This product also required a neutralizing conditioner to prevent bag rot.

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Literature Cited

(1) Brown, E. H., Brown, W. E., Lehr, J. R., Smith, J. P., Frazier, A. W., *J. Phys. Chem.* **62,** 366–7 (1958).
(2) Brown, E. H., Lehr, J. R., Smith, J. P., Brown, W. E., Frazier, A. W., *Ibid.*, **61,** 1669–70 (1957).
(3) Hein, L. B., Hicks, G. C., Silverberg, Julius, Seatz, L. F., J. AGR. FOOD CHEM. **4,** 318–20 (1956).
(4) Phillips, A. B., Young, R. D., Heil, F. G., Norton, M. M., *Ibid.*, **8,** 310 (1960).
(5) Phillips, A. B., Young, R. D., Lewis, J. S., Jr., Silverberg, Julius, *Ibid.*, **5,** 839–44 (1957).
(6) Yates, L. D., Nielsson, F. T., Hicks, G. C., *Farm Chem.* **117,** No. 7, 38–48 (1954).

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CELLULOSIC WASTES AS FERTILIZERS

Microbial Decomposition and Nitrogen Availability of Reacted Sawdust, Bagasse, and Coffee Grounds

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The Fersolin process for making sawdust and other cellulosic wastes useful for supplementing soil organic matter decreases decomposability, adds plant nutrients, and avoids microbial competition for available nitrogen. Microbial and chemical studies showed carbon-nitrogen transformations of Fersolins added to soil were less decomposable than the corresponding raw materials. Bagasse, most decomposable, gave a product showing greatest resistance increase. Reacted sawdust was most resistant. Coffee Fersolin showed a decrease in decomposability, but was less resistant than reacted bagasse or sawdust. The imparted resistance is desirable for increasing persistence in the soil as well as decreasing nitrogen demands. All the reacted products carried nitrogen in excess of the requirements of microorganisms involved in their decomposition.

TREATMENT OF SAWDUST or other cellulosic wastes with dilute sulfuric acid at high temperature decreases hydrolyzable carbohydrates and increases nonhydrolyzable lignin or ligninlike substances, giving a product more suitable for use on the soil. Wood wastes and similar residues treated by the Fersolin process (*8*) or a modification thereof (*9*), because of conversion of much of the cellulose to resistant complexes, are less readily decomposable than the raw materials. This decreases or eliminates the microbial demand for available nitrogen when such organic products are added to the soil, imparts desirable lasting qualities, and prolongs physical effects. After the heat treatment, ammonia is added to neutralize the acid, and the processed material carries more than enough nitrogen for its own decomposition. Some of the nitrogen is in organic combination. Use of phosphoric acid with the sulfuric in the primary treatment, or nutrient fortification of the product with mineral fertilizer, can enhance value and economic feasibility.

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An economical method has been developed for conversion of fine sawmill wastes into a useful agricultural product. The process, described by Farber and Hind (*10*), consists of treatment with mineral acids, addition of plant nutrients, heating, and conditioning. The finished product, generally identified as Fersolin, is an efficient fertilizer carrier and physical soil conditioner.

Appropriate analyses and soil microbial studies of raw and reacted sawdust, raw and reacted bagasse, and spent and reacted spent coffee grounds were made to evaluate these materials on the basis of their reactions under controlled conditions in the laboratory.

Materials

The soil used was collected from an area mapped as Salinas fine sandy loam, noncalcareous phase. A bulk sample representing the 0- to 6-inch horizon was passed through a 10-mesh sieve and stored in large tightly covered cans.

One-hundred-pound samples of raw and reacted sawdust, coffee grounds, and bagasse were individually well mixed and 5-pound representative subsamples were taken with a sample splitter. About 1 pound of each subsample was ground through a No. 800 screen (approximately 60-mesh) on a Weber mill for an analytical sample and for use in the microbial studies, where fine materials are required to ensure adequate mixing with small quantities of soil and reasonable agreement between replicate treatments.

Methods

Moisture was determined as loss in weight by drying samples at 105° C. for 24 hours.

Moisture capacity of the soil and organic materials was calculated from the amount of water retained by samples in Gooch crucibles wetted from below by immersion, and then allowed to drain to constant weight in a moisture-saturated atmosphere.

Exchange capacity and exchangeable cations were determined by the ammonium acetate method. This and other methods not specifically outlined are described in procedures used by the Oregon State College soil testing laboratory (*7*).

Ash was determined by ignition at 900° C.; total carbon, by dry combustion at 950° C.; total nitrogen, by the Kjeldahl method modified to include nitrates. Ammonium nitrogen was de-

termined by distilling samples in phosphate buffer solution at pH 7.4 and titrating the distillate with 0.071*N* sulfuric acid, using methyl red–bromocresol green mixed indicator. This method includes ammonium in free and exchangeable forms, each of which is available for nitrification. The distillation at pH 7.4 avoids release of ammonia from amides and other organic nitrogenous compounds. Such release occurs when drastic alkalies like magnesia and sodium hydroxide are employed and gives abnormally high results. Additional ammonia released by further distillation when the pH was increased to 12 by addition of sodium hydroxide was also determined.

Table I. Analysis of Salinas Fine Sandy Loam Soil, pH 7.6

	%
Mechanical analysis	
Fine sand	50.78
Total sand	76.04
Silt	14.59
Clay	9.36
Moisture capacity	37.0
Moisture tension	
5.0 atm., water	5.65
1.0 atm., water	7.33
0.5 atm., water	8.42
Moisture equivalent, water	8.91
0.1 atm., water	37.82
	Atm.
At 50% of moisture capacity	0.14
	%
Organic matter	1.04
Total carbon	0.84
Total nitrogen, C:N ratio, 14:1	0.06
	P.P.M.
Ammonium nitrogen	
Distilled at pH 7.4	20
Distilled at pH 12.0	0
Nitrate nitrogen	18
Phosphorus, bicarbonate soluble	18
Boron, water-soluble	1.38
	M.E./ 100 G.
Cation exchange capacity	4.75
Exchangeable cations	
Na	0.62
K	0.52
Ca	7.0
Mg	3.7

pH determinations were made with a glass electrode electrometer; for soil, readings were made on 1 to 5 distilled water suspensions from which coarse particles were allowed to settle; a 1 to 20 suspension was used for the organic materials.

Nitrites and nitrates were determined colorimetrically with appropriate filters in a Klett-Summerson photoelectric instrument, using the 1-naphthylamine–sulfanilic acid and the phenoldisulfonic acid method, respectively. The considerable difficulty encountered in obtaining clear filtrates, especially with coffee grounds and bagasse, was overcome with development of a procedure employing copper hydroxide. Cupric acetate and a slight excess of calcium hydroxide were first added to the 1 to 5 soil suspensions as well as to the 1 to 20 organic matter extracts. After filtration, ammonium carbonate was added to remove excess calcium. These second filtrates were satisfactory for nitrite determinations; for nitrates, additions of Superoxol to evaporating aliquots were required to remove final traces of color, especially with coffee extracts.

Total phosphorus was determined by the AOAC official volumetric method (*2*).

Numbers of microorganisms were estimated by plating procedures. Potato dextrose agar acidified to pH 4 with tartaric acid was used for molds, and sodium albuminate agar was employed for bacteria and *Streptomyces*.

Respiration studies were conducted in conventional manner but with a modified apparatus (*3*) designed to keep the soil under slight pressure rather than vacuum. Two hundred grams of soil (water-free basis) were used for each treatment; incubation was at 28° C. with moisture content maintained at 50% of the moisture capacity. The evolved carbon dioxide was absorbed in approximately 1*N* sodium hydroxide. Periodic determinations of the amounts evolved during a 30-day incubation were made by double titration (*7*), using a Beckman automatic titrator. The results provide an index of the rate and extent of decomposition of the raw and reacted products in comparison with dextrose and other sources of organic matter.

Comparative ammonification of the reacted products, with peptone for comparison, was studied by adding these materials at a rate equivalent to 1000 p.p.m. of nitrogen to 100-gram portions of soil and determining ammonia, nitrite, and nitrate nitrogen after 3 and 6 days' incubation in pint milk bottles. The raw materials were added in amounts equivalent to the reacted products on a weight basis. At the designated intervals, replicate bottles of control and treated soils were removed for analysis. Samples equivalent to 10 grams (water-free basis) were removed for ammonia determinations by distillation from phosphate buffer solution as mentioned above. To the remaining soil (90 grams, water-free basis) distilled water was added to give 1 to 5 dilutions and the suspensions were shaken 30 minutes. Clear filtrates obtained by the procedure previously outlined were analyzed for nitrite and nitrate.

For the nitrification experiment the various products, as well as ammonium sulfate for a standard of comparison, were added at rates equivalent to 300 p.p.m. of nitrogen to 100-gram portions of soil and incubated at 50% moisture capacity for 30 days. Ammonia, nitrite, and nitrate were then determined by the methods previously described. From these data nitrification percentages were calculated.

Incubation temperature in all cases was 28° C. Where aeration was not forced, as in the respiration experiment, the bottles of soil were covered with uniformly punctured caps to permit gaseous exchange. Moisture loss was restored by weight at frequent intervals to maintain water content at near 18.5%, or

Table II. Analysis of Raw and Reacted Materials[a]

Material	pH	Moisture Capacity, %	Total Carbon, %	Total Nitrogen, %	C:N Ratio	Ammonium Nitrogen, P.P.M. At pH 7.4[b]	Ammonium Nitrogen, P.P.M. At pH 12[c]	Nitrate Nitrogen,[b] P.P.M.	Bound Nitrogen,[d] P.P.M.	Total Phosphorus, % As P_2O_5	Total Phosphorus, % As P
Raw sawdust	4.3	495	49.7	0.08	622	40	100	0	660	0.66	0.29
Reacted sawdust (3–2–0)[e]	6.6	243	54.86	3.28	17	15,570	3400	7750	6,080	2.46	1.07
Reacted sawdust (3–2–2)[e]	6.9	148	51.53	3.16	16	15,350	2700	7575	5,975	2.85	1.24
Raw bagasse	6.1	818	44.89	0.56	80	682	200	0	4,718	0.75	0.33
Reacted bagasse (5–7–2)[e]	7.3	242	41.30	6.87	6	43,310	7600	820	16,970	6.42	2.81
Spent coffee grounds	5.1	288	50.67	2.02	25	320	200	145	19,835	0.14	0.06
Reacted coffee grounds (4–3–1)[e]	6.2	213	55.95	4.33	13	11,145	2500	360	29,295	3.53	1.54

[a] Data on water-free basis, except for pH on a 1 to 20 suspension.
[b] Water-soluble, immediately available.
[c] Ammonifiable, rapidly available.
[d] (Total nitrogen) − (ammonia nitrogen + nitrate nitrogen). Organic forms most resistant to ammonification, slowly available.
[e] Nominal analysis.

50% of the moisture capacity (Table I), an amount considered optimum for general microbial activity in the soil. For the Salinas fine sandy loam soil 18.5% moisture corresponds to a moisture tension of 0.14 atm. (Table I). For a light textured soil this is approximately equal to field capacity.

All data, except for pH, are expressed on the water-free basis. Rates of treatment, given in parts per million (p.p.m.), also are on the water-free basis. All treatments in the ammonification, nitrification, and respiration experiments were made in triplicate and the results were analyzed statistically.

Chemical and physical analyses of the soil are presented in Table I. The mechanical analysis, made by the pipet method (*12*), confirms the textural classification of the soil as a fine sandy loam. Analytical data for the various materials shown in Table II are means of triplicate determinations. Because particle size of organic matter affects rate of decomposition, mechanical analyses were made of the raw and reacted materials, fractionations being made with a stack of Tyler standard screens mechanically shaken for 30 minutes. Results for the sawdust, presented in Table III, are typical. These data are pertinent to interpreting experimental results, and judging the effects of treatments on microbial activity and possible influences on soil fertility.

Results and Discussion

Ammonification. Ammonification studies reveal the rate at which organic nitrogen becomes available. It is relatively rapid for protein nitrogen and is always accompanied under favorable conditions by nitrification, which is a slower process. The nitrite, which tends to accumulate when ammonium concentrations are high, and the nitrate are produced from ammonia and must be included in ammonification calculations. As some of the reacted materials contained ammonium and nitrate nitrogen, allowance must be made for these additions in the soil treatments.

Table IV shows that none of the organic nitrogen in any of the reacted materials was ammonified in 6 days. The values at 3 days were only slightly different and are not shown. Peptone, a protein product, was extensively ammonified, alone and in combination with the raw products; coffee grounds appreciably depressed the transformation, probably because of the oil content. Possibly, ammonification of the fortified reacted materials was retarded by the ammonium and nitrate originally contained, or microbial assimilation of available nitrogen resulted in the slight net decrease of the total at 6 days. A longer incubation period could have revealed release of ammonium from the reacted materials; this follows from the nitrates found at 30 days in the nitrification tests.

The slow rate of ammonification of the reacted materials is not undesirable. A slow release of ammonium is more suitable for agricultural use than a rapid release such as exhibited by the peptone.

Nitrification. For the nitrification study ammonium sulfate and reacted materials were added to the soil at rates equivalent to 300 p.p.m. of nitrogen. Rates for raw sawdust, bagasse, and coffee grounds were the same as for the corresponding reacted products.

Results of the nitrification study are given in Table V. The Salinas soil, although relatively low in organic matter and nitrogen, has a good nitrifying power or capacity; 86% of the added ammonium sulfate was nitrified in 30 days. A complete nitrification could be expected if limestone were added to prevent the considerable increase in acidity as

Table III. Mechanical Analysis of Raw and Reacted Sawdust

Tyler Standard Screen, Meshes/Inch	Original Samples		Weber Hammer Mill No. 800 Screen[a]	
	Raw, %	Fersolin 3-2-0, %	Raw, %	Fersolin 3-2-0, %
+5	1.9	0.2		
+10	22.5	4.8		
+20	52.6	44.2		
+40	17.4	28.4		
+60	2.8	6.2	35.0	8.5
+80	1.1	3.0	17.5	9.8
+100	0.4	1.5	10.0	6.2
+150	0.4	2.3	10.0	10.6
+200	0.3	1.9	10.0	8.0
−200	0.4	7.2	17.5	56.7
Total	99.8	99.7	100.0	99.8

[a] Approximately 60 mesh.
+ Retained on screen.
− Passing screen.

Table IV. Ammonification of Raw and Reacted Materials in Salinas Fine Sandy Loam Soil in 6 Days

Treatment[a]	Nitrogen Added, P.P.M.		Ammonification in 6 Days, P.P.M.			Total, N as NH_4^+, NO_2^-, and NO_3^-, P.P.M.	Nitrogen Ammonified[c]		pH
	As NH_4^+ and NO_3^-	Ammonifiable[b]	NH_4^+ N	NO_2^- N	NO_3^- N		P.p.m.	%	
Soil only	38	562	5	0	29	34	0	0	7.2
Peptone	0	1000	597	36	28	661	627	63	8.6
Raw sawdust	1	24	10	0	19	29	0	0	7.3
Raw sawdust + peptone	1	1000[d]	450	20	37	507	462	46	8.6
Reacted sawdust (3-2-0)	710	290	531	1	176	708	0	0	7.1
Reacted sawdust (3-2-2)	732	277	536	1	148	685	0	0	7.1
Raw bagasse	1	78	33	0	1	34	0	0	7.5
Raw bagasse + peptone	1	1000[d]	338	120	66	524	489	49	7.6
Reacted bagasse	631	369	568	19	92	679	14	4	6.7
Spent coffee grounds	4	463	32	0	2	34	0	0	6.8
Spent coffee grounds + peptone	4	1000[d]	195	117	63	375	337	34	7.0
Reacted spent coffee grounds	248	752	63	2	10	75	0	0	6.3
LSD									
0.05			20.42	2.49	8.97				
0.01			27.75	3.39	12.20				

[a] Additions of peptone and reacted materials equivalent to 1000 p.p.m. N; raw material additions equivalent to reacted materials on total weight basis.
[b] (Total nitrogen) − (ammonia nitrogen at pH 7.4 + nitrate nitrogen). See Table II.
[c] (Total N found as NH_4^+, NO_2^-, and NO_3^-) − (NH_3^+ and NO_3^- added in treatment) − total N found as NH_4^+, NO_2^-, and NO_3^- in soil only.
[d] Peptone only.

shown by the marked pH drop. The little nitrogen in raw sawdust also was extensively nitrified, but here, as the pH remained approximately neutral, it is likely that the resistant nature of the organic nitrogen can account for the incomplete transformation.

Nitrates disappeared almost completely from the soil treated with raw bagasse, probably indicating that the soils' own supply as well as any nitrate being produced was rapidly assimilated by microorganisms favored by the readily decomposable bagasse. A similar disappearance occurred with raw coffee, even though this addition carried considerably more nitrogen. In this case, probably, coffee oil not only stimulated certain nitrate-assimilating organisms but also inhibited nitrifying bacteria.

Similar considerations apply to the effects of the raw materials applied with ammonium sulfate. The ammonium was completely nitrified in the presence of raw sawdust. Not only did the sawdust exert no inhibiting effect, but it evidently exerted a protective action against the low pH that developed. Apparently only three fourths of the nitrogen was nitrified in the presence of raw bagasse, but here again appreciable assimilation of nitrate must have resulted from utilization of the accompanying readily decomposable organic matter. Raw coffee greatly reduced nitrification of ammonium sulfate, again indicating the probable inhibiting influence of the oil. Results of a subsequent study on the effects of this oil are presented in a following section.

All the nitrogen, including organic nitrogen amounting to about 30% in the reacted sawdust (3–2–0 and 3–2–2), was nitrified in 30 days. This shows a fairly rapid availability. With reacted bagasse, assuming complete nitrification of the ammonium, only one half of the organic nitrogen was nitrified, the over-all transformation being 82%. Reacted coffee showed the least nitrification of any of the reacted materials; the 22% conversion accounts for not quite complete nitrification of the ammonium alone. While here again the oil was probably inhibitive, it appears on comparing the results with those from raw coffee plus ammonium sulfate that resistance of the organic nitrogen is a major factor.

The decreases in pH were roughly proportional to ammonium available for nitrification; this reflects low buffer capacity of the soil and is not unexpected in view of the light texture and low organic matter.

Soil Respiration. A respiration experiment to study the rate and extent of decomposition of the raw and reacted materials was conducted under optimum temperature (28° C.) and moisture at 50% of the moisture capacity, for 30 days. Because available nitrogen often is a limiting factor in organic matter decomposition, treatments with ammonium nitrate to supply readily available nitrogen in two forms with each raw material were included.

Treatments with organic matter, including dextrose as a standard for comparison, were made uniformly on the basis of 2000 p.p.m. of carbon. Carbon

Table V. Nitrification of Raw and Reacted Organic Products in Salinas Fine Sandy Loam Soil

	Nitrogen Added in Treatment, P.P.M.					Analysis at 30 Days, P.P.M.						
									NO_3^- N		Nitrification at 30 Days	
Treatment	Total	NH_4^+	NO_3^-	Organic	Nitrifiable (NH_4^+ + organic)	pH	NH_4^+ N	NO_2^- N	Found	Less NO_3^- N in treatment	P.p.m.[a]	%[b]
Soil only	600	20	18	562	582	7.1	30	0.13	43	25	25	4
$(NH_4)_2SO_4$	300	300	0	0	300	5.3	85	0.27	283	283	258	86
Raw sawdust	8	1	0	7	8	7.2	22	0.13	31	31	6	75
Reacted sawdust (3–2–0)	300	142	71	87	229	5.6	38	0.50	325	254	229	100
Reacted sawdust (3–2–2)	300	145	72	83	228	5.6	20	0.15	345	273	248	109
Raw sawdust + $(NH_4)_2SO_4$	307	300	0	7	307	5.1	52	0.20	336	336	311	101
Raw bagasse	24	3	0	21	24	7.4	5	0.14	3	3	−22	−92
Reacted bagasse	300	189	4	107	296	5.6	23	0.05	271	267	242	82
Raw bagasse + $(NH_4)_2SO_4$	324	303	0	21	324	5.4	59	0.65	253	253	228	70
Spent coffee grounds	140	0	1	139	139	7.2	1	0.05	1	0	−25	−18
Reacted spent grounds	300	72	3	226	298	6.3	3	0.12	95	92	67	22
Spent grounds + $(NH_4)_2SO_4$	440	300	1	139	439	5.3	12	0.29	200	199	174	40
LSD												
0.05							9.14	0.22	26.30			8.77
0.01							12.42	0.31	35.75			11.92

[a] Net increases in NO_3^- N; amounts in treatment and soil only subtracted.
[b] Based on NH_4^+ + organic nitrogen.

Table VI. Decomposition of Raw and Reacted Products at 2000 P. P.M. Carbon in Salinas Fine Sandy Loam Soil

	Days						Apparent Decomp. at 30 Days,	
	3/4	2	4	7	14	30		
Treatment	CO_2 Evolved, Mg. C/200 G. Soil						%	pH at 30 Days
Soil only	1.5	7.8	12.3	15.8	20.2	33.4	2	7.3
NH_4NO_3	2.2	7.3	11.2	14.1	20.1	30.3	2	6.9
Dextrose	25.6	135.1	214.2	235.9	257.4	285.4	63	7.5
Dextrose + NH_4NO_3	11.8	115.4	158.7	181.2	205.4	237.5	52	7.0
Raw sawdust	3.6	12.0	17.4	22.9	34.7	78.7	11	7.6
Raw sawdust + NH_4NO_3	1.9	9.4	16.2	21.1	29.4	47.1	4	7.1
Reacted sawdust (3–2–0)	6.0	11.8	17.1	21.2	28.7	44.1	3	6.9
Reacted sawdust (3–2–2)	4.9	10.9	16.5	21.1	28.6	45.5	3	6.9
Raw bagasse	6.0	21.2	47.4	87.9	157.1	240.3	52	7.8
Raw bagasse + NH_4NO_3	6.4	20.1	42.1	71.4	137.1	212.0	45	7.5
Reacted bagasse	8.2	15.6	23.0	28.4	39.7	59.9	7	6.2
Spent coffee grounds	3.9	36.5	63.5	83.4	120.5	207.1	43	8.0
Spent grounds + NH_4NO_3	8.2	48.5	80.9	104.7	146.1	231.1	50	7.9
Reacted spent grounds	5.6	42.3	70.5	87.1	106.8	139.5	27	7.2
LSD								
0.05						1.10	0.27	
0.01						1.13	0.28	

content of the materials (Table II) ranged from 41 to 56%. The rate of treatment based on whole material (dry basis) ranged from 5 to 3.5 tons per acre $6^2/_3$ inches or 2,000,000 pounds of soil to obtain carbon equivalence, giving 4 tons per acre as the average rate. Ammonium nitrate additions were made in amounts required to adjust the carbon-nitrogen ratio of the raw organic materials to 20 to 1, this ratio being considered optimum for microbial utilization of available carbon. As a control, the average of these amounts was used for a nitrogen addition to the soil with no organic addition.

Results of carbon dioxide evolution from the respiration study are shown in Table VI. Whenever microbial activity in a soil is increased by any treatment, whether addition of organic matter or otherwise, carbon dioxide evolution from decomposition of the native organic matter is also more or less increased (*4*). Thus increased amounts of carbon dioxide from soil to which organic material is added may not be due wholly to decomposition of the addition. This error is minimized, however, with soils of low organic mater content. With the Salinas soil, therefore, apparent decomposition as determined by net increases in carbon dioxide with added organic materials can be assumed to represent a close approach to the rate and extent of true decomposition.

The rate of decomposition of raw and reacted materials shows that the latter are significantly more resistant despite the considerably greater proportion of the finest mechanical fraction in the milled samples. Typical of this extreme fineness, which should increase decomposability, is the mechanical analysis of the sawdust Fersolin shown in Table III. It appears that the reaction process has increased susceptibility to mechanical breakdown.

Decomposability of bagasse has been markedly decreased by the reaction process, the reacted product being only 7% decomposed in 30 days compared to 52% for the raw material. Only the raw bagasse, probably because of the abundance and readily decomposable nature of its carbohydrate constituents, approached glucose in rate of decomposition.

Reacted coffee decomposed relatively rapidly in comparison with the other reacted materials, but still much more slowly than raw coffee. The relatively higher decomposability of coffee, both raw and reacted, is attributable to a high native nitrogen content (2%), present largely in protein form, which is readily decomposable.

Although spent coffee grounds appear to be a very desirable raw material for a reacted product, problems have developed in field testing of coffee Fersolin (*11*). There appear to be substances in both the raw and reacted product which are toxic to some plants. In addition, mold growth becomes very abundant and an unpleasant odor develops. These factors may be eliminated by future research.

Sawdust is high in lignin and lignocelluloses, which are highly resistant to breakdown by microorganisms. Thus the carbon dioxide evolution, indicative of the decomposition, should be considerably lower than for any of the other raw materials. For the same reason, the reaction process would have a less pronounced effect in decreasing susceptibility to decomposition. Nevertheless, the reacted sawdust (3–2–0 and 3–2–2) decomposed only one fourth as extensively as the raw sawdust in 30 days. The addition of ammonium nitrate to the raw sawdust decreased the rate of decomposition to essentially that of the reacted sawdust. This is in accordance also with the nitrification results (Table VI), showing that all the nitrogen in the 3–2–0 and 3–2–2 reacted sawdusts was nitrified in 30 days.

The effects of added ammonium nitrate, as often shown in respiration experiments, appear anomalous, except with the raw coffee. Although the rate of over-all decomposition of materials of wide carbon-nitrogen ratio is generally increased, within limits of decomposability of the carbonaceous constituents, by added available nitrogen, the rate of carbon dioxide evolution falls, particularly after the first few hours. This is due to a number of complexly interrelated factors. Over-all decomposition includes loss of hydrogen and oxygen as well as of carbon dioxide; using weight loss as index, increased decomposition with ammonium nitrate or other available nitrogen sources could be shown.

As all the reacted products decomposed less rapidly than the raw materials (Table VI), and also as nitrates accumulated (Table V), the products carry more than enough nitrogen to care for requirements of microorganisms involved in the decomposition. Respiration data show that in increasing order persistence in the soil of the reacted materials would be coffee, bagasse, sawdust, with essentially no difference between the 3–2–0 and 3–2–2 sawdust.

Although the laboratory experiments indicate a rather rapid release of nitrogen from the sawdust Fersolin, field tests indicate a more gradual liberation of a significant portion of the nitrogen present, its being mineralized at a rate similar to that characteristic of a urea-formaldehyde fertilizer such as Uramite (*6*, *13*). The slower release under field conditions may be attributed to use of the relatively coarse product as obtained from the reactor. For laboratory tests the material was hammer-milled to pass an approximately 60-mesh screen (Weber No. 800); this was deemed desirable to ensure uniform samples and sufficient fineness to permit adequate mixing with small amounts of soil. Mechanical analysis of the original and milled sawdust Fersolin (3–2–0), using 100-gram random samples fractionated in a stack of Tyler standard screens on a Porter sand shaker for 30 minutes, gave the results shown in Table III. The Fersolin as received consisted largely of 10- and 20-mesh material, while 56.7% of the milled subsample used in the laboratory passed a 200-mesh screen. A sample of the 3–2–2 sawdust Fersolin gave a similar distribution of particle sizes, although the milled subsample gave a higher percentage of material passing 200 mesh.

Microbial Counts. Plate counts were made on the variously treated soil samples incubated in the respiration experiments for 30 days. This time was chosen as a period at which any effects would become reasonably per-

Table VII. Microbial Analysis of Soil from Respiration Experiment at 30 Days

Treatment	C:N Ratio of Additions	pH	Molds,[a] Thousands	Bacteria	
				Total,[a] millions	Streptomyces, %
Soil only	14	7.28	30.00	22.50	8.20
NH_4NO_3		6.91	40.35	49.85	9.56
Dextrose		7.46	92.85	57.65	11.13
Dextrose + NH_4NO_3	20	7.03	55.85	32.50	18.47
Raw sawdust	622	7.58	28.15	27.65	22.89
Raw sawdust + NH_4NO_3	20	7.11	42.85	54.50	21.44
Reacted sawdust (3–2–0)	17	6.86	24.35	26.00	32.95
Reacted sawdust (3–2–2)	16	6.86	33.65	83.85	24.35
Raw bagasse	80	7.81	41.35	31.35	24.63
Raw bagasse + NH_4NO_3	20	7.46	19.50	29.50	18.83
Reacted bagasse	6	6.21	23.65	13.65	29.17
Spent coffee grounds	25	8.03	20.50	50.00	17.99
Spent grounds + NH_4NO_3	20	7.88	20.15	42.15	25.54
Reacted spent grounds	13	7.23	22.65	44.15	13.17
LSD					
0.05			18.49	10.08	8.99
0.01			24.99	13.63	12.15

[a] Counts expressed as numbers per gram of soil, water-free basis.

sistent and indicative. More pronounced earlier effects from certain treatments undoubtedly occurred; while these are significant in ammonification and general decomposition processes, the more stabilized values developing later are indicative of any relatively permanent changes likely to result from the treatments (Table VII).

Numbers of molds and bacteria in the Salinas soil are typical for a soil low in nitrogen and with little or no fresh organic matter. Few of the treatments caused significant changes, as is evident from the LSD values. Readily soluble organic materials stimulate mold development, and such an effect resulted from the dextrose treatment. The apparent increases and decreases resulting from the other organic treatments are not statistically significant. Ammonium nitrate decreased molds with dextrose and raw bagasse; such a decline may be attributed to a marked earlier increase which largely exhausted growth-stimulating carbohydrates. With raw sawdust, on the other hand, where essentially all the carbon source is resistant and longer lasting, the ammonium nitrate resulted in an increased mold count at 30 days.

The greatest increase in numbers of bacteria was obtained with reacted sawdust 3–2–2. This result is apparently attributable to the potash carried by this product. In most cases the ammonium nitrate additions raised the bacterial count, as would be expected for a soil low in nitrogen. The high count from dextrose indicates increased growth normally following addition of a readily available carbon source. Significantly high counts with all the coffee treatments reflect the influence of their relatively high nitrogen content.

All the significant increases in *Streptomyces*' percentages are in accord with the ability of these higher forms of bacteria to attack more resistant organic residues such as cellulose and lignin. These organisms are important in humification, and they undoubtedly would play an important role in a slow, gradual transformation of the reacted materials.

While the microbial analyses reveal some statistically significant differences, in no case are these differences overwhelming. Unless the differences are of the order of tenfold or greater, either positive or negative, the results indicate that any immediate or lasting effects of the treatments on soil microorganisms are likely to be minimum.

Supplemental Studies with Coffee Grounds and Oil. As the coffee grounds appeared to contain much oil, original product samples of 40 to 50 grams in duplicate were extracted with Skellysolve B in a Soxhlet apparatus. After 7 hours' extraction, which removed little more than one half of the oil content, the samples were removed, air-dried, and hammer milled to pass an approximately 60-mesh screen (Weber No. 800). They were then further extracted until the solvent drippings were colorless, which required another 7 hours. The raw grounds were found to contain 23.44% oil, while the reacted grounds contained only slightly less (23.15%, Table VIII).

Although mineral and vegetable oils are decomposed by a variety of microorganisms, especially by species of *Pseudomonas*, oils may retard decomposition of other organic matter by forming a protective coating which interferes with moisture transfer. Certain oils are more or less germicidal, while others interfere with the activity of some germicides. Chandra and Shrikhande (*5*) found that mineralization of nitrogen in oil cakes and wool waste was retarded by fat content. Any residual caffeine in the spent coffee grounds could have had little inhibiting effect, as many soil bacteria and molds are able to decompose this substance.

In view of the observed effect of the raw spent grounds in reducing nitrification of ammonium sulfate, the influence of the extracted oil on this process as well as on ammonification and carbon dioxide evolution was determined, using the procedures previously described.

A study showed that coffee oil markedly retarded ammonification and subsequent nitrification of peptone and the soil's own nitrogen. The extracted grounds were apparently more inhibitive than unextracted spent grounds. This could be attributed to a more rapid general microbial development and nitrogen assimilation with the extracted grounds, although data (Table IX) indicate the raw grounds, including the oil, decomposed more rapidly. The reacted grounds showed appreciable ammonification.

Table VIII. Oil in Coffee Grounds

	Total Carbon, %	Kjeldahl Nitrogen, %	Oil			
			Total, %	Total carbon, %	Kjeldahl nitrogen, %	Sp. gr. 30°/4°
Spent grounds	50.67	2.02	23.44	79.00	0.05	0.938
Extracted spent grounds	46.38	2.59	...	...	...	...
Reacted grounds	55.95	4.33	23.15	79.10	0.04	0.938
Extracted reacted grounds	50.00	5.55	...	...	...	...

Table IX. Effect of Oil on Decomposition of Coffee and Other Organic Materials at 2000 P.P.M. Carbon in Salinas Sandy Loam Soil

Treatment	Days: 3/4	2	4	10	20	30	Apparent Decompn. at 30 Days, %
	CO_2 Evolved, Mg. C/200 G. Soil						
Soil only	8.4	12.2	17.3	26.7	36.6	44.5	2.6
Dextrose	77.9	200.3	289.9	335.7	361.0	377.1	83.2
Raw coffee	22.3	49.0	81.1	126.8	178.0	222.9	44.6
Extd. raw coffee	20.6	41.4	71.1	120.2	164.5	205.6	40.3
Reacted coffee	20.4	55.4	81.2	108.5	135.8	151.2	26.7
Extd. reacted coffee	13.7	20.8	29.5	42.8	58.8	78.1	8.4
Coffee oil	13.8	40.9	64.6	111.7	175.4	220.0	43.9
Dextrose + oil[a]	82.5	207.1	296.7	359.2	431.2	498.6	56.8
Wheat straw	27.0	45.8	82.1	138.3	192.2	243.0	49.6
Wheat straw + oil[a]	36.0	71.9	105.7	160.5	245.9	316.2	34.0
Uramite	8.7	22.6	34.4	62.0	82.1	96.3	13.0
LSD							
0.01	4.2	5.6	7.7	11.1	14.0	14.1	3.6
0.05	3.1	4.1	5.7	8.1	10.3	10.4	2.6

[a] Each at 2000 p.p.m. carbon, giving 4000 p.p.m. total.

Table X. Effect of Coffee Grounds and Oil on Nitrification of Ammonium Sulfate at 30 Days in Salinas Fine Sandy Loam Soil

Treatment[a]	pH	NO_3^- Nitrogen, P.P.M.	Nitrification, %
Soil only	7.2	49	
$(NH_4)_2SO_4$	5.4	183	44
Spent grounds	7.0	1	−17
Spent grounds + $(NH_4)_2SO_4$	6.0	118	22
Extracted spent grounds	7.0	1	−17
Extracted grounds + $(NH_4)_2SO_4$	5.6	182	44
Coffee oil[b]	6.4	2	−16
Coffee oil[b] + $(NH_4)_2SO_4$	6.7	2	−16
Reacted spent grounds	6.4	49	− 1
LSD			
0.05		0.12	
0.01		0.16	

[a] $(NH_4)_2SO_4$ and coffee grounds each at rates equivalent to 300 p.p.m. nitrogen.

[b] Coffee oil added equivalent to oil in spent coffee grounds at 300 p.p.m. nitrogen.

The influence of the oil on nitrification of ammonium sulfate (Table X) was even more depressive. While a marked depression occurred with the unextracted grounds, the oil-free grounds showed no interference with nitrification at the end of 30 days. Reacted coffee grounds had essentially no effect on nitrification of the soil and none of their nitrogen appeared to be nitrified.

Coffee oil added to wheat straw and to dextrose retarded decomposition of these materials. Total carbon dioxide evolution at 30 days was less from the combinations, including 2000 p.p.m. of carbon from each source, than the sums from corresponding individual treatments. Nevertheless, unextracted grounds gave more carbon dioxide than the extracted grounds. As the oil alone decomposed rapidly, its presence must have made significant contributions to the over-all carbon dioxide evolution from the unextracted grounds, both raw and reacted.

While the effect of raw grounds in reducing ammonification and nitrification is due to their oil content, the oil does not retard decomposition of the grounds.

Conclusions

Reacted sawdust, bagasse, and coffee grounds are more resistant to general decomposition in the soil than the raw materials. The increased resistance was most pronounced qualitatively with sawdust, while quantitatively it was greatest with bagasse. The increased resistance is desirable not only to decrease nitrogen demand but also to prolong persistance of soil-conditioning effects. Nitrogen in the reacted products was more than sufficient to provide an excess over the requirements for microbial decomposition, and the excess should become more or less rapidly available for plant growth. Spent coffee grounds, because of their high nitrogen content, appear to be a promising material to process into a reacted product.

Literature Cited

(1) Alban, L. A., Kellogg, M., Oregon Agr. Expt. Sta., Misc. Paper **65** (1959).
(2) Assoc. Offic. Agr. Chemists, "Methods of Analysis," 7th ed., pp. 9–10, 1950.
(3) Bollen, W. B., Lu, K. C., *Soil Sci. Soc. Am. Proc.* **21,** 35–41 (1957).
(4) Broadbent, F. E., Bartholomew, W. V., *Ibid.*, **13,** 271–4 (1948).
(5) Chandra, P., Shrikhande, J. G., *Agra Univ. J. Research* **4,** Pt. 1, 25–30 (1955).
(6) Clark, K. G., Yee, J. Y., Gaddy, V. L., Lundstrom, F. O., J. AGR. FOOD CHEM. **4,** 135–40 (1956).
(7) Cooper, S. C., *Ind. Eng. Chem., Anal. Ed.* **13,** 466–70 (1941).
(8) Farber, E., U. S. Patent **2,574,027** (1951).
(9) *Ibid.*, **2,735,756** (1956).
(10) Farber, E., Hind, R. R., *Forest Products J.* **9,** 340–3 (1959).
(11) Hind, R. R., personal communication, 1959.
(12) Kilmar, V. J., Alexander, L. T., *Soil Sci.* **68,** 15–24 (1949).
(13) Lunt, O. R., Stark, J., Sciaroni, R. H., Bryne, T., California Agr. Expt. Sta. Rept. on Project **1469** (1958).

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NITROGEN AVAILABILITY IN SOILS

Rapid Procedure for Estimating Potentially Available Soil Nitrogen under Greenhouse Conditions

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A rapid technique for determining a fraction of the more readily hydrolyzable nitrogen content of soils is described. Data are presented to support the thesis that nitrogen from this fraction was utilized by wheat grown under greenhouse conditions on seven soils of varying organic matter and total nitrogen contents.

CONSIDERABLE EFFORT has been expended to find a procedure for estimating the nitrogen-supplying power of soils, and various biological and chemical methods (*10–12*) have been proposed. However, most of these procedures are too time-consuming or unreliable for general use. In the absence of a suitable test, nitrogen fertilizer recommendations are arrived at by indirect and often erroneous means, with little or no consideration being given to the appreciable variation in the ability of different soils to supply nitrogen from their organic reserves. An accurate, rapid technique for estimating the potentially available nitrogen content of soils would therefore be useful.

The bulk of soil nitrogen is thought to be combined in organic substances that are highly resistant to decomposition, but as much as a third of the nitrogen in some soils is in protein form and may be liberated by acid or alkaline hydrolysis (*1, 4, 8*). As only traces of amino acids have been detected in soils (*7*), it appears reasonable to assume that proteins are the basic source of the soil nitrogen released by microbial decomposition.

Although plant proteins are known to vary in amino acid composition, most of the protein nitrogen present in soils has lost its original identity as a result of refabrication by soil microbes. Bremner (*1*) found the amino acid composition of the protein fraction from 10 soils to be remarkably constant, while other investigators (*2*) have reported only minor variation in the composition of proteins from podsol and prairie soils.

If the protein fraction of soils is of fairly constant composition, and proteins constitute the principal reservoir from which nitrogen is released in soils, a rapid technique for estimating a comparable fraction of the protein content of soils would be useful. Furthermore, the ease of hydrolysis of the protein fraction of soils by chemical means should afford a measure of its rate of hydrolysis by biological means. A procedure is presented for estimating the more readily hydrolyzable nitrogen fraction in soils, and testing the hypothesis that this is the source of soil nitrogen utilized by plants.

Materials and Methods

Preliminary studies indicated that soil may be differentiated on the basis of the amounts of ammonia released by