

# **Comparisons of Organic Sources for Denitrification: Biodegradability, Denitrification Rates, Kinetic Constants and Practical Implication for Their Application in WWTPs**

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## **ABSTRACT**

The aim of this paper is to present a comprehensive and critical literature review on alternative carbon sources for supporting denitrification in wastewater treatment. In addition, the results of a characterization study to compare denitrification rates and kinetics among diverse class of carbon sources (pure compounds, industrial wastes and a proprietary product) and to discuss the methods that are generally applied for denitrification evaluation, is also presented. Demonstration of the implication of the difference in denitrification rates and kinetics on BNR performance in real practice through modeling, and the identification of the key aspects of consideration for selecting an appropriate alternative carbon for denitrification in wastewater treatment, including also cost and availability, effects on effluent quality, potential issues related to storage and transportation, are also discussed.

## **KEYWORDS**

Denitrification, carbon sources, methanol, Industrial and agricultural by-products, kinetics, modeling.

## **INTRODUCTION AND OBJECTIVES**

The addition of an external carbon source to improve the stability and efficiency of the denitrification in biological nutrient removal (BNR) process is becoming crucial for many wastewater treatment plants wishing to comply the increasingly stringent limits on nitrogen. The commonly used external carbon sources include methanol, ethanol, sugar and sludge digestion supernatant. Other alternative carbon sources that have been proposed recently include MicroC™, molasses, glycerol, corn starch and others. For the last two decades methanol has been the common choice, however recently, do to its increasing price and associated safety issues, alternative carbon sources are emerging, including some proprietary products and organic by-products from other processes (industrial and agricultural wastes). Evaluation of the potentials of various carbon sources for supporting denitrification in wastewater treatment has been of interest to many facilities. There are significant amount of information from previous investigations on application of various carbon sources for denitrification. Comprehensive literature review will lead to an update of the current database on carbon sources and help identify the research area/aspects in need.

The aim of this paper is to present the results of our investigation in combination with a comprehensive and critical literature review on alternative carbon sources for supporting denitrification in wastewater treatment. Specific objectives include:

- 1) Summarize the available information on the potential of various carbon sources for denitrification;
- 2) Compare the denitrification rates and kinetics among the carbon sources and discuss the methods that are generally applied for denitrification evaluation;
- 3) Demonstrate the implication of difference in denitrification rates and kinetics on denitrification performance in real practice through modeling;
- 4) Identify the key aspects of consideration for selecting an appropriate alternative carbon for denitrification in wastewater treatment, including also cost and availability, effects on effluent quality (e.g. due to impurities) and treatment processes, potential issues related to storage and transportation.

## MATERIALS AND METHODS

### Potential Carbon sources for denitrification

The carbon sources that can potentially support denitrification can be categorized as: 1) pure chemicals like methanol, ethanol, acetate, sugar, butanol etc.; 2) purified agricultural or industrial byproducts; 3) raw industrial/agricultural byproducts as corn syrup, molasses, brewery waste and other process wastes; 4) sludge fermentation products and 5) others such as hydrogen, methane and H<sub>2</sub>S.

In addition to the information collected from literature review, carbon source that were tested by the authors include methanol, sodium acetate, one proprietary compound, MicroC™ which is a purified agricultural byproduct and four industrial wastewaters from an ice cream factory, a beet-sugar factory, a brewery and a winery.

The industrial wastes were prepared to represent the expected quality of biological treatment influent; primary effluents were collected for the dairy waste samples, lagoon neutralized effluents were collected for the beet sugar samples, because they are routinely subjected to these types of pre-treatment before biological processes. The brewery sample was collected in the equalization tank at the factory. In the experiments the samples were also adjusted to a pH of 7-8, a range suitable for biological activity. Table 1 summarizes the characteristic of the industrial wastes analyzed.

Table 1. Conventional characterization of selected industrial wastes.

Waste	pH	COD mg/l	SS mg/l	TN mg/l	TP mg/l
Dairy	4.4	5.000-11.000	508	27.5	5.5
Beet-sugar	1.1	9250	-	230	6.2
Brewery	8.2	1250-3000	-	15	-
Winery	3-6	230,000	600	-	-

## **Characterization and denitrification potential assessment methods**

The potential of a carbon source to support denitrification depends on its biodegradability. Biodegradability of a given compound is in general associated with the readily biodegradable COD fraction; for the characterization of the different influent COD fractions a number of methods have been proposed in the literature, basically divided into two groups: biological and chemical physical methods.

The biological methods are based either on oxygen uptake rates measurements, variable with the higher or lower substrate biodegradability (aerobic tests), or on nitrate concentration measurements and their time profile (NUR test) in anoxic batch tests; in both cases the electron acceptor needed for the organic fraction oxidation is measured.

The chemical-physical methods are instead based on sample filtration, preceded with or without a flocculation step, and so do not give any useful information on biomass behavior, but can give an estimate of the readily biodegradable fraction of the COD.

Table 2 summarizes some the major characterization methods proposed in literature.

**Table 2.** Characterization methods available

Kind of test	Symbol	Parameters	Reference
Aerobic batch*	AE	$\mu_A$ , $\mu_H$ , rbCOD	(Ekama <i>et al.</i> 1986)
Aerobic batch	AE <sub>KG</sub>	rbCOD	(Kappelar & Gujer 1992)
Aerobic batch	AW	rbCOD, $\mu_A$ ,	(Wentzel <i>et al.</i> 1995)
Aerobic batch	AW2	All COD fraction, $\mu_A$	(Wentzel <i>et al.</i> 1999)
Single OUR	SOUR	rbCOD	(Xu & Hasselblad 1996)
Continous flow	CF	rbCOD, upCOD	(Ekama <i>et al.</i> 1986)
Continous flow	CF <sub>SG</sub>	rbCOD	(Sollfrank & Gujer 1991)
Anoxic batch*	AN	$\mu_{HD}$ , rbCOD, sbCOD, $k_{dmax}$	(Ekama <i>et al.</i> 1986)
Anoxic batch*	AN <sub>D</sub>	$\mu_{HD}$ , $Y_H$	(Dold <i>et al.</i> 2005)
Anoxic -pH stat*	AN <sub>pH</sub>	$\mu_{HD}$ , rbCOD, sbCOD, $k_{dmax}$	(Rozzi <i>et al.</i> 1999)
Physical-chemical*	PC	rbCOD, usCOD	(Mamais <i>et al.</i> 1993)
Physical*	P	rbCOD, usCOD	(Mamais <i>et al.</i> 1993)

\*methods used in this study.

$\mu_A$ : autotrophic growth rate;  $\mu_H$ : heterotrophic maximum growth rate;  $\mu_{HD}$ : denitrifiers maximum growth rate; rbCOD: readily biodegradable COD; sbCOD: slowly biodegradable COD; upCOD: Unbiodegradable Particulate COD;  $Y_H$ : heterotrophic yield;  $k_{dmax}$ : maximum specific denitrification rate.

Denitrification rates and kinetics can be studied in continuous or batch systems. The latter systems are frequently used to study kinetics, and are preferred as they are simple and easy to operate.

The low F/M ratio anoxic batch (AN) test is commonly used because allows for the determination of denitrification rates as well as an estimate of the rbCOD and the specific growth rate in a short period of time (2-3 hours). High F/M ratio anoxic tests (AN<sub>D</sub>) are also used to determine the anoxic growth rates of denitrifiers for different carbon sources and temperatures (Dold *et al.* 2005), and to estimate the heterotrophic yield.

The use of pH-stat test has also been proposed as an alternative to the anoxic batch test (Onnis *et al.* 2006, Rozzi *et al.* 1999); in this test the nitrate used to oxidize the biodegradable COD present in the wastewater/carbon source is estimated from the amount of acid added to neutralize alkalinity produced in the denitrification reaction (approximately one mol of OH<sup>-</sup> for every mol of nitrate removed). The denitrification rate can be derived from the acid consumption rate. In our study, both acclimated and not acclimated sludges were used for the tests. Acclimated sludge were taken from the industrial wastewater treatment facility or drawn from laboratory scale SBRs set up for the experiments and fed with the specific organic source. The non acclimated sludge was drawn at a wastewater treatment plant operated with the modified Ludzak-Ettinger (MLE) scheme.

For the specific set up and procedure of the methods used refer to the respective references. The advantages and limitations of different methods for denitrification tests and data processing will be discussed.

## RESULTS AND DISCUSSION

### **Potential Carbon sources for denitrification**

Table 3A and 3B summarized the results of characterization studies, including biodegradability, denitrification rates, denitrification potential (in terms of COD/N ratio) and growth kinetics for different carbon sources found in literature as well as tested in this study.

#### ***Pure compounds***

Methanol has been the industry standard for wastewater denitrification due to historically low cost, favorable kinetics, and low cell yield. There is a wide range for the denitrification rates for methanol, since several studies have been done on this compound. This variability is in part due to the different approaches uses to conduct the test (e.g. batch versus continuous), or for the sludge used (mixed activated sludge, acclimated or not, and pure culture). The highest values for the denitrification rate were found with pure culture (Christensson *et al.* 1994), however with activated sludge lower rates are found with values between 3 and 13 mgN/gVSS/h.

Several studies comparing methanol to other pure chemicals have been conducted; methanol has been compared to glucose, sodium propionate and acetate in SBR systems. It was shown that acetate and sodium propionate were the most effective and produced higher removal rates than methanol or glucose (Tam *et al.* 1994, Tam *et al.* 1992). These results correlate with Gerber and colleagues (1986) who reported that compounds such as acetate, propionate, butyrate and lactate consistently produced higher denitrification rates than methanol or glucose. Tam *et al.*, (2002) concluded that the results could be explained biochemically. The glycolytic pathway and tricarboxylic acid (TCA) cycle are the two metabolic pathways for utilizing organic substrate as

sources of energy and carbon in most organisms. Acetyl Co-A, which is easily formed from acetic acid or acetate is the key compound of these pathways; as a result, sodium acetate is a directly utilizable substrate which is more readily metabolizable than methanol or glucose. In addition, acetate might account for 5-10% of the total COD in sewage (Henze *et al.* 1994), therefore the suitable denitrifying population with the appropriate enzyme should be already present in activated sludge, justifying the higher rates found in general for acetate compared to other the simple compounds.

Methanol utilization can only be carried out by a specific group of bacteria called methylotrophs who has the ability to use single C compounds such as methane, methanol, formate, and carbon monoxide. Organic-utilizing methylotrophs possess serine pathway through which single-C compound is assimilated into acetyl-coA. Methanol has been shown to select for highly specialized denitrifying population, consisting of facultative methylotrophs belonging to the genus *Hyphomicrobium* (Nurse 1980); some kinetics studies have also highlighted the need of a long acclimatization period for the development of a robust methylotrophs population (Hallin & Pell 1998) as well as the influence of temperature on the growth rate of these bacteria (Mokhayeri *et al.* 2006, Nichols *et al.* 2007). Other disadvantages are the safety issues associated with its transportation and storage. Lastly, prices of methanol have recently fluctuated and in some cases, shortages have occurred. Besides these issues, due to the long experience with this chemical, its lower sludge yield which translates into higher denitrification efficiency as indicated by the relatively lower g methanol/g Nitrate ratio, methanol is still currently the most widely used external carbon to enhance denitrification.

As is acetate, ethanol is easily converted by the bacterial cell into Acetyl Co-A, before entering the TCA cycle, and in several studies was found to efficiently be available for denitrification with higher rate than methanol. The price of ethanol is however strictly linked with the methanol market and is commonly more expensive; the availability of waste rich in ethanol (as pharmaceutical waste) can potentially provide a source for readily available carbon to implement in BNR system.

The lower rates associated with glucose are most probably associated to the more complex degradation pathway through which bacteria derive energy from sugars. Also Cokgor and colleagues (1998) found that the use of glucose in respirometric tests gives results difficult to interpret and attributed this fact to the complex sequence of biochemical reaction this compound undergoes.

### ***Other compounds- methane***

Several studies have shown methane could be used as a carbon source for biological denitrification. The most studied process is the AME-D, in which denitrification occurs by an association of methanotrophic and denitrifying organisms where the aerobic methanotrophs oxidize methane and release organic compounds that are used by coexisting denitrifiers (Modin *et al.* 2007).

### ***Industrial or agricultural by-products***

Initially the trend was to use industrial or agricultural wastes as external carbon sources for denitrification (McCarty *et al.* 1969, Skrinde *et al.* 1982, Monteith *et al.* 1980). In these studies several industrial wastes were tested as organic carbon sources and it was found that some organic wastes such as formaldehyde and dextrose waste were less efficiently degraded than distillery oils or methanol, but the majority of the food industry wastes exhibit very high denitrification rates and C/N ratio from 2 up to 6.

More recent studies (Bernet *et al.* 1996, Cappai *et al.* 2004, Tsonis 1997, Sage *et al.* 2006) also explored the feasibility of using residue from the food industrial processes to enhance denitrification. Huhtamäki & Huhtamäki (2003) have shown that the addition of an industrial waste carbohydrates, from a nearby food industry, improved the nitrogen removal performance at a full scale municipal WWTP in Finland.

The use of anaerobic fermentation products from the organic fraction of municipal solid waste (MSW) has also been suggested for enhance denitrification (Bolzonella *et al.* 2001), in an integrated BNR-Solid waste management system, which has already being applied in full-scale size at the Treviso (Italy) WWTP.

The results of the numerous characterization studies on industrial wastes have highlighted favorable kinetics and high biodegradability and therefore they could be potentially used in BNR plant; however some of these studies have also revealed some shortcomings associated with this practice. Foremost is the availability and consistency of these wastes: for example, the residues from the food industry are often characterized by temporal variability in quantity (seasonal operation) as well as composition (Cappai *et al.* 2004); additionally a pretreatment of the waste is usually recommended, as degreasing, pH adjustment or removal of color. As example, the dairy waste analyzed in this study and in Cappai *et al.* (2004), contained high readily biodegradable COD such as lactose and lactate, and high rates were measured, but the presence of large amount of fats, caused by a malfunctioning of the pre-treatment at the industrial facility, caused the formation of foam, and therefore poor effluent quality. To overcome these problems, the solution would be to have collection centers, where the waste from several industries (preferably with similar characteristics) are sent and where could be pretreated, homogenized, stored and shipped to the plants that request them. A detailed cost analysis would need to be performed to verify that the benefits at the plant are higher than the cost associated with the transports and pretreatment.

MicroC™ is a proprietary wastewater treatment chemical developed by Environmental Operating Solutions, Inc. and designed specifically for use as an electron donor for biological denitrification of wastewater. MicroC™ was developed as a viable alternative carbon source to methanol without the safety hazards. Since 2003, wastewater treatment facilities throughout the Northeastern United States have been using MicroC™ in a broad range of applications, including onsite, decentralized, municipal and industrial facilities. The kinetics found with this compound are comparable with methanol, however higher yield have been determined. A recent study (Cherchi *et al.* 2008) have also highlighted the fact that MicroC™ acclimated biomass contains a relatively large diversity of microorganisms, due to the complexity of the MicroC™ composition

as well as its high degradability, and have shown that even non acclimated sludge are able to use up this substrate with high rate.

Corn syrup (CS) is a product widely used in the food industry because of its high glucose content; in addition, high-fructose corn syrup (HFCS), where the major sugar is fructose, has become a popular ingredient in many sodas and fruit-flavored drinks. For its high sugar content, corn syrup has been suggested as an alternative carbon source and recently several studies on a pilot and full scale operation have been conducted (Prentice 2007, Pretorius *et al.* 2007). Prentice and coworkers conducted batch experiments as well as a full scale investigation (still ongoing) at the Henrico County water reclamation facility (WRF) in Virginia and found that the addition of corn syrup improved the nitrogen removal process, with an empirical dosing of about 7.9 gCOD/gN, and denitrification rate of about 4.4 mgN/gVSS/h. The disadvantages however are associated with the cost of CS and the variation in the COD content. Similar results, in terms of dosing and increased efficiency were obtained with HFCS (Pretorius *et al.* 2007), but lower C/N ratio were obtained by Mokhayeri *et al.*, (2006).

Glycerol, a by-product of biodiesel production, and glycerol solution such as unicarb-DN, which is made by Univar, have been recently proposed as external carbon for denitrification but very few data are available at this stage; Ramalingam *et al.*, (2006) in a pilot study conducted in collaboration with the NYCDEP using biodiesel waste found an average denitrification rate of 1.8 mgN/gVSS/h; they also highlighted the need for acclimatization of the biomass in order to efficiently removed the nitrate.

### ***Sludge-based carbon sources***

Another concept applied in BNR system is the utilization of hydrolyzed sludge (hydrolyzate) to use internal carbon sources more efficiently, avoiding the cost of external carbon. Hydrolyzed primary sludge was successfully used in a SBR system (Abufayed & Schroeder 1986) and biological, chemical and physical (thermal) hydrolysis techniques were investigated as a means of improving the bioavailability of the organic carbon in the HYPRO (hydrolysis process) project (Abufayed & Schroeder 1986, Barlundhaug & Odegaard 1996b, Barlundhaug & Odegaard 1996a, Aesoy & Odegaard 1994b).

Kristensen and Jorgensen (1990) found that hydrolysate obtained from biologically hydrolyzed sludge gave denitrification rates similar to values obtained with acetate. They also found that hydrolysate obtained from thermally or chemically hydrolyzed sludge gave rates which were approximately half the acetate value.

Primary sludge fermentation has also been applied for producing volatile fatty acids VFAs, which serve as carbon source for supporting denitrification as well as improving phosphorus removal (Christensson *et al.* 1998, Moser-Engeler *et al.* 1998, Ubay-Cokgor *et al.* 2005).

**Table 3A. Summary of kinetic rate of various carbons sources studies**

Carbon source	COD/N	Y (gVSS/gCOD)	$\mu_{max}$ (d <sup>-1</sup> )	k <sub>D</sub> (mgN/gVSS·h)	Reference	
Methanol COD: 1188000 mg/l				2.3	(Monteith et al. 1980)	
				3	(Nyberg et al. 1996)	
				8.7-13.3	(Beccari et al. 1983)	
		4.6		4.28	(Bilanovic et al. 1999)	
				5-6	(Bailey et al. 1998)	
					(Stensel et al. 1973)	
				0.52 (10°C) - 1.86 (20 °C)		
		4.7		0.4-0.5 (13°C) 1.0 (19 °C)	(Mokhayeri et al. 2006)	
				0.56 (13°C) - 6.29 (20 °C)	(Dold et al. 2005)	
		4.1 -4.5	0.23-0.25	0.77 (15°C) - 2 (20 °C)	32 (15°C) 91 (20 °C)	(Christensson et al. 1994) (pure culture)
		3.57		0.67-1 (15°C)	29	(Lee & Welander 1996)
			0.5-0.65			(Henze et al. 1995)
			0.18	0.52 (10°C) - 1.86 (20 °C)		(Tchobanoglous et al. 2003)
					2.4-3.6	(Ramalingam et al. 2007)
		3.7			1.7(10°C) - 6.72(25 °C)	(Carrera et al. 2003)
				9.6	(Peng et al. 2007)	
	4.8	0.29	0.34 (10°C) - 1.2 (20 °C)	2.3 (10°C) - 6.07(20 °C)	<b>This study</b>	
Ethanol		0.25-0.28	1.89 (15°C) - 4.8 (25 °C)	46 (15°C) 139 (20 °C)	(Christensson et al. 1994) (pure culture)	
				5.6	(Ramalingam et al. 2007)	
				10	(Nyberg et al. 1996)	
				12	(Peng et al. 2007)	
			0.22			(Hallin et al. 1996)
Acetate				4-7	(Naidoo et al. 1998)	
				2.08-3.53	(Isaacs & Henze 1995)	
				10.4	(Carucci et al. 1996)	
				7.95-10.6	(Tam et al. 1992)	
				3.2	(Karlsson 1990)	
				10-20	(Henze et al. 1994)	
				9.89	(Bilanovic et al. 1999)	
			0.32			(Muller et al. 2003)
					3.2	(Peng et al. 2007)
			0.66		3.6	(Kujawa & Klapwijk 1999)
		3.5		1.2 (13°C) 3.5 (19 °C)		(Mokhayeri et al. 2006)
	5.7	0.35		13.6	<b>This study</b>	
Acetic acid				27	(Akunna et al. 1993)	
COD: 1121000 mg/l	4		2.18(15°C)	49-76	(Lee & Welander 1996)	
				2.2-2.5	(Gerber et al. 1986)	
Butirric acid				2.0-2.1		
Propionic acid				1.7-2.1		
Formic acid				0.9-1.5		
Glucose				2.7	(Akunna et al. 1993)	
	8.9			3.8	(Prentice 2007)	
		0.38			(Muller et al. 2003)	

**Table 3B. Industrial byproducts, industrial waste, fermentation products, and methane**

Carbon source	COD/N	Y (gVSS/gCOD)	$\mu_{\max}$ (d <sup>-1</sup> )	k <sub>D</sub> mgN/gVSS·h	Reference
Hydrolyzed/ Fermented sludge				4.9-7.5 (BH)	(Hoffmann & Klute 1990)
				6.1-7.3 (BH/PA)	
				3.9-5.7 (CH)	
				0.67-3.09	(Isaacs & Henze 1995)
	6.9	0.27		11.9-15.9 (TH)	(Barlindhaug & Odegaard 1996a)
	4.5		0.61	23.6 (BH)	(Aesoy & Odegaard 1994a)
Fermented MSW	1.6-2.4			5	(Bolzonella et al. 2001)
Hydrolyzed molasses	4.3-5.8			2.9-3.6	(Quan <i>et al.</i> 2005)
Corn Syrup	4.5		1.3 (13°C) 3.7 (19 °C)		(Mokhayeri et al. 2006)
	7.9			4.4	(Prentice 2007)
Sugar solution	10.2			3	(Prentice 2007)
Olive Oil Mill	4.6-5.4				(Tsonis 1997)
Dairy waste	3.6-3.8	0.22-0.38		3.4-8	(Sage et al. 2006)
	4.7		1.91	6.21	(Onnis et al. 2006); <b>this study</b>
Winery waste				32.6	(Bernet et al. 1996)
	3.4		1.43	6.8	<b>This study</b>
Distillery Fusel Oils	2.22			13.8	(Monteith et al. 1980)
Pea blanch water	5.71			10.8	
Wines Sludge Conc.	7.3			8.6	
Methanol Still Bottoms	3.66			7.1	
National Starch	3.26			6.6	
Tomato Sludge	2.54			6.6	
Distillers Fusel Oils	5.32			6.6	
Organic Acid Waste	5.14			5.9	
Methanol Heads	2.45			5.3	
Acetic Acid Waste	1.71			5.2	
Fibres Glicol Waste	5.98			4.3	
Waste Dextrose	8.19			2.9	
Formaldehyde Waste	6.21			1.7	
Brewery waste	5.5-6.2			7.8- 8.2	
	4.2		1.08	8.18	(Onnis et al. 2006); <b>this study</b>
	3				(Skrinde et al. 1982)
Bio-Diesel				1.8-6.8	(Ramalingam <i>et al.</i> 2007)
MicroC <sup>TM</sup>				2.5 (10°)	
COD: 663000 mg/l	6.4	0.39	1.2 (10°C) 3.6 (20 °C)	4.7-6.37(20°)	<b>This study</b>
Beet-sugar waste	3.4		1.89	5.83	(Onnis et al. 2006); <b>this study</b>
Methane	4.0-5.9			25 (max)	(Thalasso et al. 1997)
	4.2			2.48-9.47	(Houbron et al. 1999)
				4.79	(Lee et al. 2001)
				2.46	(Raghoebarsing et al. 2006)

BH: biological hydrolysis; PA: post alkalization; CH: chemical hydrolysis; TH: thermal hydrolysis; FMSW: Fermented MSW

## **Characterization and denitrification potential assessment methods**

No characterization method designed specifically for the selection of alternative carbon source for denitrification exists at the moment; however anoxic batch tests have been in general preferred and applied for the kinetics parameters determination that are relevant to the denitrification process and its modeling.

### ***Characterization of bio-available COD***

Respirometric tests, especially the one that use the measurement of the oxygen uptake rate have often been used for the determination of the rbCOD, which is critical in the selection of carbon source for denitrification. To perform respirometric tests continuous flux systems as well as batch systems have been applied; continuous flux systems produce in general reliable and reproducible results, however the major drawbacks are the cost and difficult to operate. Batch system are simpler to operate, and can be performed under aerobic condition (OUR measurement) or anoxic (NUR); however the need of an acclimated sludge, requires a lab scale reactor operating in continuous or SBR mode (Ekama et al. 1986, Sollfrank & Gujer 1991, Cappai et al. 2004) or a full scale plant that use that specific carbon source.

Figure 1 shows the results obtained with four industrial wastewaters, using three different characterization methods for the determination of the readily biodegradable COD fraction.

The results clearly show that the three methods produce comparable results; therefore they can be interchangeable used for an initial characterization of the biodegradable fraction. Physical-chemical test were also performed (data not shown) and in general overestimated the rbCOD fraction producing values 10-20% higher. The batch tests, especially the two anoxic used give also information of the other fraction of the COD and the denitrification rate associated with them. For example for the dairy waste a large fraction of the COD (about 40%) was easily hydrolyzed and consumed with rate of 2.2 mgN/gVSS/h.

For the determination of the rbCOD from the NUR test a yield coefficient has to be assumed or determined. One of the major contentions of the NUR method is the assumption that the  $Y_H$  remains the same under aerobic ( $Y_H$ ) and anoxic ( $Y_{HD}$ ) conditions. Theoretical considerations using the energetics of aerobic and anoxic respiration and comparative tests with NUR and OUR yielded lower yield coefficients under anoxic conditions (Sozen *et al.* 1998).

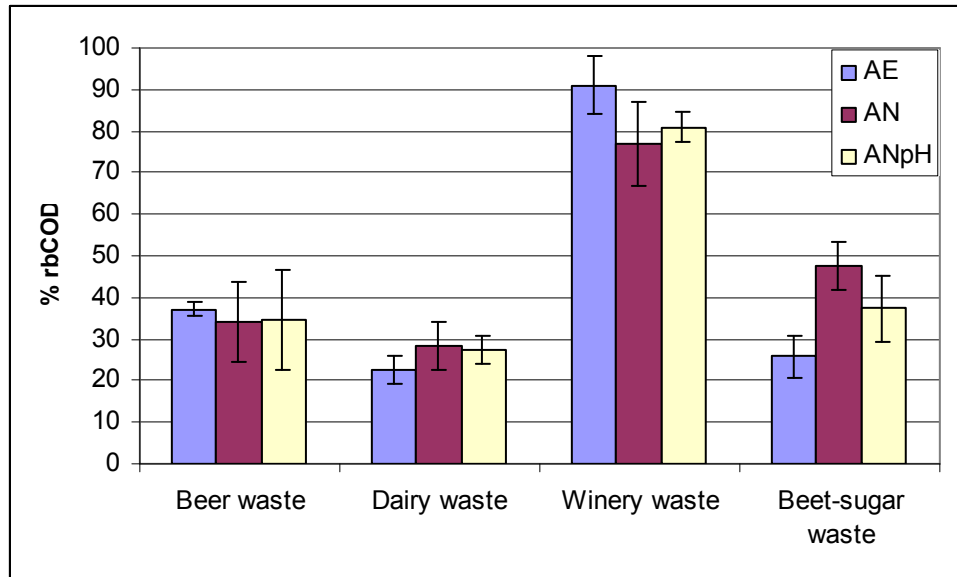


Figure 1. Percentage of rbCOD for 4 industrial wastes with three types of batch tests.

The physical-chemical test is very simple and can be used to make a first selection between different possible wastes; the following step could be to perform biological batch tests (bioassay) that give more accurate information on COD fractions and kinetics because they monitor the response of activated sludge to wastewater. Between the biological bioassays the anoxic tests are more suitable in the choosing process of an external carbon source for denitrification; in fact they allow the observation and monitoring of the biokinetic response of the denitrifying bacteria in the presence of the carbon source tested; moreover they give information regarding the denitrification potential and so allowing an estimation of the amount of the organic waste to dose during the anoxic phase.

#### *Determination of denitrification rates*

Tables 3A and 3B show that there is a variation in denitrification rates and in the other kinetic constants even if the substrates are the same; this is probably due to differences in biomass activity and operating conditions of the various reactors and experimental procedure adopted.

For the determination of the denitrification rate two anoxic batch test types were tested in this study: NUR batch test (AN) and the pH-stat method. Figure 2 show the results obtained.

The variability of the results obtained, indicated by the high error bar, is in part due to the different condition used during the test (acclimated versus non acclimated sludge), but are also associated with the variable composition of the industrial wastewaters, which are instead attributed to the production cycle of the raw material.

In general less variability was noted with the pH stat test; the reason being is the ability of this system to continuously follow the denitrification processes. More specifically, the automated titrimetric unit records in continuous the acid consumption (and therefore the nitrate uptake), whereas the nitrate concentration determined during the anoxic batch test are usually taken at regular interval of 10-20 minutes, causing the overestimation, or underestimation of the nitrate consumption rate.

To carry out the anoxic batch test a simple set up is needed, whereas a automated titrimetic unit is needed for the pH stat, even though with the latest no costly and time-consuming chemical analysis are required.

The choice of substrate to biomass ratio (F/M) forms an important part of the NUR test as it is one of the factors which defines the form of the NUR profile. High F/M ratios do not realistically represent conditions at treatment plants which generally operate at low F/M ratios. However, F/M ratios which are too low may result in substrate limitation and indistinguishable breaks.

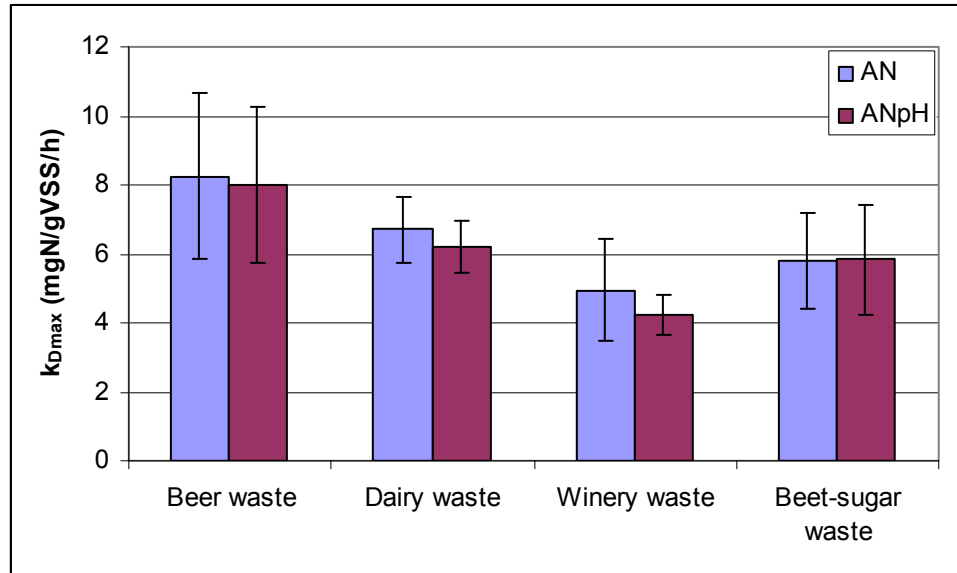


Figure 2. Denitrification rates for 4 industrial wastes with two types of batch tests.

For the correct determination of the denitrification rate, as well as of the rbCOD fraction, a significant factor is the measurement of the amount of electron acceptor utilized. When nitrite accumulate during the test, the use of only nitrate is unacceptable and need to be take into consideration (Cokgor *et al.* 1998). Nitrite accumulation could be a problem with the pH-stat system, in fact the reaction affecting the pH of the solution, and therefore the acid addition is the second step of the denitrification process (from  $\text{NO}_2$  to  $\text{N}_2$ ), therefore an accumulation of nitrite will cause a unclear acid profile which will determine errors in the estimation of the various parameters.

The results obtained with both methods are nevertheless comparable, and therefore both recommended for denitrification studies.

#### ***Determination of kinetics and stoichiometric constants***

The final goal of the characterization is the determination of kinetics and stoichiometric parameters to use as input into a simulator software, which will allow to simulate the addition of the carbon source at the actual plant.

Besides the estimation of the rbCOD fraction and the specific denitrification rate, the anoxic batch tests allow for the determination of the specific growth rate of the denitrifiers. The two

major assumptions are that the initial rate of electron acceptor consumption stays proportional to the specific growth rate and that the biomass remains constant at least at the initial phase. Under these conditions the corresponding relationship holds (Sozen et al. 1998):

$$\frac{dN_1}{dt} = \frac{(1 - 1.42 \cdot Y_{HD})}{2.86 \cdot Y_{HD}} \cdot \hat{\mu}_{HD} \cdot X_{HD}$$

Where  $dN_1/dt$  is the initial slope of the nitrate-time curve and  $X_{HD}$  is the biomass concentration of denitrifiers; a similar expression can be found using the acid consumption rate, for the pH-stat method. Using this expression to determine the specific growth rate, the fraction of denitrifiers in the mixed liquor needs to be determined; in the method proposed in literature two test in parallel are run to measure and compare the OUR and the NUR (Sozen et al. 1998).

An alternative method for the determination of the specific growth rate of the denitrifiers has been proposed by (Dold et al. 2005), which has been applied for the determination of the growth rates of methylotrophs as well as the growth rate of denitrifiers when alternative carbon sources, such as corn syrup, are used.

In this test the major assumption is that the denitrifying population is exponentially growing due to the very F/M ratio used. The test allows for the determination of the specific growth rate independently from the determination of the denitrifying population, which is instead needed in the previous anoxic batch test.

From both tests is also possible to determine through chemical analysis of both COD and nitrate the, C/N ratio, which can also be used to indirectly estimate the yield. The COD/N measured in these tests can be however affected by several factors including the possible interference of storage phenomena (luxury uptake) which can take place when a considerable amount of organic substrate is put in contact with the biomass (Dionisi *et al.* 2004, Majone *et al.* 1999), the possible activity under anoxic condition of poly-phosphorus accumulating bacteria if sludge from EBPR plant are used (Naidoo *et al.* 2000) and the reliability of the COD and nitrate measurement itself.

An important parameter which is needed to specify into any simulation software is the half saturation constant for the carbon source ( $K_S$ ). The estimation of this parameter can be done using continuous reactor running at different solid residence time (SRT) (Grady *et al.* 1998), or combining the results of multiple short anoxic batch test with different initial concentration of COD, as shown from Figure 3, obtained using MicroC™ and sludge drawn at a full scale WWTP which use MicroC™ to enhance denitrification.

A combination of anoxic batch tests run at low F/M, to determine denitrification rate, readily biodegradable fraction and ( $K_S$ ) and high F/M test, which allow the determination of the denitrifiers maximum growth rate, are needed to obtain all kinetics and stoichiometric parameters. As alternative NUR test in combination with OUR test could be used for the determination of the denitrifiers maximum growth rate.

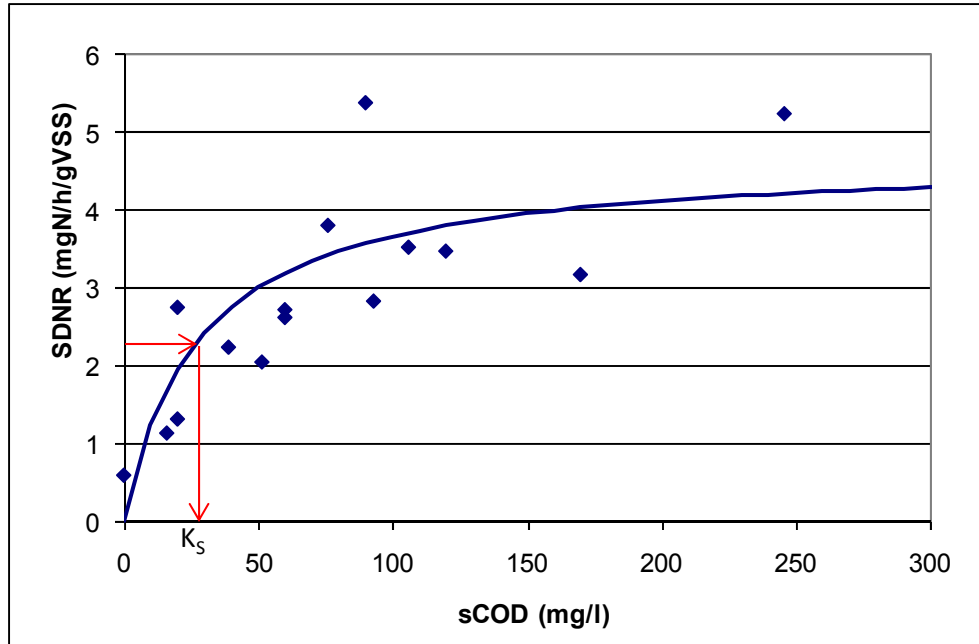


Figure 3. Estimation of the half saturation constant using the results from multiple NUR tests.

### ***Sludge source requirement***

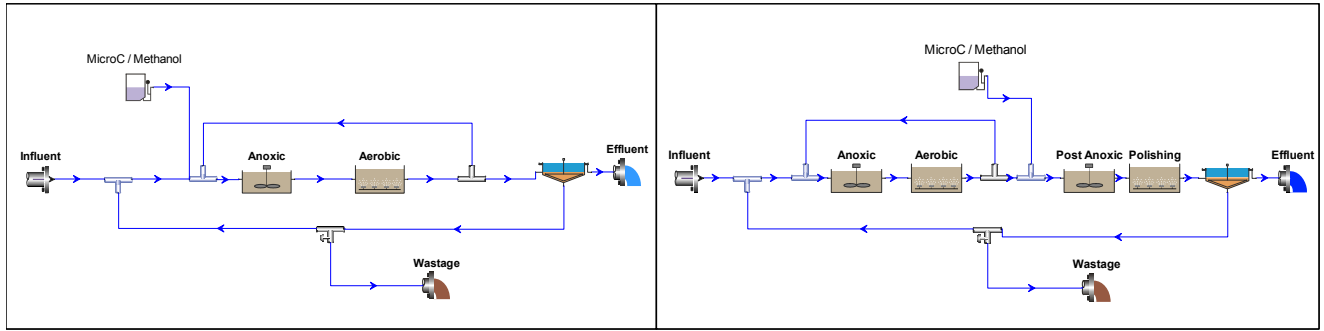
In all characterization tests described, the use of acclimated sludge is needed or highly recommended.

While it might be easy to find sludge acclimated to methanol and other chemical or proprietary products in BNR plants, more difficult would be to find it for other alternative carbon sources that are starting to be considered, as for example industrial byproduct. When available, the sludge from biological pretreatment in the industrial facility will be preferred; otherwise a lab scale reactor should be operated to obtain acclimated sludge. This latest option could be hard to put into practice, especially when numerous carbon sources need to be evaluated. The results from (Monteith et al. 1980) suggests, however, that most industrial waste are readily utilized by the bacteria already present in BNR system, therefore for a first round of screening acclimatization should not be an issue.

### **Practice Implications**

To demonstrate the implications of differences in denitrification rates and kinetics on the nitrogen removal performance in real practice, the simulator software Biowin was used to reproduce the addition of two carbon sources, methanol and MicroC<sup>TM</sup>, under different scenarios.

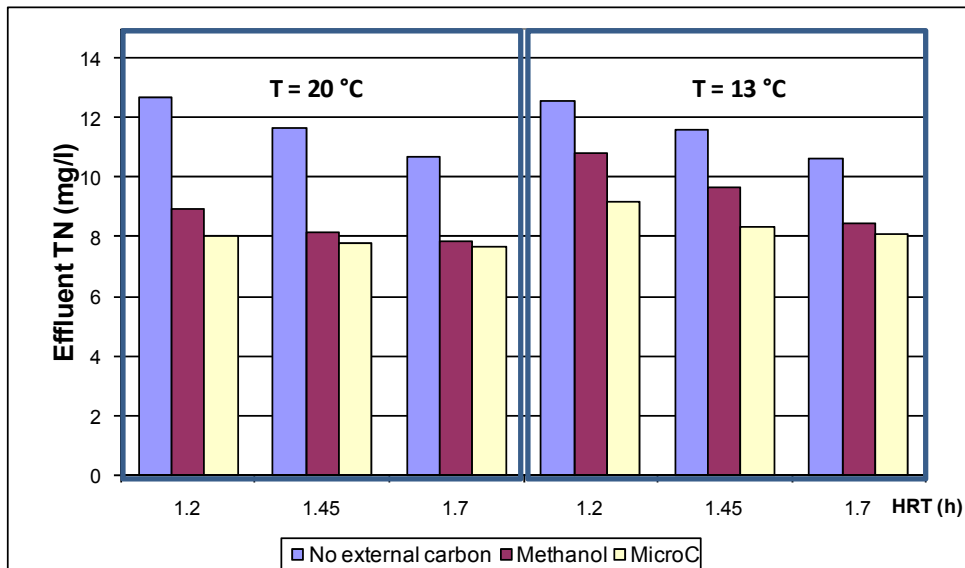
Two configurations were considered: a modified Ludzak-Ettinger (MLE) for predenitrification and a 4-stage Bardenpho (see Figure 4). For each configuration, some operational parameters were changed in order to assess their effects of on the steady-state final effluent TN concentration. The parameters that were varied included external carbon dosage, pre-anoxic and post-anoxic volumes (therefore anoxic hydraulic residence time HRT) and temperature. Table 4 summarizes the conditions and operation parameters that were used for the different scenarios analyzed.



**Figure 4. Biowin layouts for the two configuration analyzed: on the left MLE, on the right MLE plus post-denitrification.**

**Table 1.**

Design parameter			Kinetics and stoichiometry		
Configuration	MLE	4-stage Pardenpho	Parameter	MicroC™	Methanol
Influent flow rate, Q (MGD)	5	5	$\mu_{max}$ [1/d]	3.66	1.25
Temperature (°C)	<b>13 and 20</b>	<b>13 and 20</b>	Ks [mgCOD/L]	20	5
Aerobic SRT (d)	10	10	Aerobic decay [1/d]	0.08	0.06
Influent COD (mg/l)	250	250	Anoxic decay [1/d]	0.08	0.06
V anoxic (MG)	<b>0.25-0.35</b>	0.35	Yield (anoxic) [gCOD/gCOD]	0.52	0.4
V post anoxic (MG)	-	<b>0.3-0.4</b>	Temperature coefficient $\theta$	1.1	1.1
V aerobic (MG)	0.91	0.91	Aerobic growth	yes	no
V polishing (MG)	-	0.1			
Mixed liquor recycle, MLR	3Q	3Q			
RAS	0.5Q	0.5Q			



**Figure 5. Comparison of N removal performance with either MicroC or methanol as C sources, under different anoxic HRT and temperature conditions.**

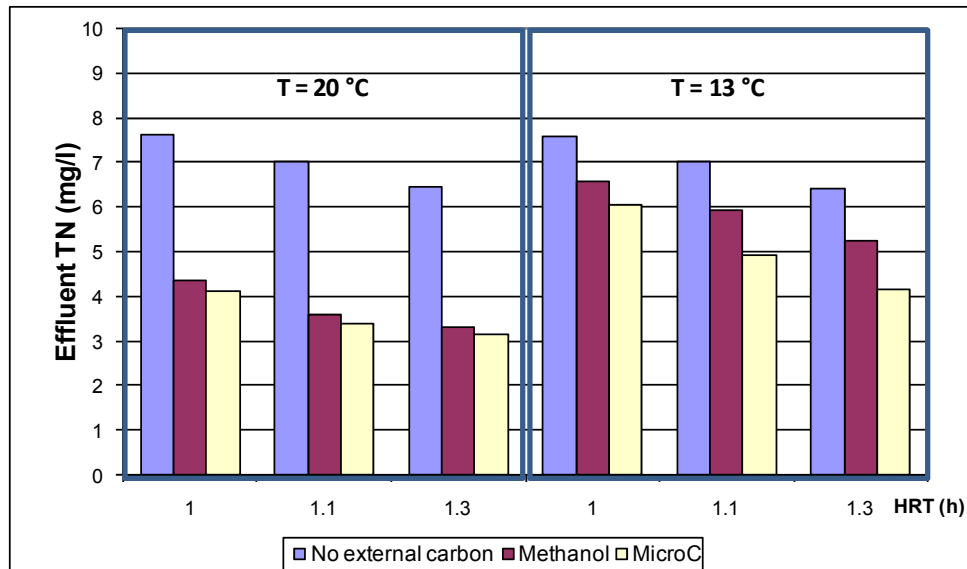


Figure 6. . Comparison of N removal performance with either MicroC or methanol as C sources, under different anoxic HRT and temperature conditions.

Figure 5 and 6 show the results of the simulation. From the results of the simulation with Biowin it can be concluded that when same amount of COD as either MicroC<sup>TM</sup> or as methanol is added to enhance pre-denitrification, MicroC<sup>TM</sup> gives slightly better performance than methanol at 20°C. Also, HRT of anoxic zone also affects denitrification performance and therefore both COD dosing and HRT should be considered when adding an external carbon for enhancing denitrification.

Temperature affects denitrification rates and kinetics of both methylotrophs and MicroC<sup>TM</sup>-utilizer bacteria. The denitrification in the system with MicroC<sup>TM</sup> supplement seems to be less sensitive to temperature drop compared to with methanol supplement. The results suggest that at 13°C, for equivalent COD added, MicroC<sup>TM</sup> performs slightly better than methanol, especially in the post-denitrification scenario. Comparison of the impact of temperature on denitrification between using MicroC<sup>TM</sup> and using methanol is more clearly demonstrated when the temperature drops to be <5°C. At this very low temperature the minimal SRT required to prevent methylotrophs from washing out from the suspended post denitrification reactor is about 4.5 days. In contrast, the minimal SRT required for keeping the MicroC<sup>TM</sup>-utilizers is less than 1.5 days. This implies that larger anoxic reactor volume is required for methanol compared to MicroC<sup>TM</sup> at extremely low temperature conditions.

## CONCLUSIONS

The literature data and results presented in this work have shown that alternative carbon sources, including industrial waste and agricultural by product have comparable rates and COD/N ratio to those of pure chemicals, with the majority been within the 3 to 5 range; lower values are reported for methanol head and acetic acid waste, whereas higher values were found for thermal hydrolyzed sludge and some other organic waste. A high COD/N ratio translate usually in higher costs if the external carbon sources need to be purchased, but also higher biomass production, which could affect the sludge handling processes at the plant.

To identify feasible alternatives to methanol a characterization of the possible candidates is required, based on the time and equipment available, different tests can be performed as shown; however the authors emphasize the need of a detailed protocol that wastewater treatment plants can employ for the characterization and preliminary selection of the more suitable carbon sources.

Following a good characterization, which include the determination of biodegradability (COD fractions), kinetics and stoichiometric parameters, the use of simulator software, such as BIOWIN, or GPS-X, can help in the selection, for an existing facility, of the most effective external carbon source, as well as the selection of optimal operational condition (carbon dosage).

For new plant the operational temperature and the selection of the necessary anoxic volume are critical for achieving good nitrogen removal. A cost/benefit analysis would be needed to determine the option of building additional reactors versus using alternate substrates.

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