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IDENTIFICATION-EFFICIENCIES OF ALTERNATE CARBON SOURCES

BY

D. L. KAPLAN
P. A. RILEY
J. PIERCE

and

A. M. KAPLAN

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)		
<p>→ Eleven industrial carbon sources were evaluated for their efficiency to supply energy for biological denitrification of high nitrates (1259 mg/liter) in a single-stage continuous flow fermenter. The determination of relative efficiency was the minimum C/N ratio (grams of carbon to grams of nitrogen) necessary to achieve at least 95% denitrification and 90% total organic carbon (TOC) removal. Methanol was the most efficient carbon source evaluated, while sweet whey, corn steep liquor, acid whey and soluble potato solids followed in order of decreasing efficiency. Three of the carbon sources failed to achieve →</p>		

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the 90% ^{Percent} reduction in TOC. Sewage sludge was unusable due to lack of available carbon.

This order of efficiency may change once other factors are considered such as cost of carbon source, transportation costs, handling costs, availability, and other factors. In the treatment of nitrate contaminated munitions process waters the use of alternate carbon sources will be needed not only for biological nitrate reduction but also for the biological cometabolism of many of the munition compounds themselves.



PREFACE

The work described in this report was performed in support of a number of tasks conducted for the U.S. Army Toxic and Hazardous Materials Agency, on the biodegradation of munitions compounds. The needs for the work were identified by the requirement for supplemental carbon in the treatment of many munitions compounds, along with the biological treatment of the nitrate contamination of process waters. The most efficient and cost effective approach to alternate pollution hazards from nitrates and hazardous organic compounds must be sought. This work is a preliminary guide to the solution of some of these concerns.

The work units which supported this effort include P112.01.01 (W-1), Biodegradation of Nitrate Esters, P112.01.01, RDX/HMX Wastewater Biodegradation, P112.02.02, ¹⁴C-Tagged RDX/HMX Biodegradation, P112.03.05, Ball Powder Biological Control Technology, and P112.03.06, Nitramine Propellant Wastewater.

The authors wish to thank Tineka Jaeger, Chris Kilbridge and Andrew Grenon of the Natick R&D Center for their technical assistance.

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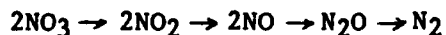
DENITRIFICATION - EFFICIENCIES OF ALTERNATE CARBON SOURCES

INTRODUCTION

The removal of nitrate from waste water is necessary to avoid contamination of waterways. The hazards associated with nitrate contamination of waters include the acceleration of eutrophication, toxic effects on wildlife, potential reactions with chlorine to yield toxic chloramines and human toxicity as expressed in infant methemoglobinemia. These hazards prompted the establishment in 1962 of a 10 mg/liter limit for nitrate-nitrogen contamination of drinking water (45 mg/liter nitrate).

A number of physical-chemical techniques have been utilized to remove contaminating nitrates from wastewaters including selective ion exchange, algal harvesting, reverse osmosis and ammonia stripping. However, biological denitrification has proven to be one of the most economical and effective means of nitrate removal through nitrate reduction.

Biological denitrification may be defined as dissimilatory nitrate reduction where nitrate serves as the terminal electron acceptor in the oxidation of an organic substance. Nitrogen gas is the final product in the case of dissimilatory denitrification for the production of energy via the respiratory transport chain. Nitrate serves as the terminal electron acceptor instead of oxygen. The sequence of electron transfers coordinated with the cytochrome system in the cell involves a sequence of reducing steps; nitrate, nitrite, nitric oxide, nitrous oxide, and nitrogen gas as depicted below.

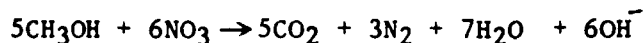


Dissimilatory denitrification, which provides energy for the cell, must be distinguished from assimilatory denitrification which results in the reduction of nitrate to ammonia to serve in cellular synthesis. In addition, it has been shown that a dissimilatory pathway exists which produces ammonia and does not produce energy. The function of this pathway has been postulated as detoxification or as an alternate electron sink. Many facultative bacteria, mainly heterotrophs, are capable of denitrification. Autotrophic denitrifying bacteria have also been identified which have the capacity to oxidize inorganic energy sources such as hydrogen and sodium sulfide.

A number of books and review articles have been published on the subject of denitrification which have included discussions on the role of oxygen, pH, temperature, organic matter, waste treatment systems, and denitrification activity in aqueous, soil and sediment systems.^{1,2,3,6}

Most high nitrate industrial wastewaters, such as those produced in the fertilizer, explosive and nuclear fuel processing industries do not contain sufficient electron donors to provide the energy for the reduction of all the nitrate to nitrogen gas. Therefore an external source of carbon is necessary to provide the energy to promote this reduction.

Numerous system designs have been shown to effectively utilize biological denitrification in the removal of nitrates from wastewaters. Currently, bed reactors, bio discs, glass filtration, batch systems and continuous cultures are the most prominent methods in use. Methanol has long been used in biological denitrification as an external carbon source, with the stoichiometric relationship as illustrated below:



In addition to these requirements, carbon is also needed for bacterial growth.

However, the large volumes of methanol needed to reduce high nitrate levels and the current price have prompted the consideration of alternate carbon sources to provide the energy to drive the denitrification process. Preferably, industrial wastes that are inexpensive, stable, homogeneous, and produced in a convenient geographical location could provide this alternate carbon on a cost-efficient basis.

A number of reports have discussed the use of supplemental carbon sources other than methanol (both single organic substrates and complex industrial wastes) for the energy requirements in denitrification. None of these reports, however, has dealt with high concentrations of nitrate and complex industrial wastes. In addition, the baseline requirements for comparison of efficiency of denitrification and carbon removal for the various carbon sources have not been rigorously defined.

Christensen and Harremoes (1977) reviewed reports on the use of supplemental carbon in the denitrification process.¹ McCarty, Beck and Amant (1969) used consumptive ratios (ratio of the total quantity of an organic chemical consumed during denitrification to the stoichiometric requirement for denitrification and deoxygenation alone) to evaluate relative efficiencies of carbon sources. The authors used batch and continuous systems with relatively low nitrate concentrations (25 mg per liter) and single organic carbon source (methanol, acetate, ethanol, acetone, sugar).⁴ They found approximately equal consumptive ratios for all these sources with the exception of the sugar. Based on these findings with costs factored in, methanol was determined to be the optimal carbon source of those investigated. High nitrate removal (over 95%) was achieved for the most part, while removal of soluble organic carbon was around 70%.

Skrinde and Bhagat (1982) investigated a number of carbon sources (methanol, spent sulfite liquor, yeast, corn silage, acid whey) in a continuously fed fluidized activated carbon filter receiving between 18 and 42 mg per liter nitrate.⁷ The authors utilized a chemical oxygen demand (COD) to nitrate ratio to evaluate efficiency and determined methanol to be the most efficient with a ratio of 2.5/1. The ratios for the yeast and sulfite liquor were 2.8/1 and 2.9/1, respectively. Nitrate removal efficiencies never reached 95% COD and BOD removal efficiencies ranged between 38 to 79% and 32 to 85%, respectively.

Monteith, Bridle, and Sutton (1980) used batch systems to evaluate 30 industrial carbon sources.⁵ The authors measured denitrification rates to compare carbon sources in relation to methanol. We question the validity of using short-term batch nitrate reduction rate studies to evaluate comparative efficiencies. In operational continuous systems the rates of nitrate reduction will be instantaneous once the system is established. Using denitrification rates the authors found many of the carbon sources exhibited higher rates than methanol. Consumptive ratios were also determined and many of the carbon sources had ratios below methanol. There was no apparent correlation between consumptive ratios and denitrification rates for the various carbon sources. The authors also found that carbon loads above those required for denitrification affected the consumptive ratio.

Toit and Davies (1973) evaluated methanol and lactate in a continuous multistage system with between 300 and 37.5 mg per liter nitrate nitrogen. With methanol, nitrate removal efficiencies were 34 to 96.4% and COD removal was 32.6 to 88.6%.⁸ With lactate these efficiencies were 18.3 to 98.0% and 76.2 to 95.1%, respectively. The authors also found settled domestic sewage was unsuitable as a carbon source for denitrification.

None of these reports has addressed the objectives of this paper, utilizing continuous flow, single stage, denitrification systems receiving high nitrate loads (1259 mg/liter nitrate or 285 mg/liter nitrate-nitrogen) to compare efficiencies of industrial carbon sources; the criteria for comparison being the minimum C/N at which 95% denitrification and 90% TOC removal are achieved. If alternate carbon sources are used to drive the denitrification process, this must not be at the expense of increasing effluent TOC.

In addition, waste waters contaminated with nitrates often contains hazardous organic compounds which must be treated concurrently. Many of these compounds undergo biodegradation only through cometabolism (nonspecific enzymatic degradation where no energy is obtained for the microorganisms and alternate carbon is required). Treatment systems dealing with both nitrates and these types of organic waste products would gain a double benefit with the addition of these carbon sources.

METHODS AND MATERIALS

The various carbon sources analyzed included the following: (1) reagent grade methanol; (2) nutrient broth (Difco, Detroit, MI); (3) dehydrated soluble potato solids, process effluent generated by the manufacture of starch (U.S. Department of Agriculture, Philadelphia, PA and Colby Starch Co-operative, Priscil, ME); (4) anaerobic digest obtained from a municipal sludge treatment plant (Nut Island Sewage Treatment Plant, Boston MA; this digest was analyzed as raw sludge and sludge treated by acid hydrolysis); (5) corn steep liquor (a concentrated solution of maize solubles obtained from the lactic fermentation process during the steeping of maize prior to wet milling, Grain Processing Corporation, Muscatine, IA), (6) soluble fish condensates, (Sharpley Laboratories Inc., Fredericksburg, VA, volatile components condensed from the

steaming operation utilized in the production of fish meal); (7) acid whey (H.P. Hood Inc., Boston, MA); (8) brewery spent grain (solid waste from the brewing process, National Feed of New England, Merrimack, New Hampshire), (9) sweet whey (a free-flowing powder resulting from spray drying sweet fresh cheese whey which is pasteurized either before or during the manufacturing process, and (10) sugar beet molasses (a sugar beet processing waste product, Great Western Sugar Company, Loveland, CO through the Beet Sugar Development Foundation, Fort Collins, CO). Table 1 lists nutritional data for some of the carbon sources. Acid hydrolysis of the anaerobic sewage digest was accomplished with hydrochloric acid (approximately 1N final concentration) and autoclaving for one hour. The mixture was neutralized with sodium hydroxide, centrifuged for 20 min at 10,000 rpm and passed through glass wool.

The continuous cultures were run at room temperature. All of the systems except for the nutrient broth contained the following salts per liter of filtered lake water: $MgSO_4 \cdot 7H_2O$, 500 mg; NaCl, 50 mg; $CaCl_2$, 15 mg; $FeCl_3 \cdot 6H_2O$, 10 mg; $CuSO_4 \cdot 5H_2O$, 10 mg; $MnSO_4 \cdot H_2O$, 10 mg; $NaMoO_4 \cdot 2H_2O$, 1 mg; K_2HPO_4 , 0.87 g; and KNO_3 2.05 g. The lake water was obtained from Lake Cochituate, Natick, MA and generally had a pH between 6 and 7 and less than 25 mg/liter total organic carbon (TOC). Total organic carbon content was determined with a Beckman 915B Tocamaster with Matheson Ultra Zero as carrier gas at 300 mLs per minute. Samples, 20 uL, were delivered with a Hamilton CR-200 200 uL constant rate syringe. Before total organic content was determined, continuous culture influent and effluent samples were centrifuged at 12,000 rpm for 20 minutes then filtered through a Nylon 66 0.45 micron filter (Rainin Instrument Co. Woburn) (MA) with a glass filter unit (Millipore Corp. Bedford, MA).

Nitrates, pH and oxidation-reduction (E_h) readings were recorded once a week using a Corning Model 130 pH meter. Nitrates were determined using an Orion model 93-07 nitrate electrode (Corning Research, Inc., Cambridge, MA). E_h was monitored with an Orion platinum redox electrode. A Corning calomel reference electrode and Corning pH electrode were used to determine pH readings. Ammonia was determined with an Orion specific ion electrode less frequently.

Head space gases were obtained with a gas-tight syringe through rubber septa. The continuous systems were closed off from surrounding air for at least one hour and then sampled. The 0.5 mL gas samples were analyzed on a Hewlett-Packard gas chromatograph model 5880A with a thermal conductivity detector. The injector and detector temperatures were 150°C and 275°C, respectively. Helium carrier gas flowed at 30 mL per minute through a 2.44 m by 0.32 cm stainless steel column containing Carbosieve S, 120/140 mesh. Programmed runs were initiated with an oven temperature at 35°C for five minutes, followed by a program rate of 15°C per minute to a final temperature of 175°C for 15 minutes.

TABLE 1. Analysis of Carbon Supplements used in the Denitrification Systems

A. Acid Whey

Protein	12.2%
Nonprotein Nitrogen	0.6%
Fat	0.5%
Lactose	63.0%
Moisture	6.1%
Ash	10.7%
Titratable Acidity	0.39%
pH	4.6

Data Source: Whey Products Institute Chicago, Illinois, acid whey source: H.P. Hood Inc., Boston, MA

B. Corn Steep Liquor

Solids	48%
Protein	21%
Lactate Acidity	13%
Reducing Sugars	2%
Amino Nitrogen	1%
Ash	9%
Phosphate (P ₂ O ₅)	4%
Sulfur	1%
pH	4.0 -4.2

Data and corn steep liquor source: Garton, Sons and Company, Ltd. via Claire Smith, Grain Processing Corporation, Muscatine, IA

**TABLE 1. Analysis of Carbon Supplements used in the Denitrification System
(cont'd)**

C. Sugar Beet Molasses

Solids	82%
Sucrose	51%
Nitrogenous Compounds	13%
Ash	11%

Data source: "Beet: Beet Sugar Technology" in a personal communication with the Beet Sugar Development Foundation, Fort Collins, CO., sugar beet molasses source: Great Western Sugar Company Loveland, CO through the Beet Sugar Development Foundation of Fort Collins, CO

D. Dehydrated Soluble Potato Solids

Minerals	19.6%
Moisture Content	2.6%
True Proteins	11.5%
Amino Acids, Amides	21.4%
Total Sugars	34.8%
Glucose	14.6%
Fructose	13.7%
Sucrose	6.5%
Citric Acid	4.0%
Other	8.7%

Data source: U.S. Department of Agriculture, Philadelphia, PA, dehydrated soluble potato solids source: U.S. Department of Agriculture, Agriculture Research Service Northeast Regional Research Center, Philadelphia, PA

**TABLE 1. Analysis of Carbon Supplements used in the Denitrification System
(cont'd)**

E. Sweet Whey

Lactose	74%
Moisture	3%
Fat	1%
Ash	8%

Data and sweet whey source: Cuba Cheese Inc.,
Cuba, New York

F. Volatile Fish Condensates

Solids	57.4%
Protein	41.4%
Moisture	42.6%
Ash	5.1%
Fat	0.17%

Data and volatile fish condensate source:
Sharpley Laboratories, Inc.,
Fredericksburg, VA

Continuous flow systems were run in either BioFlo Model C30 bench-top fermenters (New Brunswick Scientific, New Brunswick, NJ) or in modified 500 mL Erlenmeyer flasks. The BioFlo systems used 1500 mL reaction vessels and the medium was stirred slowly. The modified Erlenmeyer flasks, 500 mL, were fitted with a 24/40 ground glass joint, an overflow tube, and a 35 cm long glass tube, 5 mm I.D. suspended by a teflon adaptor to deliver nutrient solution to the bottom of the reaction vessel. Nutrient solution was delivered continuously to the reaction vessels by a Rainin Rabbit peristaltic pump (Woburn, MA).

Throughout the study the concentration of the carbon source was adjusted while the concentration of nitrate remained constant. As these C/N adjustments were made a minimum of two to three weeks equilibration time was allowed for the cell populations to reach carrying capacity. Most carbon sources were studied for extended periods because of these long equilibration times.

RESULTS

When testing a carbon source, the objective was to determine the lowest carbon to nitrogen ratio at the point where 95% denitrification and 90% disappearance of total organic carbon was achieved. Of the 11 carbon sources tested, however, only 5 reached this objective. Table 2 lists the carbon sources tested along with the lowest C/N ratio at which optimum results were observed. Category a of Table 2 contains those carbon sources which exhibited a denitrification efficiency of 95% or greater while also utilizing 90% to 97% of the total organic carbon (Figures 1 through 5). The carbon sources in category b of Table 2 reached a denitrification efficiency comparable to category a, however only 80% to 90% of the total organic carbon was utilized (Figures 6 through 8).

Category c of Table 2 lists the carbon sources with which there were difficulties in evaluation. Volatile fish condensate showed a preliminary optimum C/N of 2.5 but the supply of this medium was exhausted before confirmation was obtained (Figure 9). Sewage sludge digest was not usable due to the fact that the C/N ratio did not surpass 5.1, regardless of the amount of digest added. Figure 10 shows that at the 5.1 maximum C/N ratio denitrification reached only approximately 70%. In all cases, except with acid hydrolyzed sewage sludge digest, once the carbon level was reached to maintain maximum denitrification, additional carbon loads did not inhibit denitrification. The additional carbon sometimes added TOC in the effluents due to incomplete utilization of available carbon. Figure 11 illustrates that with acid hydrolyzed sewage digests at a C/N of 2.1, denitrification is 95% and the total organic carbon drop is approximately 85%. These levels are maintained until the C/N reaches 2.8 at which point denitrification and organic carbon consumed decreased rapidly to a final denitrification efficiency of only 25% at a C/N of 5.5.

Experimental results for pH, retention time, percent denitrification, percent TOC removal and C/N ratio for each of the systems are presented in Table 3 and Figures 1 to 11. The data presented in these figures represents moving averages of three neighboring values. All systems were maintained at room temperature, 20°C to 23°C. The pH readings of the effluent samples were generally in the slightly alkaline range, indicative of active denitrification. Generally a four-day retention time \pm one day was maintained throughout these studies. At this retention time flow rates were sufficient to prevent clogging of tubing by biomass while not so rapid as to result in excessive wash-out of cells.

It is interesting to observe that if TOC removal efficiency is omitted from consideration, then the relative efficiencies of denitrification (minimum C/N at which 95% denitrification is achieved) are similar (Table 4) to those found in Table 2. These findings may have application in cases where a two-stage system is utilized, such that excessive TOC is removed in the second stage.

TABLE 2. Efficiencies of Alternate Carbon Sources Evaluated in the Denitrification Process.

<u>Carbon Source</u>	<u>C/N</u> ¹
a. 95% denitrification, 90% TOC ² removal	
Methanol	1.1
Sweet Whey	1.4
Acid Whey	1.4
Corn Steep Liquor	1.6
Soluble Potato Solids	1.7
b. 95% denitrification, 80% TOC removal	
Nutrient Broth	1.7
Brewery Spent Grain	2.3
Sugar Beet Molasses	3.6
c. Others	
Acid Hydrolyzed Sewage Sludge Digest	2.1 ³
Volatile Fish Condensate	2.5 ⁴
Sewage Sludge Digest	--- ⁵

¹Ratio of grams of carbon to grams of nitrogen in media

²Total organic carbon

³At C/N ratios above 2.1 the percent TOC removal decreased from 87% (see Figure 11).

⁴Insufficient medium to complete study

⁵95% denitrification and 80% TOC removal never achieved.

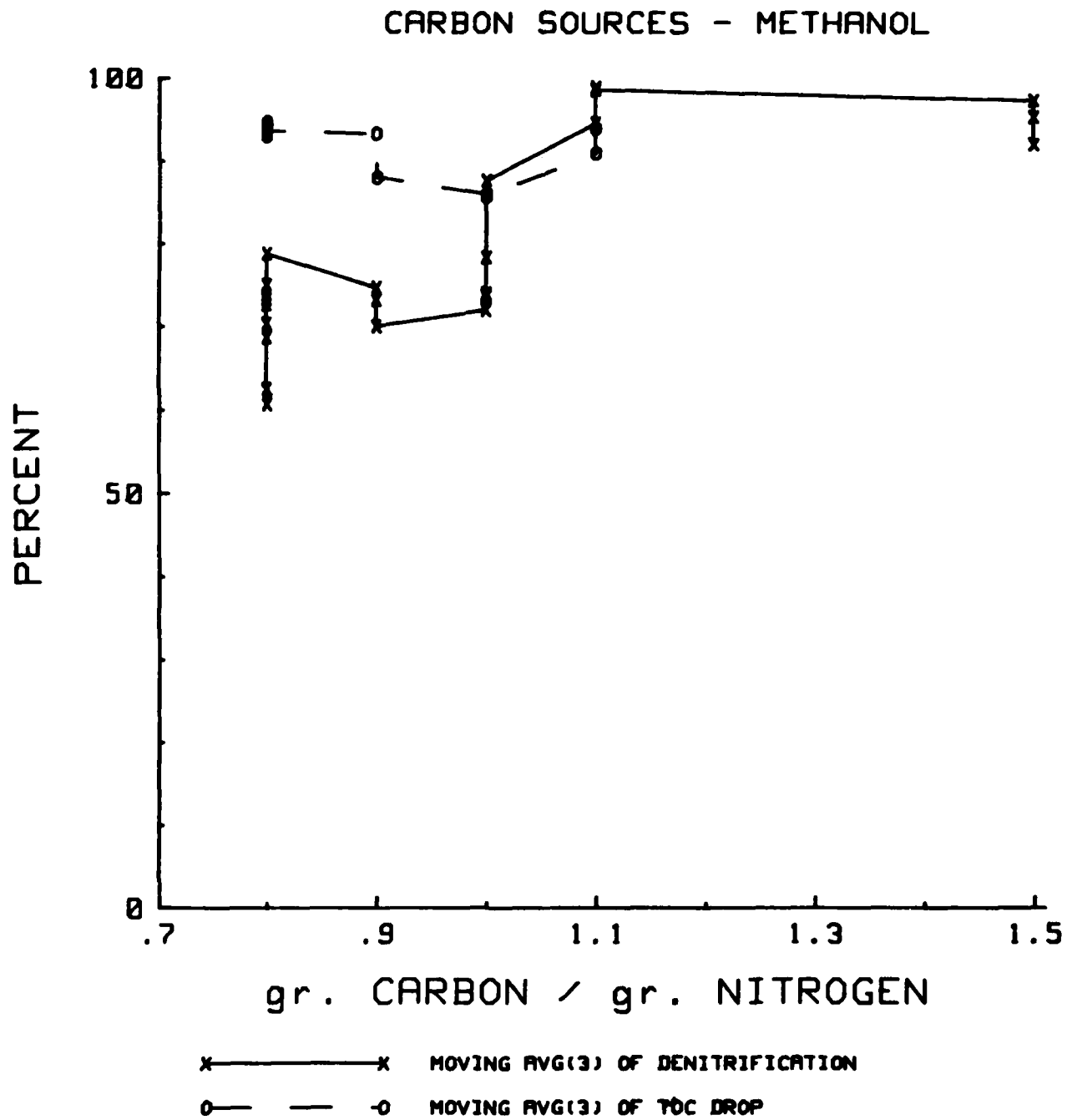


Figure 1. Percent denitrification and total organic carbon removal with methanol

CARBON SOURCES - SWEET WHEY

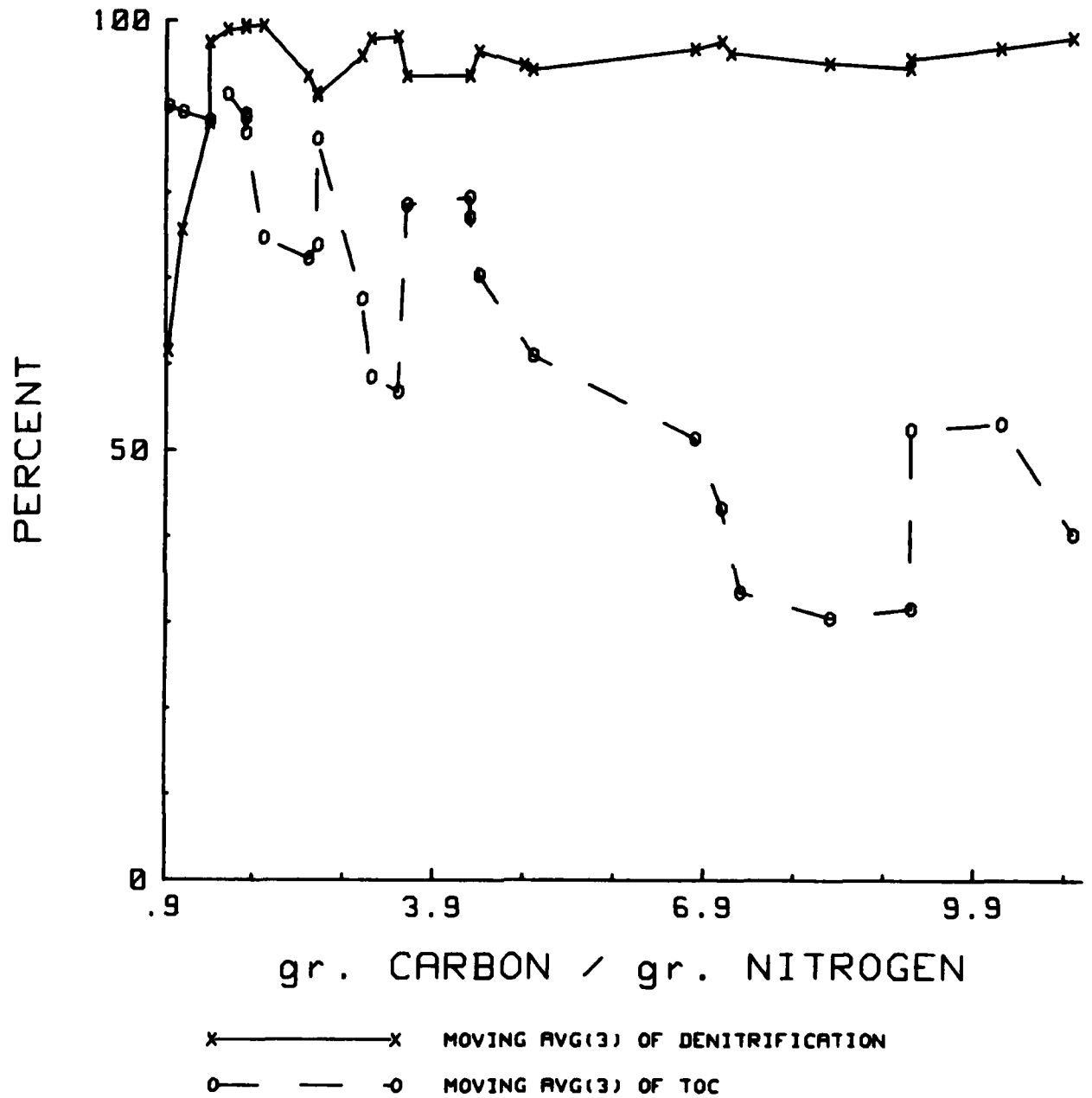


Figure 2. Percent denitrification and total organic carbon removal with sweet whey

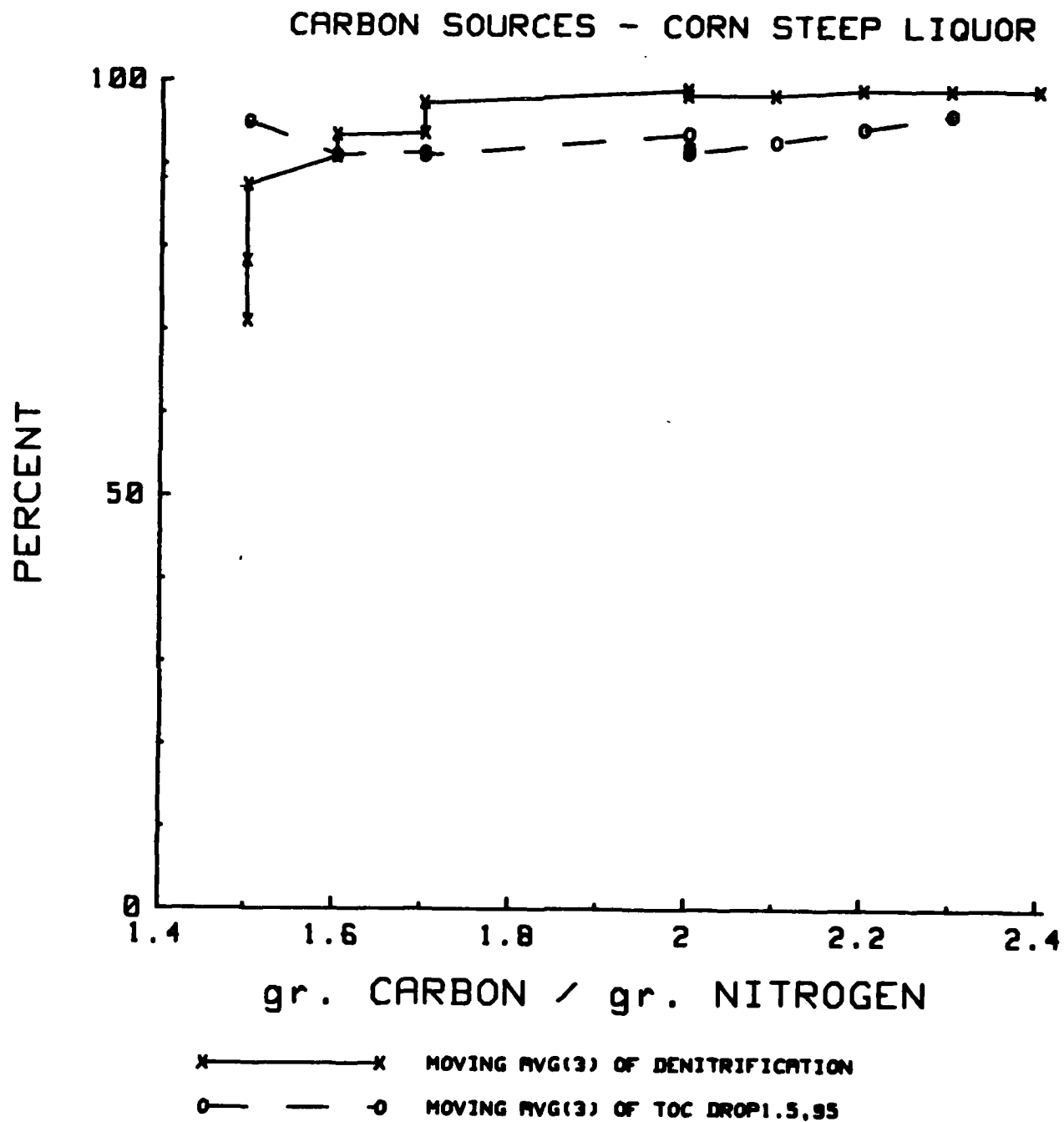


Figure 3. Percent denitrification and total organic carbon removal with corn steep liquor

CARBON SOURCES - ACID WHEY

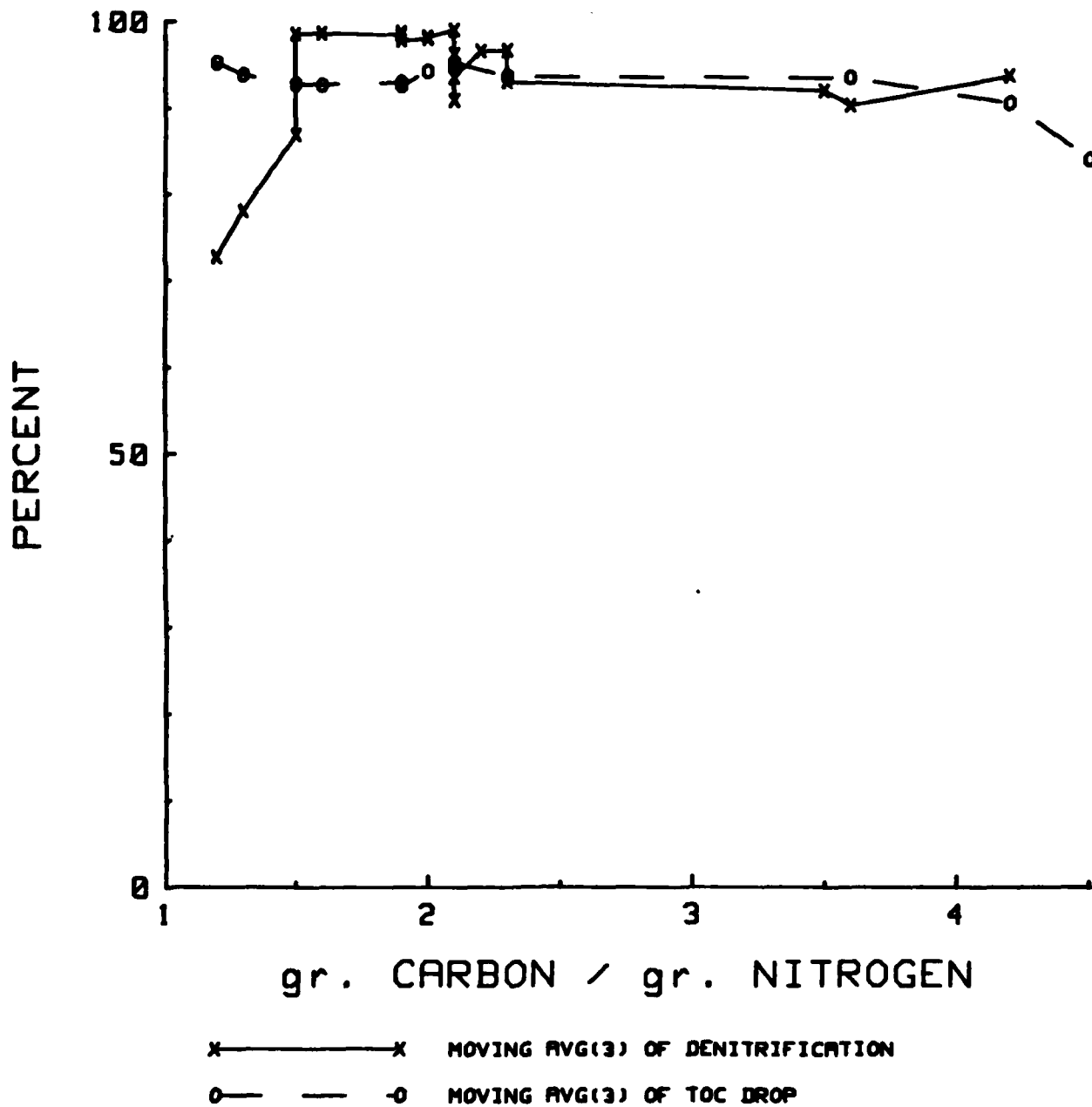


Figure 4. Percent denitrification and total organic carbon removal with acid whey

CARBON SOURCES - POTATO SOLIDS

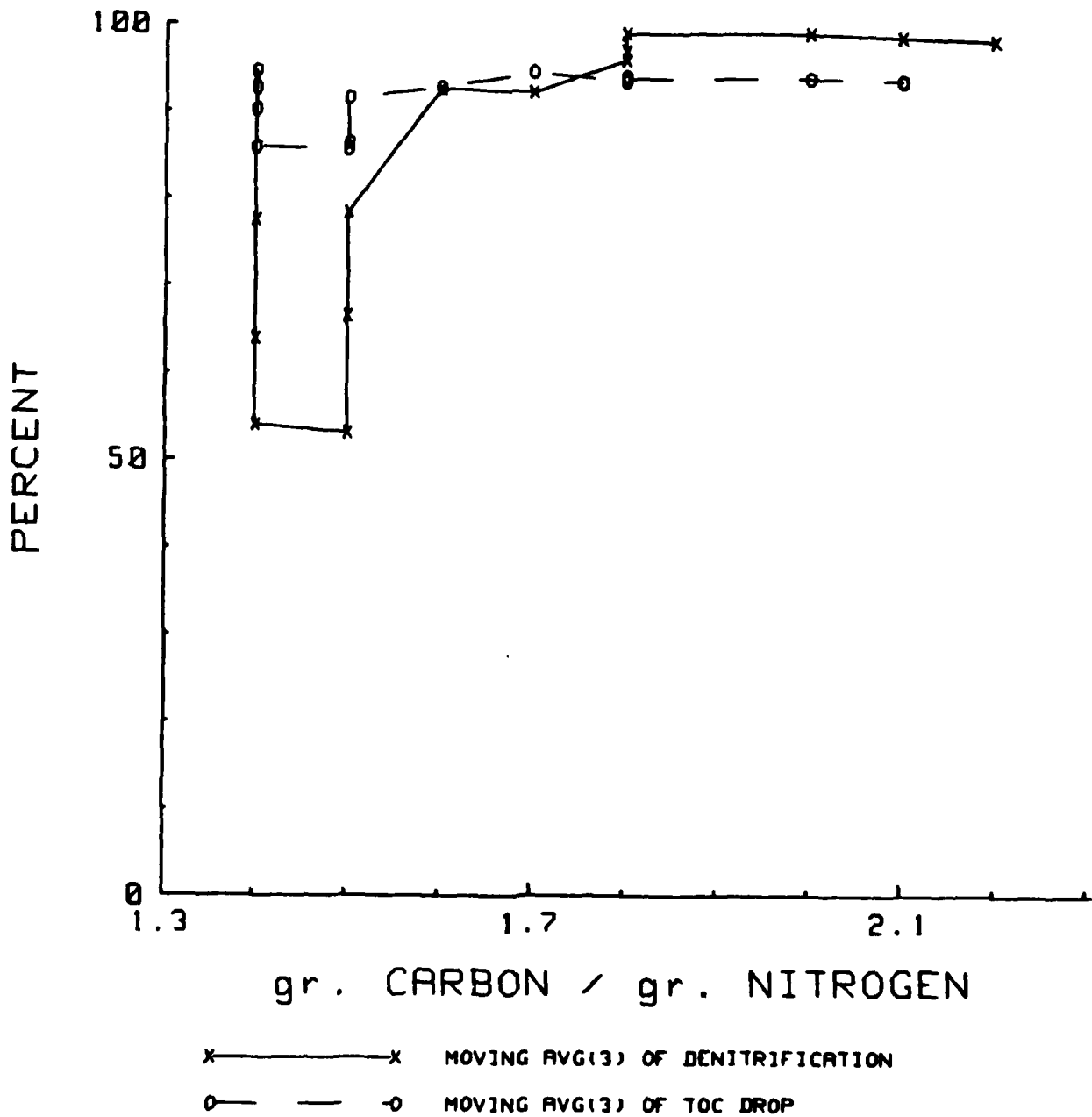


Figure 5. Percent denitrification and total organic carbon removal with potato slides

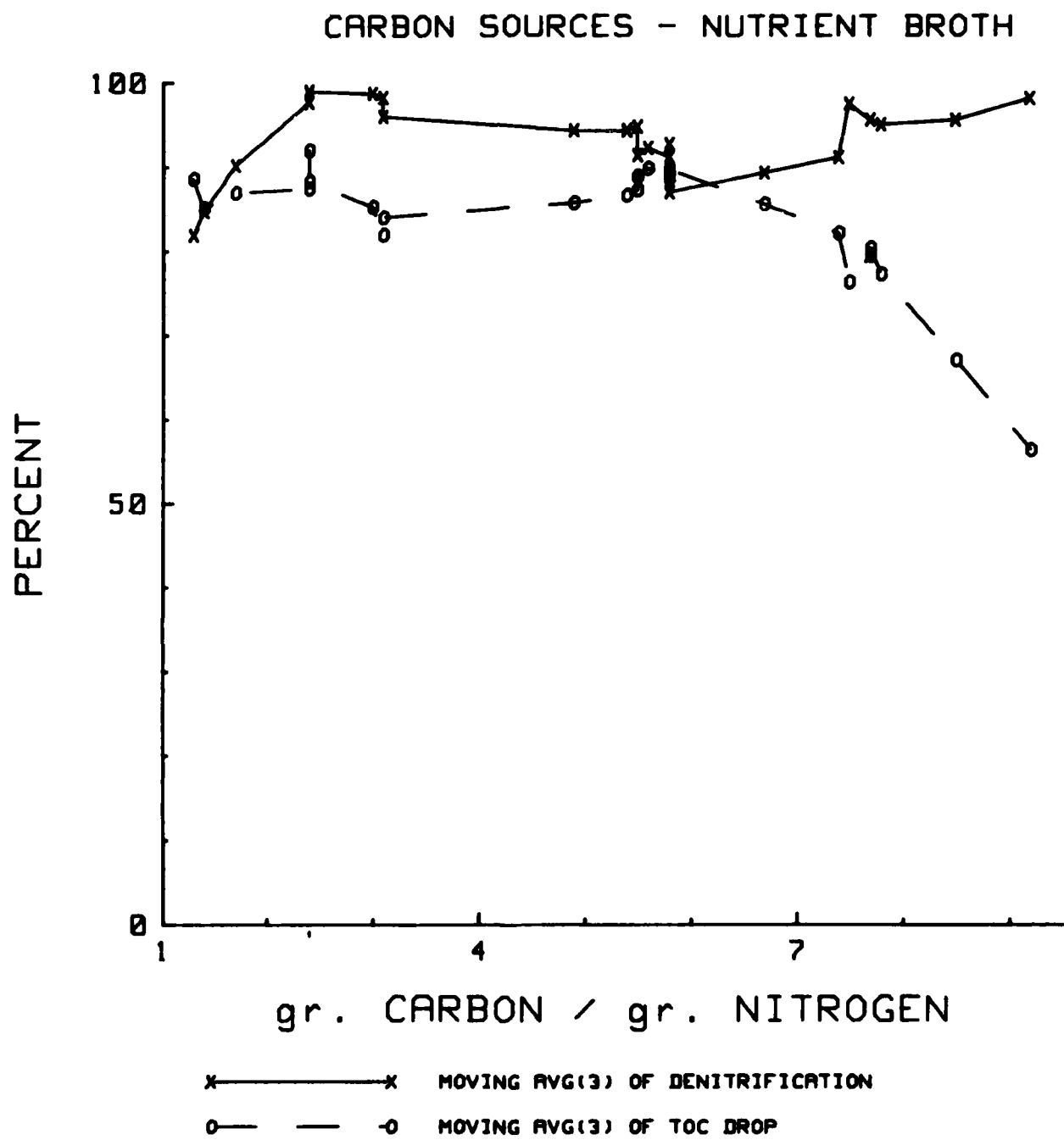


Figure 6. Percent denitrification and total organic carbon removal with nutrient broth

CARBON SOURCES - SPENT BREWERY GRAIN

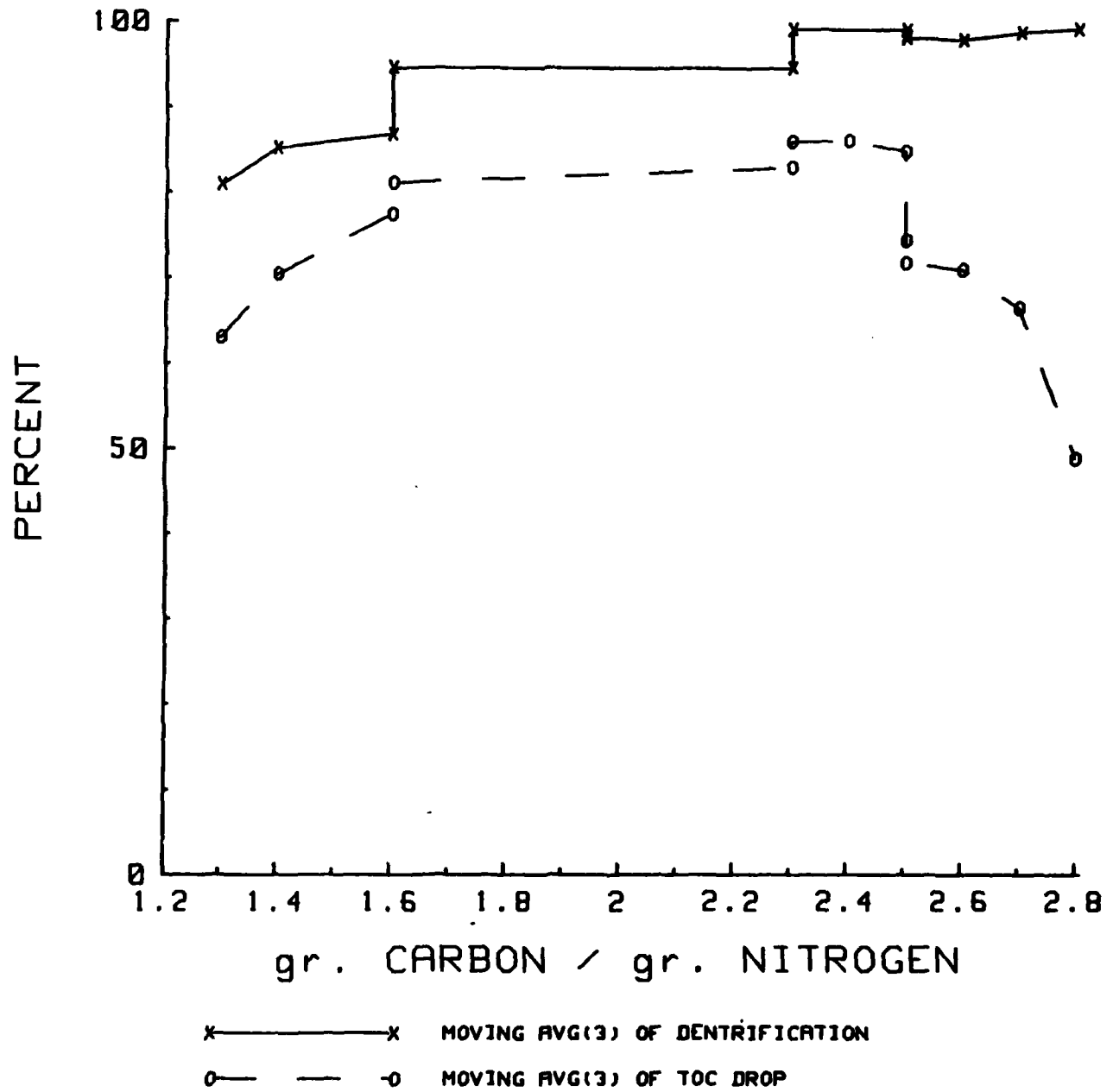


Figure 7. Percent denitrification and total organic carbon removal with spent brewery grain

CARBON SOURCES - SUGAR BEET MOLASSES

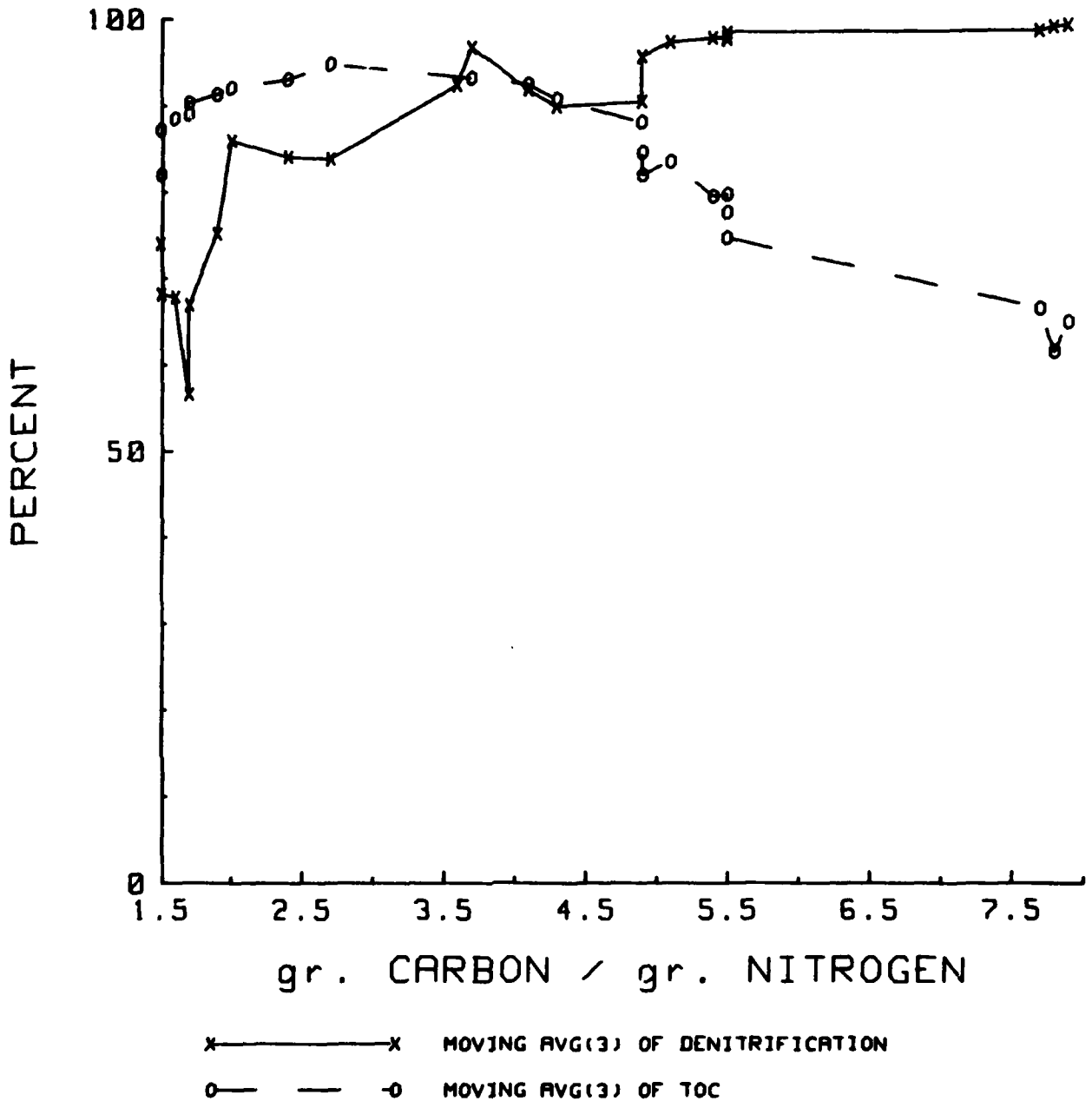


Figure 8. Percent denitrification and total organic carbon removal with sugar beet molasses

CARBON SOURCES - HYDROLIZED SLUDGE

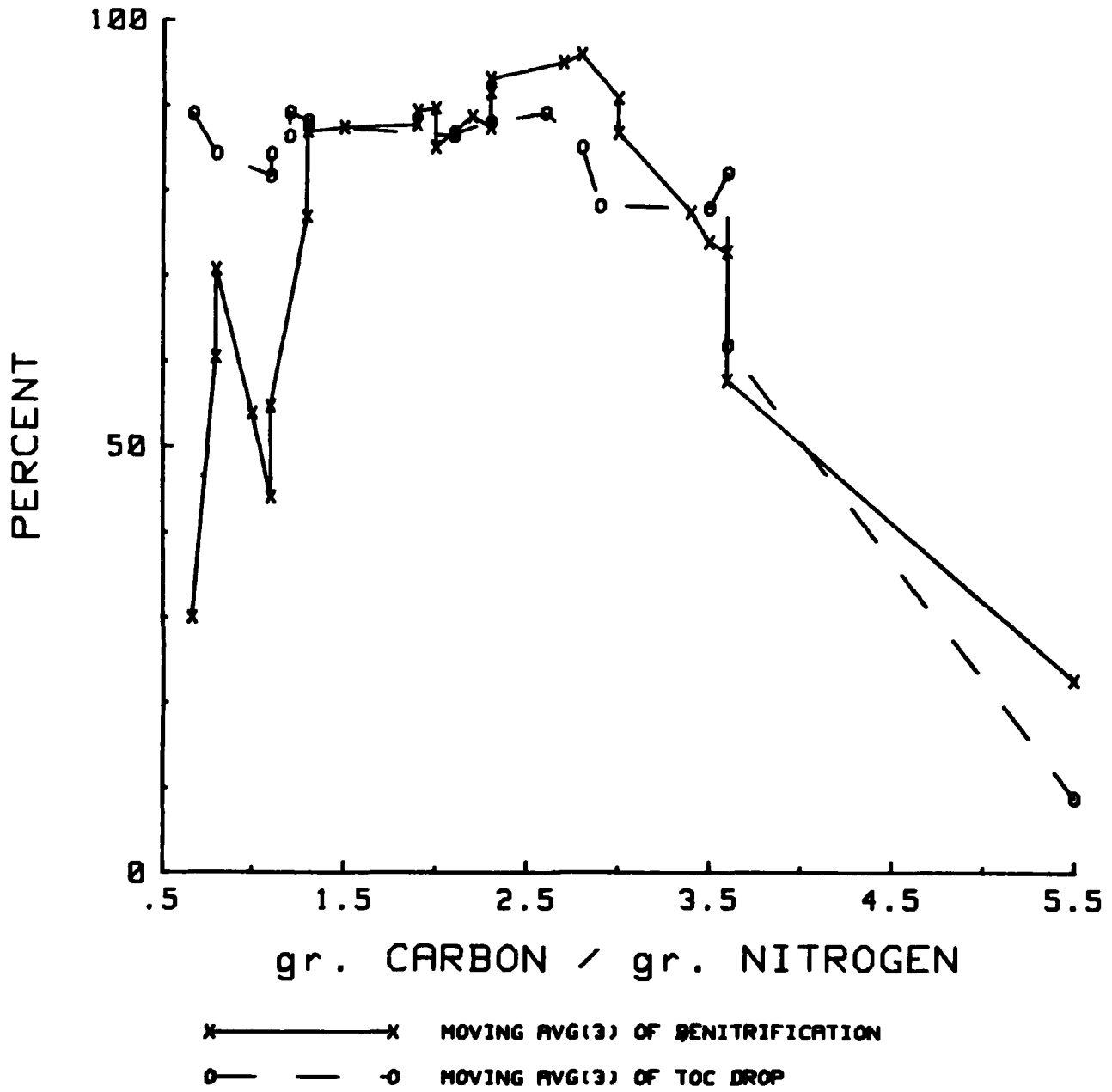


Figure 9. Percent denitrification and total organic carbon removal with hydrolyzed sludge

CARBON SOURCES - FISH STICK

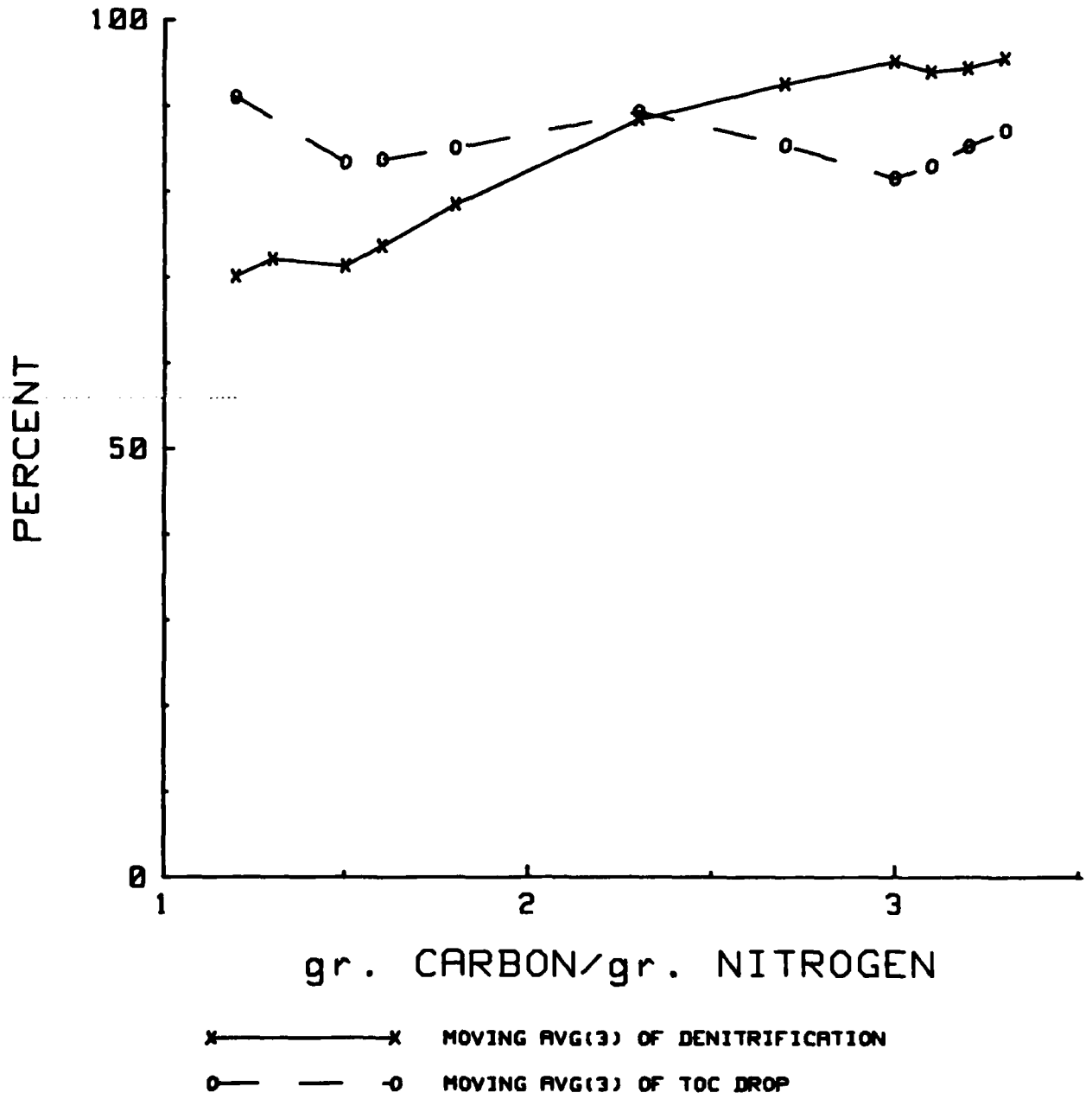


Figure 10. Percent denitrification with total organic carbon removal with fish stick

CARBON SOURCES - RAW SLUDGE

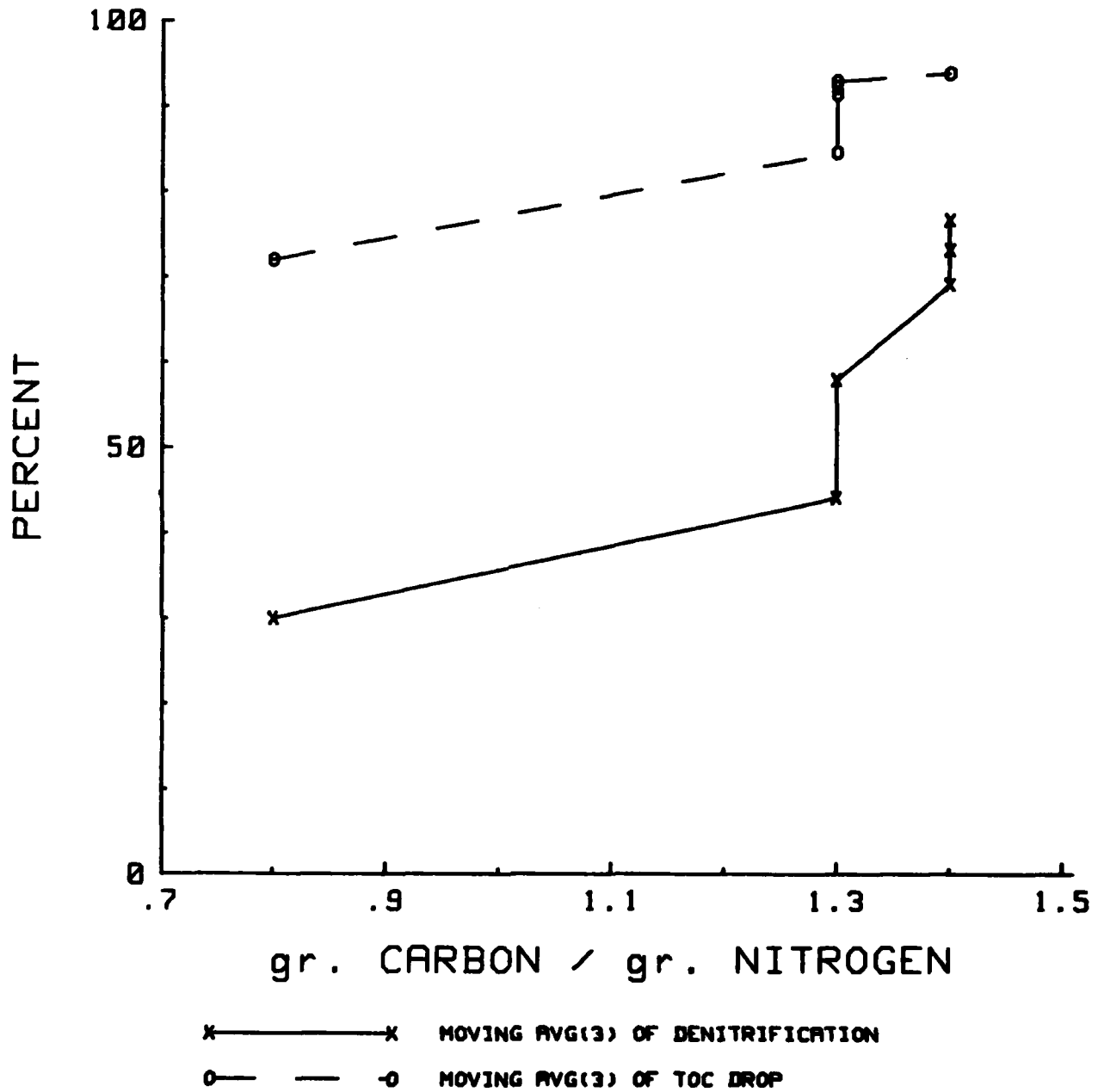


Figure 11. Percent denitrification and total organic carbon removal with sewage sludge

TABLE 4. Efficiencies of Alternate Carbon Sources
Without Consideration of TOC Removal

<u>Carbon Source</u>	<u>C/N</u> ¹
Methanol	1.1
Sweet Whey	1.4
Acid Whey	1.4
Corn Steep Liquor	1.6
Brewery Spent Grain	1.6
Soluble Potatoe Solids	1.7
Nutrient Broth	1.7
Acid Hydrolyzed Sewage Sludge Digest	2.1
Volatile Fish Condensate	2.5 ²
Sugar Beet Molasses	3.6
Sewage Sludge Digest	--- ³

¹ Ratio of grams of carbon to grams of nitrogen in media. Minimum at which denitrification surpassed 95%.

² Insufficient medium to complete study.

³ 95% denitrification never achieved.

DISCUSSION

Our results show that none of the carbon sources was as effective as methanol in providing the energy to drive denitrification. Based on stoichiometric relationships, 5/6 mole of methanol are required to reduce one mole of nitrate completely to molecular N_2 . However, an additional 30% over this amount is needed to satisfy the requirements for bacterial growth. While the complex wastes appear not to have the oxidizing potential of methanol, some of the carbon sources tested exhibited an efficiency only slightly lower than that of methanol. If economic factors are considered, these sources may in fact be more cost efficient than methanol.

The carbon sources in category a of Table 2 showed the best results relative to methanol. A review of the components in these wastes leads to some conjectures as to why these four sources are preferable. Corn steep liquor and soluble potato solids contain protein in amounts greater than 20%. Since the deamination of amino acids yields a variety of carboxylic acids which can subsequently serve as electron donors, these proteins can fuel the denitrification process. Sugars are also used as an energy source for biological denitrification. Acid whey and sweet whey contain 63% and 74% sugar, respectively, indicating this is a main energy source for the bacteria utilizing these wastes for denitrification.

The sources listed in category b of Table 2 show adequate denitrification, however, more carbon per each nitrate reduced is needed to achieve the desired level of denitrification. Apparently, the carbon contained in this category of carbon sources is not as readily assimilable as for the carbon sources in category a. This is reflected by the higher TOC remaining in effluent samples.

The results from sewage sludge digest indicate that the needed carbon nutrients were not in forms readily available to the bacteria. This problem was partially eliminated through acid hydrolysis treatment of the sludge.

Acid hydrolyzed sewage sludge was unique in that after reaching an optimum C/N the denitrification decreased with subsequent increasing C/N. A possible explanation is that with time and increasing carbon concentration the biomass in the reaction vessel became so dense that the incoming medium was not distributed evenly, resulting in the decreased denitrification. During the dismantling it was noted that the reaction vessel contained a semisolid mass not found in other systems. An alternative explanation may be the presence of inhibitory compounds that would demonstrate an effect as the concentration of sludge was increased.

TABLE 3. Data Collected from Continuous Flow Denitrification Systems with Alternate Carbon Sources

A. Acid Whey

Time (Days)	C/N ¹	% Denitrification ²	% TOC Removal ³	pH	Retention Time (days)
1-11	2.0 + 0 (1) ⁵	91.0 + 0 (1)	84.0 + 0 (1)	7.4 + 0 (1)	4.5 + 0.2 (2)
12-25	2.3 + 0 (1)	86.5 + 7.8 (2)	93.0 + 0 (1)	7.7 + 0.1 (2)	4.4 + 0.5 (3)
26-47	6	99.0 + 0 (3)	91.0 + 0 (1)	7.6 + 0.5 (3)	4.0 + 0.5 (6)
48-83	2.1 + 0 (2)	94.2 + 7.2 (5)	96.5 + 3.5 (2)	7.9 + 0.2 (5)	4.2 + 0.6 (16)
84-99	2.2 + 0 (2)	99.0 + 0 (1)	93.0 + 0 (2)	7.7 + 0 (1)	4.3 + 0.5 (10)
100-139	1.9 + 0 (3)	95.3 + 8.2 (4)	93.6 + 0.7 (3)	8.0 + 0.3 (5)	4.5 + 0.4 (19)
140-155	2.0 + 0 (1)	96.5 + 0 (1)	95.0 + 0 (1)	8.2 + 0 (1)	4.1 + 0.2 (11)
156-173	1.5 + 0.1 (2)	96.6 + 1.1 (3)	92.9 + 1.3 (3)	8.1 + 0 (3)	4.2 + 0.3 (9)
174-191	1.3 + 0.1 (2)	67.6 + 7.4 (2)	93.7 + 2.3 (2)	8.1 + 0.2 (2)	3.8 + 0.3 (8)
192-208	3.3 + 1.4 (2)	95.0 + 1.8 (2)	93.8 + 0.3 (2)	8.1 + 0.3 (2)	3.5 + 0.1 (8)

B. Corn Steep Liquor

1-25	1.7 + 0.1 (2)	89.6 + 4.4 (4)	96.5 + 0.7 (2)	7.8 + 0.2 (4)	4.5 + 0.3 (6)
26-77	2.4 + 0.0 (2)	95.8 + 7.6 (6)	90.0 + 6.6 (3)	7.6 + 0.5 (6)	4.4 + 0.4 (18)
78-112	2.0 + 0.1 (4)	98.6 + 1.1 (5)	92.8 + 1.7 (4)	7.7 + 0 (5)	4.3 + 0.7 (21)
113-152	1.5 + 0.1 (2)	85.1 + 11.8 (5)	92.0 + 5.4 (4)	7.6 + 0.2 (6)	4.4 + 0.5 (15)

C. Volatile Fish Condensates

1-19	2.3 + 0 (1)	94.0 + 7.3 (3)	92.1 + 0 (1)	8.4 + 0.1 (3)	4.0 + 0.1 (7)
70-75	3.1 + 0.2 (5)	95.3 + 3.2 (8)	83.9 + 3.6 (5)	7.9 + 0.1 (8)	3.8 + 0.5 (10)
76-78	4.6 + 0 (1)	—	92.4 + 0 (1)	7.8 + 0 (1)	3.2 + 0 (1)

TABLE 3. Data Collected from Continuous Flow Denitrification Systems with Alternate Carbon Sources (cont'd)

D. Sweet Whey

Time (Days)	C/N ¹	% Denitrification ²	% TOC Removal ³	pH	Retention Time ⁴ (days)
1-18	4.4 ± 0.1 (2)	99.3 ± 0.3 (3)	70.5 ± 7.8 (2)	7.4 ± 0.2 (3)	5.0 ± 0.7 (11)
19-56	1.4 ± 0 (3)	98.0 ± 2.1 (4)	78.0 ± 23.8 (3)	7.8 ± 0.3 (4)	4.4 ± 0.4 (26)
57-91	2.8 ± 0.3 (3)	93.1 ± 6.5 (5)	84.6 ± 5.0 (4)	7.4 ± 0.2 (5)	4.0 ± 0.5 (19)
92-126	4.9 ± 0.2 (4)	89.0 ± 5.2 (4)	76.1 ± 12.5 (3)	7.3 ± 0.2 (5)	4.5 ± 0.6 (17)
127-195	9.6 ± 1.0 (5)	95.5 ± 1.7 (9)	49.4 ± 14.5 (4)	6.2 ± 0.4 (9)	4.8 ± 0.8 (14)
196-223	7.1 ± 0.2 (4)	97.6 ± 0.4 (3)	43.3 ± 5.6 (3)	6.6 ± 0.2 (4)	5.4 ± 1.1 (8)
224-251	3.4 ± 0.2 (3)	98.2 ± 1.9 (3)	56.8 ± 30.2 (3)	7.0 ± 0.1 (2)	4.1 ± 1.2 (7)
252-302	1.8 ± 0.1 (5)	99.2 ± 0.4 (6)	88.3 ± 2.4 (5)	8.1 ± 0.2 (5)	5.1 ± 0.6 (15)
303-320	1.0 ± 0.1 (2)	75.9 ± 17.9 (3)	86.0 ± 5.7 (2)	8.1 ± 0.2 (3)	5.5 ± 0.5 (12)

E. Methanol

1-34	1.5 ± 0 (1)	84.2 ± 20.2 (5)	—	8.1 ± 0.1 (4)	4.1 ± 0.5 (18)
35-65	1.1 ± 0 (1)	72.8 ± 17.9 (3)	—	8.5 ± 0.1 (4)	3.6 ± 0.3 (16)
66-105	0.8 ± 0 (4)	66.0 ± 9.4 (6)	94.3 ± 1.3 (4)	8.3 ± 0.1 (6)	3.7 ± 0.4 (17)
106-147	1.1 ± 0 (1)	76.6 ± 5.0 (5)	—	8.4 ± 0.1 (5)	4.0 ± 0.3 (15)
148-202	0.9 ± 0 (2)	77.2 ± 7.6 (7)	92.5 ± 0.7 (2)	8.4 ± 0.3 (7)	5.5 ± 1.0 (19)
203-239	1.1 ± 0 (6)	98.9 ± 0.2 (4)	90.0 ± 6.6 (6)	8.3 ± 0.2 (5)	4.0 ± 1.4 (8)

F. Brewery Spent Grain

1-18	2.6 ± 0.1 (2)	97.0 ± 1.3 (3)	68.4 ± 8.8 (2)	8.4 ± 0.2 (3)	6.4 ± 0.4 (5)
19-51	2.6 ± 0.3 (2)	99.2 ± 1.3 (3)	69.1 ± 17.8 (3)	7.9 ± 0.1 (3)	6.4 ± 0.4 (5)
52-86	2.4 ± 0 (4)	99.0 ± 0.5 (4)	81.7 ± 2.1 (4)	8.1 ± 0.1 (2)	6.1 ± 1.2 (8)
87-108	1.4 ± 0.1 (2)	81.4 ± 5.8 (3)	67.1 ± 5.8 (2)	8.2 ± 0.1 (3)	3.6 ± 1.2 (12)
109-128	1.6 ± 0 (2)	93.6 ± 6.9 (3)	80.6 ± 5.1 (2)	8.3 ± 0.1 (3)	4.6 ± 0.5 (12)

TABLE 3. Data Collected from Continuous Flow Denitrification Systems with Alternate Carbon Sources (cont'd)

G. Sugar Beet Molasses

Time (Days)	C/N ¹	% Denitrification ²	% TOC Removal ³	pH	Retention Time ⁴ (days)
1-15	1.6 + 1 (2)	73.5 + 0.7 (2)	84.0 + 2.8 (2)	7.6 + 0.6 (2)	3.4 + 1.2 (11)
16-40	1.6 + 0.1 (3)	60.6 + 15.3 (4)	90.5 + 1.5 (3)	8.0 + 0.2 (3)	4.1 + 0.9 (14)
41-54	2.1 + 0.1 (2)	91.9 + 8.5 (2)	91.0 + 2.8 (2)	8.4 + 0 (2)	3.9 + 0.5 (4)
55-90	3.7 + 0 (1)	95.4 + 5.4 (5)	95.9 + 2.2 (2)	8.6 + 0.1 (4)	4.8 + 0.5 (18)
91-120	2.4 + 0 (2)	81.3 + 7.8 (3)	95.1 + 1.3 (2)	8.3 + 0.2 (3)	3.7 + 0.5 (14)
121-147	4.3 + 0 (3)	92.7 + 7.1 (4)	93.1 + 3.4 (3)	8.2 + 0.3 (4)	3.4 + 0.5 (11)
148-208	4.9 + 0.8 (5)	97.0 + 1.6 (9)	80.6 + 5.7 (6)	7.7 + 0.2 (9)	3.6 + 0.5 (12)
209-243	5.5 + 0 (4)	97.9 + 0.8 (4)	79.8 + 6.3 (4)	7.5 + 0.1 (4)	4.5 + 1.1 (10)
244-288	7.8 + 0.1 (3)	99.0 + 0.2 (5)	61.4 + 2.9 (4)	7.5 + 0.3 (5)	4.5 + 0.7 (11)

H. Sewage Sludge Digest

1-29	0.8 + 0.1 (4)	33.7 + 9.6 (5)	74.5 + 3.7 (4)	8.0 + 0.2 (5)	3.8 + 1.0 (20)
30-55	1.4 + 0.1 (2)	78.6 + 10.0 (2)	93.3 + 1.1 (2)	8.3 + 0.1 (3)	4.0 + 0.4 (9)
56-96	1.3 + 0 (1)	54.9 + 17.0 (5)	92.4 + 4.0 (2)	8.2 + 0.2 (5)	4.1 + 0.5 (18)

I. Soluble Potato Solids

1-21	5.5 + 0 (1)	89.9 + 8.3 (3)	78.8 + 0 (1)	7.7 + 0 (3)	5.1 + 0.4 (8)
22-100	1.4 + 0.1 (8)	79.9 + 21.2 (1)	89.2 + 4.3 (8)	7.9 + 0.1 (11)	4.2 + 0.7 (17)
101-140	2.1 + 0.1 (3)	96.1 + 5.7 (5)	93.4 + 0.5 (3)	8.0 + 0.2 (4)	5.7 + 1.2 (10)
141-171	1.8 + 0.5 (4)	96.7 + 5.5 (4)	93.0 + 2.0 (4)	8.3 + 0.1 (3)	5.7 + 0.9 (7)
172-210	1.7 + 0.1 (3)	87.8 + 6.8 (6)	94.4 + 1.7 (3)	8.0 + 0.2 (6)	4.6 + 0.6 (19)

TABLE 3. Data Collected from Continuous Flow Denitrification Systems with Alternate Carbon Sources (cont'd)

J. Acid Hydrolyzed Sewage Sludge Digest

Time (Days)	C/N ¹	% Denitrification ²	% TOC Removal ³	pH	Retention Time ⁴ (days)
1-11	0.7 ± 0 (1)	21.0 ± 12.7 (2)	89.0 ± 0 (1)	8.1 ± 0.1 (2)	5.4 ± 1.7 (3)
12-39	1/4 ± 0 (1)	67.3 ± 19.4 (3)	88.5 ± 2.1 (2)	8.3 ± 0.2 (4)	5.3 ± 0.9 (7)
40-68	2.3 ± 0 (1)	93.0 ± 5.3 (3)	88.0 ± 0 (1)	8.2 ± 0.1 (2)	4.6 ± 0.5 (11)
69-81	2.0 ± 0.1 (2)	94.5 ± 0.7 (2)	87.0 ± 1.4 (2)	8.1 ± 0.1 (2)	5.3 ± 1.6 (6)
82-88	2.4 ± 0 (1)	92.5 ± 0 (1)	86.0 ± 0 (1)	8.0 ± 0 (1)	3.0 ± 0.6 (4)
89-102	2.9 ± 0.1 (2)	96.3 ± 0.4 (2)	89.5 ± 0.7 (2)	7.5 ± 0.6 (2)	5.1 ± 0.6 (7)
103-125	3.0 ± 0 (1)	94.7 ± 1.1 (2)	—	8.0 ± 0 (1)	5.1 ± 1.9 (8)
126-137	2.7 ± 0 (1)	92.8 ± 0.3 (2)	88.3 ± 0 (1)	8.3 ± 0.1 (2)	5.1 ± 2.2 (6)
138-158	3.7 ± 0 (1)	84.4 ± 6.8 (3)	88.4 ± 0 (1)	8.0 ± 0.1 (3)	3.8 ± 0.2 (11)
159-173	3.5 ± 0 (1)	68.8 ± 1.8 (2)	78.7 ± 13.3 (2)	8.0 ± 0.1 (2)	4.9 ± 1.8 (5)
174-188	0.7 ± 0 (1)	74.8 ± 0.4 (2)	82.3 ± 0 (1)	7.8 ± 0.3 (2)	5.6 ± 0.9 (8)
192-196	1.2 ± -0 (1)	60.7 ± 0 (1)	81.5 ± 0 (1)	8.1 ± 0 (1)	5.0 ± 0.5 (3)
197-206	0.6 ± 0 (1)	51.7 ± 35.6 (2)	81.1 ± 0 (1)	8.2 ± 0.1 (2)	5.0 ± 0.6 (2)
213-231	5.9 ± 0 (1)	53.4 ± 43.5 (2)	8.8 ± 0 (1)	8.6 ± 0.1 (2)	3.1 ± 0 (1)

K. Nutrient Broth

1-114	5.6 ± 0.5 (10)	90.7 ± 7.8 (14)	89.3 ± 3.1 (10)	8.6 ± 0.1 (13)	3.9 ± 0.8 ((3)
115-161	8.3 ± 0.6 (5)	95.8 ± 2.2 (6)	68.5 ± 14.1 (5)	8.3 ± 0.2 (5)	4.8 ± 1.2 (9)
162-195	7.5 ± 0.2 (4)	97.8 ± 1.0 (4)	72.0 ± 8.6 (4)	8.4 ± 0.1 (4)	4.1 ± 0.8 (8)
196-235	3.0 ± 0.1 (4)	97.5 ± 2.2 (6)	81.9 ± 2.9 (3)	8.4 ± 0.1 (6)	4.3 ± 0.4 (19)
236-256	1.5 ± 0.2 (3)	84.7 ± 9.4 (3)	85.1 ± 4.3 (3)	8.4 ± 0.1 (3)	3.4 ± 0.5 (14)
257-284	2.4 ± 0.1 (4)	98.8 ± 0.6 (4)	91.4 ± 3.2 (4)	8.4 ± 0.1 (4)	4.0 ± 0.7 (18)

¹Ratio of grams of carbon to grams of nitrogen.

²Percent efficiency based on the reduction of nitrate between influent and effluent samples.

³Percent efficiency based on the reduction in total organic carbon between influent and effluent samples.

⁴Volume of system divided by flow rate in days.

⁵Average ± 1 standard deviation (Number of data Points).

⁶No data.

The possibility existed that ammonia might be formed (assimilatory denitrification pathway) instead of nitrogen gas during the reduction of nitrate. Ammonia readings were taken from the systems to evaluate this possibility. The levels of ammonia detected were not considered significant, with the exception of the nutrient broth system. From previous investigations we found that the high ammonia levels formed in the nutrient broth system were attributable to the nutrient broth nitrogenous components and not the nitrate in the feed. If the assimilatory pathway is active in the continuous systems studied apparently only a low percentage of the nitrate is following this pathway. If the assimilatory pathway were more active, we would expect to see much higher levels of ammonia in the media above that which is incorporated into cellular biomass. Since this was not the case it is clear that the dissimilatory pathway predominated. In addition, head space gas analysis failed to reveal any volatile nitrogen products other than nitrogen gas.

From other studies carried out in the same fermenters and with some of the same carbon sources in this laboratory we have found only trace levels of nitrite (< 1 mg/liter). Also, no significant levels of nitrous oxide were detected.

Biological denitrification can be the most cost effective method for alleviating nitrate-contamination from process waters. The primary nitrogen product in an active dissimilatory denitrification system is nitrogen gas. To provide the energy for this process a carbon source must be present. This supplemental carbon will serve as the electron donor to reduce the nitrate and provide the energy for growth and maintenance of cells as well as serve as a substrate to promote the cometabolic biotransformation of otherwise recalcitrant compounds.

In this study we have rated the efficiency of various carbon sources. This rating has been defined, for the purpose of this study, as the minimum C/N ratio at which efficient denitrification (> 95%) and TOC removal (> 90%) is achieved. Theoretically therefore, the more efficient the carbon source the more readily metabolizable the carbon that makes up that carbon source. Conversely, the lower the efficiency the more complex or unavailable the carbon, and therefore on an equivalent basis more of this carbon source will be required to achieve the same degree of nitrate reduction in these continuous systems. This unused carbon will be reflected as TOC in the system effluents and as higher BOD or COD containing wastes.

The results of this study clearly show that methanol is the most efficient carbon source of those evaluated, although a number of other carbon sources appear to be relatively efficient as well. Once other considerations are factored in (i.e., cost of the carbon source, transport to treatment site, geographic location, handling costs, dependability of supply) some of these other carbon sources in category a, or possibly in category b, may in fact prove more acceptable than methanol.

CONCLUSIONS

A number of industrial carbon sources were evaluated for their efficiency to drive the denitrification of high nitrate (1259 mg/liter nitrate or 285 mg/liter nitrate-nitrogen) wastes in a single stage continuous flow fermenter. The basis for comparison of carbon sources was the minimum ratio of grams of carbon to grams of nitrogen necessary to achieve at least 95% denitrification and 90% total organic carbon (TOC) removal for each carbon source. Methanol was found to be the most efficient of the 11 carbon sources evaluated, with sweet whey, corn steep liquor, acid whey and soluble potato solids following in order of decreasing efficiency. Three carbon sources, nutrient broth, brewery spent grain and sugar beet molasses, failed to reach 90% TOC removal. Sewage sludge digest contained insufficient available carbon to promote efficient denitrification. Any of the top five most efficient carbon sources may prove acceptable once considerations of cost, availability, transport and handling are considered. The importance of alternate carbon sources for the cometabolism of hazardous organic compounds provided the background upon which this work was based.

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