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B. L. Onisko
T. Wydeven, Ames Research Center, Moffett Field, California

National Aeronautics and
Space Administration

Ames Research Center
Moffett Field, California 94035

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Abstract

In an attempt to verify the results reported previously concerning the phytotoxicity of wet-oxidation (wet-ox) products, lettuce solids were suspended in water and then heated to 548 K for 3.6 ksec (1 hr) under 4.1×10^7 Pa (400 psig at 294 K) oxygen pressure and 1.52×10^8 (1500 psig at 548 K) total pressure. Such treatment resulted in oxidation of 80% of the initial organic carbon to carbon dioxide. Thirty-three percent of the remaining organic carbon was present in acetic acid. Organic nitrogen in the feed was decreased 90% by the wet-ox treatment. Ammonia and nitrogen gas were the main nitrogen products. Analysis of the liquid product of wet-ox indicated that most of the minerals essential for plant growth were present. However, when tested using a lettuce-root growth-rate assay, the solution was toxic. This toxicity was not due to excessive salt or ammonia or to an improper pH. Analysis of the wet-ox solution revealed the presence of silver and chromium, thus implicating reactor corrosion as the cause of the phytotoxicity. Both cation and anion exchange resins removed the silver and the toxicity of the liquid effluent, indicating silver as the toxic component. Uptake of both silver and chromium by lettuce roots correlated with diminished root growth. Toxicity of the solution from wet-ox was not observed when precautions were taken to minimize contact of the liquid in the reactor with the metal reactor components.

Introduction

Wet oxidation (wet-ox) is a waste treatment process in which organic matter, either suspended or dissolved in water, is heated to 523-573 K (250°-300°C) in the presence of oxygen at an elevated pressure

(Zimmerman 1958 and 1961). It is of considerable interest that thermal energy can be obtained from the process without initial drying of the organic fuel (Flynn 1976; Othmer 1978). Thus, wet fuels, which typify biological materials, can be oxidized by wet-ox with the evolution of usable heat. Ammonia is a major product of the wet-ox of nitrogenous materials (Wheaton 1967; Jagow 1972; Onisko 1981), which makes the process an attractive component for photothermal energy conversion from a biomass system (Figure 1) and for Controlled Ecological Life Support Systems (CELSS) for use in space exploration. Recovering nitrogen as ammonia rather than nitrogen gas enables the cyclic production of biomass without the need for the reduction of nitrogen gas, an energy-intensive process.

However, past attempts to use the wet-ox solution for growing plants hydroponically has had limited success (Agre et al. 1966; Zamota et al. 1971). The solution was found to be toxic to higher plants. It was the intent of this study to confirm the results of prior studies and to determine if the growth rate of lettuce roots (a representative biomass) was impaired by the wet-ox solution.

Materials and Methods

Representative Biomass: Lettuce (Lactuca sativa L.) seeds, the tip burn resistant strain of Grand Rapids, Mich., lot number 323, were obtained from the Ball Seed Co. (Mountain View, Calif.).* Lettuce

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*The use of trade names of manufacturers in this report does not constitute an official endorsement of such products or manufacturers, either expressed or implied, by the National Aeronautics and Space Administration.

plants were grown from the seeds using polyethylene containers (two plants per container) filled with 10^{-3} m^3 (1 ℓ) of half-strength modified Hoagland's solution (Hammer 1978). The plants were supported with a black polyethylene cover containing polyester wicks as described by others (Prince et al. 1978). The containers were placed in a growth chamber maintained at 298 K and surrounded by ambient carbon dioxide and humidity. Cool-white fluorescent tubes provided continuous illumination (photosynthetically active radiation) of 120-160 $\mu E/m^2$ sec measured at container level. Airflow over the plants varied from 0.05-0.33 m/sec. Water loss from the hydroponic nutrient solution was periodically replaced with deionized water, and the entire nutrient solution was replaced when the solution conductivity dropped below 50% of its initial value. Four weeks after germination, mature plants (roots and tops) were harvested and dried at 353 K, then ground and passed through a No. 20 sieve (840-μm opening) to provide material for wet-ox studies.

Wet Oxidation Reactor: A high-pressure reactor made of No. 316 stainless steel (Model 9521, Parr Instrument Co., Moline, Ill.) was used for the wet-ox studies. A thermocouple well was sealed to the top of the reactor with a silver metal gasket. The reactor could be stirred during oxidation at 10 rps with a No. 316 stainless steel stirrer. When stirring was not desired, the entire stirrer assembly was removed. A cylindrical 9×10^{-4} m^3 (900 ml) Pyrex glass liner was used in some of the experiments to contain the feed solution and keep it from contacting the stainless steel reactor wall. In all experiments the reactor was charged with 3×10^{-4} m^3 (300 ml) of deionized water and pressurized with oxygen to 4.1×10^7 Pa (400 psig) at 294 K. In experiments in which lettuce was oxidized, 1.5×10^{-2} kg of dried ground lettuce solids were added to the reactor.

Lettuce solids of known chemical composition were suspended in water in the wet-ox reactor. The reactor was sealed and then pressurized with oxygen to 4.1×10^{7} Pa at 294 K before heating it to 548 K. The temperature of the reactor was maintained at 548 K for 3.6 ksec before it was cooled and the contents were removed. In two experiments a Pyrex liner located inside the reactor contained the lettuce slurry. In all cases, wet-ox of the lettuce slurry produced a clear, colorless liquid containing a small amount of a white precipitate.

Wet-ox Solution Treatment: After the wet-ox of lettuce, the reactor contents were cooled and the pH of the product solution was adjusted to 6.5 by the addition of a dilute potassium hydroxide or dilute sulfuric acid solution. The pH of Hoagland's solution, a commonly used nutrient solution for growing plants hydroponically, is 6.5. An ethylenediaminetetraacetic acid (EDTA) solution (9.27×10^{-6} m^3 (9.27 ml)) was added to some of the liquid effluent samples from the wet oxidation reactor before adjusting the pH and filtering and analyzing the samples. The EDTA solution was prepared by dissolving 6.25×10^{-4} kg of EDTA in 6.4×10^{-5} m^3 (64 ml) of 0.1 M KOH solution and then diluting the solution to 2.5×10^{-4} m^3 (250 ml). EDTA was added in an attempt to dissolve, by chelation, some of the metal compounds in the precipitate which remained after the wet-ox of lettuce. The effect of EDTA addition on the analysis of the liquid is shown in Table 1. All of the samples were filtered before chemical analysis and lettuce-root growth-rate studies.

Some of the filtered samples were also passed through an ion exchange column before chemical analysis and before using them in the lettuce-root growth-rate assay. Certain samples (3×10^{-4} m^3 (300 ml)) were passed through 0.15 m × 0.24 m columns of either an anion exchange resin (BioRad AG 1-X8, 100-200 mesh, hydroxide or chloride form) or a cation exchange resin (BioRad AG 50W-X2, 200-400 mesh, proton form). Samples were eluted from the columns with deionized water. Effluent peaks were detected and then collected by monitoring the electrical conductivity of the eluate. The pH of the chromatographed samples was then adjusted to 6.5 with dilute aqueous KOH or H_2SO_4 before use in the lettuce-root growth-rate assay.

Lettuce-Root Growth-Rate Assay: The lettuce-root growth-rate assay used here was similar to that described by Berry (1978). Fifty lettuce seeds were placed in a 10^{-3}-m^3 (1-ℓ) Erlenmeyer flask containing 2.5×10^{-4} m^3 (250 ml) of test solution. The flasks were allowed to remain at 296 K for 4 days under a continuous illumination of 150 $\mu E/m^2$ sec (photosynthetically active radiation), obtained with cool-white fluorescent lights. Air that had been filtered through glass wool and activated carbon was continuously bubbled through the solutions during the assay. At the end of the growth period, the seedlings were harvested and the root length from the hypocotyl to the root tip was measured. For calculation of mean root length and standard errors (Table 2), seedlings with a root length of less than 4 mm were excluded. The pH and electrical conductivity of all the growth-rate test solutions were measured before seed imbibition and at the end of the test period. At the end of the growth-rate tests, water lost by evaporation was replaced before measuring the pH and the electrical conductivity of the solutions.

Chemical Analysis: Ammonia and organic nitrogen were determined as described previously (Anon. 1971). Organic and inorganic carbon were determined with a Beckman Instruments Model 915 Total Organic Carbon Analyzer. Organic acids were analyzed by gas chromatography on a fused silica Carbowax 20M column (J&W Scientific, Inc., 30 m × 0.32 mm) with a helium carrier gas flow rate of 3.3×10^{-8} m^3 sec (2 ml/min) and an oven temperature programmed from 343 K to 453 K at 0.05 K/sec following an initial isothermal period at 343 K for 240 sec. Samples were acidified to pH 2 with HCl before injection. The N_2 and CO_2 produced by the wet-ox of lettuce was measured as described elsewhere (Onisko et al. 1981). Elemental analyses for C, H, N, O, S, and P were performed by Huffman Laboratories (Wheat Ridge, Colo.). Other analyses shown in Table 1 were performed by the Soil and Plant Laboratory (Santa Clara, Calif.). The trace metal analysis data shown in Table 3 were obtained by Dr. John Carden using inductively coupled plasma optical emission spectrometry (ICPOES), and that in Figure 5 was obtained by Dr. Wade Berry using optical emission spectrometry (Alexander and McAnulty 1981).

Results and Discussion

Organic carbon was found in the aqueous phase from the wet oxidation of lettuce and the amount indicated that 80% of the organic carbon in the starting sample was oxidized. Acetic acid accounted for 33% of the unoxidized organic carbon content of the solution. Propionic acid accounted for 5% of the organic carbon content of the reactor product. Formic acid could not be detected, since a flame ionization detector was used in the gas chromatographic analysis of the organic acids

(Merriman et al. 1966). No other organic acids were detected. No inorganic carbon was detected in solution and there was only a trace amount of carbon found in the precipitate obtained from the reactor. Gas chromatographic analysis of the product gas from the wet-ox of lettuce showed carbon dioxide to be the only carbon-containing product. In summary, most of the organic carbon in lettuce was oxidized to carbon dioxide; about one-third of the remaining organic carbon was present as acetic acid.

The organic nitrogen content of the lettuce feed was decreased by approximately 90% during wet-ox. Since the nitrate-nitrogen content before and after wet-ox remained essentially unchanged, nitrate was not a product of wet-ox. The nitrogenous products found before and after oxidation are shown in Figure 2.

Mineral elements in the solution from the wet-ox of lettuce were also measured (Table 1). Na, K, Mg, Cl, and sulfate were recovered in good yield; Fe, Mn, Zn, Ca, and P were not. Presumably, most of the multivalent cations were precipitated as insoluble phosphates. Iron was found in solution in extremely low yield (0.5%). Adding EDTA in an attempt to solubilize iron before filtration (1 mole EDTA/1 mole Fe in the initial charge) did not increase iron recovery.

When the Pyrex glass liner was used, boron was found in considerably higher than quantitative yields. Evidently, the wet-ox conditions resulted in the leaching of boron from the liner. The leaching of silicon from Pyrex during wet-ox was reported earlier (Onisko 1981).

After EDTA addition, filtration, and pH adjustment to 6.5, the reactor solution was tested for phytotoxicity using a lettuce-root growth-rate assay (Berry 1978). Lettuce seeds were exposed to the test solution for 346 ksec (4 days), after which root length was measured (see Figure 3). A series of dilutions of this solution (3×10^{-4} m^3 total) was then made, and these diluted samples were tested for phytotoxicity. Germination was not observed for the most concentrated solution. However, when the solution was diluted 100-fold, root growth rate similar to that of the control solutions was found (see Table 2).

Several possible causes of the observed phytotoxicity were eliminated from consideration. Solution pH was not the cause of phytotoxicity, since the pH did not change by more than one unit in any of the flasks containing seedlings. Neither elevated ammonia nor high total salt concentration were the cause of phytotoxicity, since in the most concentrated solution (7.7-fold dilution of the reactor product), ammonia nitrogen was only 110 ppm and the electrical conductivity of the solution was 1800 mho/cm. A control solution (see Table 2, "NH_4^+ Hoagland's") containing 105 ppm ammonia nitrogen and an electrical conductivity of 1650 μmho/cm showed no toxicity. This latter solution, a modified Hoagland's solution, was prepared by substituting calcium chloride and ammonium sulfate for calcium nitrate in Hoagland's solution. The amounts of calcium chloride and ammonium sulfate added were such that the calcium and nitrogen concentrations remained the same as in Hoagland's solution. Furthermore, a modified Hoagland's solution containing additional ammonium sulfate (175 ppm ammonia nitrogen and an electrical conductivity of 2250 μmho/cm) was also nontoxic.

Another possible cause of phytotoxicity that was considered was metal contamination of the wet-ox solution due to corrosion of the metal reactor. To test this hypothesis, deionized water was treated in the reactor without the addition of lettuce solids, and the product was filtered and diluted before lettuce-root growth-rate studies. No germination was detected for the most concentrated solution (see Figure 3) whereas in untreated deionized water, germination was observed, although the root growth rate was somewhat variable (Table 2). Water processed with the Pyrex liner, but in contact with the stainless steel stirrer, allowed better root growth, although not as good as that found with the control solutions. Water processed with the Pyrex liner and the stirrer removed allowed control-level root growth rate. These data supported the hypothesis that corrosion products from the metal reactor and stirrer were the cause of phytotoxicity.

A phytotoxic sample of water from the wet-ox reactor was treated with two ion-exchange resins in an attempt to determine if the toxic contaminant was an anion or a cation. Treatment with either the cation (H^+ form) or the anion (OH^- or Cl^- forms) exchange resins completely removed the toxicity of the wet-ox solution (Figure 4). This result cannot be explained by the presence of both toxic anions and toxic cations in the reactor effluent, since treatment with either of the resins should have left residual toxicity in the product. More likely, the toxic metal ion was removed by both resins, perhaps by different removal mechanisms (see below).

Analysis of the wet-ox solution was done by ICPOES. As shown in Table 3, both silver and chromium were found in a sample of deionized water heated with oxygen in the wet-ox reactor. Ni, Mo, V, As, Be, and Cu were not detected (see Table 3 footnotes for detection limits).

Purification of deionized water treated in the wet-ox reactor with the cation exchange resin removed only the silver; the chromium content of the sample remained unchanged. (Chromium was probably not removed by the cation exchange resin because it was present in solution as the CrO_3^{2-} anion.) The removal of silver and not chromium, coupled with the lack of phytotoxicity of this sample, demonstrated that silver and not chromium was responsible for the phytotoxicity of the wet-ox solution. Furthermore, treatment with the anion exchange resin (in both the OH^- and Cl^- forms) removed both chromium (presumably CrO_3^{2-}) and silver. Precipitation of silver as silver oxide (or chloride) may explain this result. Removal of both metals by the anion exchange resin explains the reduced phytotoxicity of this sample of the wet-ox solution.

Also shown in Table 3 are the chromium and silver contents of a water sample treated in the reactor with a Pyrex liner to avoid contact of the liquid with the metal reactor wall. The chromium and silver contents were reduced by the use of the Pyrex liner. Removal of the No. 316 stainless steel stirrer further reduced metal contamination of the wet-ox solution and reduced the phytotoxicity (Figure 3).

The trace metal content of lettuce seedlings germinated and grown in diluted samples of the toxic wet-ox water sample was also determined. When the seed cotyledons were analyzed, no silver (2 ppm) or chromium (1 ppm) was detected. However, both metals were accumulated in the roots (see Figure 5). As the toxic water sample from the reactor was diluted, the concentration of both silver and chromium in the roots decreased. Sufficient dilution allowed normal root growth rate. Upon dilution of the sample from 10 to 30 times that of its initial concentration, a large increase in root growth rate was observed (Figure 5). Interestingly, the roots from these dilutions showed the same chromium uptake but a diminished silver uptake as roots from more concentrated samples. This result again indicates that silver and not chromium was the phytotoxic component in the wet-ox solution.

Summary

Wet oxidation has significant potential for a photothermal energy conversion cycle of biomass (see Figure 1) and for waste treatment in CELSS. This process has several advantages over dry incineration or combustion. First, the biomass need not be dried before oxidation and therefore several different fuels (e.g., algae and food processing wastes) can be used with wet-ox. Secondly, ammonia is the predominant nitrogenous product of wet-ox rather than nitrogen gas, which is produced by incineration. The recycling of this ammonia in continued biomass production would improve the energy efficiency if wet-ox was used in a biomass energy system. For example, 18% of the total energy used for agricultural production in the United States in 1970 was used to produce fertilizer (Steinhart and Steinhart 1975). Finally wet-ox, unlike dry incineration, does not suffer from the emission of gaseous nitrogen oxides and sulfur oxides, the release of which apparently causes potentially severe ecological disturbances (Ember 1981). The advantages of wet-ox cited here for a biomass energy system also apply to CELSS in space.

The results of this study have shown that corrosion products from the wet-ox reactor can reduce the growth rate of lettuce roots and presumably the growth of healthy higher plants. Therefore, if healthy plants are to be grown hydroponically on the products of wet-ox, precautions must be taken in selecting components for a wet-ox reactor and the conditions of wet-ox in order to minimize corrosion.

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Table 1

Chemical Analysis of the Filtrate from the Wet Oxidation Products of Lettuce

Species	Recovery in the filtrate as a per cent of the initial amount			
	With Pyrex liner		Without Pyrex liner	
	Before EDTA addition	After EDTA addition	Before EDTA addition	After EDTA addition
Organic C	17	17	19	19
Organic N	12	12	9.5	9.5
NH_3-N[a]	58	59	53	55
N_2-N[a]	20	20	15	15
NO_3^--N	95	91	83	81
S[b]	71	71	52	42
P[c]	22	22	10	14
Na	130	120	102	106
K[d]	108	107	105	106
Fe	0.5	[e]	0.5	0.5
Mg	86	81	86	86
Ca	16	24	12	12
Cl	98	98	98	98
Cu	52	52	93	31
Zn	11	6	8	[e]
Mn	29	20	20	13
B	1,110	1,100	150	200

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[a] Percent of initial organic nitrogen.

[b] SO_4^{2-} sulfur; calculated after correcting for the S added for pH correction.

[c] PO_4^{3-} phosphorus.

[d] Calculated after correcting for the K added for pH correction.

[e] Below the sensitivity limit.

Table 2

Controls for the Lettuce-Root Growth-Rate Assay

Replicate	Root length, mm ±SE(#)[a]		
	Deionized H_2O	NO_3^- Hoagland's	NH_4^+ Hoagland's
1	31 ±1 (37)	28 ±2 (32)	28 ±1 (40)
2	18 ±1 (26)	33 ±1 (44)	N/A
3	18 ±1 (38)	26 ±1 (37)	
4	19 ±1 (40)	30 ±1 (35)	
5	29 ±2 (36)	29 ±2 (35)	

[a] Number of seedlings with root length greater than 4 mm.

Table 3

Chromium and Silver Content of Wet Oxidation Samples

Sample	In contact with	Stirred at 10 rps with a No. 316 stainless steel stirrer	Treated with	Cr, ppm	Ag, ppm
Deionized H_2O (control)	-	-	-	0.00	0.00
Deionized H_2O heated to 275°C for 1 h with P_{O_2} = 400 psi (RT)	No. 316 stainless steel	yes	-	0.26[a]	0.31[a]
	No. 316 stainless steel	yes	Anion exchange resin (OH^-)	0.00	0.00
	No. 316 stainless steel	yes	Anion exchange resin (Cl^-)	0.00	0.00
	No. 316 stainless steel	yes	Cation exchange resin (H^+)	0.23	0.00
	Pyrex liner	yes	-	0.18	0.02
	Pyrex liner	no	-	0.05	0.01

---

[a] This sample was also analyzed for Ni, Mo, V, As, Be, and Cu; these elements were not detected at detection limits of 0.01, 0.01, 0.0007, 0.08, 0.003, and 0.008 ppm, respectively.

Figure Captions

Figure 1. Flow diagram of a photothermal energy conversion cycle based on the wet oxidation of a biomass system. Small rectangles represent heat exchangers.

Figure 2. Comparison of the amounts of nitrate nitrogen, organic nitrogen, ammonia nitrogen, and nitrogen gas found before and after wet oxidation of lettuce solids. See text for oxidation conditions and analytical methodology.

Figure 3. Lettuce-root growth-rate assay results using a series of dilutions of the following solutions: Products of the wet oxidation of lettuce exposed to the reactor surface (—··—) or Pyrex liner (—·—) during oxidation; water treated in the reactor using either no liner with the stirrer in the solution (— —), a Pyrex liner with the stirrer in the solution (— — —), or a Pyrex liner with the stirrer removed (——).

Figure 4. Lettuce-root growth-rate assay results using a series of dilutions of the following solutions: water treated in the wet oxidation reactor with no liner and with the stirrer in the solution and before (——) or after treatment with either cation exchange resin (— — —), OH^- anion exchange resin (—·—), or Cl^- anion exchange resin (—··—).

Figure 5. Root length and Ag and Cr concentrations in the roots of young lettuce plants as a function of dilutions of water treated in the wet oxidation reactor.

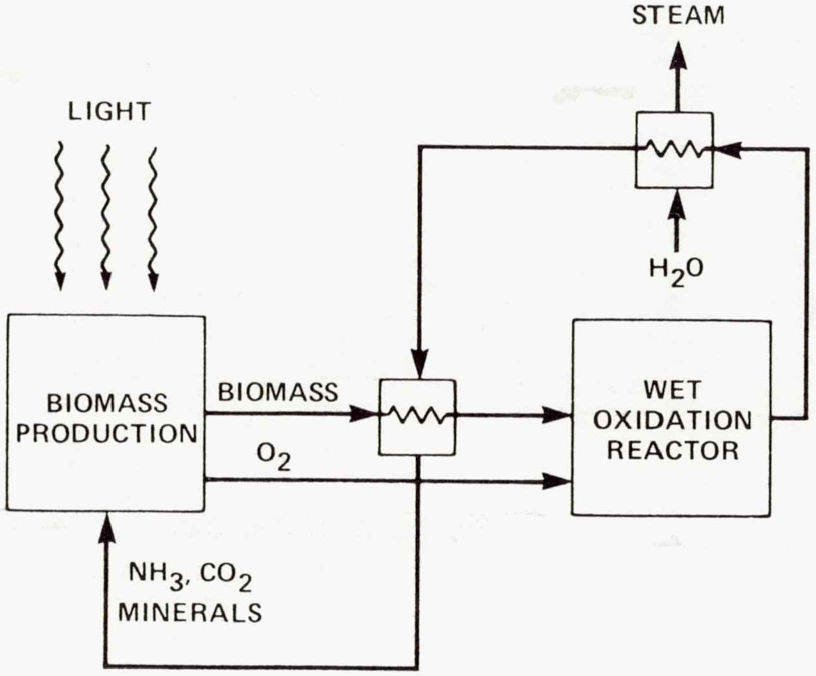

STEAM
LIGHT
H_2O
BIOMASS PRODUCTION
BIOMASS
O_2
WET OXIDATION REACTOR
NH_3, CO_2
MINERALS

Fig. 1

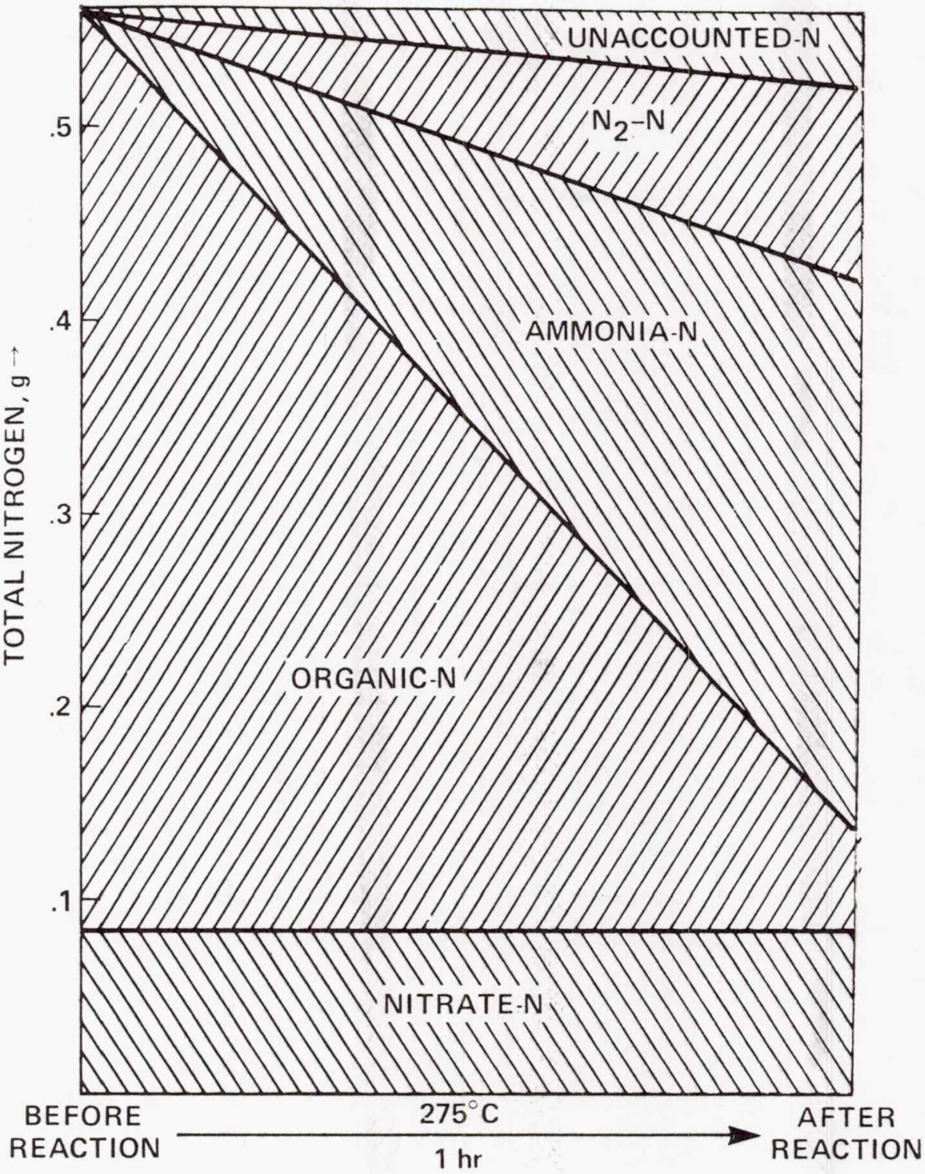
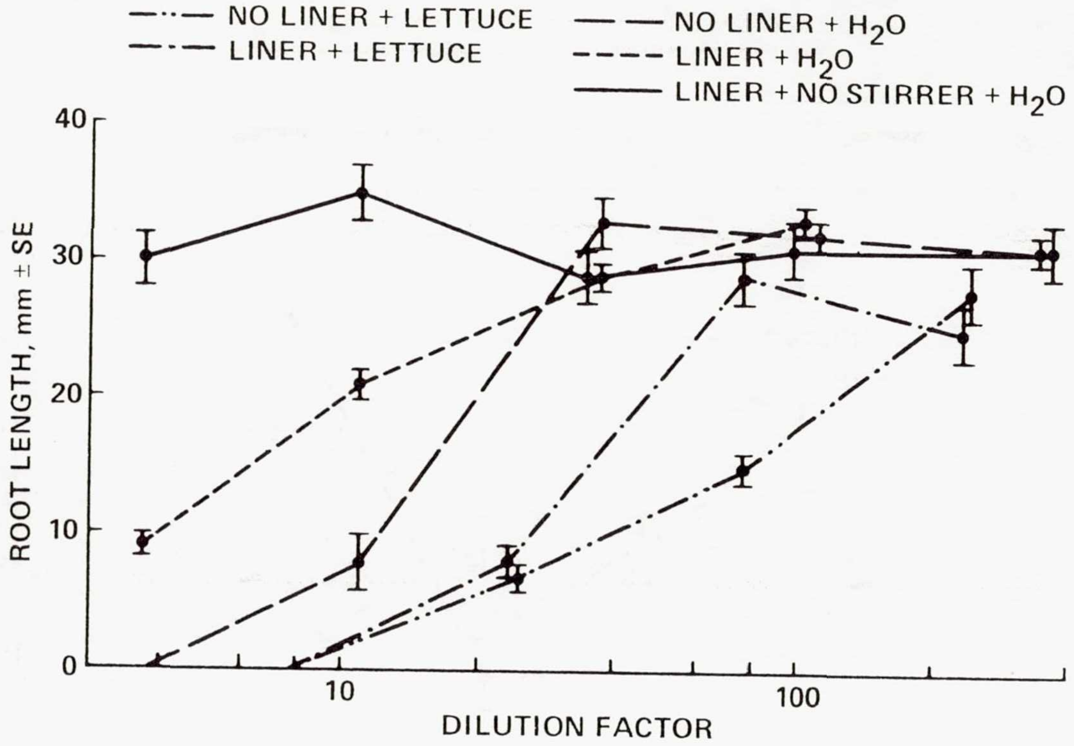

UNACCOUNTED-N
N_2-N
AMMONIA-N
ORGANIC-N
NITRATE-N
TOTAL NITROGEN, g →
.5
.4
.3
.2
.1
BEFORE REACTION
275°C
1 hr
AFTER REACTION

Fig. 2



—··— NO LINER + LETTUCE
—·— LINER + LETTUCE
— — NO LINER + H_2O
- - - - LINER + H_2O
—— LINER + NO STIRRER + H_2O
ROOT LENGTH, mm ± SE
0
10
20
30
40
10
100
DILUTION FACTOR

Fig. 3

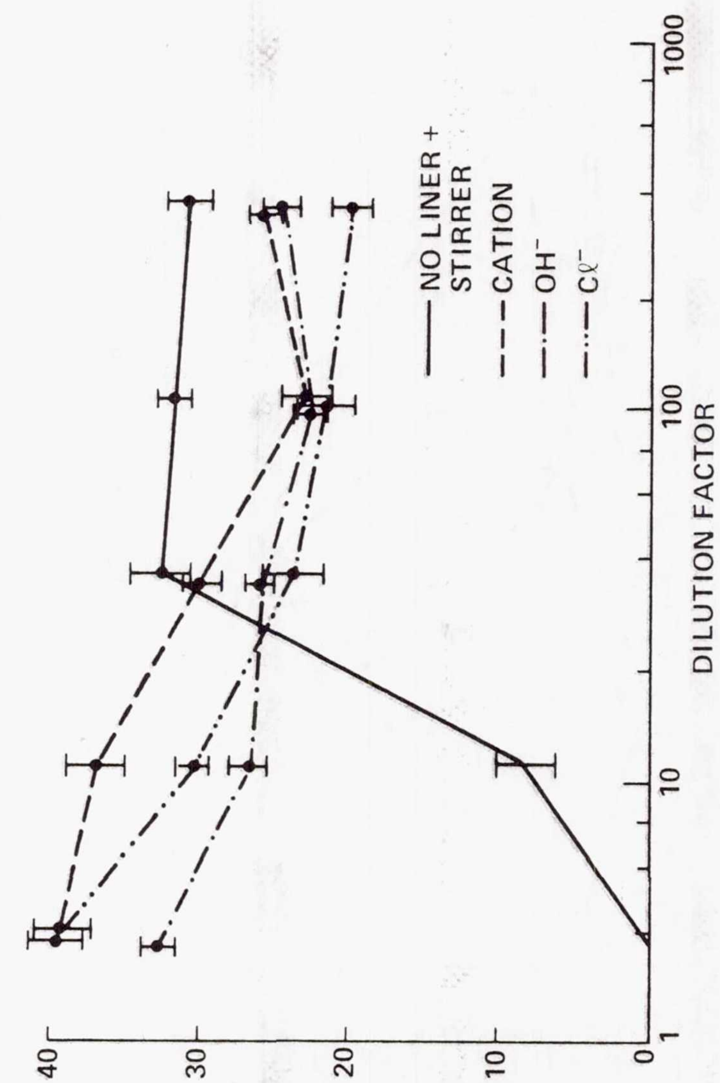

ROOT LENGTH, mm ± SE
0
10
20
30
40
1
10
100
1000
DILUTION FACTOR
NO LINER + STIRRER
CATION
OH⁻
Cℓ⁻

Fig. 4

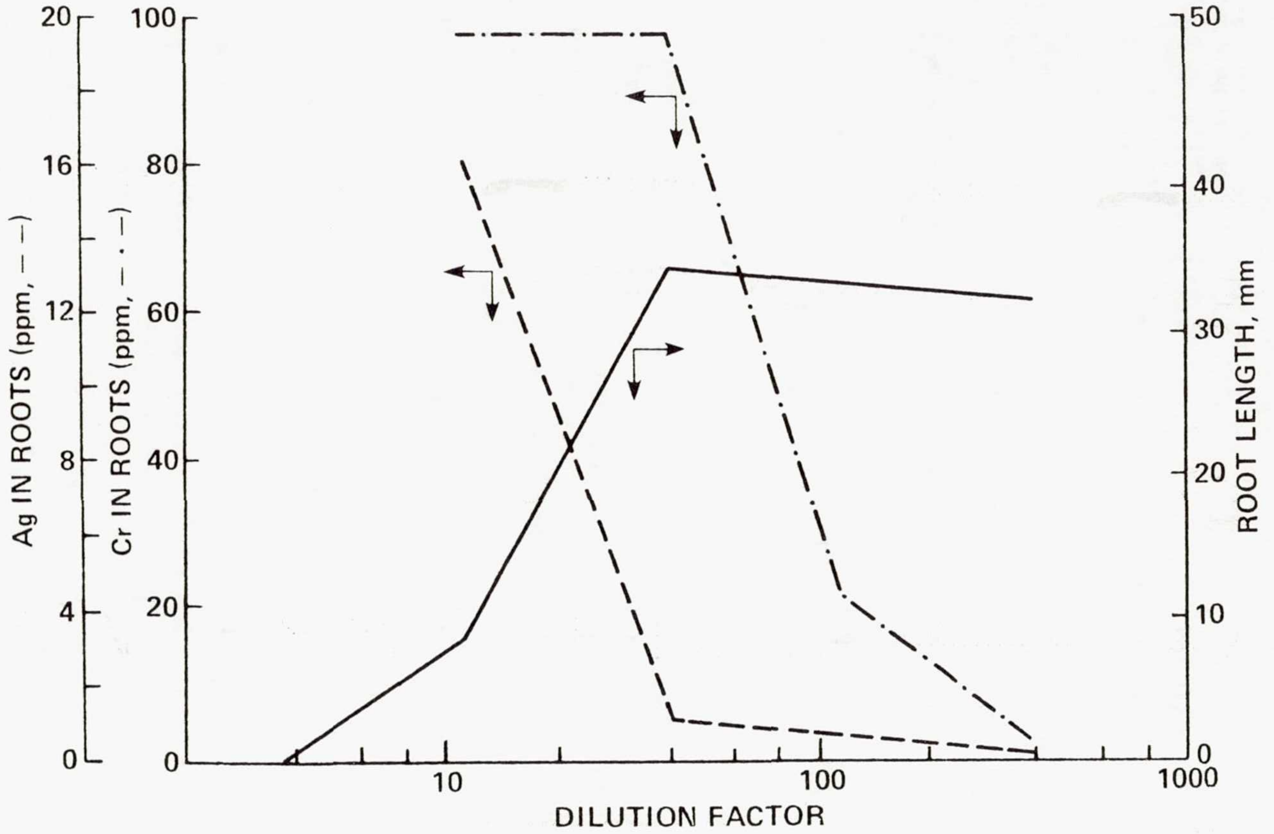

Ag IN ROOTS (ppm, – –)
Cr IN ROOTS (ppm, – · –)
ROOT LENGTH, mm
DILUTION FACTOR
20
16
12
8
4
0
100
80
60
40
20
0
50
40
30
20
10
0
10
100
1000

Fig. 5

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