

Denitrification of High Nitrate Loads — Efficiencies of Alternative Carbon Sources

David L. Kaplan, Patricia A. Riley, Jennifer Pierce and Arthur M. Kaplan

US Army Natick Research Development and Engineering Center Science and Advanced Technology Directorate, Natick, MA 01760-5020, USA

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ABSTRACT

Eleven industrial carbon source nutrients were evaluated for their efficiency in supplying energy for biological denitrification of high nitrate (1259 mg liter) waters in single-stage continuous flow fermenters. The defined criterion for comparison was the minimum carbon-to-nitrogen ratio necessary to achieve at least 95% nitrate reduction and 90% total organic carbon (TOC) removal. Methanol was the most efficient carbon source of those evaluated. Some of the carbon sources studied failed to achieve a 90% reduction in TOC. The relative rankings in efficiency of the various carbon sources may change once consideration is given to cost, transportation, handling and availability.

INTRODUCTION

A number of physical-chemical techniques have been utilized to remove contaminating nitrates from wastewaters, including biological denitrification, selective ion exchange, algal harvesting and reverse osmosis. Biological denitrification has proved to be one of the most economical and effective means of nitrate removal through nitrate reduction (Christensen and Harremoes, 1977; Delwiche, 1981; Payne, 1981; Knowles, 1982).

Most high nitrate industrial wastewaters, such as those produced in the fertilizer, explosive and nuclear fuel processing industries do not

contain electron donors at sufficient concentrations 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. Methanol has long been used in biological denitrification as an external carbon source. However, the large volumes of methanol needed to reduce high nitrate levels and the attendant cost have prompted the consideration of alternative carbon sources. Preferably, industrial wastes that are inexpensive, stable, homogeneous, and produced in a convenient geographical location could provide this alternative 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.

Christensen and Harremoes (1977) reviewed reports on the use of supplemental carbon in the denitrification process. McCarty *et al.* (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 l^{-1}) and simple organic carbon sources (methanol, acetate, ethanol, acetone, sugar). 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 l^{-1} nitrate. The authors utilized a chemical oxygen demand (COD)-to-nitrate ratio to evaluate efficiency and determined methanol to be the most efficient carbon source, 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%, and COD and BOD removal efficiencies ranged from 38 to 79% and 32 to 85%, respectively.

Monteith *et al.* (1980) used batch systems to evaluate 30 industrial carbon sources. The authors measured denitrification rates to compare carbon sources in relation to methanol; they 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 multi-stage system with between 37.5 mg l^{-1} and 300 mg l^{-1} nitrate-nitrogen. With methanol, nitrate removal efficiencies were 34–96.4% and COD removal was 32.6–88.6%. With lactate these efficiencies were 18.3–98.0% and 76.2–95.1%, 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 l^{-1} nitrate or 285 mg l^{-1} nitrate-nitrogen) to compare efficiencies of industrial carbon sources, the criterion for comparison being the minimum carbon-to-nitrogen ratio (C/N) at which 95% denitrification and 90% TOC removal are achieved.

In addition, wastewaters contaminated with nitrates often contain hazardous organic compounds which must be treated concurrently. Many of these compounds undergo biodegradation only through co-metabolism (nonspecific enzymatic degradation of one compound not providing energy for microorganisms through the metabolism of another compound which provides energy). Treatment systems dealing with both nitrates and these types of organic compounds would gain a double benefit with the addition of these carbon sources.

MATERIALS AND METHODS

The various carbon sources analyzed included the following: (1) reagent-grade methanol; (2) nutrient broth (Difco, Detroit, MI, USA); (3) dehydrated soluble potato solids, process effluent generated by the manufacture of starch (US Department of Agriculture, Philadelphia, PA, USA and Colby Starch Co-operative, Priscil, ME, USA); (4) anaerobic digest obtained from a municipal sludge treatment plant (Nut Island Sewage Treatment Plant, Boston, MA, USA; 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, USA); (6) soluble fish condensates (Sharpley Laboratories Inc., Fredericksburg, VA, USA);

volatile components condensed from the steaming operation utilized in the production of fish meal); (7) acid whey (H. P. Hood Inc., Boston, MA, USA); (8) brewery spent grain (solid waste from the brewing process; National Feed of New England, Merrimack, NH, USA); (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, USA, through the Beet Sugar Development Foundation, Fort Collins, CO, USA). Table 1 lists nutritional data for some of the carbon sources.

Acid hydrolysis of the anaerobic sewage digest was accomplished with hydrochloric acid (approximately 1.0 M final concentration) and autoclaving for 1 h. The mixture was neutralized with sodium hydroxide, centrifuged for 20 min at 10 000 rpm and filtered through glass wool.

Continuous flow systems were run in either BioFlo Model C30 bench-top fermenters (New Brunswick Scientific, New Brunswick, NJ, USA) 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 ID, 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, USA).

The continuous cultures were run at room temperature (20–23 °C). All of the systems, except for the nutrient broth, contained the following salts per liter of filtered lake water: MgSO₄·7H₂O, 500 mg; NaCl, 50 mg; CaCl₂, 15 mg; FeCl₃·6H₂O, 10 mg; CuSO₄·5H₂O, 10 mg; MnSO₄·H₂O, 10 mg; NaMoO₄·2H₂O, 1 mg; K₂HPO₄, 0.87 g; and KNO₃, 2.05 g. The lake water was obtained from Lake Cochituate, Natick, MA, USA, and generally had a pH between 6 and 7 and a TOC content of less than 25 mg l⁻¹.

Continuous cultures were inoculated with organisms from activated sludge (Marlborough Easterly Sewage Treatment Plant, Marlborough, MA, USA), anaerobic digest (Nut Island Sewage Treatment Plant, Boston, MA, USA), and garden soil. Samples (1 ml) of the two sludges were combined with 1 g of soil, diluted with 50 ml of lake water, mixed, and gravity-filtered through filter paper. A 1 ml aliquot of this filtered mixture was used as an inoculum in the continuous cultures. This inoculum contained between 2.3×10^3 and 3.5×10^3 CFU ml⁻¹.

TOC was determined with a Beckman 915B Tocamaster with Matheson Ultra Zero as carrier gas flowing at 300 ml min⁻¹. Samples (20 µl) were delivered with a Hamilton CR-200 200 µl constant-rate

TABLE 1
Analysis of Carbon Supplements used in the Denitrification Systems

<i>Acid whey^a</i>		<i>Corn steep liquor^b</i>	
Protein	12.2%	Solids	48%
Nonprotein nitrogen	0.6%	Protein	21%
Fat	0.5%	Lactate acidity	13%
Lactose	63.0%	Reducing sugars	2%
Moisture	6.1%	Amino Nitrogen	1%
Ash	10.7%	Ash	9%
Titrate acidity	0.39%	Phosphate (P ₂ O ₅)	4%
pH	4.6%	Sulfur	1%
		pH	4.0-4.2
<i>Sugar beet molasses^c</i>		<i>Dehydrated soluble potato solids^d</i>	
Solids	82%	Minerals	19.6%
Sucrose	51%	Moisture content	2.6%
Nitrogenous compounds	13%	True proteins	11.5%
Ash	11%	Amino acids, amides	21.46
		Total sugars	34.8%
		Glucose	14.6%
		Fructose	13.7%
		Sucrose	6.5%
		Citric acid	4.0%
		Other	8.7%
<i>Sweet whey^e</i>		<i>Volatile fish condensates^f</i>	
Lactose	74%	Solids	57.4%
Moisture	3%	Protein	41.4%
Fat	1%	Moisture	42.6%
Ash	8%	Ash	5.1%
		Fat	0.17%

^aData Source: Whey Products Institute Chicago, Illinois. Acid whey source: H. P. Hood Inc., Boston, MA, USA.

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

^cData source: 'Beet: Beet Sugar Technology' in a personal communication with the Beet Sugar Development Foundation, Fort Collins, CO, USA. Sugar beet molasses source: Great Western Sugar Company Loveland, CO through the Beet Sugar Development Foundation of Fort Collins, CO, USA.

^dData source: US Department of Agriculture, Philadelphia, PA, USA. Dehydrated soluble potato solids source: US Department of Agriculture, Agriculture Research Service Northeast Regional Research Center, Philadelphia, PA, USA.

^eData and sweet whey source: Cuba Cheese Inc., Cuba, New York, USA.

^fData and volatile fish condensate source: Sharpley Laboratories, Inc., Fredericksburg, VA, USA.

syringe. Before TOC was determined, continuous culture influent and effluent samples were centrifuged at 12 000 rpm for 20 min and filtered through a nylon 66 0.45 μm filter (Rainin Instrument Co., Woburn, MA, USA) with a glass filter unit (Millipore Co., Bedford, MA, USA).

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, USA). 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 1 h 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 min⁻¹ through a 2.44 m \times 0.32 cm stainless steel column containing Carbosieve S, 120/140 mesh. Programmed runs were initiated with an oven temperature at 35°C for 5 min, followed by a program rate of 15°C to a final temperature of 175°C for 15 min.

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 maximum levels. Most carbon sources were studied for periods of six to ten months 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 organics were achieved. Of the 11 carbon sources tested, however, only five 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 90–97% of the TOC (Figs 1–5). The carbon sources in category (b) of Table 2 reached a denitrification efficiency comparable with category (a); however, only 80–90% of the TOC was utilized (Figs 6–8).

Category (c) of Table 2 lists the carbon sources with which there were

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

Carbon source	C/N ^a
(a) 95% denitrification, 90% TOC ^b 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 ^c
Volatile fish condensate	2.5 ^d
Sewage sludge digest	— ^e

^aRatio of grams of carbon to grams of nitrogen in media.

^bTotal organic carbon.

^cAt C/N ratios above 2.1 the percent TOC removal decreased from 87%.

^dInsufficient medium to complete study.

^e95% denitrification and 80% TOC removal never achieved.

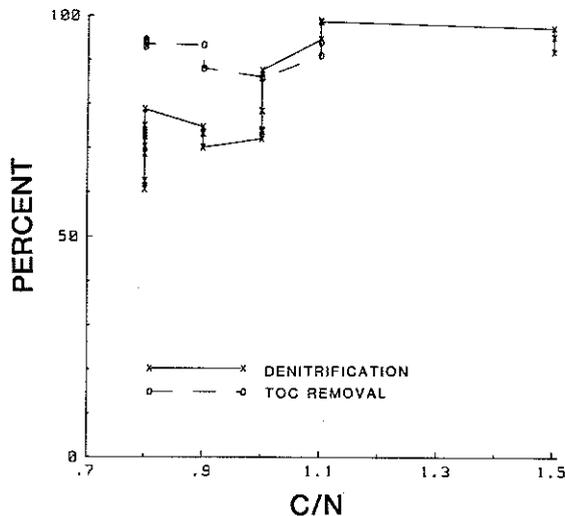


Fig. 1. Percentage denitrification and total organic carbon removal with methanol.

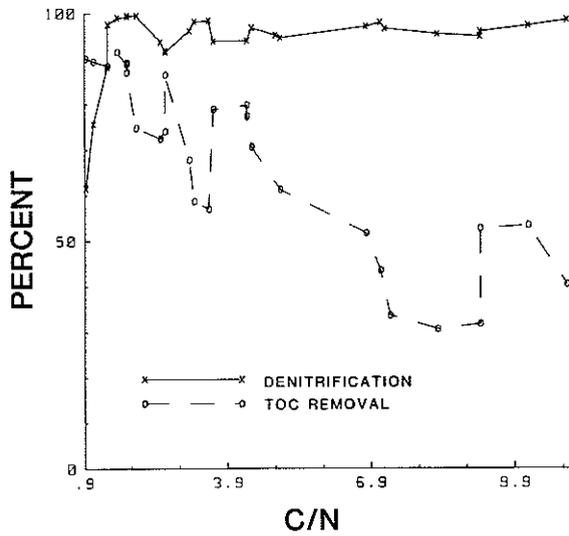


Fig. 2. Percentage denitrification and total organic carbon removal with sweet whey.

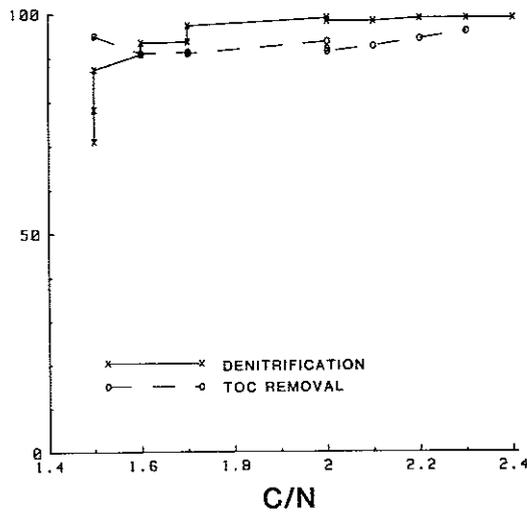


Fig. 3. Percentage denitrification and total organic carbon removal with corn steep liquor.

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 (Fig. 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

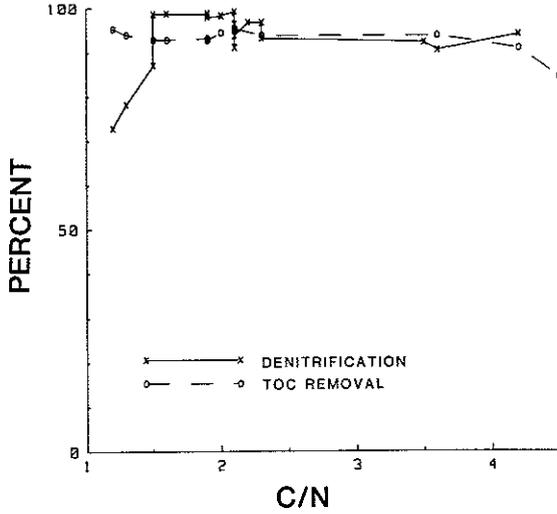


Fig. 4. Percentage denitrification and total organic carbon removal with acid whey.

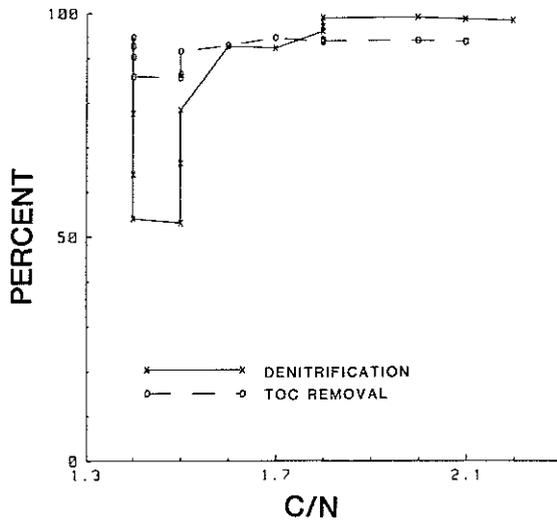


Fig. 5. Percentage denitrification and total organic carbon removal with potato solids.

TOC in the effluents due to incomplete utilization of available carbon. Figure 11 illustrates that, with acid-hydrolyzed sewage digests at a C/N ratio 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,

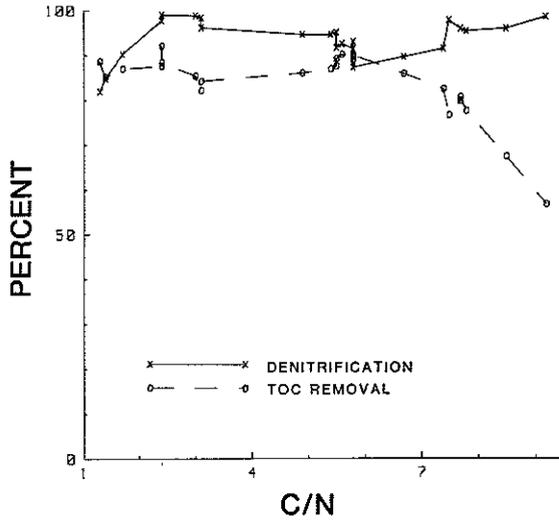


Fig. 6. Percentage denitrification and total organic carbon removal with nutrient broth.

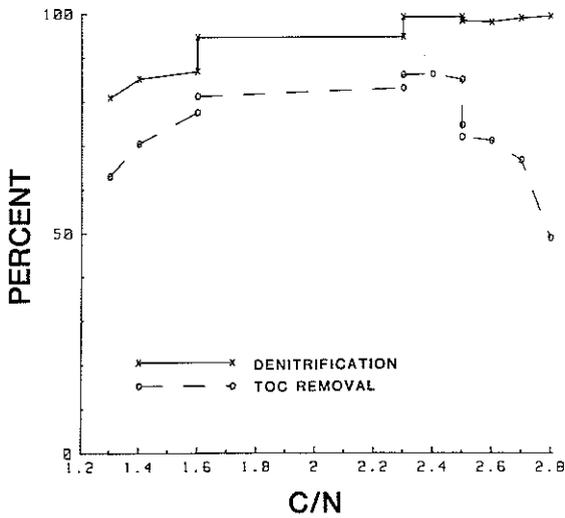


Fig. 7. Percentage denitrification and total organic carbon removal with spent brewery grain.

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.

The pH readings of the effluent samples were in the slightly alkaline range, and approximately a four-day retention time was maintained throughout these studies.

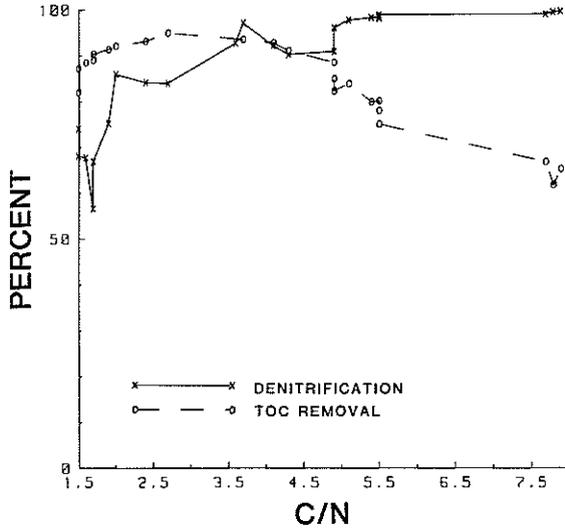


Fig. 8. Percentage denitrification and total organic carbon removal with sugar beet molasses.

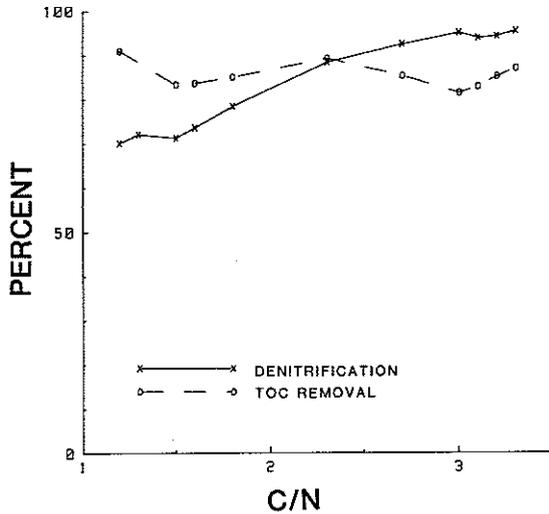


Fig. 9. Percentage denitrification and total organic carbon removal with fish stick.

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 3) to those found in Table 2. These findings may have application in cases where a two-stage system is utilized, such that excessive TOC would be removed in the second stage.

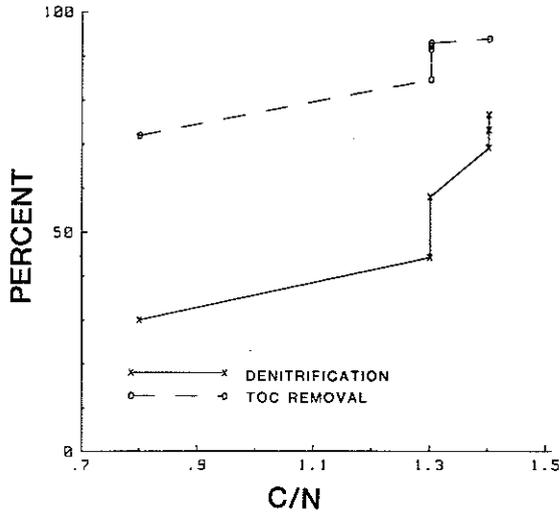


Fig. 10. Percentage denitrification and total organic carbon removal with sewage sludge.

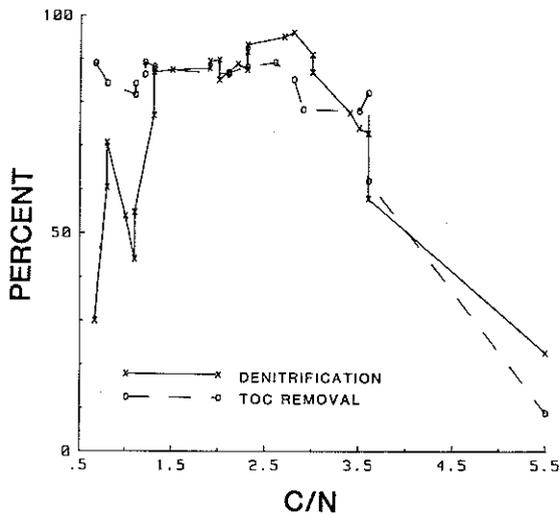


Fig. 11. Percentage denitrification and total organic carbon removal with hydrolyzed sludge.

TABLE 3
Efficiencies of Alternate Carbon Sources Without
Consideration of TOC Removal

<i>Carbon source</i>	<i>C/N^a</i>
Methanol	1.1
Sweet whey	1.4
Acid whey	1.4
Corn steep liquor	1.6
Brewery spent grain	1.6
Soluble potato solids	1.7
Nutrient broth	1.7
Acid hydrolyzed sewage sludge digest	2.1
Volatile fish condensate	2.5 ^b
Sugar beet molasses	3.6
Sewage sludge digest	— ^c

^aRatio of grams of carbon to grams of nitrogen in media. Minimum at which denitrification surpassed 95%.

^bInsufficient medium to complete study.

^c95% 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, $\frac{5}{8}$ mol of methanol is required to reduce 1 mol of nitrate completely to nitrogen gas. However, an additional 30% over this amount is needed to satisfy the requirements for bacterial growth. While the complex wastes do not appear to have the oxidizing potential of methanol, some of the carbon sources tested exhibited efficiencies only slightly lower than that of methanol. If economic factors are considered, these sources may in fact be more cost-effective than methanol.

The carbon sources in category (a) of Table 2 showed the best results relative to methanol. A review of 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 for each nitrate reduced is needed to achieve the desired level of denitrification. Apparently, the carbon contained in this category of carbon source is not as readily assimilable as 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 carbon was not in forms readily available to the bacteria. This problem was partially eliminated through acid hydrolysis treatment of the sludge. It would be expected that raw sewage might provide sufficient available carbon for this purpose (Randall, 1984).

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 of the system it was noted that the reaction vessel contained a semi-solid mass not found in the other systems. An alternative explanation may be the presence of inhibitory compounds that would demonstrate an effect as the concentration of sludge was increased.

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 nutrient broth systems could be attributed to the nitrogenous components in the nutrient broth and not the nitrate in the feed. If the assimilatory pathway was active in the continuous systems studied, all of the ammonia produced was incorporated into biomass. 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 (less than 1 mg l^{-1}).

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 serving as a substrate to promote the co-metabolic biotransformation of otherwise recalcitrant compounds. Continuous systems were successfully established in these studies without the benefit of specialized cultures, which would indicate that it is the nature of the substrate that controls the performance of the system.

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 (greater than 95%) and TOC removal (greater than 90%) is achieved. Theoretically, therefore, the more efficient the carbon source the more readily metabolizable the carbon in 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 therefore as higher BOD- or COD-containing wastewaters.

Based on the results presented, sweet whey, acid whey, corn steep liquor and soluble potato solids can be considered as optimal alternative sources to methanol, although the cost-benefit analysis would be largely influenced by availability in the immediate geographic vicinity of need and the current costs for purchase, transport and handling. Other factors to consider are that the quality of the alternative carbon source may vary from source to source as well as the dependability of supply. It is clear that sewage sludge digest cannot be considered for the applications discussed here; however, work needs to be done on raw sewage to evaluate its potential for supplying the necessary source of carbon in a denitrification system.

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