on glucose*	Morchella spp. on *Mg-WSL**	Agaricus compestris (Fruiting Bodies)*	*FAO* Reference
Thr	2.99	3.55	4.14	2.98	4.65	2.68	3.37	2.85	4.32	3.08	2.8
Val	3.04	3.73	4.14	3.36	5.08	2.94	3.86	2.91	4.61	3.18	4.2
Met	1.01	1.05	1.18	0.90	1.73	0.69	1.41	0.51	1.64	1.00	2.2
Ile	2.87	2.90	2.91	2.70	4.15	2.40	3.45	2.40	3.76	2.61	4.2
Leu	5.57	4.78	6.05	5.12	6.68	5.03	6.06	3.59	6.18	4.27	4.8
Phe	1.90	3.30	3.92	2.51	3.92	2.28	2.82	2.05	3.57	2.56	2.8
Trp	1.48	**	**	0.86	**	0.98	1.43	**	**	**	1.4
Lys	3.46	5.07	6.94	3.84	6.94	3.02	5.38	4.79	6.78	4.79	4.2

*this work
**not reported

protein content of *Morchella* spp. on *MgWSL* (48.0%) is slightly less than that of commercial morel mushroom flavouring (51.0%, ref. 12) and *Morchella hybrida* on soybean wheys (48.3%-54.7%, ref. 7) but it competes with fresh meadow mushrooms (46.3%).

Fat content was found to be 2.45% and 4.38% for *Morchella* spp. and *Morchella crassipes* respectively, grown on *Mg-* and NH_3-*WSL*. Values reported in the literature were 0.8% for *Morchella hybrida* grown on *WSL*[2] and 7.55% for *Morchella crassipes* grown on glucose[12]. It seemed that *Morchella crassipes* has the highest fat content (7.55%) of the five morel mushrooms studied (Table 3) and the amount of total lipid in each species depends highly upon the substrate used as a culture medium.

Analyses for mycelial carbohydrate by anthrone method gave values of 13.7% for *Morchella esculenta* on *Mg-WSL* to 39.2% for *Morchella crassipes* on NH_3-*WSL*, in comparison with 19.2% for meadow mushrooms.

Ash values were found to be as high as 18.0% for *Morchella esculenta* on *Mg-WSL*, or very low as 5.9% for *Morchella crassipes* on NH_3-*WSL*. Similar figures were found in literature, i.e. 18.2% for *Morchella crassipes* cultivated on glucose to 6.44% for *Morchella* spp. which was also cultivated on glucose[12]. This variation could be probably attributed to non-uniform and non-efficient of the mycelia that contain a hollow core which contains liquid of varying composition.

The essential amino acids of *MMM* on *WSL* are shown in Table 4 together with values obtained from other sources. The mushrooms which might be of nutritional interest on the basis of the essential amino acids content are *Morchella* spp. on *Mg-WSL*, *Morchella crassipes* on NH_3*WSL* and *Morchella esculenta* on *Mg-WSL*. The spectrum of essential amino acids is comparable to the *FAO* standard, except for methionine and isoleucine which are of low concentration in almost all fungi. The *MMM* grown on *WSL* have only slightly higher quality in comparison with commercial morel mushroom flavoring, but it is much more valuable in comparison with fresh meadow mushrooms.

Strong mushroom flavor (aroma and taste) is conserved in the freeze-dried powder samples. It was found that *Morchella crassipes* on *Mg-WSL* have a stronger flavor than that of *Morchella* spp. on *Mg-WSL*, and *Morchella esculenta* on *Mg-WSL* have a more pleasant flavor than *Morchella* spp. grown on glucose medium.

Characteristics of the cultivation of MMM on WSL

A very low aeration rate is required for growth of *MMM* (0.25 vvm). If the aeration rate is higher, the pellet size decreases and growth may take place in a more dispersed form. Litchfield et al[10] claimed that the yield of mycelia is lower when the growth is dispersed. Large pellets are preferable because their recovery and purification are simplified. One fermented broth containing small pellets of about 1 mm diameter had been left to sediment by gravitation and it was found that all of the pellets settled down within less than 2 hours resulting in a clear supernatant. Usually in large fermentors such as the 5 gallon bottles, the pellets are of large size (0.5 - 1.0 cm diameter) and are expected to be even larger in industrial-scale fermentors. It is, therefore, assumed that even a better settling rate may be obtained in the latter case. This is a great advantage in comparison with the yeast grown on *WSL*.

Another interesting point for industrial-scale production is that the sterilization of the culture medium is not necessary if the *WSL* is pumped directly from the digesters to the fermentors. Liquor from the digesters are sterile since the digesters usually operate at 257°F - 320°F and 90 - 110 psi for 6 to 12 hours. Thus resterilization would not be required. The only pretreatment required before fermentation is steam stripping in order to eliminate residual SO_2 dissolved in the *WSL*. This step has been found necessary in all experiments, as SO_2 tended to inhibit growth. Sulphur dioxide stripping was also used by Cirillo et al[4] in mushroom fermentation on *WSL*.

The cultivation of *MMM* on *WSL* yields a high protein content product and at the same time contributes to the treatment of the waste. A part of the fermentable sugars in *WSL* is consumed for mycelial growth. The advantage of this system as compared to some other biotreatment systems is also in the fact that *Morchella esculenta* is able to take up both hexoses and pentoses[6] which are main carbohydrates present in *WSL*. From this standpoint, it competes with *Candida utilis* used for single cell protein production from *WSL*, which may remove both hexoses and pentoses, and is more effective than the yeast *Saccharomyces cerevisiae* used for alcohol fermentation from *WSL*, which uses only hexoses which are present.

Conclusion

Three species of morel mushrooms: *Morchella* spp., *Morchella crassipes* and *Morchella esculenta* grew well on both *Mg-WSL* and NH_3-*WSL*. Their protein content is as high as that of the meadow mushrooms, and their essential amino acid spectrum is comparable with that of the *FAO* standard. A strong mushroom flavor is easily conserved in the freeze-dried powder samples.

The cultivation of *MMM* on *WSL* was possible and it would appear that strain improvements for growth on more concentrated liquors at higher growth rates are the real developmental challenges.

References

(1) Mueller, J. C., Pulp and Paper Mag. of Can., 71, 72 (1970).
(2) Reusser, F., Spencer, J. F. T. and Sallans, H. R., Appl. Microbiol., 6, 1 (1958).
(3) Wiley, A. J., Oubey, G. A., Lueck, B. F. and Hughes, L. P., Ind. Eng. Chem., 42, 1830 (1950).
(4) Cirillo, V. P., Hardwick, W. A. and Seeley, R. D., U.S. Patent 2,928,210 (1960).
(5) Augustin, F., Deska Mykol, 21, 45 (1967).
(6) Brock, T. D., Mycologia, 43, 402 (1951).
(7) Falanghe, H., Smith, A. K. and Rackis, J. J., Appl. Microbiol., 12, 330 (1964).
(8) Fron, M. G., Acad. Sci. Paris, Compte Rendus Hebd. Séances, 140, 1187 (1905).
(9) Janardhanan, K. K., Kaul, T. N. and Husain, A., J. Food Sci. Technol., 7, 197 (1970).
(10) Litchfield, J. H., Overbeck, R. C. and Davidson, S. S., Agric. Food Chem., 11, 158 (1963).
(11) Szuecs, J., U.S. Patent 2,761,246 (1956).
(12) Litchfield, J. H., Vely, V. G. and Overbeck, R. C., J. Food Sci., 28, 741 (1963).
(13) Litchfield, J. H., Biotechnol. Bioeng., 9, 289 (1967).
(14) Morris, D. L., Science, 107, 254 (1948).
(15) Furman, N. H., Standard Methods of Chemical Analysis, 6th ed., D. Van Nostrand Co., Inc., 1, 736 (1962).
(16) Kosaric, N., Ph.D. Thesis, The University of Western Ontario, Canada (1969).
(17) Jacobs, M. B., The Chemical Analysis of Food and Food Products, 3rd ed., D. Van Nostrand Co., Inc., 30 (1965).

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