3608

APPLIED MICROBIOLOGY, Nov. 1974, p. 845-850
Vol. 28, No. 5
Copyright © 1974 American Society for Microbiology
Printed in U.S.A.

Fermentation of Feedlot Waste Filtrate by Fungi and Streptomycetes

B. A. WEINER AND R. A. RHODES

Northern Regional Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Peoria, Illinois 61604

Received for publication 6 June 1974

The soluble and dispersed nitrogen and carbon components in the filtrate fraction of cattle feedlot waste are a potential nutrient source from which single-cell protein could be produced for animal feeds. The ability of more than 200 fungi and streptomycetes to grow in this liquid was determined; these included isolates from the waste and associated sources, as well as organisms maintained in the Culture Collection of the Agricultural Research Service in Peoria, Ill. Utilization of waste nutrients was measured by changes in nitrogen content and chemical oxygen demand. Only 20% of the organisms were able to grow appreciably in the filtrate. Of these, dry-weight yields varied from 0.6 to 2.7 g of mycelium per liter; from 21 to 50% of the nitrogen in the filtrates was used during growth, whereas chemical oxygen demand levels diminished from 4 to 60%. In general, streptomycetes isolated from the feedlot used nutrients from the filtrates better than fungi did. Addition of readily available carbon sources such as glucose or whey significantly increased (as much as sixfold) cell yields of selected organisms and promoted better utilization of nitrogen (from two- to threefold); the effect on chemical oxygen demand varied (0 to 33% increase).

Livestock production centers must cope with huge quantities of waste which offer a pollution hazard and a disposal problem (6, 7, 13, 15). Biological treatment by oxidation ditches and lagoons to stabilize the waste before land disposal is the most common system used (6, 9). We have enumerated and identified the microbial groups in feedlot waste (FLW; 8, 12), but not the ability of individual aerobic organisms to grow on feedlot pollutants. Organisms grown on waste pollutants might provide a source of microbial protein for animal feed while decreasing the pollution potential of the material. In a survey of isolates from waste and others from our Culture Collection, we sought filamentous organisms that could reduce pollutants and filter easily for cell recovery. More than 200 fungi and streptomycetes were studied for their ability to use nitrogen and organic material in the waste, the latter being measured by chemical oxygen demand (COD). The production of cell mass and the effect of adding glucose and dairy whey to waste filtrates also were investigated.

(This paper was presented at the 1974 Annual Meeting of the American Society for Microbiology in Chicago, Ill.)

MATERIALS AND METHODS

Source of microorganisms. Samples were taken from pens of a cattle feedlot located near Peoria, Ill. (12). Fungi were isolated from plates of Mycophil medium (pH 7.0; Bioquest, Div. of Becton, Dickinson and Co., Cockeysville, Md.) to which has been added 0.2 mg of dihydrostreptomycin sulfate and 330 U of penicillin G per ml. A salts-starch agar (11) amended with cycloheximide (0.5 mg/ml) was used to isolate streptomycetes.

Preparation of liquid waste for fermentation. Feedlot manure (21 to 40% solids) was diluted with distilled water to a solids content of 15%. After it was mixed to break up lumps, 15% (wt/vol) diatomaceous earth was added to aid separation. Filtrate obtained by suction filtration through filter paper (Whatman 54) served as substrate for the fermentation studies. The pH ranged between 6.0 and 6.8. The filtrate presented a qualitatively predictable substrate without the particles which would make equivalent samples difficult to obtain.

Survey of organisms. Initially the ability of organisms to grow in FLW filtrate was evaluated in two ways. (i) Streptomycetes isolated from feedlot sites and fungi from the Agricultural Research Service Culture Collection were first grown on agar prepared with FLW filtrate as the sole nutrient source. Those which visibly grew well were further tested in liquid fermentations. (ii) Fungi isolated from feedlot sites,

Purchased by Agricultural Research Service, U.S. Dept. of Agriculture, for official use

together with streptomycetes and fungi selected from (i), were grown in sterile FLW filtrate in single, shaken test tubes (10 ml in tubes 25 by 150 mm). Inoculum was provided by 1-week-old plate and slant cultures. Tubes were shaken on a rotary shaker (200 rpm, 5-cm displacement) at 28 C. After 7 days, fermentation samples were brought back to volume with distilled water, and the mycelium was recovered by vacuum filtration through Whatman 54 paper. Cell masses were dried at 103 C overnight. Filtrate was analyzed for nitrogen (2), COD (1), and total carbohydrate (4).

Flask fermentations. Organisms selected from the preliminary survey were further evaluated in duplicate flask fermentations. Flasks containing FLW filtrate (50 ml in a 300-ml Erlenmeyer flask) were inoculated with 2% (vol/vol) washed and blended mycelium. The mycelial inoculum was grown in a medium of 2% glucose, 0.1% peptone, 0.1% yeast extract, and 2% malt extract. Flasks were incubated on a rotary shaker at 28 C; the contents were analyzed for nitrogen and COD as were the tube cultures.

RESULTS

Survey. Streptomycetes isolated from FLW were streaked on agar plates made with waste filtrates. Of 59 cultures, 35 grew well and were transferred to FLW filtrate in tubes. Eight isolates produced more than 2 g of cell mass per liter in the liquid. Filtrates of these were lower in nitrogen and COD content than initial levels by 37 to 50% and 53 to 60%, respectively. Mycelium weight ranged from 2.0 to 2.4 g/liter (Table 1).

Only 17 of 170 fungi from the Agricultural Research Service Culture Collection, streaked on waste filtrate-agar, grew well; the test organisms represented 14 different genera. The 17 fungi were inoculated into tubes of FLW filtrate. Nitrogen and COD levels were diminished below initial values by 31 to 58% and 4 to 52%, respectively. Cell mass ranged from 0.7 to 1.9 g/liter.

Isolates of fungi from feedlots were grown on FLW filtrates supplemented with 0.6% glucose (Table 2). They produced more cell mass and lowered COD and nitrogen levels further than fungi grown in waste liquid without added glucose.

Nutrient additions. *Trichoderma viride* Persoon ex S. F. Gray NRRL 3652 and *Fusarium aquaeductuum* (Radlkofer and Rabenhorst pro parte) Lagerheim NRRL 2503 grew poorly on FLW filtrate in shaken flasks and were ineffective in reducing COD and nitrogen (Table 3). Addition of glucose increased cell mass and decreased nitrogen and COD levels more.

Fusarium oxysporum grew twice as well as *T. viride* and *F. aquaeductuum* and was 2 to 3 times as effective in diminishing pollution potential. *F. oxysporum*, an isolate from FLW, reduced COD levels by one-half in 1 week of fermentation (Table 4). Addition of glucose yielded more cell mass and greater use of nitrogen from the waste; however, residual COD was not lowered.

Common nutrients that might be limiting were added to FLW filtrate; these mixtures were fermented for 1 week with *F. oxysporum*. Levels of COD were diminished by half in all flasks (Table 5). Nitrogen content was lowered by 37% with FLW filtrate without additives. Glucose alone and in combination with peptone and phosphate diminished nitrogen and COD levels by 44 and 47%, respectively, and produced more mycelium than in the control flasks. Addition of ammonium ion diminished nitrogen levels by 28% as compared with 37% for controls, but phosphate supplement allowed

TABLE 1. *Ranges in cell mass and reductions of COD[a] and nitrogen in FLW[b] filtrate by fungi and streptomycetes*

Organism	Source[c]	Cell mass (g/liter)	Nitrogen (mg/ml)		COD (mg of O_2/liter)	
			Initial	Final	Initial	Final
Fungi	Agricultural Research Service Culture Collection (17)	0.7	0.45	0.31	7,250	6,900
		1.9		0.19		3,500
Fusarium oxysporum NRRL 5836	FLW (1)	2.7	1.04	0.66	17,200	8,700
Streptomycetes	FLW (4)	2.0	0.38	0.25	7,500	3,500
		2.4		0.19		3,100
Streptomycetes	Pen core (3)	2.3	0.38	0.21	7,500	3,200
		2.4		0.19		3,000
Streptomycetes	Runoff (1)	2.1	0.38	0.24	7,500	3,200

[a] COD, Chemical oxygen demand.
[b] FLW, Feedlot waste.
[c] Numbers represent strains or isolates tested.

TABLE 2. *Ranges in cell mass and reductions of COD[a] and nitrogen by fungi grown in FLW[b] filtrate supplemented with glucose*

Isolates from feedlot sites[c]	Cell mass (g/liter)	Nitrogen (mg/ml)		COD (mg of O_2/liter)	
		Initial	Final	Initial	Final
Aspergillus (9)	2.7	0.67	0.42	15,200	6,700
	4.8		0.33		5,000
Alternaria (1)	4.2		0.34		4,400
Fusarium (2)	2.4		0.44		6,850
	4.9		0.25		5,750
Trichoderma (1)	2.3		0.46		7,450

[a] The COD is increased by 1,067 mg of O_2/liter for each 1,000 mg of glucose added; 97% of this value is measured by a method given by the American Public Health Association (1).
[b] FLW without glucose had a COD of 8,800 mg/liter; 0.6% glucose was added.
[c] Numbers represent isolates tested.

TABLE 3. *Growth of Trichoderma viride NRRL 3652 and Fusarium aquaeductuum NRRL 2503 on FLW filtrate supplemented with glucose*

Organism	Glucose (%)	Incubation (days)	Cell mass (g/liter)	Nitrogen (mg/ml)		COD (mg of O_2/liter)[a]	
				Initial	Final	Initial	Final
Trichoderma viride NRRL 3652	0	3	0.68	0.47	0.41	7,650	6,500
		7	0.86		0.37		6,300
	0.5	3	2.60		0.26	12,100	4,600
		7	3.72		0.28		4,700
	1.0	3	4.79		0.19	17,800	4,300
		7	3.78		0.25		4,600
Fusarium aquaeductuum NRRL 2503	0	3	0.36	0.47	0.46	8,700	8,800
		7	1.64		0.37		5,200
	0.5	3	2.43		0.30	14,800	9,100
		7	3.73		0.18		5,200
	1.0	3	2.07		0.18	19,600	16,400
		7	5.65		0.18		5,150

[a] COD increased by 1,067 mg of O_2/liter for each 1,000 mg of glucose added; 97% of this value is measured by a method given by the American Public Health Association (1).

TABLE 4. *Growth of Fusarium oxysporum NRRL 5836 in FLW filtrate supplemented with glucose*

Glucose (%)	Cell mass (g/liter)	Nitrogen (mg/ml)		COD (mg of O_2/liter)[a]	
		Initial	Final	Initial	Final
0	2.7	1.04	0.65	17,200	8,800
0.1	3.5		0.58	18,250	8,450
1.0	6.0		0.56	27,600	9,200
3.0	13.3		0.37	48,300	10,700

[a] COD increased by 1,067 mg of O_2/liter for each 1,000 mg of glucose added; 97% of this value is measured by a method given by the American Public Health Association (1).

a nitrogen uptake of only 20%. Whereas mycelium production with addition of NH_4^+ was little different from that with peptone, added phosphate gave the least cell mass.

Filtrates obtained from heated FLW, as compared with unheated material (Table 5), contained more nitrogen (78%) and organic material (40%, measured as COD). *F. oxysporum*, grown 1 week on filtrate from heated FLW, took up one-third more nitrogen and one-tenth less COD substances than in unheated liquid. Mycelium production was the same with both filtrates.

When a sterile mixture of dairy and FLW

TABLE 5. *Effect of treatment or supplementation of FLW filtrates on growth and utilization of pollutants by Fusarium oxysporum NRRL 5836*[a]

Additive or treatment[b]	Cell mass (g/liter)	Nitrogen		COD		pH
		Mg/ml	Decrease (%)	Mg of O_2/liter	Decrease (%)	
None, not inoculated		1.04		17,200		6.3
None, inoculated	2.7	0.65	37	8,800	49	8.7
Peptone (0.1%)	3.0	0.70	33	8,900	48	8.9
KH_2PO_4 (0.1%)	2.5	0.83	20	9,000	48	8.8
Glucose (0.1%)	3.5	0.58	44	8,500	51	8.2
Peptone, KH_2PO_4, and glucose (each 0.1%)	3.6	0.56	47	8,400	51	9.0
$(NH_4)_2SO_4$(0.1%)	2.9	0.75	28	9,200	46	8.9
Sterile FLW filtrate-whey (2.5:1, vol/vol)						
Frozen control		0.83		28,500		
Fermented		0.32	61	5,350	81	
Unsterile FLW filtrate-whey (2:1, vol/vol)						
Frozen control		0.76		25,800		
Fermented		0.24	62	1,050	96	
Not heated						
No inoculum		0.91		16,900		6.2
Fermented	1.8	0.53	42	7,400	56	
Heated						
No inoculum		1.61		23,700		6.4
Fermented	1.9	1.10	32	15,000	58	8.9

[a] Fermentations were run for 7 days.

[b] Additions are final concentrations in flasks (wt/vol). Sterile system, autoclaved before inoculation; unsterile not autoclaved. Heated system was prepared by heating raw FLW at 100 C for 1 h before filtration; unheated used standard preparation.

liquids (FLW filtrate-whey, 2:1, vol/vol) in flasks was inoculated with *F. oxysporum*, 10.3 g of cell mass per liter was made in 1 week. Initial COD and nitrogen levels were lowered by 85 and 73%, respectively (Table 5).

F. oxysporum inoculated into the mixed culture of unsterile dairy liquid and filtrate from FLW overgrew other organisms. After 7 days of growth, nitrogen and COD materials were diminished 62 and 96%, respectively (Table 5). Sterile mixtures, similarly fermented by this organism, had an equivalent amount of nitrogen but less organic material.

Dairy whey was fractionated by dialysis in an Amicon apparatus and combined with waste filtrate in ratios of 1:2 (Table 6). *F. oxysporum* produced 6.4-fold greater cell mass on this mixture (10.3 g/liter) than on FLW filtrate alone (1.6 g/liter). Although nitrogen uptake was improved, comparable final COD values indicate that admixture with whey did not increase utilization of FLW filtrates. Dialysates of whey gave similar results. Thus, increased yields of mycelium from combined waste are attributable to usable nutrients in whey. Amendment of FLW filtrate with 1.7% lactose gave two-thirds the cell mass and showed decreases of nitrogen and COD material comparable to that for combined waste liquids.

DISCUSSION

As expected, a large proportion of streptomycetes isolated from FLW grew well as streaks on FLW filtrate-agar. Such growth may reflect survival and adaptation in a limiting environment. However, limited microbial growth in FLW in situ was indicated by the relatively constant number of organisms found in waste at a feedlot regardless of season (12).

Streptomycetes which were isolated from the feedlot reduced pollutants in waste filtrates with modest yields of cells (Table 1). Fungi which were similarly isolated and grown on FLW filtrate with glucose also reduced nitrogen and COD levels, but formed more mycelium (Table 2). Fungi that were not adapted to FLW nor supplemented with glucose reduced pollut-

TABLE 6. *Effect of dairy whey fractions added to FLW filtrate on growth of Fusarium oxysporum NRRL 5836*[a]

Flask content[b]	Cell mass (g/liter)	COD (mg of O_2/liter)			Nitrogen (mg/ml)			pH	
		Initial	Final	Decrease (%)	Initial	Final	Decrease (%)	Initial	7th Day
FLW filtrate-water	1.6	7,300	3,500	52	0.47	0.22	53	6.1	8.9
Whey-water	15.1	54,700	3,100	94	0.79	0.51	35	4.7	8.5
FLW filtrate-whey	10.3	28,400	4,400	85	0.77	0.21	73	5.1	8.5
FLW filtrate-Amicon whey dialysate[c]	8.9	45,200	3,400	93	0.45	0.14	69	5.2	8.5
FLW filtrate-Amicon whey residue[c]	2.1	7,100	4,000	44	0.55	0.39	29	6.3	8.9
FLW filtrate-water plus 1.7% lactose[d]	6.8	41,100	6,000	85	0.42	0.15	64	6.2	8.0
FLW filtrate-residue of dialyzed whey	2.2	7,300	4,500	38	0.52	0.47	10	6.4	8.9
FLW filtrate-water (2:1) plus 0.27% casein[d]	2.3	7,100	4,100	42	0.58	0.47	19	6.3	8.9

[a] Fermentations were run for 7 days.
[b] Mixtures were in ratios of 2:1.
[c] Amicon whey residue is the component of whey which does not pass an Amicon P-M membrane and includes those materials of about 10,000 molecular weight and larger; Amicon whey filtrate is the component which passes through this membrane.
[d] Lactose and casein supplements approximate one-third the concentrations in whey.

ants less well and produced less cell mass (Table 1).

FLW filtrate contains 3 mg of carbohydrate and 0.5 mg of N per ml. Assuming that carbohydrate was the major nonprotein carbonaceous material in FLW, the calculated carbon-to-nitrogen ratio of FLW filtrate is 2.4. This ratio was compared with 6.6 and 7.6, which values are based on the elemental composition of mycelium (10). Nitrogen levels of *Aspergillus niger* mycelium have been shown to be a function of initial nitrogen content of medium (14). Foster (5), reporting elemental data by Porges, gave a carbon-to-nitrogen ratio of 18 for fungi. A suitable carbohydrate source is often crucial for growth of molds (3). Consequently, carbohydrate supplements were supplied to microorganisms isolated from a feed lot and to others selected from the Agricultural Research Service Culture Collection.

Selected fungi exhibited varied ability to take up pollutants from waste filtrates as a function of glucose amendent. Although *T. viride*, grown in FLW filtrate with glucose, reduced COD levels below those found without supplement, utilization of COD by *F. aquaeductuum* and *F. oxysporum* was either unaffected or impaired by glucose addition. Less uptake of organic material with increasing glucose content suggested that diminished utilization occurred because of a sparing action. In contrast, uptake of nitrogen was invariably increased by addition of glucose to the waste.

Addition to FLW filtrate of nutrients common to microbial media did not greatly affect uptake of nitrogen or COD substances (Table 5). Liberation of waste nutrients by heating FLW (before filtration) affected subsequent pollutant utilization even less. Evidently, *F. oxysporum* is so acclimated to waste substrates that common supplements give little benefit.

Two dissimilar wastes that complemented deficiencies in nutrient composition were combined. For example, fermentation of dairy-whey in combination with FLW filtrate resulted in a high uptake of nitrogenous and organic materials (Table 6). It is likely that dairy whey complemented feedlot filtrate by providing lactose because waste was deficient in total carbohydrates.

Liquids resulting from fermentation of supplemented waste filtrate are not suitable for release to surface waters because of residual nitrogen and COD levels. However, stabilized fermentation liquids could be used for flushing feedlot surfaces and for irrigation. In conditions of protein shortages, the fungal mycelium of those fermentations might prove useful in animal feeds.

LITERATURE CITED

1. American Public Health Association. 1971. Standard methods for the examination of water and waste water, 13th ed. American Public Health Association, Inc., Washington, D.C.
2. Association of Official Analytical Chemists. 1971. Methods of analysis, 11th ed. Association of Official Analytical Chemists, Washington, D.C.
3. Burkholder, P. R., and I. McVeigh. 1940. Growth of *Phycomyces blakesleeanus* in relation to varied environmental conditions. Amer. J. Bot. 27:634–640.
4. Dubois, M., K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith. 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28:350–356.
5. Foster, J. W. 1949. Chemical activities of fungi, p. 79. Academic Press Inc., New York.
6. Loehr, R. C. 1968. Pollution implications of animal wastes—a forward oriented review, p. 79. Federal Water Pollution Control Administration, U.S. Department of

the Interior. Robert S. Kerr Water Research Center, Ada, Okla.
7. Loehr, R. C., and R. W. Agnew. 1967. Cattle wastes—pollution and potential treatment. J. Sanit. Eng. Div., Proc. Amer. Soc. Civil Eng. 93(SA4):55–72.
8. McCalla, T. M., and F. G. Viets, Jr. 1969. Chemical and microbial studies of wastes from beef cattle feedlots. Proc. Pollut. Res. Symp., Nebr. Agr. Exp. Sta.
9. Miner, J. R., L. R. Fina, J. W. Funk, R. I. Lipper, and G. H. Larson. 1966. Stormwater runoff from cattle feedlots, p. 27–32. Proc. Nat. Symp., Amer. Soc. Agr. Eng., St. Joseph, Mich.
10. Porter, J. R. 1946. Bacterial chemistry and physiology, p. 359. John Wiley and Sons, Inc., New York.
11. Pridham, T. G., P. Anderson, C. Foley, L. A. Lindenfelser, C. W. Hesseltine, and R. B. Benedict. 1967. A selection of media for the maintenance and taxonomic study of *Streptomycetes*. Antibiot. Annu. 1956–1957: 947–953.
12. Rhodes, R. A., and G. R. Hrubant. 1972. Microbial population of feedlot waste and associated sites. Appl. Microbiol. 24:369–377.
13. Smith, S. 1965. Pollution-caused fish kills in Kansas. Kansas Water News, State Water Resources Board 8:8–14.
14. Steinberg, R. A. 1939. Optimum solutions as physiological reference standards in estimating nitrogen utilization by *Aspergillus niger*. J. Agr. Res. 58:717–732.
15. Walker, W. R. 1970. Legal restraints on agricultural pollution, p. 233–241. *In* Relationship of agriculture to soil and water pollution. Cornell University Conference on Agricultural Waste Management, New York.

GPO 809–490