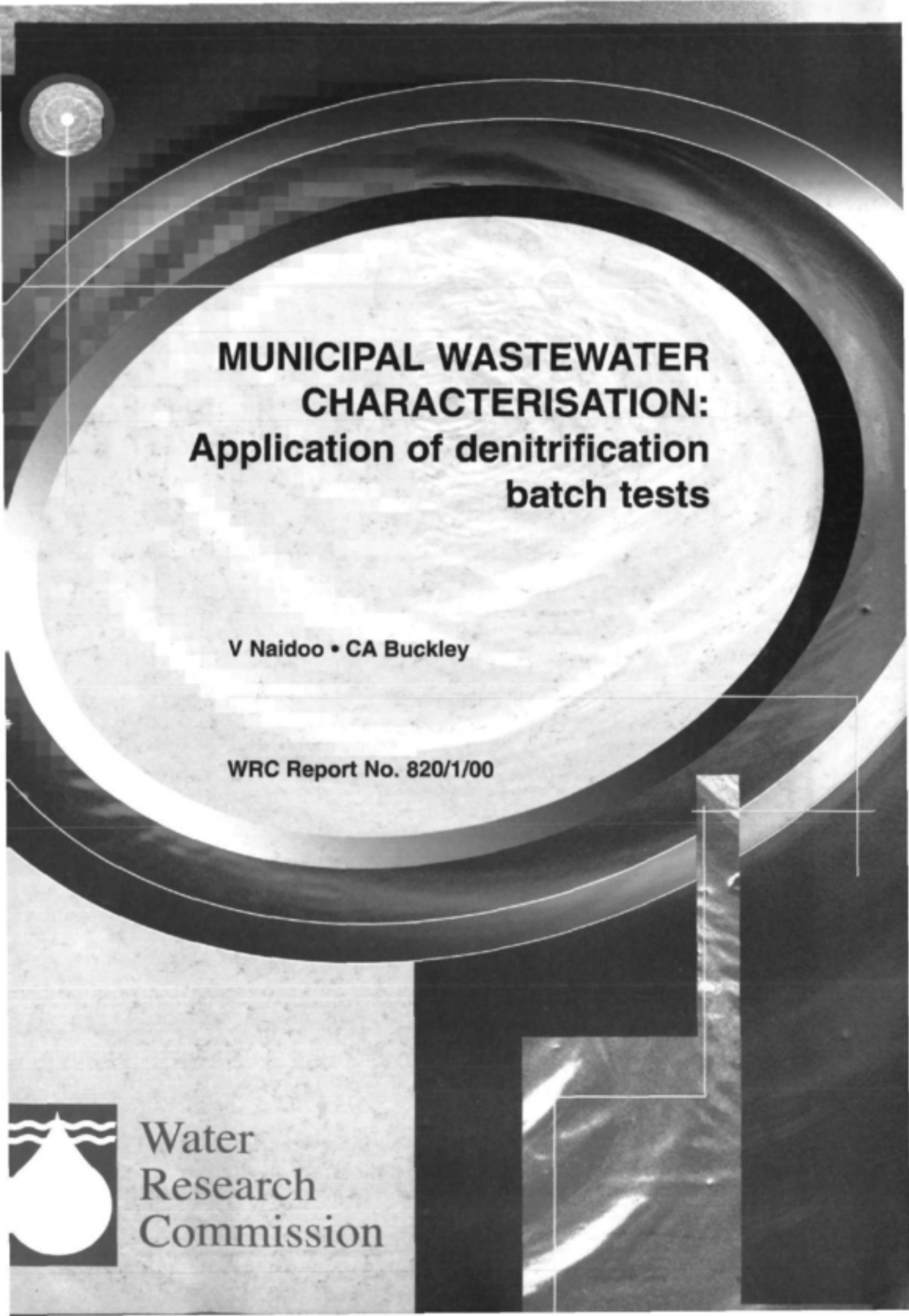

MUNICIPAL WASTEWATER CHARACTERISATION: Application of denitrification batch tests

V Naidoo • CA Buckley

WRC Report No. 820/1/00

Water
Research
Commission

MUNICIPAL WASTEWATER CHARACTERISATION:
Application of denitrification batch tests

Report to the Water Research Commission

by

V Naidoo and CA Buckley
Pollution Research Group
University of Natal

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Executive Summary

1. Background

The biological treatment of wastewater has evolved significantly from simple single sludge systems practicing organic carbon removal to ones which now include either nitrification/denitrification (N/DN) and / or phosphorus (P) removal. The inclusion of more biological processes have increased the complexity of current wastewater systems which has subsequently led to the development of more complex mathematical models. The operation of plants can be assessed and improved by the use of mathematical modelling tools which require accurate input data. Thus, knowledge of the wastewater characteristics is an important step towards the optimum modelling, design and operation of present and future plants. However, for these tools to be effective, the input data needs to be accurate which is dependent on the current methods used to determine them.

Wastewater is a complex substrate consisting of compounds of differing biodegradability. The organic matter is discussed in terms of chemical oxygen demand (COD). Biokinetically, these compounds have been divided into readily biodegradable (RBCOD), slowly biodegradable (SBCOD) and unbiodegradable substrate groups. Compounds with intermediate biodegradability i.e. compounds which fall between the RBCOD and SBCOD groups, have been termed readily hydrolyzable organic substrates (RHCOD). The readily biodegradable and readily hydrolyzable COD fractions of wastewater can be determined by respirometric tests such as the oxygen utilization rate (OUR) and nitrate-N utilization rate (NUR) tests.

2. Project Objectives

The primary aims of this project were as follows :

- Study the protocol of NUR batch tests and apply it to a range of wastewater and sludge samples with the aim to : (1) assess, (2) understand, and (3) make recommendations which could improve the procedure and make it easily applicable on-site.
- Characterize a variety of municipal wastewater samples by the :
    - *nitrate-N utilization rate test - biological respirometry*
    - *physico-chemical methods (settling, centrifugation, filtration and coagulation)*
- Study the utilization of an experimental readily biodegradable COD substrate, acetate, under anoxic conditions.

- Perform exploratory investigations to :
    - *clarify the impact of enhanced biological phosphorus removal (EBPR) sludge (biomass) samples on wastewater characterization*
    - *Determine the influence of storage on wastewater characteristics*
    - *Assess the influence of sludge acclimatization on wastewater characterization*

3. Approach

Samples from 22 wastewater treatment plants, most of which were located in France were tested. The majority of the samples tested were from France because the first two years of this study were done at the CIRSEE-Suez-Lyonnaise-des-Eaux laboratory in Paris, France. Samples from a variety of plants with different characteristics were tested in order to determine the variability in the readily biodegradable COD concentrations. Samples were also taken from Berwick (England), Rostock (Germany), Brno (Czech Republic), and Orense (Spain) because these plants are operated by Suez-Lyonnaise-des-Eaux and the samples were made available to the CIRSEE-Suez-Lyonnaise-des-Eaux laboratories in Paris. Only a limited number of South African wastewater samples from the Durban region in Kwa-Zulu Natal were tested due to time constraints. The four South African wastewater treatment plants tested were Darvil, Southerns, Northerns, and Kwa-Mashu.

All the tests were done using the nitrate-N utilization rate method to determine the biodegradable COD concentrations in raw wastewater samples. Specific denitrification rates were also calculated from the NUR profiles. In addition, NUR tests were done using acetate as a sole exogenous substrate with sludge samples taken from a variety of wastewater treatment plants. These tests were used to calculate the yield coefficient (indirectly) and mass balances from which the accuracy of the nitrate-N utilization rate test was assessed.

4. Results and Discussion

Data obtained from the NUR tests were used to calculate the readily biodegradable COD (RBCOD) and a second biodegradable COD fraction, the readily hydrolyzable COD (RHCOD) fraction. The SBCOD, however, could not be determined directly from the 6 h NUR batch tests.

- The RBCOD fractions ranged between 7 and 25 %, with an average of 13 %, of the total COD concentration of raw wastewater. The majority of these results fell within the 10 to 20 % (of the total COD) range, which was expected.

- The intermediate fraction, the RHCOD, was found to range between 5 and 29 % of the total COD concentration, with an average of 16 %. The range of the RHCOD fractions suggests that this fraction is either very variable or that the NUR test does adequately or accurately characterize this fraction.

It is highly likely that the second biodegradable fraction is a component of the RBCOD from the influent raw wastewater. This hypothesis is substantiated by tests done with acetate as substrate. Results suggest that a significant part of the added acetate (as COD) was stored and the second phase is in fact an *apparent or residual* phase brought about by the consumption of the stored or accumulated acetate products. This is suggested in two ways : (1) the calculation of the yield coefficient is lower and closer to the 0.5 mgCOD/mgCOD values, cited in the literature, when the COD calculated from phases 1 and 2 are considered, and (2) in some cases, the acetate mass balances were found to be approximately 100 % when phases 1 and 2 were used to calculate the amount of acetate utilized under anoxic conditions.

The physico-chemical and biological characteristics obtained for European (n = 36) and South African (n = 4) wastewater samples were found to be fairly comparable.

- The average total COD concentrations of the European and South African wastewater samples were found to be 749 ± 247 mg/l and 788 ± 155 mg/l, respectively.

- The average physico-chemically determined readily biodegradable (Ss) fraction which was calculated by difference between the coagulated soluble fraction (Sco) and the inert soluble fraction (Si) were comparable which suggests that the wastewater samples from Europe and South Africa have a similar RBCOD content. These results were compared with the average RBCOD and RHCOD values obtained from NUR tests. The RBCOD values from the NUR tests for both European and South African wastewater samples were found to be lower than the soluble Ss fraction.

- However, the sum of the average RBCOD and RHCOD fractions (*i.e.* 27 %) obtained with European wastewater samples were highly comparable to the average physico-chemically derived Ss fraction (*i.e.* 27 %). The sum of the average RBCOD and RHCOD fractions (*i.e.* 26 %) for the South African wastewater samples was slightly lower than the Ss value (*i.e.* 21 %) obtained after coagulation tests.

The South African wastewaters appear to have similar characteristics to the European wastewaters. However, these conclusions are based on limited comparative results especially since wastewater samples from only 4 South African wastewater treatment plants were tested.

An experimental readily biodegradable organic substrate, sodium acetate, was tested under anoxic conditions. Approximately 50mg/l sodium acetate as COD to the reactors at the start of the NUR experiments. The data from NUR tests with acetate were analyzed by considering phase 1 (NOx 1) and the sum of phase 1 and phase 2 (NOx 1 and NOx 2) and acetate utilization was calculated by using a yield coefficient of 0.63 mgCOD/mgCOD. The following trends were observed :

1. < 100 % acetate recovery (NOx 1),
2. ca. 100 % acetate recovery (NOx 1),
3. ca. 100 % acetate recovery (based on sum of NOx 1 and NOx 2),

4. > 100 % acetate mass balance (based on NOx 1 and NOx 2).

The results obtained from NUR tests with acetate were used to formulate several conclusions on acetate utilization during denitrification.

- Firstly, from acetate mass balances it was found that acetate may be used exclusively for denitrification (100 % acetate was accounted for). In this case, the sludge contains a significant proportion of denitrifying organisms and few or no polyphosphate accumulating organisms. This observation was made only when non-EBPR (enhanced biological phosphorus removal) sludge samples were used.

- Secondly, acetate mass balances which were found to be < 100 % suggest that acetate could be used for denitrification and the production of storage products like polyhydroxyalkanoates (PHA's). These sludge samples probably contained a higher proportion of polyphosphate accumulating organisms, which competed for the available acetate in the bulk liquid. This observation was made for both EBPR and non-EBPR sludge samples.

- Thirdly, acetate could be used for denitrification by denitrifiers and for polyhydroxyalkanoate synthesis by denitrifying polyphosphate accumulating organisms. The stored PHA's in the denitrifying polyphosphate accumulating organisms are subsequently re-utilized during denitrification. This secondary utilization is manifested in the second denitrification phase and is supported by the observation of phosphorus uptake. These results showed that wastewater samples high in volatile fatty acids (VFA's) were also subject to denitrifying polyphosphate accumulating organism activity even though the sludge was sampled from non enhanced biological phosphorus removal systems (non EBPR).

Several of the NOx profiles revealed either 2 or 3 rates due to the control of the substrate to biomass ratio (S/X : ≤ 0.1 mgO$_2$ / mgO$_2$).

- Majority of the samples (i.e. 85 %) tested produced initial maximum specific denitrification rates (k_1) between 3 and 6 mgN/gVSS.h.

- The intermediate denitrification rate (k_2) was found to vary between 2 and 3 mgN/gVSS.h.

- Denitrification rates (k_3) obtained from utilization of influent and endogenous slowly biodegradable COD (SBCOD) varied between 1.0 and 1.5 mgN/gVSS.h. This latter rate is significantly higher than the endogenous denitrification rates cited in the literature. One of the reasons for these higher rates could be be linked to the re-use of stored or accumulated products by the microorganisms.

In addition, a comparative study was undertaken to determine the RBCOD both of wastewaters with enhanced biological phosphorus removal sludge samples and non-EBPR sludge samples. It was found that the RBCOD values derived by NUR tests with EBPR sludge were consistently lower (4 to 5 %) than those with non-EBPR sludge. Thus, the NUR tests with EBPR sludge resulted in a 4 to 5 % underestimation of the RBCOD fraction of raw wastewater samples. This loss in RBCOD to polyphosphate accumulating organisms appears to be linked to the influent raw wastewater acetate concentration.

Other studies showed that the storage of sludge and wastewater samples for up to 24 h did not change the characteristics of the raw wastewater sample. However, complications may arise if the samples are stored for 72 h. In addition, it was shown that, in some cases, it is possible to characterize wastewater samples with an unacclimatized sludge sample.

5. Conclusions

The conclusions from this study are as follows:

- these tests showed that the RBCOD fraction could be adequately characterized using the NUR method. Thus, while the results showed that the NUR is a useful tool for wastewater characterization, it will continue to be a more tedious characterization tool than the oxygen utilization rate test, until the NUR test is automated.

- the accuracy of the tests appears to be compromised when enhanced biological phosphorus removal sludge samples are used in the NUR tests. It was found that non-EBPR sludges can also consume about 4 - 5 % of the acetate that is present in the system for the production and replenishment of storage compounds. Fortunately, for the raw wastewater samples tested, the acetate component of the RBCOD fraction was small and therefore, did not significantly affect the RBCOD results.

- mechanisms such as substrate accumulation and storage may also impact on substrate removal and hence, the determination of the readily biodegradable COD component of municipal wastewater samples.

6. Recommendations

These studies led to the following recommendations:

- For the NUR method to become more widely used, an automatic system needs to be developed to monitor denitrification. While it is currently possible to do the NUR tests on-site, the withdrawal of liquid samples for analysis remains one of the major drawbacks of this method. Advances in nitrate electrode sensitivity and accuracy and development of on-line titrimetic procedures may provide the solution.

- Studies done with wastewater and particularly with acetate, led to the following postulation: *The bacteria present in the system have the propensity to rapidly accumulate and / or store some of the acetate / RBCOD added to the reactor. Once the acetate / RBCOD becomes limiting, these bacteria are able to re-use the accumulated / stored compounds under anoxic conditions. This is manifested in the observation of the short, intermediate phase in NUR tests*. This postulation, if true, may have a significant influence on the way future biological wastewater treatment systems are modelled. It also has far reaching consequences on the way respirometric techniques are viewed as characterization tools. This hypothesis could be validated by using radio-labelled acetate and following acetate consumption and nitrate utilization. If future studies on accumulation and storage phenomenon show that it does influence the biodegradable fraction derived by the respirometric techniques, then past comparisons between physico-chemical and respirometric methods need to be re-evaluated.

- One of the suggestions in this study was that the RHCOD fraction was a component of the RBCOD. This could be tested by doing an anaerobic P release test to determine the RBCOD content. The RBCOD consumed can be determined from this test and can be compared to the RBCOD and RHCOD calculated from NUR tests.

7. *Technology Transfer*

- The principal researcher was seconded for 2 years to the laboratories of CIRSEE-Suez-Lyonnaise-des-Eaux, Paris, France. It therefore, possible to draw on the experience (technical and academic) of the members, Vincent Urbain, Herve Harduin, Philippe Ginestet and Jean-Marc Audic, of the biological group at CIRSEE. It also fostered better communication and co-operation between the Water Research Commission and CIRSEE-Suez-Lyonnaise-des-Eaux.

- This knowledge gained from this project is now being used in a spin-off project which aims to develop an **automatic anoxic test** for the determination of wastewater fractions and the study of denitrification kinetics. A Masters student is currently testing a nitrate electrode and a titrimetric procedure with the open and closed oxygen utilization rate test.

- **CONFERENCE PRESENTATIONS**

1. Urbain V., **Naidoo V.**, Guellil A., and Block JC. *Municipal Wastewater Characterization using Physico-chemical and Biological Methods*, WEFTEC Asia 98, Conference, Raffles City Convention Center, Singapore, 8-11 March, 1998.

2. Urbain V., **Naidoo V.**, and **Buckley CA**. *Characterization of Wastewater Biodegradable Organic Fraction : Accuracy of the Nitrate-N Utilization Rate Test.* Water Environment Federation Conference, Orlando, USA, 3-7 October 1998.

3. **Naidoo V.**, Urbain V., and **Buckley CA**. *Characterization of Wastewater and Activated Sludge from European Municipal Wastewater Treatment Plants using NUR Tests.* IAWQ 19th Biannual, International Conference, Vancouver, Canada 21-26 June 1998.

4. **Naidoo V.**, **Foxon, K.**, Urbain V., Ginestet P., and **Buckley CA**. *Different Phosphorus Release Patterns and the Impact on the Characterization of Municipal Wastewater Fractions using the NUR Test.* BIOY2K Conference, Grahamstown, South Africa, 23-28 January 2000.

5. **Naidoo V**., Urbain V., Ginestet P., and **Buckley CA**. *Reliability of the Anoxic Respirometric Technique for Wastewater Characterization using EBPR and non-EBPR Sludge.* WISA 2000 Conference, Sun City, South Africa, 28 May to 1 June 2000.

- **POSTER PRESENTATIONS**

Naidoo V., Harduin H., Urbain V., and **Buckley CA**. *Factors Influencing Denitrification Batch Kinetic Studies*. Biotech SA 1997, Second Grahamstown Conference, Biotechnology and Development in Southern Africa, Rhodes University, 21-24 January 1997

- **PAPERS PUBLISHED**

Naidoo, V., Urbain, V., and **Buckley, C.A**., *Characterization Of Wastewater and Activated Sludge from European Municipal Wastewater Treatment Plants using the NUR Test*, Water Science and Technology, **38** (1), pp. 303-310, 6 October 1998, ISSN: 0273-1223/98

- **PhD THESIS**

Naidoo V., *Municipal Wastewater Characterization: Application of Denitrification Batch Tests.* PhD Thesis, University of Natal, Durban, South Africa, 1999.

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Abbreviations

ATP	adenosine triphosphate
BNR	biological nutrient removal
BEPR	biologically enriched phosphorus removal
Bio-P	biological phosphorus removing sludge
COD	chemical oxygen demand (mgO_2/l)
CV	coefficient of variation
DO	dissolved oxygen
DPAO	denitrifying poly-accumulating organism
FAD	flavin adenine dinucleotide
$FADH_2$	flavin adenine dinucleotide (reduced)
g	gaseous
h	hour
K	Kelvin
k_1	first rate (mgN/gVSS.h)
k_2	second rate (mgN/gVSS.h)
k_3	third rate (mgN/gVSS.h)
min	minute
mV	millivolts
μm	micrometer
N	Nitrogen
N/DN	Nitrification/denitrification
N/A	not applicable
n.d.	not determined
n.o.	not observable
NOx	nitrates and nitrites as N (mg/l)
NUR	Nitrate utilization rate
OUR	Oxygen utilization rate
P	Phosphorus (mg/l)
PAO	poly-accumulating organism
p.e.	population equivalents
PHB	polyhydroxybutyrate
PHA	polyhydroxyalkanoate
RBCOD	readily biodegradable COD (mg/l)
RHCOD	readily hydrolyzable COD (mg/l)
rw	raw wastewater
S	substrate
Ss	truly soluble 'readily biodegradable' fraction
SBCOD	slowly biodegradable COD
Sce	COD of supernatant after centrifugation

$Sf_{0.45}$	COD of supernatant after 0.45 µm filtration
Sco	COD fraction after coagulation
Sp	particulate COD (after filtration)
St	total COD
Sns.	COD of supernatant after 2h settling
T	temperature in degrees Celsius
t	time
TSS	total suspended solids
V	volume
Vt	total volume
Vww	volume of raw wastewater
Vx	volume of sludge
VFA	volatile fatty acid
VSS	volatile suspended solids
WTP	wastewater treatment plant
Xt	biomass
Xf	filtered fraction of biomass
Y_{HD}	yield coefficient (anoxic)
Y_{H}	yield coefficient (aerobic)

Chapter One

Introduction

1.1 . BACKGROUND

One of the principle aims of present day wastewater treatment plants is the removal of organic carbon (COD) from wastewaters (Henze *et al.*, 1997). This has been achieved by effecting several steps in the treatment process such as primary settling, biosorption, biodegradation, followed by a secondary settling step to remove the sludge flocs (Figure 1-1). This eventually results in the production of residual organic carbon that can be released into the river systems. Furthermore, the activated sludge process has shown that under certain selector conditions (aerobic, anaerobic and / or anoxic) it is also able to efficiently remove nitrogen and phosphorus. Nitrogen can be transformed and removed by biologically mediated nitrification (aerobic process) and denitrification (anoxic process). Nitrification is a process which converts ammonia to nitrites and then to nitrates while denitrification results in the transformation of nitrates and nitrites to nitrogen gas. Biological phosphorus removal is enhanced by the presence of anaerobic and aerobic zones, and polyphosphate accumulating organisms. Both denitrification and phosphorus removal efficiency are dependent on the biodegradability of the available organic carbon substrate (COD).

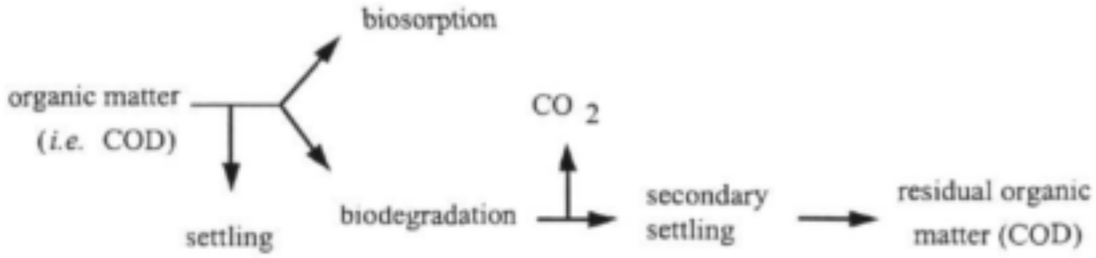


Figure 1-1: Basic outline of organic matter (COD) removal mechanisms in municipal wastewater treatment systems.

Biodegradability of organic carbon of raw wastewaters

Studies on full-scale activated sludge plants showed that denitrification in a plug-flow primary anoxic reactor occurred in two linear phases. The rapid initial rate was followed by a slower denitrification rate which was hypothesized to arise from the utilization of two different biodegradable COD fractions (Ekama *et al.*, 1979). The first phase, which was a rapid, short phase, was linked to the utilization of a readily biodegradable COD fraction (RBCOD). This fraction consisted of small molecules that could pass directly through the cell wall of the organisms for metabolism.

The second phase was longer and produced a slower rate. This phase was attributed to the slowly biodegradable COD fraction (SBCOD) which consists of larger complex molecules that cannot pass directly through the cell wall of the microorganism. The compounds which belong to the SBCOD division requires several hydrolytic steps before they can be taken up and utilized by the heterotrophic bacteria in activated sludge. Hydrolysis is facilitated by extracellular enzymes which break down the larger complex molecules into smaller simpler compounds. The second slower denitrification rate is limited by the rate of hydrolysis rather than the rate of metabolism (Wentzel *et al.*, 1992; Henze *et al.*, 1994).

Once the slowly biodegradable COD of the wastewater has been exhausted the bacteria will utilize endogenous respiration products. The latter occurs when the organic carbon substrate concentration is low. Consequently, the bacterial cells die and through lysis release cell material, which consists of unbiodegradable and biodegradable components. The biodegradable fraction is first adsorbed and hydrolyzed, before being utilized by the bacteria (Randall *et al.*, 1992; Wentzel *et al.*, 1992). These observations have led to greater interest in the characteristics of wastewaters and the methods employed to characterize them.

Wastewater characteristics

Organic matter in municipal wastewater has a very complex composition. Acetate may account for 2 to 10 % of the COD and all the other organic compounds occur in concentrations that are small. As a totality however, these compounds are important for overall reaction rates and removal capacities (Henze *et al.*, 1994; Henze *et al.*, 1995). Optimal and efficient use of municipal wastewater as organic carbon sources in biological nutrient removal (BNR) systems requires a knowledge of the biodegradability of wastewater. This has led to a need to separate and to define wastewater fractions for the purposes of studying, understanding and optimizing organic carbon utilization in biological processes.

Wastewater can be characterized by physical and chemical methods into its soluble and particulate fraction. However, the division of wastewater into soluble and particulate components does not provide sufficient information on which to base process simulations for biological processes, as the observed biokinetic responses are linked to the biodegradability of the substrate present (Ekama *et al.*, 1979; Isaacs and Henze, 1995; Skrinde and Bhagat, 1982; McCarty *et al.*, 1969). The influent wastewater has been classed biologically as biodegradable, unbiodegradable, and active biomass (as COD) by the IAWQ Task Group on modelling of activated sludge processes (Wentzel *et al.*, 1995; Henze *et al.*, 1995). The biodegradable fraction is divided into the readily biodegradable (RBCOD) and slowly biodegradable (SBCOD) fractions. The readily biodegradable COD fraction is further sub-divided into an acetate fraction and fermentable COD fraction. In addition, there have been suggestions that the SBCOD be divided into the rapidly hydrolyzable (RHCOD) and slowly hydrolyzable fractions (Orhon *et al.*, 1997). The division of the RBCOD and SBCOD are made entirely on the biokinetic response of bacteria to fractions of different biodegradability in the wastewater.

Since physico-chemical methods are more rapid and easier to conduct, several studies have been done with the objective of finding a physical and/or chemical method comparable to the biokinetic response of activated sludge to the RBCOD and SBCOD fractions (Wentzel *et al*., 1995; Torrijos *et al*., 1994; Bortone *et al*., 1994; Mamais *et al*., 1993). Different chemical and physical separation methods affect the size distribution of organics in a given wastewater. Therefore, care must be taken in choosing a fractionation method (Henze and Harremoes, 1990; Mamais *et al*., 1993; Pouet and Grasmick, 1994). Dold *et al*. (1986) found that membranes with a molecular weight threshold of < 10 000 daltons gave RBCOD values comparable to that derived from biological methods. However, Bortone *et al*. (1994) showed that this comparability did not apply to industrial wastewater. Mamais *et al*. (1993) showed that the application of a coagulation method combined with filtration gave comparable results to biologically determined RBCOD fractions if the readily biodegradable fraction is considered to consist of a truly soluble fraction and a truly soluble inert fraction. However, this approach does not consider the possibility of soluble readily hydrolyzable COD (RHCOD) *i.e.* this method does not distinguish between the state of biodegradability (readily biodegradable or rapidly hydrolyzable) (Orhon and Çokgör, 1997).

To date several biological methods such as oxygen utilization rate (OUR) and nitrate-N utilization rate (NUR) (continuous and batch) tests have been employed successfully to determine the readily biodegradable fractions. Although the potential of the NUR method is recognized, the use of the method in studies has been largely neglected since it is more time-consuming and tedious than the OUR method. The NUR test is also often referred to as the anoxic batch test and is similar to that of the aerobic batch test (oxygen utilization rate-OUR) method. In the anoxic batch test, the nitrate concentration will initially decrease at a constant rapid rate reflecting the utilization of the readily biodegradable fraction (RBCOD) from the wastewater. This initial rapid rate is analogous to the initial high OUR in aerobic batch systems. The decrease in nitrate is linear when the substrate is in excess. Once the RBCOD from the influent is depleted, the denitrification rate is reduced to the rate of utilization of RBCOD generated by hydrolysis of complex molecules and particulate material. This second rate is analogous to the second OUR plateau in the aerobic batch test.

One of the major points of contention of the NUR method is the choice of yield coefficient, Y_H (mg COD / mg biomass as COD). Currently, the aerobic yield coefficient of 0.63 (mgO_2/mgO_2) is also used for anoxic conditions. However, recent work by Sozen *et al*. (1998), Sperandio *et al*. (1997) and Çogkör *et al*. (1998) have highlighted the need to use a lower yield coefficient for anoxic reactions. An anoxic yield coefficient of 0.5 (mgO_2/mgO_2) has been cited for acetate while a range of values from 0.5 to 0.61 (mgO_2/mgO_2) have been cited for domestic wastewater. Another factor which influences the biological characterization of wastewater by the NUR method is the presence of polyphosphate accumulating (Poly-P / bio-P) bacteria. The role of these organisms in enhanced biologically phosphorus removal (EBPR) systems has been well discussed (Wentzel *et al*., 1992 ; Mino *et al*., 1998). These organisms are known to take up volatile fatty acids (VFA's) for polyhydroxyalkanoates (PHA) synthesis with simultaneous phosphorus release (Hascoet and Florentz, 1985; Mostert *et al*., 1988; Gerber *et al*., 1986 and Wentzel *et al*., 1992). Anoxic polyphosphate accumulating organism activity is an important factor to consider when characterizing the wastewater according to the NUR method. This aspect will be discussed further in **Chapter 2**, section 2.4.4.3.

Finally, the treatment of wastewater has evolved significantly and the inclusion of more biological processes for wastewater treatment have increased the complexity of current wastewater treatment systems which has subsequently led to the development of more complex mathematical models. The accurate simulation of these processes requires accurate input data. Thus, the knowledge of the wastewater characteristics is an important step towards the successful modelling, design and operation of present and future plants.

1.2 . OBJECTIVES

The primary aims of this project were as follows:

- Since the NUR method has not been extensively evaluated as a respirometric method, the primary aim was to comprehensively evaluate the NUR batch test protocol and apply it to a range of wastewater and sludge samples with the aim to : (1) assess, (2) understand, and (3) make recommendations which could improve the procedure and make it more easily applicable on-site.

- Characterize raw wastewater by the NUR test *i.e.* data acquisition from different wastewaters with differing characteristics. In addition, physico-chemical methods such as filtration and coagulation were used to compare and understand the relationship between biologically and physico-chemically derived fractions.

- Study the utilization of an experimental readily biodegradable COD substrate, acetate, under anoxic conditions.

- Perform exploratory investigations to :

    - *clarify the impact of EBPR sludges on wastewater characterization*

    - *investigate how storage of sludge and wastewater samples can impact on the wastewater characteristics*

    - *assess the influence of sludge acclimatization on the accuracy of the NUR tests*

1.3 . REPORT OUTLINE

This report entitled *Municipal Wastewater Characterization: Application of denitrification batch tests* is divided into 7 chapters. A schematic representation is provided in Figure 1-2.

Chapter 1 gives a brief introduction to the field of wastewater treatment, and in particular the importance of substrate biodegradability on the efficiency of the denitrification process, wastewater characterization and the method that was applied to characterize the wastewaters sampled for this study. The major objectives of this study have also been outlined.

Chapter 2 focuses on the literature of wastewater characterization and denitrification. The review endeavours to understand the mechanisms, the process and some of the factors which may influence the organisms capable of denitrification. This chapter also deals with wastewater characteristics and the divisions as well as the methods which may be used to determine the wastewater fractions. Since the objective of this project was to use the NUR method to study the biological fractions, a more comprehensive review of the NUR method is provided.

The experimental approach and methodology are discussed in ***Chapter 3***. This chapter deals with the NUR protocol, assessing the method and the changes made to the original method outlined by Ekama *et al.* (1986). The results from the NUR tests are presented and discussed in ***Chapter 4***, ***Chapter 5***, and ***Chapter 6***. ***Chapter 4*** deals with the utilization of an experimental substrate, acetate, under anoxic conditions. ***Chapter 5*** presents and discusses the results and trends in the wastewater characteristics of numerous different wastewater treatment plants. The trends and correlations of the maximum, second and third specific denitrification rates (k_2 and k_3) are also discussed. ***Chapter 6*** deals with secondary experiments that were done to investigate the influence of several factors on wastewater characteristics using the NUR method. These include the use of unacclimatized sludge samples or stored wastewater samples for characterization tests and the impact of using enhanced biological phosphorus removal sludge samples for wastewater characterization. The conclusions and recommendations are discussed in ***Chapter 7***.

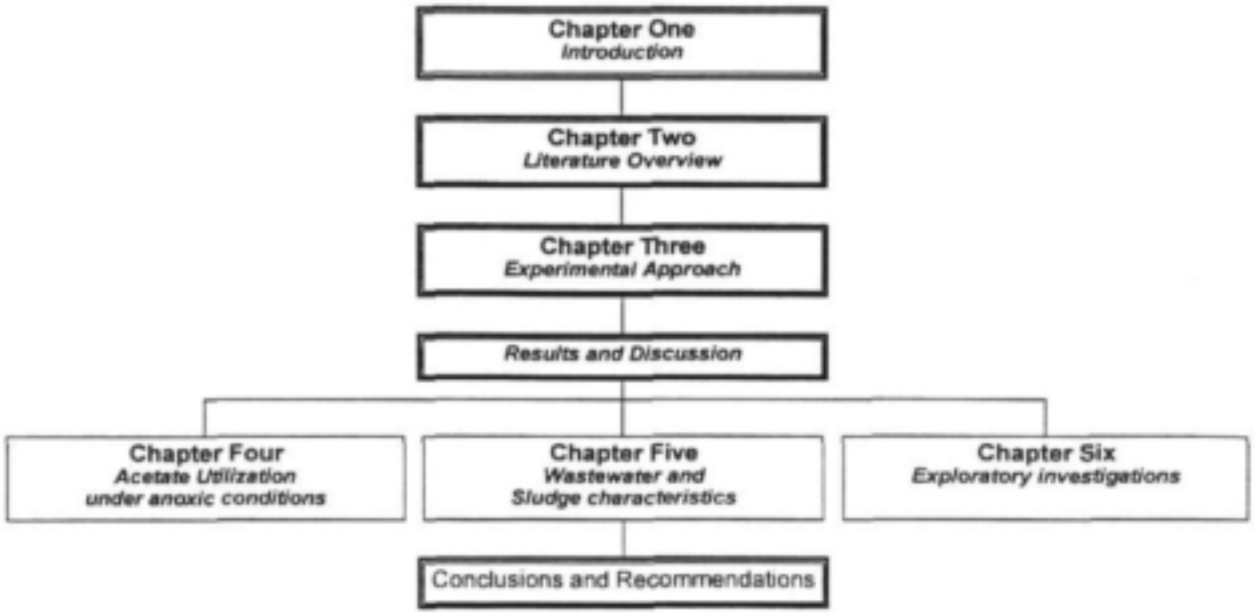


Figure 1-2: Schematic representation of report chapters.

Chapter Two

Denitrification and Wastewater Characterization : An Overview

This chapter is divided into 3 sections, the first of which deals with the process of denitrification (also referred to as anoxic respiration). It contains a generalized description of denitrification and the biochemical reactions involved when nitrate or nitrites act as the final electron acceptor. Since this project investigates the utilization of organic carbon under anoxic conditions, it will concentrate mainly on the heterotrophic denitrifying biomass found in activated sludge systems of wastewater treatment plants. This review also looks the current characterization of municipal wastewaters and some of the methods employed for the determination of these fractions which are used in modelling and simulation studies of wastewater treatment processes. In particular, attention has been paid to the readily biodegradable and slowly biodegradable COD fractions, and the nitrate-N utilization rate method.

2.1. DENITRIFICATION

Denitrification is just one pathway in the nitrogen cycle. It is a biochemical reaction (equation 2-1) effected by microorganisms which transform nitrates/nitrites to the gaseous form, nitrogen. This reaction couples the transport of electrons by the respiratory chain to energy production via oxidative phosphorylation (Knowles, 1972 ; Payne, 1981).

$$2NO_3^{-\ (+5)} \rightarrow 2NO_2^{-\ (+3)} \rightarrow 2NO_{(g)}^{\ (+2)} \rightarrow N_2O_{(g)}^{\ (+1)} \rightarrow N_{2(g)}^{\ (0)} \qquad (2\text{-}1)$$

where : (#) the values in the brackets refer to the oxidation states of the nitrogen atom for each nitrogenous compound.

(g) denotes gaseous species.

2.1.1 Microbiology

Heterotrophs obtain their energy and carbon requirements from the transformation or breakdown of organic carbon substrates. This is termed metabolism which can be divided into anabolism and catabolism. The former is the enzymatic biosynthesis of complex cellular materials of the organism. Catabolism is the enzymatic degradation of complex organic molecules to smaller ones. The organic molecules serve as electron donors and the electrons removed are transferred through a sequence of processes to the terminal electron acceptor. During this process chemical energy is released in the form of the energy rich molecule called adenosine triphosphate (ATP). This chemical energy is used by the organism for the growth part of metabolism (*i.e.* synthesis of new cell material, anabolism) and maintenance of, for example, cellular functions (*i.e.* catabolism). Catabolism includes both respiration (aerobic and anoxic) and fermentation. This review will concentrate largely on anoxic respiration (denitrifying) processes.

The *denitrifiers* can be subdivided into those organisms that are capable of the complete dissimilatory nitrate reduction process *(i.e.* nitrate to nitrogen gas) and those microorganisms that can carry out one or more of the reaction steps (*i.e.* nitrate to nitrite or nitrite to nitrous oxide) (Henze, 1992). For example, some *Bacillus* species are capable of nitrate and nitric oxide reduction but are unable to reduce nitrites or nitrous oxide, while some species of *Pseudomonas* have been shown to accumulate nitrous oxide as a terminal product instead of dinitrogen and initiate denitrification with nitrites rather than nitrate (Payne, 1981 ; Randall *et al.*, 1992). These microorganisms are termed *partial denitrifiers*. Those microorganisms that are only able to reduce nitrates to nitrites are termed *nitrate reducers*. Thus, within the heterotrophic biomass in activated sludge system there are different proportions of these microorganisms present (Table 2-1). This may explain the accumulation of the intermediate nitrite in some instances since if a bacterial species *e.g. Comamonas testosteroni* is only able to reduce nitrates then nitrites will accumulate. It was also shown that some species that are capable of both nitrate and nitrite reduction still accumulate nitrites since the nitrate reduction rate is faster than the nitrite reduction rate (Betlach and Tiedje, 1981).

Table 2-1: Some examples of facultative anaerobic bacteria capable of complete and partial denitrification, and nitrate reduction (Fass, 1994).

Microorganism	**Gram stain**	**Characteristics**
Agrobacterium radiobacter	negative	Reduction of NO_3^- and NO_2^- to N_2
Agrobacterium tumefaciens	negative	Reduction of NO_3^- and NO_2^- to N_2
Comamonas testoteroni	negative	Reduction of NO_3^- to NO_2^- only
Alcaligenes faecalis	negative	Uses NO_2^- only and not NO_3^-
Cytophage johnsonae	negative	Uses NO_2^- only and not NO_3^-
Aquaspirillium itersonii	negative	Denitrification stops at N_2O
Chromobacterium violaceum	negative	Denitrification stops at N_2O
Roseobacter denitrificans	negative	Denitrification stops at N_2O
Pseudomonas fluorescens	negative	Denitrification stops at N_2O

2.1.2 Biochemistry

Aerobic and anoxic respiration by heterotrophic bacteria involves the oxidation of organic substrates like carbohydrates, proteins and lipids to end products such as CO_2 and H_2O. By the process of respiration the bacteria are able to produce energy which becomes available to the micro-organism through a series of internally mediated oxidation-reduction reactions. This involves electron and proton transfers from an organic substrate through a number of intermediate enzyme complexes to the final electron acceptor (nitrates in this study). Two types of molecules, energy transport molecules and electron and proton transport molecules, are coupled to redox reactions to produce energy. The energy transport molecules of interest are adenosine triphosphate (ATP) and guanosine triphosphate (GTP) while the electron and proton transport molecules include nicotinamide adenine dinucleotide ($NADH_2$) and flavin adenine dinucleotide ($FADH_2$) (Lehninger, 1975 ; Casey *et al.*, 1993)

Respiratory metabolism can be divided into 4 stages (Figure 2-1):

2.1.2.1 Stages in anoxic respiration

During the first stage complex organic molecules are hydrolyzed to simpler ones. Carbohydrates are degraded to sugars, proteins to amino acids, and lipids to fatty acids (Figure 2-1). In stage 2, the end products of stage 1 are degraded further to form acetyl-Coenzyme A (acetyl Co-A) and carbon dioxide. This step involves different biochemical pathways. For example, amino acid breakdown can result in the formation of Acetyl Co-A either with or without pyruvate formation. Some amino acids are not converted to acetyl Co-A but enter the third stage *i.e.* the tricarboxylic acid (TCA) cycle directly. Carbohydrate degradation can occur via a number of different pathways. The most common of which is the Embden-Meyerhof pathway which can be divided into 2 stages. The first stage (activation stage) involves the phosphorylation of simple sugars to glyceraldehyde-3-phosphate at the expense of ATP. In the second stage glyceraldehyde-3-phosphate is converted to pyruvate via a series of dehydrogenations. NADH and ATP are formed via substrate phosphorylation during this stage (Lehninger, 1975 and Casey *et al.*, 1993).

Long chain fatty acids are hydrolyzed by β-oxidation to volatile fatty acids (VFA's) which are further degraded to acetyl Co-A, NADH, and $FADH_2$ at the expense of ATP (Figure 2-2). Acetate passes through the cell membrane via active transport. Once in the cell, acetate enters into the TCA cycle as acetyl Co-A. Propionate, butyrate and valerate undergo several reactions before forming acetyl Co-A. During the transformation of propionate to acetyl Co-A several intermediates such as succinate, fumarate, malate and pyruvate are formed. Butyrate is transformed to 2 moles of acetyl Co-A by β-oxidation while valerate is transformed to acetyl Co-A and propionyl Co-A. The latter product is further degraded via the same mechanism described for propionate (Fass, 1994).

Acetyl Co-A which is the final end-product of stage 2 enters the tri-carboxylic (TCA) cycle where acetyl Co-A is oxidized to form 2 molecules of CO_2, eight protons, 4 pairs of electrons and 1 guanidine tri-phosphate (GTP) (Figure 2-3). In the final stage (stage 4) the electrons and protons produced in stages 2 and 3 pass via electron and proton carrier enzymes to a final electron acceptor (*i.e.* NO_3^- in anoxic respiration). During this process adenosine tri-phosphate (ATP) is formed via oxidative phosphorylation. The complexes of the electron transport pathway are arranged within the membrane (Lehninger, 1975 and Casey *et al.*, 1993).

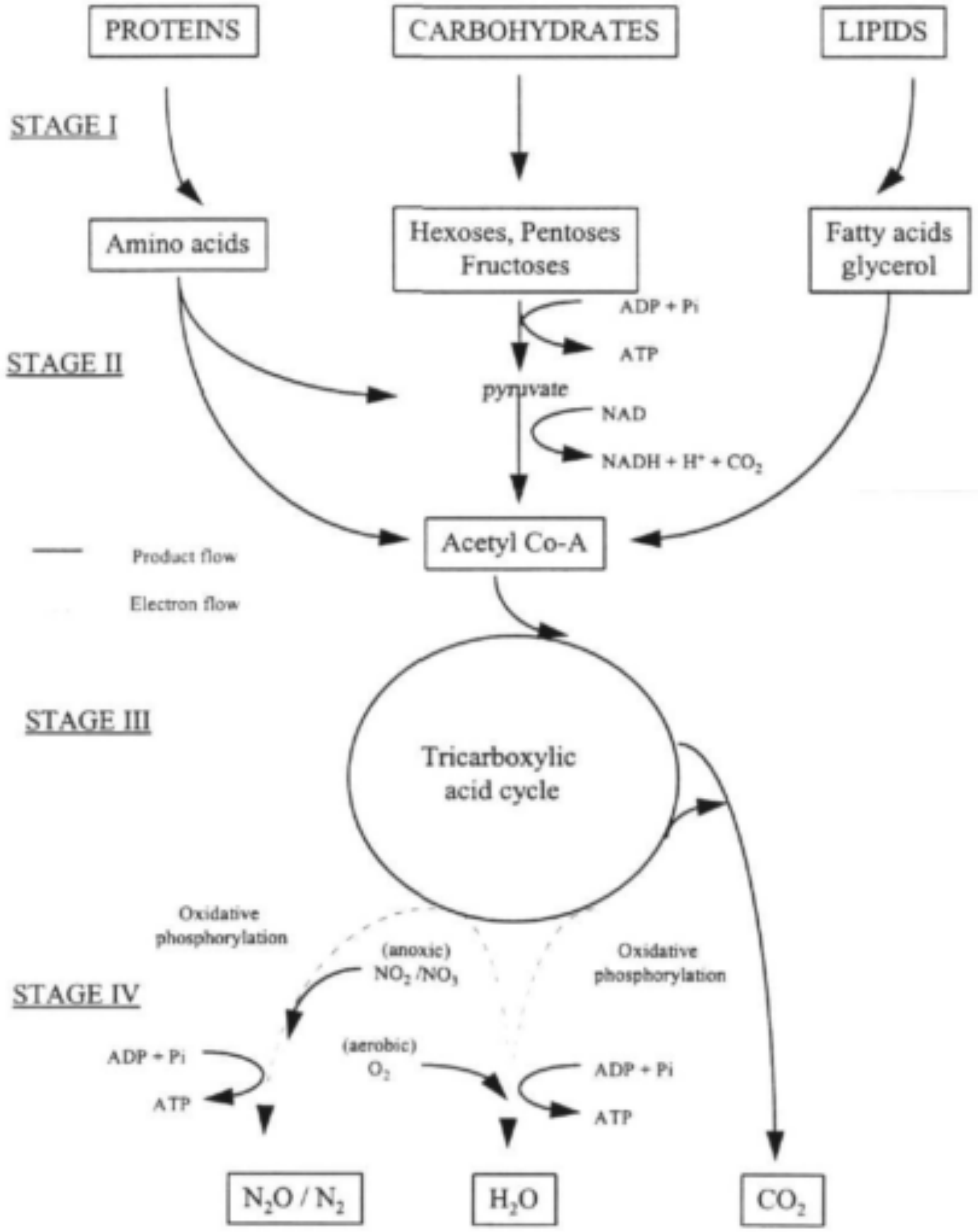


Figure 2-1: Illustration of anoxic respiratory stages showing the substrate degradation and metabolic pathways (Casey et al., 1993, adapted from Lehninger, 1975).

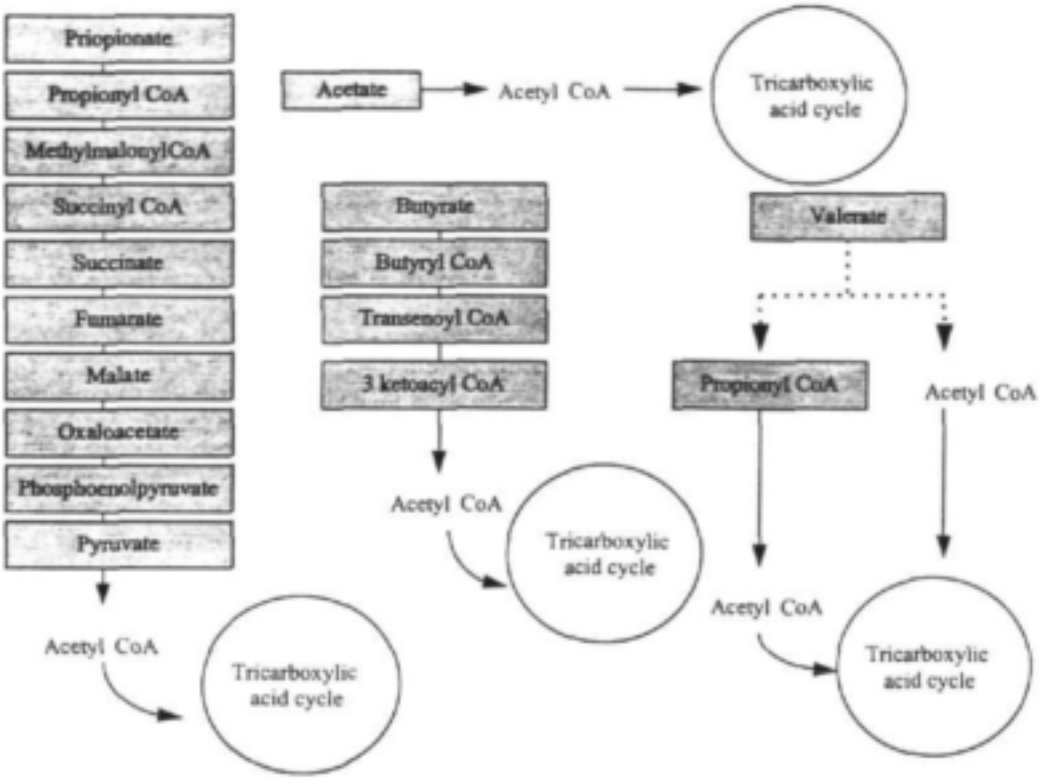


Figure 2-2: Metabolic pathways for the degradation of volatile fatty acids, acetate, propionate, butyrate and valerate to acetyl Co-A (from Fass, 1994).

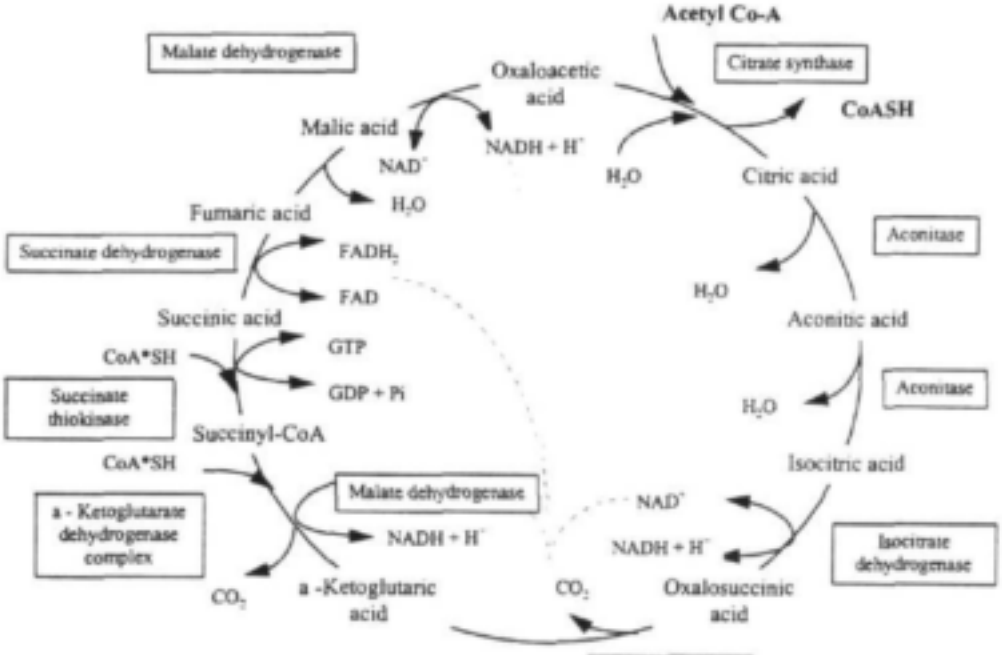


Figure 2-3: The tricarboxylic acid (TCA) cycle (from Casey et al., 1993).

2.1.2.2 Enzymes involved in denitrification

Denitrification is catalyzed by 4 enzymes which reduce nitrates to dinitrogen. This sub-section lists the enzymes and their functions. The main characteristics of these enzymes are presented in Table 2-2 (Hochstein and Tomlinson, 1988 ; Casey *et al.*, 1993, and Fass, 1994).

- *Nitrate reductases*

Nitrate reductases catalyze the reduction of nitrates to nitrites and couple this reduction to the translocation of protons. Due to the location of this enzyme (*i.e.* inside the cytoplasmic membrane) nitrate has to be translocated across the membrane. Several mechanisms have been suggested. In *Paracoccus denitrificans* nitrate uptake is thought to occur by facilitated diffusion. Two other nitrate uptake systems have been proposed : one operates in symport with protons ; while the other operates as a NO_3^- / NO_2^- antiport (Figure 2-4). The former initiates nitrate uptake in the absence of nitrite when the antiporter system is inoperative while the latter serves to maintain a low intracellular concentration of nitrite. In addition, the antiporter system provides a mechanism for export of nitrite to the location of the nitrite reductase which appears to be a periplasmic enzyme in *Paracoccus denitrificans*. It has also been suggested that the nitrate reductase complex forms a nitrate-specific channel which provides access to the active site of nitrate reductase (Boogerd *et al.*, 1983 ; Hochstein and Tomlinson, 1988; and Casey *et al.*, 1993).

- *Nitrite reductase*

Nitrite reductase reduces nitrite which originates from the bulk solution or from the reduction of nitrate to nitric oxide. The reduction of nitrite on the periplasmic side of the cytoplasmic membrane necessitates the transport of nitrite from the cytoplasm where it is formed, to the periplasm where it is reduced. This transport occurs as part of the NO_3^- / NO_2^- antiport mechanism described for the translocation of nitrate across the cytoplasmic membrane (Alefounder and Ferguson, 1982; Boogerd *et al.*, 1981; and Hochstein and Tomlinson, 1988).

- *Nitric Oxide reductase*

The function of nitric oxide reductase is to reduce nitric oxide (NO) to nitrous oxide (N_2O). However, this is the least characterized of the enzymatic steps of denitrification since nitric oxide is rarely detected during denitrification. Although this enzyme is said to occur on the periplasmic side of the membrane, there are varying opinions (Stouthamer, 1988; and Casey *et al.*, 1993).

- *Nitrous Oxide reductase*

This enzyme reduces nitrous oxide (N_2O) to dinitrogen (N_2) which is released from the cell. This enzyme is associated with the periplasmic side of the membrane and is a soluble copper containing enzyme which is considered to be between 80 and 145 kD (Boogerd *et al.*, 1981, Casey *et al.*, 1993; and Fass, 1994).

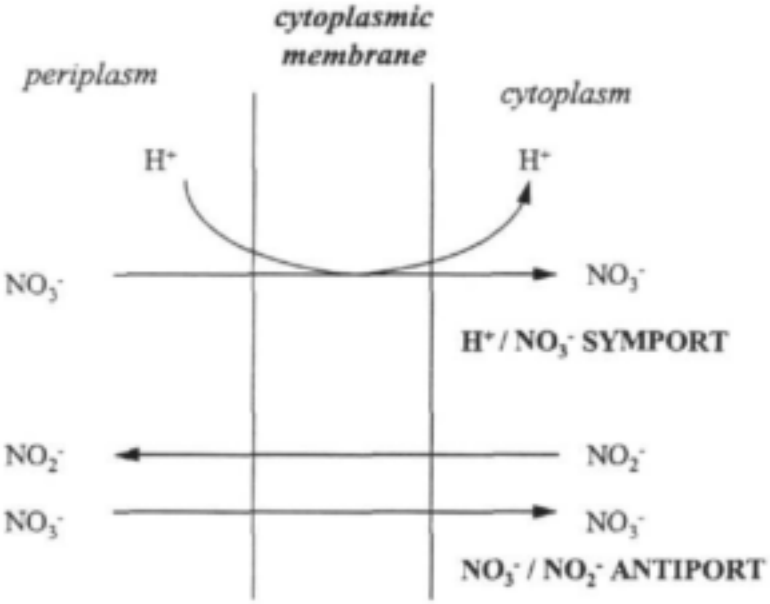


Figure 2-4: Mechanism of nitrate transport systems across the cytoplasmic membrane (Casey et al., 1993, redrawn from Stouthamer, 1988).

Table 2-2: Important characteristics of the four reductase enzymes involved in transforming nitrates to nitrogen gas (Fass, 1994).

Enzyme	**Nitrate reductase**	**Nitrite reductase**	**Nitric reductase**	**Nitrous reductase**
Reaction	$NO_3^- \rightarrow NO_2^-$	$NO_2^- \rightarrow NO$	$NO \rightarrow 0.5N_2O$	$0.5N_2O \rightarrow 0.5N_2$
e^{-s} transferred	2	1	1	1
Location	cytoplasm	periplasm	periplasm	periplasm
Composition	Mo, Fe, S	protein + Cu hemeprotein + cyt c-d	Cytochrome b+c	Soluble + Cu
Molecular Mass (kD)	100 to 200	70 to 150 90 to 140	< 55	80 to 145

(Mo - molybdenum ; Fe - iron ; S - sulphur ; Cu - copper ; e^{-s} - electrons ; kD - kilodaltons)

2.1.3 Factors influencing denitrification

This section deals briefly with four factors that influence heterotrophic denitrification. These include oxygen, temperature, pH and organic carbon availability and type.

2.1.3.1 Oxygen

The presence of dissolved oxygen (DO) acts as a strong inhibitor on denitrification as it prevents the expression of the necessary enzymes for the electron transfer (Van Haandel *et al.*, 1981; Karnaros and Lyberatos, 1998). It was shown for *Pseudomonas denitrificans* that nitrate reduction was the least sensitive while the reduction of N_2O and / or NO was almost completely inhibited by dissolved oxygen (Karnaros and Lyberatos, 1998). The inhibition is reversible once the oxygen concentration decreases (Fass, 1994). It has also been observed that denitrification is possible at DO concentrations as high as 6 mg/l. However, studies have shown that an increase in DO from 0.2 to 2.0 mg/l results in a decrease in denitrification rates from 50 to 10 % of the anoxic values. Thus, although denitrification is possible in the presence of low concentrations of dissolved oxygen, it is not beneficial to the denitrification process. It is suggested that in suspended cultures the dissolved oxygen concentration should be below 0.5 mg/l to prevent the preferential utilization of dissolved oxygen as an electron acceptor. This difference in dissolved oxygen levels for denitrification could be due to varying techniques for measurement of DO, and by the fact that the measured bulk liquid dissolved oxygen concentration does not represent the actual DO concentration within the sludge floc. Most researchers agree, however, that if the micro-environment is anoxic then denitrification will proceed even if the bulk solution (*i.e.* the macro-environment) contains detectable dissolved oxygen concentrations (Skrinde *et al.*, 1982 ; Abufayed, 1983 ; Randall *et al.*, 1992).

2.1.3.2 Temperature

The denitrification rate is a function of temperature and is described by a bell-shaped curve, *i.e.* the denitrification rate increases with an increase in temperature, reaches a maximum and then drops when the temperature is increased further (Abufayed, 1983). Since reaction rate is usually evaluated at 20°C, Lewandowski (1982) and Metcalf and Eddy (1991) expressed the relationship between measured values and the reaction rate at 20°C with equation 2-2. The denitrification process can also occur thermophilically at 50 to 60 °C. In this case the nitrate removal rate is approximately 50 % greater than at the mesophilic range of 35 °C (Henze *et al.*, 1997).

$$R_T = R_{20}\,\theta^{T-20} \qquad (2\text{-}2)$$

R_T = denitrification rate at temperature T (°C)

R_{20} = denitrification rate at temperature 20 °C

θ = temperature coefficient

2.1.3.3 pH

The same general dependency is exhibited by pH as was discussed for temperature *i.e.* bell shape. Various pH optima (7.0, 7.4, 7.5, 7.6) and pH ranges have been cited in the literature (Dodd and Bone, 1975 ; Christensen and Harremoes, 1977 ; Wang *et al.*, 1995 ; and Urbain *et al.*, 1997). Batch studies conducted by Dodd and Bone in 1975 at pH values of 7.0 ; 7.5 ; 8.0 and 8.5 showed that denitrification occurred optimally at pH 7.5. They also reported that the activity of nitrite reductase diminishes quicker when the pH value rises above the optimum. Thus, at a pH of 8.5, nitrite was found to accumulate. A similar observation was made by Urbain *et al.* in 1997. Wang *et al.* (1995) showed that cultures of *Ps. denitrificans*, grown at 30 °C reduced nitrate optimally at a pH between 7.4 and 7.6 and nitrite at a pH between 7.2 and 7.3. A pH of 7.5 is generally used for denitrification studies since it has been shown that for a pH above 7.3, N_2 gas is the end product. At a pH below 7.3 nitrous oxide occurs as an end product while for a pH below 5.0 nitric oxide can account for approximately 20 % of the total gas produced (Christensen and Harremoes, 1977). Furthermore, Urbain *et al.* (1997) found that biomass adapted to non optimum pH values (*i.e.* 7 > pH > 8.5) gave better denitrification rates than the non-adapted biomass (Figure 2-5). These results showed that bacteria have the ability to adapt with time to a non-optimum pH.

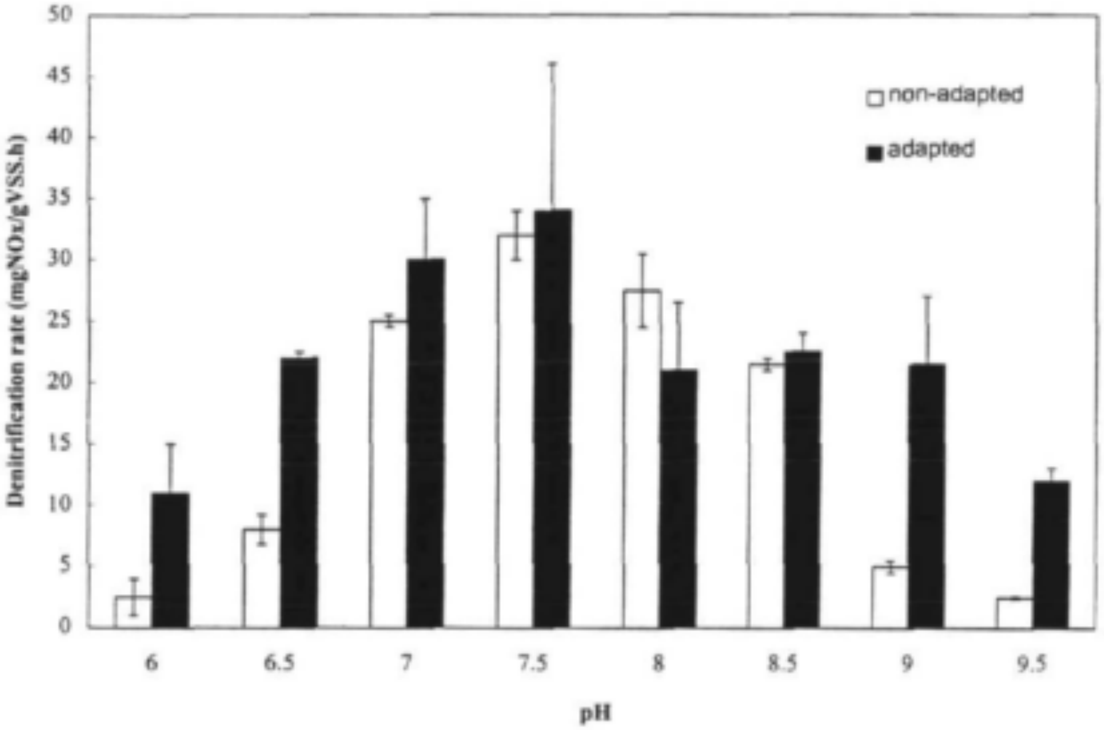


Figure 2-5: Influence of pH on specific maximum denitrification rates for adapted and non-adapted biomass (Urbain et al., 1997).

During denitrification a pH increase is expected. However, the magnitude of the increase is dependent on the buffering capacity of the wastewater (Christensen and Harremoes, 1977). The control of pH is also important if complete denitrification is to occur. Therefore, optimization of the denitrification kinetics should require pH regulation between 7.0 and 9.0 (Henze *et al.*, 1997).

2.1.3.4 Organic carbon substrates

Several studies have highlighted that the biodegradability of substrates strongly influences denitrification rates (McCarty *et al.*, 1969 ; Monteith *et al.*, 1980 ; Isaacs and Henze, 1995 ; Henze and Harremoes, 1990). In municipal wastewater, the substrates have been divided loosely into 2 groups : a readily biodegradable and a slowly biodegradable COD fraction (Ekama *et al.*, 1979). The second slower denitrification rate appears to be limited by the hydrolysis rate rather than the rate of metabolism (Wentzel *et al.*, 1992). Henze *et al.* (1994) stated that the dominating rate limiting factor in nutrient removal processes is the organic carbon source, in other words, the rate of hydrolysis of higher molecular weight compounds to readily biodegradable compounds will limit the denitrification rate while the addition of readily biodegradable carbon to a carbon limited sludge will speed up the denitrification rate. However, once the readily biodegradable carbon material (hydrolyzate) is utilized the denitrification rate falls back to the rate limited by the rate of utilization of the endogenous respiration products which are subsequently adsorbed, hydrolyzed and utilized by the bacteria in the sludge floc when the exogenous substrates become limiting (Randall *et al.*, 1992 ; Wentzel *et al.*, 1992). This type of energy source for denitrification in which cell death and lysis occurs, was first proposed by Wuhrmann in 1964 who reported that by the time the influent carbon source reached the anoxic zone all the readily biodegradable substrate had been exhausted. The remaining carbon source available to the anoxic reactor consisted of slowly biodegradable organic carbon and therefore, the denitrification rates were relatively low (Van Haandel *et al.*, 1981).

2.1.3.4.1 Internal Carbon Sources

Initially the trend was to use industrial or agricultural wastes as external carbon sources for denitrification (McCarty *et al.*, 1969 ; Skrinde and Bhagat, 1982). The cost involved in such schemes, however, has led to more studies being conducted on the potential of internal carbon sources within wastewater treatment works for enhancement of denitrification rates. An organic carbon source is defined as internal when present or derived from the influent wastewater.

The concept of using hydrolyzed sludge (hydrolyzate) in denitrifying systems was introduced to use internal carbon sources more efficiently. In 1986, Abufayed and Schroeder reported that under non-carbon limiting (COD/N > 6.3) conditions, hydrolyzed primary sludge in a sequencing batch reactor system could be used for complete nitrogen removal. The same concept was applied in the HYPRO (hydrolysis process) project which was developed through the collaboration between research institutions and companies from Denmark, Sweden and Norway to attempt to solve the problem of carbon limitation in denitrification systems. It is based on pre-precipitation of the organic matter, sludge hydrolysis and biological nutrient removal. Biological, chemical and physical (thermal) hydrolysis techniques were investigated as a means of improving the bioavailability of the organic carbon (Henze and Harremoes,1990 ; Smith and Goransson, 1992 ; Æsoy and Ødegaard, 1994).

A comparative study between the organic carbon sources, acetate and hydrolyzate, obtained in the HYPRO project was conducted by Isaacs and Henze (1995). They investigated the controlled addition of these carbon sources to batch reactors, under carbon limiting and non-carbon limiting conditions. At COD/N ratios less than

1.86 the denitrification rate was dependent on the COD (acetate) concentration. At a COD/N ratio of 7.5, however, acetate was well in excess of that required to denitrify all the nitrate present. Under carbon limiting conditions denitrification rates of 1.8 to 1.9 mgN/gVSS.h were obtained and a rate of 3.4 mgN/gVSS.h was obtained when carbon was in excess.

Hydrolyzate as a carbon source gave similar results to those observed for acetate. At COD/N ratios greater than 5.4, fast initial denitrification rates of ca. 2.4 mgN/gVSS.h were produced. These rates were followed by slow rates of ca. 0.6 mgN/gVSS.h, respectively, which compared favourably with the rate of 0.7 mgN/gVSS.h for endogenous denitrification. Two distinct phases were observed when acetate was limiting while non-limiting conditions produced a single linear phase. For hydrolyzate, however, return to the original denitrification rate occurred more gradually. It was suggested that the latter effect was owing to the fact that hydrolyzate is more complex than acetate and is composed of carbon compounds of varying degrees of biodegradability. Similar trends were reported for acetate and hydrolyzate in an alternating nitrification-denitrification pilot-scale system (Isaacs and Henze, 1995).

Table 2-3 compares the different denitrification rates at 20°C obtained under varying operating conditions. The literature cites several more examples of denitrification rates. However, the conditions under which they were calculated are either not listed or not fully described. Table 2-3 also shows that there is a variation in denitrification rates even if the substrates are the same, which is probably due to differences in biomass activity and operating conditions of the various reactors. It should be noted that the rates represented here are not as a function of active biomass but are expressed in terms of mgN/gVSS.h. Therefore, these kinetic rates fall outside the paradigm of wastewater modelling.

2.1.3.4.2 External Carbon Sources

Monteith *et al.* (1980) tested several industrial wastes as organic carbon sources. They found that some organic wastes such as formaldehyde and dextrose waste were less efficiently degraded than distillery oils or methanol. Tam *et al.* (1992) used three external carbon sources (methanol, glucose and acetate) in SBR systems. It was shown that acetate was the most effective (98 % NO_x-N removal) and produced higher removal rates than methanol or glucose (Tam *et al.*, 1992). These results correlate with Gerber *et al.* (1986) who reported that compounds such as acetate, propionate, butyrate and lactate consistently produced higher denitrification rates than methanol, glucose or citrate.

Tam *et al.* (1992) concluded that the results could be explained biochemically. The glycolytic pathway and tri-carboxylic 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. Therefore, sodium acetate is a directly utilisable substrate which is more readily metabolizable than methanol or glucose. Sodium acetate enters the pathways directly while methanol must undergo a condensation process to form 3-C or 4-C intermediates before entering the TCA cycle.

Table 2-3 : Denitrification rates (k_1) at 20 °C obtained with activated sludge fed with different organic carbon sources and at different COD/N ratios.

carbon source	reactor type	COD/N	k_1 (mgN/gVSS.h)	Reference
Raw wastewater	continuous	2	1.50 to 2.10	Hoffmann and Klute (1990)
Carbon from BH	continuous	2	4.90 to 7.50	Hoffmann and Klute (1990)
Carbon from BH/PA	continuous	2	6.10 to 7.30	Hoffmann and Klute (1990)
Carbon from CH	continuous	2	3.90 to 5.70	Hoffmann and Klute (1990)
Hydrolyzate	continuous	4 to 5	2.65	Isaacs and Henze (1995)
Acetate	continuous	6 to 7	3.09 to 3.53	Isaacs and Henze (1995)
Acetate	continuous	5	3.20	Karlsson (1990)
Acetate	SBR	3	7.95	Tam *et al.* (1992)
Acetate	SBR	6	10.60	Tam *et al.* (1992)
RBCOD	batch	11	10.40	Carucci *et al.* (1996)
RBCOD	batch	3.7	4.20	Carucci *et al.* (1996)
Acetate	batch	0.8	2.20	Isaacs and Henze (1995)
Acetate	batch	1.9	2.08	Isaacs and Henze (1995)
Acetate	batch	7.5	3.94	Isaacs and Henze (1995)
Hydrolyzate	batch	1.3	0.67	Isaacs and Henze (1995)
Hydrolyzate	batch	2.6	2.78	Isaacs and Henze (1995)
Hydrolyzate	batch	5.2	1.97	Isaacs and Henze (1995)
Hydrolyzate	batch	10.4	3.09	Isaacs and Henze (1995)

BH - biological hydrolysis ; PA - post alkalinization ; CH - chemical hydrolysis

(Denitrification rates at 20°C are calculated from equation 2-4 using a θ value of 1.04).

2.1.3.5 The P release/uptake phenomenon in activated sludge systems

Rapid removal of nitrates from solution in wastewaters is governed to a large extent by the concentration and type of biodegradable organic carbon substrate that is made available to the denitrifiying bacteria. Phosphorus removing bacteria in wastewater systems also rapidly take up readily biodegradable organic carbon substrates. Thus, phosphorus removing bacteria and denitrifiers will compete for the available readily biodegradable organic carbon present. If complete denitrification is the primary aim of a wastewater treatment system, then the presence of phosphorus removing bacteria can have a major impact on removal rates. The following sub-section will briefly discuss the process of biological phosphorus removal in activated sludge systems.

Biologically enriched phosphorus (P) removal has been well documented (Wentzel *et al.*, 1985 ; Wentzel *et al.*, 1989a ; Wentzel *et al.*, 1989b and Wentzel *et al.*, 1992 ; Kerrn-Jespersen and Henze, 1993 ; Mino *et al.*, 1998 ; Brdjanovic *et al.*, 1998a,b,c,d ; Meinhold *et al.*, 1999). Biological phosphorus removal from wastewater can be achieved by stoichiometric coupling to microbial growth or enhanced storage in the biomass as polyphosphate (Van Loosdrecht *et al.*, 1997b). In the anaerobic phase, biological phosphorus removing bacteria take up carbon sources (short chained fatty acids) and store them in the form polyhydroxyalkanoates (PHA). The energy required is generated by the conversion of glycogen and polyphosphate. The degradation of polyphosphate results in its release into the bulk solution (Figure 2-6). In the subsequent aerobic or anoxic phase the internal pool of polyhydroxyalkanoates is oxidized and used for growth, phosphate uptake, glycogen synthesis and maintenance (Figure 2-7) (Van Loosdrecht *et al.*, 1997a ; Brdjanovic *et al.*, 1998a). Thus, in an enhanced biological phosphorus removal (EBPR) system, the behaviour of the 3 storage pools viz: PHA, poly-P, and glycogen, in cells is highly dynamic and is determined by their conversion during the anaerobic and aerobic (or anoxic) phase (Brdjanovic *et al.*, 1998b).

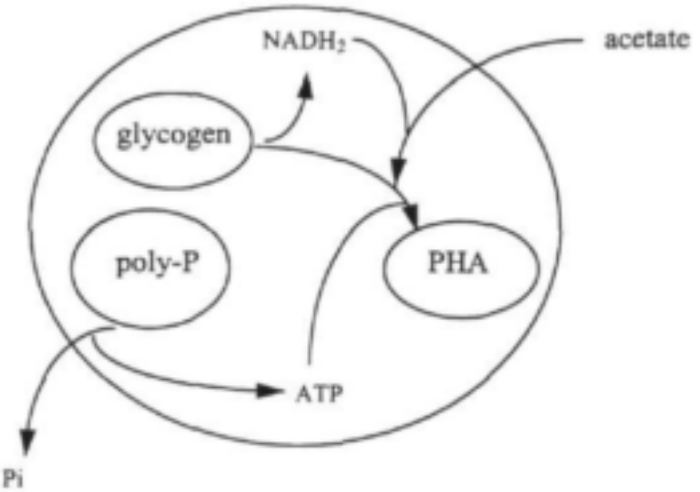


Figure 2-6 : Metabolic processes of polyphosphate accumulating organisms involved in anaerobic phase of phosphorus removal systems (poly-P - polyphosphate ; PHA - polyhydroxyalkanoate) (from Van Loosdrecht et al., 1997).

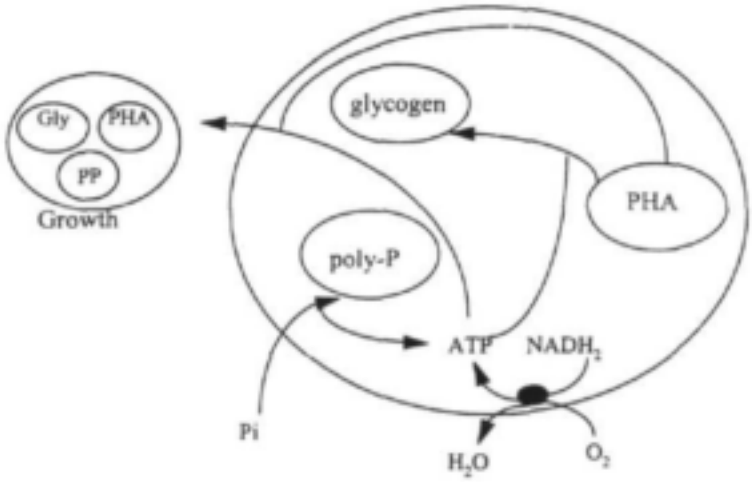


Figure 2-7 : Metabolic processes of poluphosphate accumulating organisms involved in anaerobic/aerobic phosphorus removal (Gly - glycogen ; PP - polyphosphate ; PHA - polyhydroxyalkanote) (from Van Loosdrecht et al., 1997).

2.1.3.5.1 Polyhydroxyalkanoates (PHA)

Initially, polyhydroxybutyrate (PHB) was recognized as the storage polymer in the anaerobic phase (Clayton *et al.*, 1991 ; Wentzel *et al.*, 1995). It was later verified that the PHB-like polymer contains 3-hydroxybutyrate (3HB) and 3-hydroxyvalerate (3HV) as monomeric building units. These polymers are now referred to as polyhydroxyalkanoates (PHA) in general. Polyhydroxyalkanoates have been verified to be co-polymers composed of these 4 units. When acetate is the sole substrate, then 3HB is the major unit in the PHA formed (Barker and Dold, 1997 ; Shuler and Jenkins, 1997 ; Mino *et al.*, 1998).

Polyhydroxyalkanoate is a more reduced compound than acetate, therefore the conversion of acetate, a favourable substrate for enhanced biological phosphorus removal, to PHA requires reducing power. Two possibilities exist for the generation of this reducing power in bacterial cells. In the Comeau-Wentzel model it is suggested that the required reducing power is produced by partial oxidation of acetyl CoA through the tri-carboxylic acid cycle. In the Mino model the reducing power is considered to be derived from degradation of intracellularly stored glycogen (Satoh *et al.*, 1992 ; Van Loosdrecht *et al.*, 1997a ; Mino *et al.*, 1998). Several of the experimental results seem to support the Mino model :

- The theoretically developed stoichiometry for the Mino model adequately explains the experimentally observed stoichiometry of anaerobic acetate uptake, polyhydroxyalkanoate formation, glycogen utilization and carbon dioxide (CO_2) production (Mino *et al.*, 1998).

- Bordacs and Chiesa (1989) used radioactively labelled acetate and showed that only a very small portion of the radioactivity was found in the CO_2 generated under anaerobic conditions. This indicated that the acetate taken up was not metabolized through the tri-carboxylic acid cycle.

- Pereira *et al.*, (1996) demonstrated that acetate taken up anaerobically was converted to PHA which is subsequently converted to glycogen in the aerobic phase which further supplies the carbon source for PHA formation and CO_2 generation in the anaerobic phase.

However, it is also likely that there may be a partial functioning of the tricarboxylic acid cycle. Bordacs and Chiesa (1989) and Pereira *et al.*, (1996) found that a small fraction of the labelled carbon in acetate was released as CO_2. Based on redox balance considerations, Pereira *et al.*, (1996) concluded that the reducing power generated in the observed degradation of glycogen was insufficient to account for the polyhydroxyalkanoate production. These are strong indications that a small fraction of acetate is metabolized through the tricarboxylic acid cycle under anaerobic conditions supplying a minor part of the reducing power for polyhydroxyalkanoate formation.

The anaerobic polyhydroxyalkanoate production is dependent on substrate loading while the aerobic polyhydroxyalkanoate consumption depends on the PHA level inside the biomass. The polyhydroxyalkanoates that are not used will accumulate in the cell until a saturation level is reached. Once this level is reached no further acetate uptake will occur under anaerobic conditions (Brdjanovic *et al.*, 1998d ; Meinhold *et al.*, 1999). In biological phosphorus removal systems the aerobic solids retention time (SRT) should be long enough to oxidize the amount of polyhydroxyalkanoate stored in the cell during the anaerobic phase. Thus, the minimally required solids retention time depends on the polyhydroxyalkanoate conversion kinetics and the cell PHA storage capacity. It was also found that the doubling of the SRT from 8 to 16 d at 10 °C strongly increased the content of storage polymers in the biomass (Brdjanovic *et al.*, 1998c). Brdjanovic *et al.* (1998d) developed a model that was able to adequately predict the minimally required SRT in a sequencing batch reactor system.

It was also shown that the PHA consumption was strongly influenced by temperature during long term experiments *i.e.* microorganisms exposed to a change in temperature for a relatively long time (couple of weeks). It was concluded that temperature impact on the stoichiometry and phosphorus (P) uptake process rate was marginal. However, a strong temperature effect on metabolic processes such as PHA consumption and growth was observed *i.e.* it was observed that the conversion rate of storage polymers decreased with a decrease in temperature (Brdjanovic *et al.*, 1998c).

2.1.3.5.2 Polyphosphate (Poly-P)

Under anaerobic conditions energy is required for transport of external substrates into the cell, conversion of substrates to PHA and related metabolism, and maintenance. Poly-P is considered to be the energy storage polymer for anaerobic substrate uptake. As mentioned previously, during the anaerobic phase short chained fatty acids (like acetate) are taken up by the bacterial cells with a concommitant release of phosphorus into the bulk liquid (Figure 2-8). The appearance of phosphorus in the bulk liquid is as a result of the degradation of internal reserves of polyphosphates to provide the energy necessary for production of storage compounds like polyhydroxyalkanoates. It should be noted that phosphorus release is not limited to the anaerobic phase and has also been observed in the aerobic phase when acetate was present. However, phosphorus release in the aerobic zone could lead to a deterioration in overall efficiency of the EBPR system (Brdjanovic *et al.*, (1998b). The cells internal poly-P supplies are replenished during the aerobic phase by taking up phosphorus (i.e. phosphorus uptake) from the bulk liquid (Figure 2-8) (Sorm *et al.*, 1997 ; Mino *et al.*, 1998).

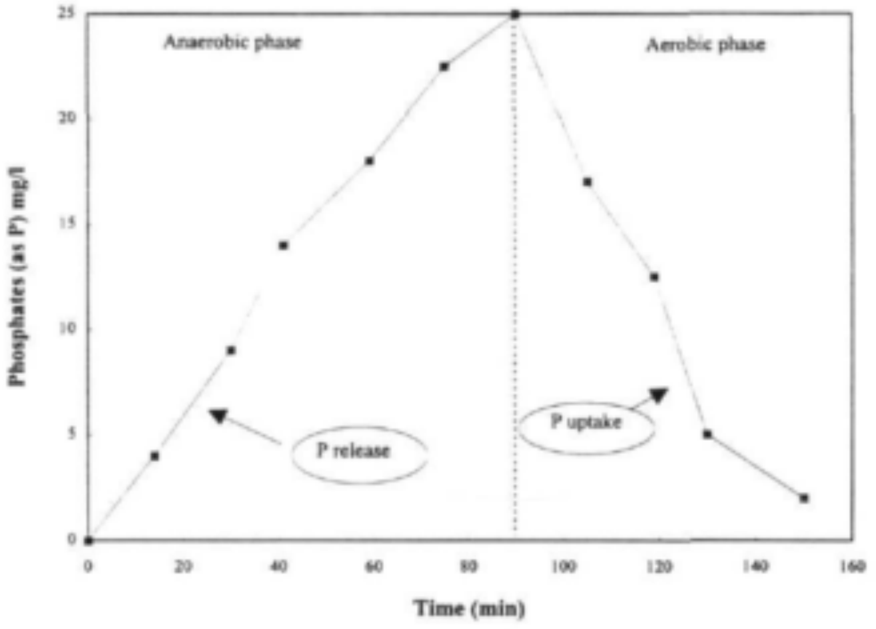


Figure 2-8 : The course of ortho-phosphate concentration under anaerobic and aerobic conditions during experiments using the Dephanox process (adapted from Sorm et al., 1997).

It has been shown that the enzyme, AMP-phosphotransferase, catalyzes the reaction :

$$(Pi)_n + AMP \rightarrow (Pi)_{n-1} + ADP.$$

This enzyme appears to be responsible for the energy conservation in bacteria which are capable of phosphorus removal. One of the strange phenomena observed in enhanced biological phosphorus removal systems is the variation in the ratio of carbon source taken up to phosphorus released. It has been reported that a lower pH gave a lower P-release/acetate uptake ratio with a variation of 0.25 to 0.75 P-mol/C-mol (Mino *et al.*, 1998). Brdjanovic *et al.* (1997a) further suggested that polyP (energy source) would be limiting at high pH since more energy is required for acetate transport through the membrane at high pH. Moreover, this variation indicates that the dependency on poly-P as energy source can vary due to the balance between production and consumption of energy in the cell. Energy requirements for the PHA formation metabolism depends on the metabolic pathways used (Mino *et al.*, 1998).

It was shown that excessive aeration leads to a quick full depletion of the already relatively low PHA content of the bio-P cells present at the end of the standard aerobic phase. After the system is returned to normal operation the phosphorus uptake is strongly affected due to the dependence of phosphorus uptake on the PHA content of the bacterial cells. The aerobic phosphorus uptake depends not only on the polyhydroxyalkanoate concentration but also on polyphosphate content of the cells. Under aerobic starvation conditions glycogen cannot replace PHA's for phosphorus uptake and is only used for maintenance. During this period no oxygen consumption due to decay processes has been observed (Brdjanovic *et al.*, 1998b). Since the phosphorus release is hardly affected, the net result is a decreased phosphorus removal efficiency after a period of excessive aeration.

2.1.3.5.3 Glycogen

Glycogen according to the Mino model is a key substrate for the generation of the reducing power required for polyhydroxyalkanoate synthesis. Brdjanovic *et al.* (1997a) reported that when excess acetate is fed to biological phosphorus removing sludge, the anaerobic uptake of acetate stops not because of polyphosphate limitation or polyhydroxyalkanoate saturation but because of glycogen exhaustion. This suggests that glycogen can be the limiting substance in the anaerobic substrate uptake phase under shock loading conditions. Glycogen is metabolized via the Entner-Doudoroff (ED) pathway and is also known to be anaerobically metabolized via the succinate-propionate pathway (Mino *et al.*, 1998).

There are several different methods to measure the glycogen content of bacterial cells. However, these analytical methods measure not only glycogen but also total carbohydrates and glucose which could lead to possible overestimation of glycogen. Recently two methods have been proposed for the measurement of glycogen. Schulze *et al.* (1995) used an enzymatic method for glycogen determination while Brdjanovic *et al.* (1997a) proposed a batch experiment in which the sludge is exposed to excess acetate feeding under anaerobic conditions and the maximum acetate uptake rate is measured for glycogen determination, the stoichiometric relation between acetate uptake and glycogen consumption is applied assuming glycogen is limiting.

2.1.3.5.4 Microbiology

The microbial population of sewage sludge is complex consisting of microorganisms which have been grouped according to function e.g. COD removal by ordinary heterotrophic organisms (OHO's), nitrification by autotrophic nitrification organisms (ANO's) and denitrification by facultative heterotrophic organisms (FHO's) (Ekama and Wentzel, 1999). The biological phosphorus bacteria are collectively referred to as polyphosphate accumulating organisms (PAO's). PAO isolates should have the anaerobic acetate metabolisms (acetate uptake and its conversion to polyhydroxyalkanoates for storage coupled with hydrolysis of stored polyphosphate and consequently the release of phosphorus under anaerobic conditions) (Mino *et al.*, 1998). Initially it was thought that *Acinetobacter spp.* was primarily responsible for enhanced biological phosphorus removal. However, it was later demonstrated by a fluorescent antibody staining technique and the application of 16s-rRNA targeted oligonucleotide probe techniques that the number of *Acinetobacter spp.* was significantly smaller (< 10 % of the total population) (Wagner *et al.*, 1994 ; Bond *et al.*, 1995). The reported predominance of *Acinetobacter spp.* in EBPR systems can be explained by the culture dependent methods that were initially used to identify these polyphosphate accumulating organisms. The development and use of gene probe techniques showed that the classical culture dependent techniques for bacterial enumeration was and is strongly selective for *Acinetobacter spp*. Some of the polyphosphate organisms isolated were found to accumulate polyphosphates under aerobic conditions while taking up glucose and casamino acids but they did not take up acetate. In addition, these microorganisms were found to have quinone 9 (Q-9) which is different to the Q-8 or MK-8 normally found in polyphosphate removing sludge. Other organisms isolated from EBPR sludges were found to accumulate phosphorus while taking up acetate but they differed morphologically to polyphosphate accumulating organisms (Mino *et al.*, 1998).

Conventionally, it has been assumed that enhanced biological phosphorus removal sludges with high phosphorus removal capabilities would be enriched with a single dominant group of microorganisms. However, there is evidence to suggest that the microbial community of the EBPR process is diverse (Bond *et al.*, 1995). It was shown by electron microscopy and genetic techniques that even under very selective conditions (i.e. one carbon source - acetate, controlled temperature and pH, and a long steady operation of the process) there was more than one type of organism present (Mino *et al.*, 1998 ; Brdjanovic *et al.*, 1998a).

The enhanced biological phosphorus removal (EBPR) process is generally a relatively stable process in practice. However, factors such as excessive rainfall, too high loading, shortage of potassium, excessive aeration and high nitrate loading can affect the efficiency of the process. In some laboratory-scale experiments where P removal efficiencies decreased, it was found that a different group of bacteria dominated and these were found in glucose fed reactors and named G-bacteria or glycogen accumulating organisms (GAO's). These organisms take up organic substrates in the anaerobic zone without P release. GAO proliferation is thought to be influenced by factors such as presence of glucose in the wastewater, long SRT and HRT, and improper seeding. Thus, two types of microbial populations are described in the literature as being responsible for anaerobic storage of acetic acid in activated sludge processes ; the polyphosphate accumulating organisms (PAO's) and glycogen accumulating non-polyphosphate organisms (GAO's) (Brdjanovic *et al.*, (1998a,b).

In polyphosphate accumulating organisms glycogen is only converted to deliver the reducing power (NADH) required for acetate reduction to polyhydroxybutyrate while in glycogen accumulating organisms internally stored glycogen would provide the energy as well as the reducing power necessary for anaerobic substrate uptake. Glycogen is therefore, the key storage compound. GAO's have the ability to produce energy through utilization of glycogen without disturbing the redox balance in the cell. The surplus in reducing equivalents obtained in this way is balanced by formation and polymerization of propionyl-CoA into polyhydroxybutyrate or polyhydroxyvalerate. Thus, the metabolism of GAO's is similar to PAO's except glycogen is thought to be the sole energy (ATP) source for the GAO's while PAO's release phosphorus via polyphosphate cleavage (Mino *et al.*, 1994 ; Liu *et al.*, 1997 ; Van Loosdrecht *et al.*, 1997 ; and Schuler and Jenkins, 1997). However, GAO's and PAO's are morphologically different and GAO's are Gram negative organisms. In addition, GAO's contain Neisser positive stains only on their cell walls while PAO's contain strongly Neisser positive granules inside the cell.

The next decisive step in BNR processes is probably going to come from understanding the population dynamics of the systems better. This will lead to the exclusive culturing of these organisms so that the biological phosphorus removal programme is optimized and made more efficient (Ekama and Wentzel, 1999). Furthermore, the measurement of the different active biomass fractions e.g. the ordinary heterotrophic organisms and the polyphosphate accumulating organisms, will allow for model kinetic rates to be validated with observation. Currently, the ordinary heterotrophic organisms for a 12 d sludge age nitrification/denitrification system can be measured in batch tests (Wentzel et al., 1995). However, PAO's cannot be measured.

2.1.3.5.5 *Anoxic Phosphorus Removal*

It was initially thought that PAO's could not grow or accumulate phosphorus under anoxic conditions, or that only a small percentage were capable of it. However, it has been shown that poly-accumulating organisms are able to grow and accumulate phosphorus anoxically (Hascoet and Florentz, 1985 ; Mostert *et al.*, 1988 ; Kuba *et al.*, 1993 ; Kerrn-Jespersen and Henze, 1993 ; Meinhold *et al.*, 1999). Gerber *et al.* (1986) clearly showed (albeit to argue the case against nitrates in the anaerobic reactor) that the phenomenon of P release from sludge acclimatized to enhanced P removal, is primarily dependent on the nature of the substrate and not the anaerobic state *per se*. They showed that the presence of acetate and propionate resulted in rapid phosphorus release under anoxic conditions and that the disappearance of these compounds coincided with a pronounced reduction in phosphorus release (Figure 2-9). It was also shown that during the anoxic phase P release was effectively prevented for substrates such as butyric acid, lactic acid, citric acid, succinic acid, glucose, ethanol, methanol, and settled aewage (Gerber *et al.*, 1986).

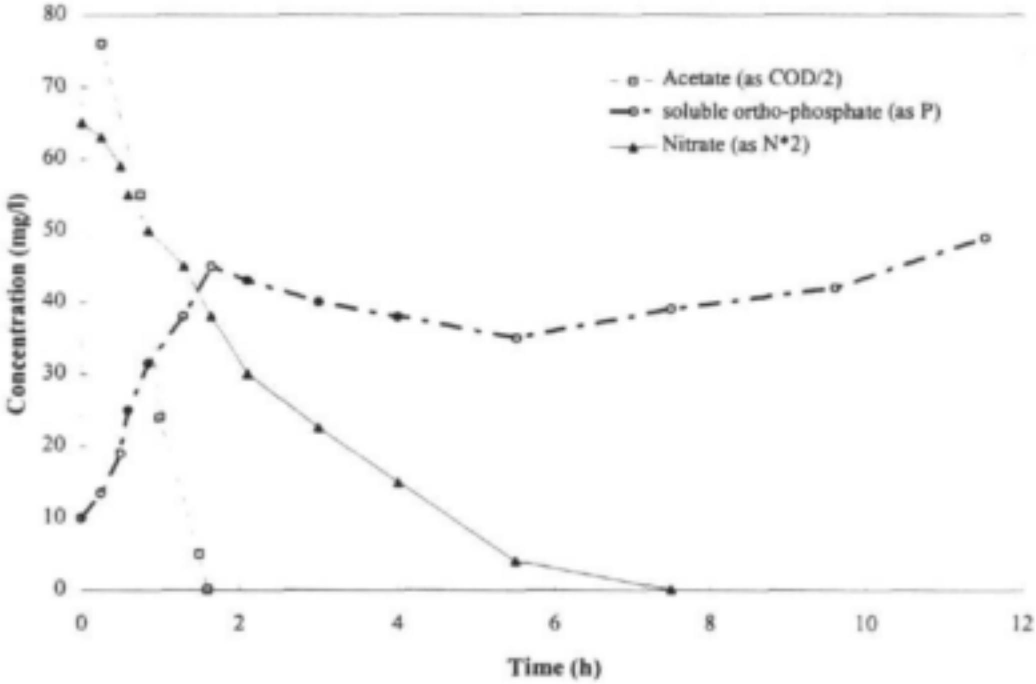


Figure 2-9 : Sequential periods showing phosphorus release and uptake under anoxic conditions (presence of nitrate) followed by phosphorus release under anaerobic conditions (absence of nitrate) (adapted from Gerber et al., 1996).

In addition, Van Loosdrecht *et al.* (1997) postulated that in the presence of nitrates and acetate the substrate is converted to PHA's instead of being used for growth since the formation of storage materials seems to be a basic characteristic of microorganisms in systems with feast/famine conditions such as those that occur in wastewater treatment plants. When the readily biodegradable COD (acetate) is depleted, PHA's are used as substrates to produce new biomass and restore polyphosphate and glycogen (Chuang *et al.*, 1996 ; and Van Loosdrecht *et al.*, 1997).

The energy production efficiency with nitrate expressed in terms of mol ATP/mol NADH is estimated to be 40 % lower than that with oxygen. Consequently a 20% lower cell yield value was reported for an anaerobic-anoxic EBPR process (Murnleitner *et al.*, 1997). Kerrn-Jespersen and Henze (1993) reported that anoxic P uptake appeared to occur at a slower rate than under aerobic conditions. They suggested that the PAO's consisted of 2 groups : (1) a portion which can utilize either oxygen or nitrate as an electron acceptor, and (2) a portion that is able to use only oxygen. This was corroborated recently by studies on anoxic phosphorus removal by Meinhold *et al.*, (1999) which pointed strongly to the existence of 2 populations of PAO's. While the division of the polyphosphate organisms into 2 groups explained the different P uptake rates under different electron acceptor conditions, it is by no means conclusive since other factors such as PHB concentration can also influence P uptake. Batch tests by Sorm *et al.* (1997) demonstrated that the occurrence of anoxic P uptake could be initiated and stimulated by process conditions *i.e.* populations acclimatized to anoxic conditions showed significantly higher anoxic phosphorus uptake than populations exposed to only aerobic conditions. This could be due to a difference in microbial populations or enzymatic induction.

The organisms capable of anoxic phosphorus removal have been termed denitrifying poly-accumulating organisms (DPAO's). Denitrifying capabilities of PAO's is important for 2 reasons:

- In mathematical modelling - behaviour of phosphate and nitrogenous compounds like nitrite, nitrate and ammonia can be predicted only by introducing denitrifying PAO's. In the activated sludge model II (ASM2) the denitrification capability of PAO's is not considered and glycogen is not included as a compound (Meinhold *et al.*, 1999).

- The available amount of COD in the wastewater is a crucial limiting factor for both EBPR and denitrification. Anoxic phosphorus removal can achieve enhanced biological phosphorus removal and denitrification at the same time and save significant amounts of COD (Filipe and Daigger, 1997 ; Meinhold *et al.*, 1999). However, it is thought that PAO's that denitrify do not accumulate in biological nutrient removal systems since they grow at a disadvantage due to their lower utilization efficiency for stored PHA's under anoxic conditions (Murnleitner *et al.*, 1997 ; Filipe and Daigger, 1997). P removal is reported to be reduced under anoxic conditions, requiring about 50 % more COD because of the energetics (Ekama and Wentzel, 1999).

2.1.4 Denitrification Kinetics

2.1.4.1 Kinetic equation

Denitrification kinetics can be described by a complex Monod type expression (equation 2-3). μ_{max} is the maximum growth rate achievable when $S > K_s$ and the concentration of all other essential nutrients are available. K_s is the value of the limiting nutrient concentration at which the specific growth rate is half its maximum value.

$$\mu = (\mu_{max} . S) / (K_s + S) * S_{NO3} / (K_s + S_{NO3}) \qquad (2\text{-}3)$$

However, when the substrates (organic carbon and nitrate) are not limiting, a zero order expression (equation 2-4) can be used (Van Haandel *et al*., 1981 and Henze *et al*., 1997). The kinetic reaction describing denitrification can be expressed by :

$$dN/dt = -rX_v \quad (2\text{-}4a)$$

$$dN/dt = -rX_a \quad (2\text{-}4b)$$

where

N = nitrate concentration (mgNO_3-N/l)

dN/dt = denitrification rate (mgN/l/h)

t = time (h)

X_v = volatile solids concentration (g/l)

X_a = active biomass concentration (g/l)

r = specific denitrification rate (mgN/gVSS.h)

This equation indicates that the nitrate versus time relationship is linear (zero order reaction) and is independent of the nitrate concentration. The denitrification rate is only a function of the volatile solids concentration (Eqn 2-4a). The specific denitrification rate can be expressed more accurately as a function of the active biomass concentration (Eqn 2-4b) because a proportion of the VSS are inert organics and does not contribute to denitrification. The rates obtained in this study are expressed as a function of VSS and are used to distinguish between the different biodegradable fractions. These rates are not a function of the active biomass fraction and cannot be used in the models.

2.1.4.2 Kinetic parameters

The major parameters affecting the denitrification process kinetics are nitrate (electron acceptor), organic carbon (electron donor) type and concentrations, cell residence time and physico-chemical conditions such as pH, oxygen and temperature. Electron donor and nitrate removals are interdependent as the removal of one will result in the removal of the other. The type of electron donor will affect the nitrate reduction rate, cell yield (mass of organisms produced per unit mass of substrate utilized) and nitrite accumulation rates (in batch systems). It has been proposed that zero order kinetics are followed until the nitrate-nitrogen concentration reaches 1 mg/l and thereafter, the rates are thought to follow first order kinetics (Payne, 1981). Table 2-4 lists some of the denitrification kinetic constants that are used to model denitrification processes (Henze *et al*., 1995 ; Metcalf and Eddy, 1991 and Henze *et al* ., 1997). In these studies a yield coefficient value of 0.63 mgO_2/mgO_2 is used rather than 0.66 mgO_2/mgO_2 which is used in models. However, 0.63 mgO_2/mgO_2 is sufficiently close to 0.66 mgO_2/mgO_2 not to affect the results.

Table 2-4 : Denitrification rate constants for denitrification (Henze et al., 1995 ; Metcalf and Eddy, 1991 and Henze et al ., 1997).

Reaction rate constants	**Symbol**	**Unit**	**Constant**
Maximum specific growth rate	μ_{max}	d^{-1}	3 to 6
Decay constant	b	d^{-1}	0.05 to 0.4
Saturation constant, COD	$K_{s,COD}$	mgCOD/l	10 to 20
Saturation constant, nitrate*	$K_{s,NO3}$	mgN/l	0.2 to 0.5
Hydrolysis constant	k_{hX}	mgCOD/mgCOD. d^{-1}	0.15 to 0.4
Maximum yield constant	Y_{max}	mgCOD/mgCOD	0.4 to 0.66

* Metcalf and Eddy lists this value as low as 0.06 mgN/l

Yield coefficient : The yield coefficient is defined as the ratio of the organic carbon used for synthesis over the total amount of organic carbon consumed. Of all the parameters liable to affect the wastewater characterization results using anoxic respiration, the yield coefficient is the most difficult to assess and control. This parameter can be measured in several ways :

1) Direct measurement *i.e.* directly measuring the amount of biomass produced and the amount of organic matter consumed. The imprecision of this method stems from the inability to accurately measure the active biomass growth during the test, especially when there are small variations.

2) Indirect measurement *i.e.* measure the total amount of electron acceptor consumed (NO_3) and the total amount of organic carbon consumed. This method is reliable when specific carbon sources are used *i.e.* when the concentration of these carbon sources can be measured directly by specific analytical methods. However, this is no longer relevant when domestic wastewaters are tested since the measurements would accounts for only 50 to 70 % of the total RBCOD. The use of the global parameter, COD is suggested based on the hypothesis that the filtered COD is representative of the organic matter consumed. However, the inaccuracies of this method is rooted in both the hypothesis and the COD analytical method itself (Nogueira *et al*., 1998).

3) Sperandio *et al.* (1998) suggested the use of CO_2 evolution rate for determining the heterotrophic yield. They suggested that instead of defining the Y_H on the basis of energetic exchanges by COD balances, it was possible to express it in terms of carbon conversion (Y_H^c), in mass of carbon produced per mass of carbon consumed (ΔCs).

4) High observed yield coefficients (ratio between oxidized and removed substrate) suggests that storage may be a significant mechanism in substrate removal. However, accumulation and biosorption can also cause high observed yields. The observed yield is also likely to change during a dynamic response because of competition for substrate which will depend on the time scale and the substrate to biomass ratio. The observed yield shows a general trend to decrease as the sludge age decreases.

Intermittently fed sludges typically exhibit faster substrate uptake and higher observed yields than continuously fed ones. This difference in yields has been explained by the presence of those microorganisms that are most able to store substrates quickly during imposed transient conditions (Majone *et al.*, 1999). Cech and Chudoba (1983) demonstrated that in addition to utilization, both the accumulation and storage mechanisms are acting when sludge is intermittently fed while only storage is possible for the continuously fed sludge. It is hypothesized that the stored products are initially produced at a constant rate and then at a decreasing rate when the saturation of the maximum storage capacity is approached. This can cause both the overall and observed storage yield to vary with time (Majone *et al.*, 1999).

Substrate to biomass ratios : Grady *et al.* (1996) indicated that there is a greater variability in kinetic parameter estimates found in the literature which is due to differences in the ratio of the initial substrate and initial biomass (S/X) ratios which range from below 0.025 to higher than 20. S represents a carbon and energy source for biosynthesis while X represents a source of carbon and energy consumption. If the ratio is very large significant changes can occur in the culture during the assay which would not be reflective of the original culture. A large ratio will reflect the characteristics of the fastest growing species rather than the original culture. If the ratio is very small it is possible for the parameters to be representative of the kinetics manifested in the source environment. For all S/X values in between these two extremes, bacteria will achieve only a partial change in physiological state and the extent of the change depends on the ratio.

Chudoba *et al.* (1985) reported that when the S/X (theoretical oxygen demand / volatile suspended solids) ratio is sufficiently low (below 2) the substrates are removed linearly and no significant cell multiplication is observed. Under high S/X conditions more energy is spent for cell multiplication and a greater part of the substrate is oxidized. This results in a higher production of microbial polymers for mixed culture organisms. Furthermore, low biomass concentrations are indicated by sigmoidal growth curves which are essentially growth and consumption curves with mixed substrates (Chudoba *et al.*, 1985). Therefore, for biodegradation with the aim to obtain kinetic constants it is necessary to work at low S/X ratios to prevent mixed culture organisms from substantial cell multiplication.

Recent studies by Majone *et al.*, (1996) using activated sludge fed continuously, and intermittently, showed the the S/X ratio did not play a major role in determining the type and extent of the response. They reported that both cultures showed that storage of polyhydroxybutyrate was in general the main mechanism of substrate removal. The biomass dominated by floc-forming bacteria showed a very fast response to the substrate spike with a high observed yield. They showed that storage of PHB is the main part of the observed yield when starvation is low and is a minor part when starvation is high. When the yield decreases, growth becomes the main mechanism of substrate removal.

Wastewater systems are normally operated at low S/X ratios (feast/famine conditions). Therefore, for this project, the batch reactors were operated at S/X ratios < 0.1 mgCOD/mgCOD. This ratio ratio was also applied to ensure that suitable NUR profiles were obtained. The experimental aspects of the S/X ratio are discussed further in **Chapter 3**, 3.2.1.3.

2.2. WASTEWATER CHARACTERISTICS

Global analytical parameters such as biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are used routinely to assess the organic content of wastewaters. In this investigation, the chemical oxygen demand (COD) measurement is used since it allows for the calculation of mass and electron balances which is not possible with BOD or total organic carbon (TOC) measurements. *In this project* mgO_2 *and mgCOD are interchangeable.* The organic carbon in the influent can be divided into biodegradable COD, non-biodegradable COD (particulate and soluble) and active biomass (also referred to as the active mass fraction). The mixed liquor can also be divided into the above but a distinction needs to be made between the active, endogenous, and inert sludge fraction.

2.2.1 Influent wastewater COD fractionation

The total organic matter content in wastewater can be measured as COD. In the University of Cape Town (UCT) Model, this is referred to as Sti (total influent substrate) but in the literature it may also be referred to as St, C_T, C_{Ti} or C_{TCOD}. The total COD of the influent wastewater can be divided into the biodegradable, unbiodegradable and active biomass fractions. These can be further divided into the readily and slowly biodegradable, and unbiodegradable fractions. According to Wentzel *et al.* (1995) the influent wastewater can be divided into five fractions : the readily biodegradable(Ss), the slowly biodegradable (Xs), the particulate inert (Xi), the soluble inert (Si) and the active mass fraction (X_H) (Figure 2-10 ; equation 2-5). The Ss fraction has since been divided into the S_A (acetate) and S_F (fermentable) fractions. This division was made largely to improve the models for enhanced biological phosphorus removal (EBPR) systems. The slowly biodegradable (Xs) fraction division has been taken a step further by Orhon and Çokgör (1997) to include the rapidly hydrolyzable COD and slowly hydrolyzable COD fractions (Figure 2-11). Thus it is possible to combine these two figures to provide a more detailed profile of wastewater COD which includes the S_A, S_F and X_H fractions (Figure 2-12). Based on this synopsis it is now possible to discuss these fractions more comprehensively.

$$St = (S_A + S_F) + S_i + X_i + X_S + X_H \qquad (2\text{-}5)$$

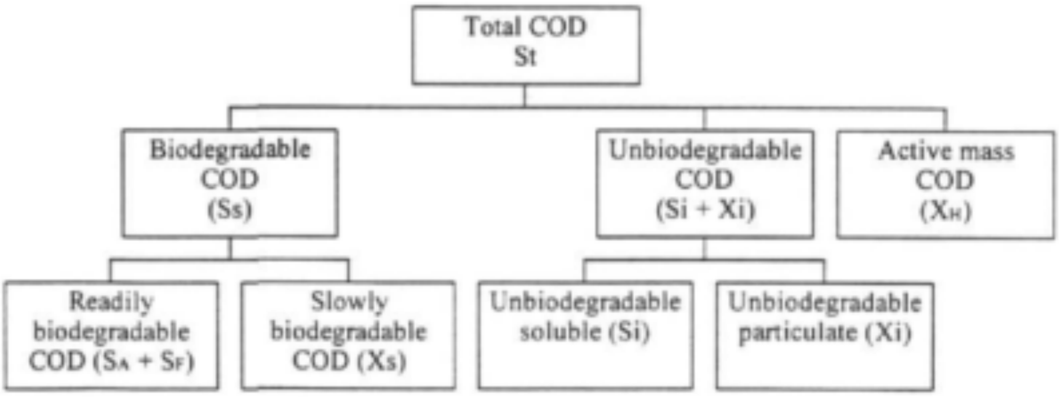


Figure 2-10: Division of influent COD into its component fractions (from Wentzel et al., 1995)

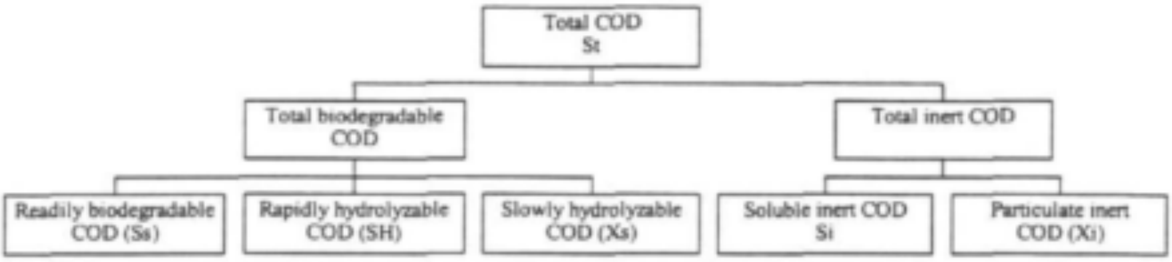


Figure 2-11: Division of influent COD into its component fractions (from Orhon and Cokgör, 1997).

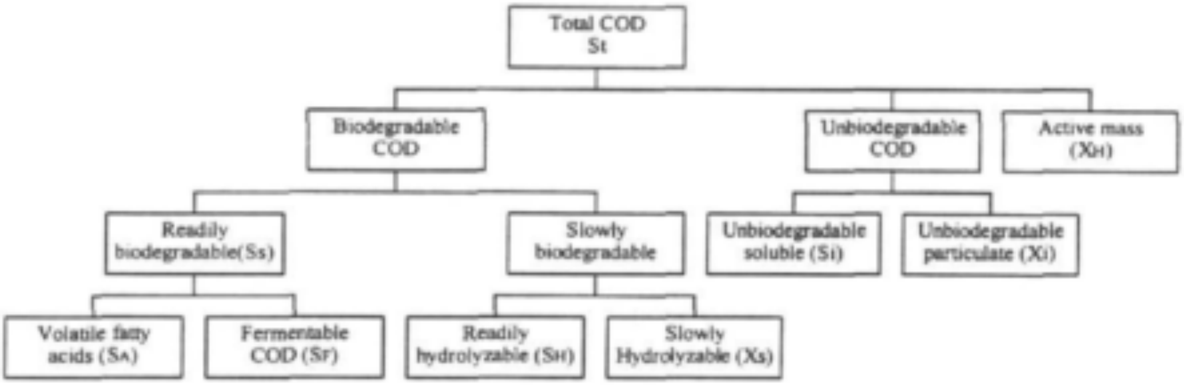


Figure 2-12: Division of influent COD into its component fractions (modified from Wentzel et al., 1995 and Orhon and Cokgör, 1997).

2.2.1.1 Biodegradable COD

The biodegradable fraction is divided into a readily biodegradable (soluble) COD (Ss) and slowly biodegradable (particulate) COD (Xs). This division is a biokinetic one. Investigations by Stern and Marais in 1974 showed that in the primary anoxic reactor, the rate of denitrification occurred in two linear phases (in Van Haandel, 1981). In the secondary anoxic reactor there is a single linear phase due to endogenous / adsorbed organic carbon utilization. In terms of the rate of utilization of nitrate-N they found that the single rate in the secondary anoxic reactor is about two-thirds that of the slow second rate in the primary anoxic reactor. It was therefore, hypothesized that the two linear phases were linked to the biodegradability of the organic carbon substrate, a readily biodegradable COD fraction and a slowly biodegradable one. In the secondary anoxic reactors of plug flow systems the single linear phase is due to the utilization of adsorbed SBCOD generated from organism death *i.e.* endogenous respiration. Further investigations verified that under dynamic loading conditions *eg.* plug flow, short solids retention time cyclic loading and batch tests, two distinct rates of utilization were observed for either oxygen (Ekama *et al.*, 1986) or nitrate (Van Haandel *et al.*, 1981 ; Ekama *et al.*, 1986) as an electron acceptor. Subdivision of this fraction is required if denitrification or phosphorus removal are included in the design or the system response is simulated with a dynamic model.

2.2.1.1.1 Readily biodegradable COD (RBCOD)

The RBCOD (or Ss fraction) consists of small simple molecules that pass directly through the cell wall (via passive or active uptake) for synthesis (growth) and catabolism (energy). The growth from RBCOD utilization is expressed according to the Monod equation linking the specific growth rate of the active mass to the RBCOD concentration. The reaction rate associated with RBCOD catabolism is rapid. This component of wastewater may be represented as a fraction (f_s) of the total COD :

$$S_s = f_s \, St \qquad (2\text{-}6)$$

Recently the readily biodegradable COD was further subdivided into the fermentation products (S_A) and fermentable biodegradable COD (S_F) (Mbewe *et al.*, 1995 ; Henze *et al.*, 1997 ; and Orhon and Cokgör, 1997). As discussed earlier, this division is largely required for accurate design of EBPR systems. The RBCOD (Ss) can be determined by biological methods, aerobic or anoxic, continuous or batch (Ekama *et al.*, 1986). The S_A fraction can be determined by chemical methods or gas chromatography. Thus, the S_F fraction can be determined by difference.

$$S_s = S_A + S_F \qquad (2\text{-}7)$$

Volatile fatty acids (S_A)

This fraction consists of fermentation products considered to be acetate. The acetate fraction is classed as S_A and comprises 2 to 10 % of the total COD (Table 2-5). In reality this fraction comprises a range of fermentation products (VFA's). The volatile fatty acids are present in the influent wastewater but can also be generated in the anaerobic reactor by acid fermentation. The rate of VFA uptake is so rapid that it can be assumed that all volatile fatty acids in the influent will be sequestered in the anaerobic reactor by polyphosphate accumulating organisms (if present).

Readily (fermentable) biodegradable fraction (S_F)

This fraction consists of fermentable, readily biodegradable (F-RBCOD) organic substrates. This fraction of the soluble COD comprises 10 to 20 % of the total COD and is considered to be directly available for biodegradation by heterotrophic organisms (Table 2-5). It is assumed that S_F (or F-RBCOD) may serve as a substrate for fermentation and therefore, does not include the fermentation products. The S_A are generated by acid fermentation of S_F by the heterotrophs in the anaerobic reactor. The volatile fatty acids generated can then be sequestered by polyphosphate accumulating organisms. The rate of the fermentation reaction is slower than the sequestration rate and the amount of F-RBCOD fermented to VFA's depends on the influent F-RBCOD concentration and wastewater treatment system design.

2.2.1.1.2 Slowly biodegradable COD (SBCOD)

The slowly biodegradable COD (SBCOD) is taken up more slowly and metabolized at rates that are about 10 % of the rate of RBCOD metabolism. This COD fraction is thought to be consist of complex organic molecules which cannot pass directly through the cell wall. The utilization of this organic carbon material involves enmeshment and adsorption to activated sludge flocs. This is followed by the extracellular enzymatic breakdown of the complex compounds to simpler molecules which are able to pass through the cell wall. The molecules are then metabolized by the microorganism for growth and metabolism. The overall reaction rate is limited by the hydrolysis rate of the adsorbed organic carbon rather than the rate of metabolism (Ekama *et al*., 1986 ; Wentzel *et al*., 1992).

This latter suggestion is supported by Henze *et al.* (1994) who stated that the dominating rate limiting factor in nutrient removal processes is the organic carbon source. The rate of hydrolysis of higher molecular weight compounds to readily biodegradable COD will limit the denitrification rate. The hydrolysis of these slowly biodegradable substrates is assumed to be catalyzed by extracellular enzymes. There are two hypothesis with regard to the locality of these extracellular enzymes :

- Large molecules are adsorbed to the surface of the biomass where hydrolysis is mediated by the cell bound extracellular enzymes. This was accepted and adopted in the UCT Model *i.e.* these hydrolysis products pass directly to the microorganism.

- According to the IAWPRC Model the organics are hydrolyzed by extracellular enzymes and are released in the bulk liquid (Dold *et al*., 1991).

Rohold and Harremoes (1993) and Larsen and Harremoes (1994) investigated this phenomenon in biofilm reactors with molasses and starch, respectively, as slowly biodegradable substrates. They reported that the extracellular enzymatic breakdown of non-diffusible organics occurs in the bulk liquid and that the enzymes are washed out of the system when the residence time is decreased. However, San Pedro *et al.* (1994) found that starch disappeared from the bulk liquid solution within a two 2 h period in suspended growth systems. This indicated a rapid adsorption to the biomass which suggests that the SBCOD becomes adsorbed to the biomass before hydrolysis. This also suggests that the observation of substrate disappearance from the bulk liquid does not necessarily imply immediate substrate utilization by the bacteria. They observed a difference in utilization rates for starch and intracellular glycogen due to differences in the hydrolysis rates. It is observations such as these that has resulted in some researchers suggesting that the slowly biodegradable COD fraction can be further sub-divided into smaller fractions according to their rate of hydrolysis (Henze, 1992). Although the SBCOD fraction was originally defined as particulate (Dold and Marais, 1986), this fraction is now said to cover a wide range of particle sizes from soluble to colloidal and larger organic particles. This provides the basis of the recent approach to sub-divide this group into the rapidly hydrolyzable COD (S_H) and slowly hydrolyzable COD (X_S) (Orhon and Çokgör, 1997). The rapidly hydrolyzable COD is generally assumed to be soluble, so that it may be defined for municipal wastewaters by means of a mass balance equation (2-8).

$$S_H = S_t - S_i - S_s - (X_H + X_i + X_s) \qquad (2\text{-}8)$$

Endogenous respiration also provides a source of slowly biodegradable COD which occurs when the organic carbon substrate concentration is low and absent. Consequently, the bacterial cells die and release cell material, which are unbiodegradable and biodegradable (Randall *et al.*, 1992 ; Wentzel *et al.*, 1992). Unlike the SBCOD from the influent wastewater, the endogenous denitrification rate is dependent on the respiration rate of the bacteria using the stored food reserves or substrate released from endogenous decay and not on the rate of hydrolysis (Randall *et al.*, 1992).

For denitrification, the rate of denitrification depends on whether RBCOD or SBCOD serves as electron donor (substrate) and the relative proportion of these two materials will influence the amount of nitrogen removed. Phosphorus removal, however, is dependent on the available VFA (S_A) fraction. In conventional wastewater treatment systems, the electron donor (organic carbon source) for denitrification consists of largely slowly biodegradable COD.

2.2.1.2 Unbiodegradable COD

The unbiodegradable (inert) COD can be divided into unbiodegradable soluble COD (S_i) and unbiodegradable particulate COD (X_i). These organic compounds cannot be further degraded in the wastewater treatment plants under normal operating conditions.

2.2.1.2.1 Unbiodegradable soluble

Raw wastewater contains a certain proportion of inert soluble organic compounds. During the nutrient removal process more soluble organics are produced and thus the final inert soluble concentration should be higher than that in the influent (Henze, 1992). The total effluent soluble COD includes the unbiodegradable organic compounds which originate from the wastewater and soluble residual COD generated as soluble metabolic products (Sp). Therefore the effluent unbiodegradable (S_R) generally contains more soluble unbiodegradable COD than the wastewater. The generation of soluble metabolic products is modelled by means of growth-associated or decay-associated processes (Orhon and Cokgõr, 1997).

This is not considered in the UCT Model which hypothesizes that for unbiodegradable soluble material at steady state for systems with a sludge age of 10 to 20 d the mass of unbiodegradable material that enters the system is equal to the mass of unbiodegradable that leaves the system. The soluble unbiodegradable materials (S_i) pass out in the secondary effluent as the COD effluent. This is done by accepting that the effluent soluble COD concentration (< 0.45 µm filtered COD) (Suse) is equal to the influent unbiodegradable soluble COD (S_i) (Dold *et al.*, 1991). It is therefore, assumed that no soluble unbiodegradable organics are generated during biological treatment in the biological reactor. This has been accepted as a reasonable assumption based on years of study. Henze et al. (1995) suggested a different method for the determination of the soluble inert fraction. It consisted of removing an aliquot from the mixed liquor from a continuously fed completely mixed reactor operating at a SRT in excess of 10 d and aerating it in a batch reactor (Orhon and Çogkor, 1997). Hence, the major set-back of both these methods is the inability of these methods to differentiate between the soluble inert COD of the effluent and the soluble residual fraction of microbial products which may or may not be biodegradable.

$$S_I = S_R - S_F \qquad (2\text{-}9)$$

2.2.1.2.2 Unbiodegradable particulate

The unbiodegradable particulate material follows two paths :

- becomes enmeshed in the sludge, and
- settles out in the secondary clarifier and is retained in the system to accumulate as unbiodegradable organic settleable solids (VSS).

At steady state the mass of unbiodegradable particulates entering the system is balanced by the mass of particulate inert compounds leaving via sludge wastage. Thus, the mass of inert particulates in the system is equal to the mass of unbiodegradable particulate fed per day multiplied by system sludge age. The unbiodegradable particulate organic material is generated by the bacteria during the treatment process. This material is referred to as *endogenous residue*. The generated particulate unbiodegradable organic material occurs as a result of microbial metabolic activity during the endogenous decay or death-regeneration phase (Ekama *et al*., 1986 ; Henze *et al*., 1995).

Unlike the soluble RBCOD fraction which is exposed to biological treatment for as long as the liquid remains in the system *i.e.* the hydraulic residence time (HRT), the SBCOD fraction is exposed to biological treatment for as long as the solids are retained in the system *i.e.* solids retention time (SRT). Therefore, even though the utilization of SBCOD is about 10 % that of RBCOD because the SRT in most systems is usually more than 10 times longer than the HRT, the SBCOD is completely utilized. Modelling has shown that all the SBCOD is completely utilized for SRT's > 2 to 3 d and at temperatures of about 20 °C. At lower temperatures, longer SRT's are required (Mbewe *et al*., 1995).

Table 2-5: Typical ranges for the wastewater fractions (from Henze et al., 1995).

Symbol	**Fraction**	**% of total COD**
S_F	readily biodegradable fermentable fraction	10 to 20
S_A	Volatile acids (acetate)	2 to 10
S_I	Inert, non-biodegradable soluble	5 to 10
X_I	Inert, non-biodegradable particulate	10 to 15
X_S	Slowly biodegradable fraction	30 to 60
X_H	Heterotrophic biomass	5 to 15

2.2.1.3 Active mass fraction (X_H)

Some of these organisms can grow aerobically and anoxically (denitrification) and others may be active anaerobically. They are responsible for the hydrolysis of particulate substrates X_S and the removal of the soluble organic carbon (Henze *et al.*, 1995). In South Africa, the sewers are generally short (retention time < 6 h) and anaerobic, and it is therefore, considered unlikely to support active biomass generation. However, European wastewaters can contain a significant heterotrophic active mass fraction *i.e.* up to 20 % of the total COD (Henze, 1989 and Kappelar and Gujer, 1992). Seeding of this fraction into the activated sludge system can have a significant impact on modelling and design. Therefore, the active mass is included as an influent COD wastewater fraction.

2.3. CHARACTERIZATION METHODS

As discussed earlier the accuracy of the input data determines the reliability of models simulating wastewater treatment processes. The accuracy of the input data, however, is largely dependent on methods used to determine the wastewater fractions such as RBCOD. To date it is largely the biological methods (oxygen and nitrate-N utilization rate) which are considered reliable for depicting the biological state of the influent substrate. Since the RBCOD is modelled as simple, soluble compounds, physical and chemical methods have been tested in order to find a method which is as comparable and reliable results as the biological respirometric tests.

2.3.1 Physical and chemical methods

It has been suggested that the difference in biokinetic response to the RBCOD and SBCOD is due to differences in molecular size. RBCOD consists of small molecules which can easily pass into the microbial cells. SBCOD, however, comprises complex molecules which require extracellular breakdown before cell utilization. This has led to a need to separate and to define wastewater fractions for the purposes of studying, understanding and optimizing organic carbon utilization.

Methods used for separating wastewater fractions include: sedimentation, centrifugation, filtration and precipitation. Filtration methods with several pore sizes have been investigated. It has been found that membranes with a molecular weight limit of less than 10 000 Daltons gave RBCOD concentrations similar to that determined in biological respirometric tests. However, it has also been reported that with textile wastewater, these membranes gave RBCOD values lower (13 % of total COD) than that derived in batch bioassays (20 % of total COD) (Bortone *et al.*, 1994 ; Wentzel *et al.*, 1995). Dold *et al.* (1980) assessed 0.45 µm filters and found that a small fraction of the SBCOD of domestic wastewater passed through the filter. This resulted in an overestimation of RBCOD fraction. Torrijos *et al.* (1993) found that wastewater passed through a 0.1 µm filter gave a true indication of the RBCOD fraction.

Several other researchers have attempted to classify the soluble fraction. Their results and methods vary. For example, Pouet and Grasmick (1994) have divided wastewater into four fractions based on different fractionation techniques (see Table 2-6). The cut-off utilized for the characterization of the soluble fraction varies from < 0.001 μm (Pouet and Grasmick, 1994) ; <0.01 or <0.03 (Henze and Harremoes, 1990) and 0.45 μm (Henze *et al.*, 1995). This fraction comprises approximately 24 to 30 % of the wastewater (Pouet and Grasmick, 1994 ; Henze and Harremoes, 1990) (Table 2-6). Henze and Harremoes (1990) cited colloidal particles sizes between 0.01 and 10 μm and 0.03 and 1.5 μm, while Pouet and Grasmick (1994) classified this fraction as 0.001 to 1 μm. In addition, another fraction called the supracolloids was classified as 1 to 100 μm in size. It is also these differences in sizes that have led to variations in the percentage of these fractions found in wastewater (Table 2-6). The difference is partly due to the difference in wastewater composition. However, there does appear to be a lack of standardization with regard to classification of wastewater fractions according to size. The major shortfall of these physical separation methods is the inability to differentiate between biodegradable and unbiodegradable compounds.

Table 2-6: List of the different components of raw wastewater and the percentage of each fraction.

	Pouet and Grasmick (1994)		**Henze and Harremoes (1990)**	
Fraction	**size (µm)**	**wastewater (%)**	**size (µm)**	**wastewater (%)**
soluble	<0.001	30	< 0.01	24
colloidal	0.001 to 1	35	0.01 to 10	19
supracolloidal	1 to 100	included in colloidal fraction	>10	-
settleable	>100	35	-	57

Mamais *et al.* (1993) reported that it was possible to differentiate between the biodegradable soluble and unbiodegradable soluble components by subtracting the soluble component of the effluent wastewater from the *truly soluble* component of the influent wastewater. This method is based on the rationale that membrane filtration of a sample that has been coagulated with $ZnSO_4$ at pH 10.5, will produce a filtrate containing only *truly soluble* (COD_{sol}) organic matter. The filtrate contains both biodegradable and unbiodegradable COD. Thus, the unbiodegradable fraction (S_i) has to be quantified independently which requires effluent from a continuous system or the measurement of filtered COD in a 10 day batch test (Wentzel *et al.*, 1995).

$$S_s\,(RBCOD) = COD_{sol} - S_i \qquad (2\text{-}10)$$

S_s = influent readily biodegradable soluble COD

COD_{sol} = influent truly soluble COD *i.e.* after coagulation

S_i = influent inert COD

The S_i component is considered equal to the *truly soluble* (COD_{sol}) effluent COD of an activated sludge system treating the influent at a sludge residence time greater than three days. Therefore, S_i can be determined by performing a COD_{sol} measurement on the effluent after coagulation and COD_{sol} by performing the same test on the influent. The difference between the two provides the S_s value (Eqn 2-10). Results of S_s which were obtained from the flocculation and the biological method were highly comparable (Table 2-7) (Mamais *et al.*, 1993).

Table 2-7: Comparison of readily biodegradable COD (Ss) values from the physico-chemical (floc) and biological method for different wastewater sources (Mamais et al., 1993).

Wastewater source	Floc COD_{sol}	floc S_I	S_s (floc)	S_s (biological)
	mgO_2/l			
primary effluent 1	99	37	62	65
primary effluent 2	84	52	32	32
raw wastewater 1	63	41	23	22
primary effluent and acid digester centrate	163	53	110	119

A disadvantage of this method is the necessity to quantify the inert fraction independently which is time-consuming procedure (Wentzel *et al.*, 1995). In addition, this S_I unbiodegradable COD value may be a false since this fraction may also contain soluble microbial products produced by the biomass which are biodegradable. Another point of contention may be the definition of the soluble fraction. While Mamais *et al.* (1993) have hypothesized that the soluble fraction of wastewater contains only RBCOD and inert soluble, Orhon and Çogkör (1997) contend that the soluble fraction consists of RBCOD, inert soluble and readily hydrolyzable COD. In this case the RBCOD calculated by the method presented above would result in an overestimation of the RBCOD fraction.

2.3.2 Biological methods for wastewater characterization

Several biological methods have been discussed in the literature for the study of biological processes and the use of these methods for the determination of wastewater components (Ekama *et al.*, 1986). Since this study is aimed primarily at the determination of the RBCOD fraction by using the NUR method, this method will be discussed more comprehensively.

2.3.2.1 The OUR method

The aerobic batch test monitors the oxygen uptake rate (OUR) which indicates the amount of oxygen consumption per unit time, per unit reactor volume resulting from microbial activity. The initial OUR, which may stay constant for 1 to 3 h if a suitable substrate to biomass (S/X) ratio is applied, is associated with the utilization of readily biodegradable organic compounds. Once the readily biodegradable compounds are consumed, the OUR drops to a lower level. The lower OUR is associated with the utilization of slowly biodegradable substrate and endogenous respiration products (Ekama *et al.*, 1986; Orhon and Çokgör, 1997). An example of the observed OUR profile is presented in Figure 2-13.

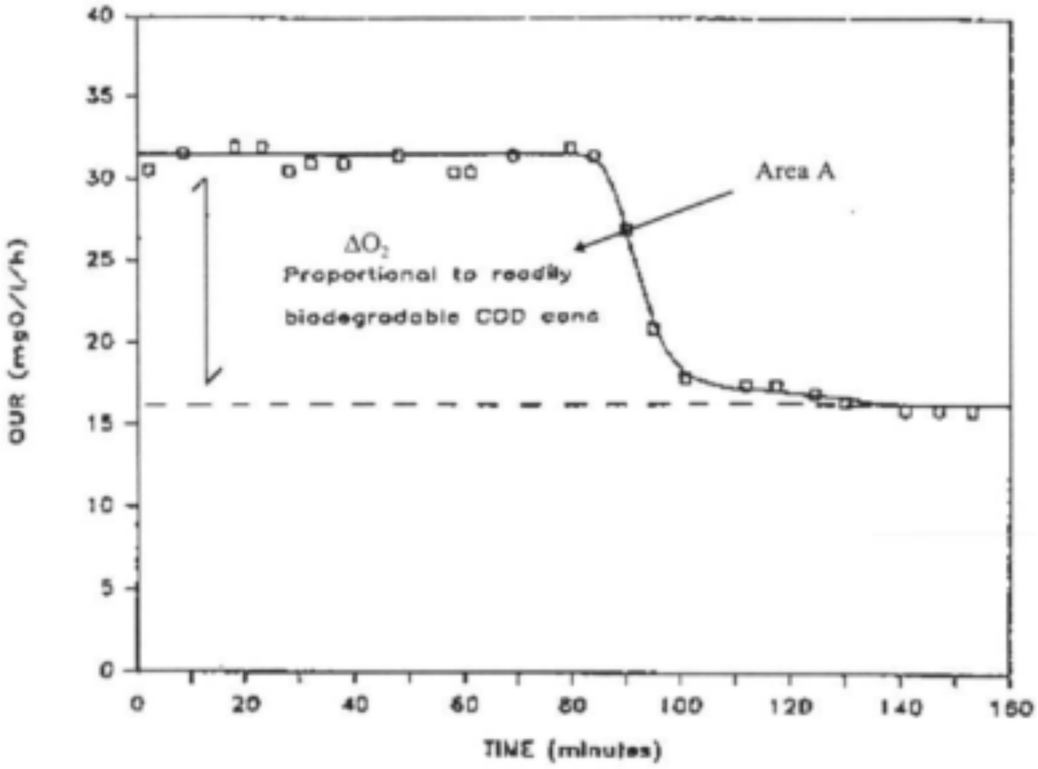


Figure 2-13: An example of an OUR curve in an aerobic batch test used to calculate the readily biodegradable COD fraction (Ekama et al., 1986).

The readily biodegradable COD may be calculated from the following relationship :

$$Ss = [1 / (1-Y_H)] \times \Delta O_2 \qquad (2\text{-}11)$$

where ΔO_2 is the area under the OUR curve (Area A) and Y_H is the heterotrophic yield coefficient. For municipal wastewaters the stoichiometrically derived value is 0.64 mgO_2/mgO_2, but values of 0.63 and 0.66 mgO_2/mgO_2 are also used (Ekama *et al.*, 1986; Henze *et al.*, 1995; Orhon and Çokgör, 1997).

2.3.2.2 The NUR Test

Denitrification kinetics can be studied in continuous or batch systems. The latter system is frequently used to study kinetics as it is simple and easy to operate. The anoxic batch test described by Ekama *et al.* (1986) was referred to as the nitrate utilization rate method (NUR). This test is similar to that of the aerobic batch test (oxygen utilization rate-OUR) method which was first developed for the study of nitrification.

At the start of the NUR test nitrate is added and is monitored over a period of approximately 4 to 5 h. In the absence of oxygen, nitrate serves the same function as oxygen *i.e.* as electron acceptor. In these tests nitrite (NO_2^-) was not considered as little or no nitrite accumulation was found in the samples taken. Therefore NO_3-N vs time curves were plotted and the equation considered only the nitrate (NO_3^-) concentration.

Theory

In the anoxic batch test, the nitrate concentration will initially decrease at a constant rapid nitrate utilization rate reflecting the utilization of the readily biodegradable fraction (RBCOD) from the wastewater. This initial rapid rate is analogous to the initial high OUR in aerobic batch systems. The RBCOD consists of simple soluble molecules that can be taken up rapidly by the organisms and metabolized for energy and cell synthesis. The decrease in nitrate concentration is linear. Once the RBCOD from the influent has been depleted, the denitrification rate is reduced to the rate of utilization of slowly biodegradable compounds (SBCOD) which has to undergo hydrolysis of the complex molecules and particulate material before being used. This second rate is analogous to the second OUR slope in the aerobic batch test (see Figure 2-13).

The results from an anoxic batch test can be used to calculate the readily biodegradable fraction (RBCOD) which is related to the decrease in the nitrate (electron acceptor) concentration. This is given by the intercepts with the vertical axis of straight lines drawn through the initial rapid and second slower rates of denitrification *i.e.* ΔNO_3^- (Figure 2-14).

The readily biodegradable COD (RBCOD) can be calculated by using the values derived from a nitrate-nitrogen-time plot and equation 2-12. Equation 2-12 relates the electron acceptor disappearance (ΔNO_3^-) in the batch reactor to the COD consumed by the heterotrophic organisms.

$$COD = [2.86/(1-Y_H)] \text{ x } \Delta NO_3 \text{ x } [(V_{ww} + V_{ml})/V_{ww}] \qquad (2\text{-}12)$$

S_H = readily biodegradable COD concentration (mg COD/l)

V_{ww} = the volume of the wastewater (l)

V_{ml} = the volume of the mixed liquor / sludge (l)

Y_H = yield coefficient (mgCOD/mgCOD)

The yield, Y_H, is the proportion of substrate (as COD) directly incorporated in the biomass. The term $(1-Y_H)$ is related to the electron flow from the organic carbon source to the terminal electron acceptor, NO_3^- in this case. It is hypothesized that for every one mg of COD used for growth, $(1-Y_H)$ mg of COD is used for catabolism. The aerobic yield coefficients of 0.66 mgO_2/mgO_2 and 0.63 mgCOD/mgCOD are also used for anoxic processes. The former value is used by Ekama *et al.* (1986) while 0.63 mgCOD/mgCOD is suggested in Henze *et al.* (1995). However, it should be noted that results published by Sperandio *et al.* (1997) and Sozen *et al.* (1998) have suggested that the Y_H value is lower for anoxic processes (about 14 % lower than the aerobic values).

The value 2.86 in equation 2-12 relates the electron acceptor capacity of nitrate to oxygen. The removal of nitrogen is as a result of biological redox reactions where the biodegradable organic material serves as an electron donor and nitrate (and nitrite if observable) serves the same function as oxygen *i.e* electron acceptor.

The equivalence between oxygen and nitrate is evident from the following half reactions (Van Haandel *et al.*, 1981).

For O_2 $e^- + 1/4\ O_2 + H^+ \rightarrow 1/2\ H_2O$ (2-13)

For NO_3^--N $e^- + 1/5\ NO_3^- + 6/5\ H^+ \rightarrow 1/10\ N_2 + 3/5\ H_2O$ (2-14)

For NO_2^--N $e^- + 1/3\ NO_2^- + 4/3\ H^+ \rightarrow 1/6\ N_2 + 2/3\ H_2O$ (2-15)

In transferring the electrons from the donor to the acceptor (O_2, NO_3^-, NO_2^-) there are approximately equal changes of free energy per electron transferred. This is irrespective of the donor or acceptor (McCarty, 1964). From equations 2-13 and 2-14 the transfer of one electron equivalent involves the reduction of 1/4 mol of O_2, or 1/5 mol of NO_3^--N. Thus,

$$32/4\ g\ O_2 \equiv 14/5\ g\ NO_3^-\text{-}N$$

In other words, 1 mg of NO_3^-N = 2.86 mg O_2 (or COD). Similarly for nitrites, 1mg NO_2^--N ≡ 1.71 mg O_2. Thus, stoichiometrically the electron acceptor capacity of nitrate (as N) is 2.86 times that of oxygen.

The term 2.86 / (1-Y_H) relates to the mass of nitrate utilized to the mass of COD consumed by the heterotrophic microorganisms under anoxic conditions. Accepting a yield coefficient of 0.63 mgCOD/mgCOD, the equation can be simplified:

$$COD\ (mgO_2/l) = 7.7 \times \Delta NO_3 \times [(V_{ww} + V_{ml})/V_{ww}] \qquad (2\text{-}16)$$

The readily biodegradable COD fraction (f_s) with respect to the total COD (St) is calculated as follows:

$$f_s = COD_{calculated}/St \qquad (2\text{-}17)$$

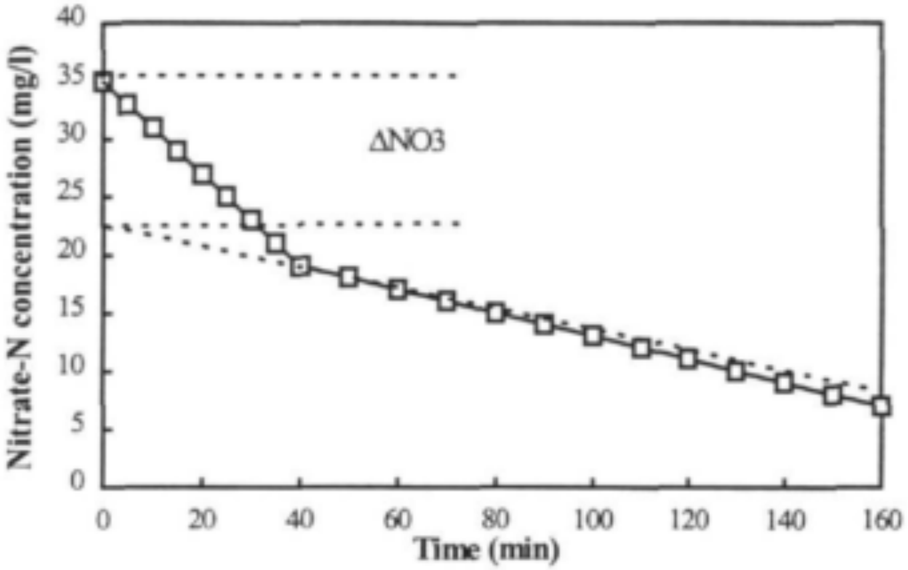


Figure 2-14: Nitrate-nitrogen-time response in an anoxic batch test for determining the RBCOD fraction. The S/X ratio of the test is 0.37 gCOD/gVSS or 0.25 gCOD/gCOD (from Ekama et al., 1986).

Evaluation of the NUR method

The reliability of the RBCOD concentration in wastewaters is of great theoretical and practical importance. This parameter is the only substrate component directly utilized for microbial growth in the current activated sludge models. In addition, it allows for the accurate calculation of the SBCOD which represents the bulk of the influent COD content. This fraction is often the critical model component for the modelling and design of activated sludge systems. However, recently work by several authors have discussed several points of contention with regard to the NUR test and RBCOD determination (Orhon and Çokgör, 1997 ; Sozen *et al.* 1998 ; Çokgör *et al.*, 1998 ; Nogueira *et al.*, 1998 and Sperandio *et al.*, 1997).

- ***Yield coefficient,***

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 repiration yielded lower yield coefficients ($Y_{HD} < Y_H$) under anoxic conditions. Y_{HD} values of 0.50 to 0.61 gcell COD /g COD were derived on an energetic consideration basis (Sozen *et al.*, 1998). This was confirmed by comparative tests with NUR and OUR which showed that the NUR derived RBCOD values for municipal wastewaters were consistently higher than the OUR derived values by an average value of 1.14. This 14 % overestimation correlates well with the results from Sperandio *et al.* (1997) using CO_2 evolution rates for heterotrophic yield determination which showed that the anoxic yield is approximately 15 % (i.e. 0.85 $Y_{Haerobic}$) lower than the aerobic one. Tests with acetic acid gave anoxic yields of ca. 0.54 mgCOD/mgCOD and aerobic yields of 0.66 mgCOD/mgCOD. However, it is equally important to note that Ekama *et al.* (1986) found the NUR and OUR methods to be comparable and that the yield coefficient may vary.

Substrate profiles vs time have been used under the assumption that autocatalytic growth will cause substrate uptake at an increasing rate whereas substrate uptake at a constant rate has been assumed as an indirect evidence of storage. A high yield coefficient suggests the occurrence of storage, accumulation or biosorption. These yield coefficients are likely to change during transient periods in an activated sludge process since competition for substrate is high. High yields (0.71 mgCOD/mgCOD) have been calculated for acetate. Normally the yield is 0.5 mgCOD/mgCOD for acetate and for bacteria growing without storage is observed. Thus, the increase in yield was hypothesized to be due to storage (Majone *et al.*, 1999).

- ***Nitrite accumulation and correction***

An equally significant factor is the determination of the amount of electron acceptor utilized. When NO_2^- accumulation is appreciable then consideration of only NO_3^- is unacceptable. Orhon *et al.* (1997) showed that NO_2^- accumulation can occur and this will influence the change in NOx (Figure 2-15). In these cases, the electron equivalence of the RBCOD consumption is best represented by the following expression (Çokgör *et al.*, 1998) :

$$N = NO_3\text{-}N + 0.6\ NO_2\text{-}N \qquad (2\text{-}18)$$

The 0.6 conversion factor can best be explained by the oxidation half reactions (2-14) and (2-15) which show that for nitrates 5 electrons are required for complete oxidation to N_2 while for nitrites only 3 electrons are required. Thus, 3/5 is equal to 0.6. Cokgor *et al.*, 1998 showed that for NUR tests the erratic nature of the data could be smoothed to linear trends after NO_2^- correction.

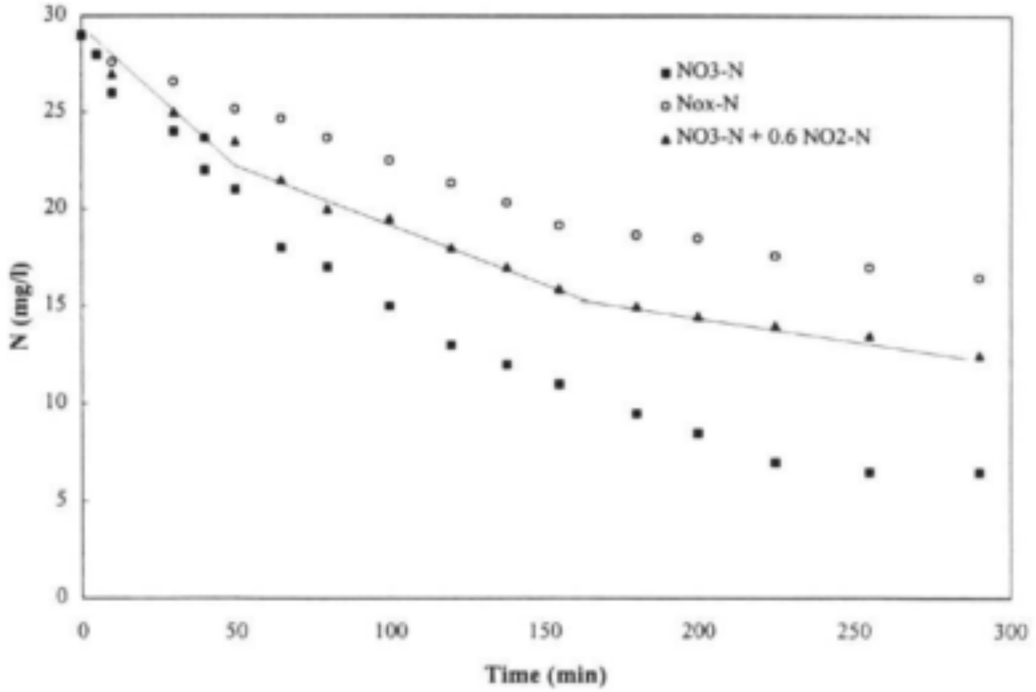


Figure 2-15: Calculation of NOx when NO_2^- accumulation occurs (Çokgör et al., 1998).

- ***S/X (F/M) ratios and NUR profiles***

The choice of the initial substrate to biomass (St/X_T) ratio for anoxic batch test should provide a defined nitrate-N vs time profile with easily distinguishable breaks (Ekama *et al.*, 1986). This needs to be determined by trial and error. For example, initial S/X of 0.45 to 0.60 (mgCOD / mgVSS) with synthetic substrates were found to be too high for the completion of the NUR tests. Therefore, the initial COD was only partially consumed at the end of the experiment. S/X ratios of 0.13 to 0.22 (COD/VSS) were found to be adequate for appropriate NUR profiles (Çokgör *et al.*, 1998).

Tests by Çokgör *et al.* (1998) also showed that mixed synthetic substrates were not reduced at a single overall rate but at appreciably different rates. They suggested that the storage of some of the RBCOD may also exert some influence on the differing rates. They also found for a 2 to 3 h test the second change in rate was not necessarily the endogenous respiration rate as was initially expected. Instead the change in rate was linked to the influent COD fraction with a lower biodegradation rate. However, it is important to note that Majone *et al.* (1999) reported that OUR profiles showed a tailing phenomenon after the RBCOD utilization which was considered to be linked to the reuse of storage compounds.

- ***Biological phosphorus (bio-P) removing sludge***

It is known that nitrate has a negative effect on the biological phosphorus removal system since denitrification removes some of the readily biodegradable organic material which is supposed to be taken up and stored in the polyphosphate accumulating organisms (PAO's) (see section 2.1.3.5). Thus, some of the RBCOD is removed (Henze *et al.*, 1997). However, when considering factors which influence the NUR test, the same argument may be made against PAO's *i.e.* the presence of PAO's reduces the amount of RBCOD available to the denitrifying bacteria. Therefore, calculation of the RBCOD based on amount of electron acceptor consumed would result in the underestimation of the RBCOD when a significant fraction of PAO's are present in the activated sludge. Substrate removal mechanisms such as accumulation and storage may also affect the RBCOD determination. Substrate can be taken up by microorganisms and maintained in an unchanged form or transformed to low molecular weight metabolic intermediates. This is referred to as accumulation. In addition, under dynamic conditions storage becomes an alternative mechanism for substrate removal even in the absence of any external limitation for the growth (Majone *et al.*, 1996 ; Majone *et al.*, *1*999).

Chapter Three

Experimental Approach and Development

This chapter defines and justifies the experimental approach adopted for the realization and assessment of the nitrate-N utilization rate batch tests. This section deals with the assessment and interpretation of the NUR profiles.

3.1. MATERIALS AND METHODS

3.1.1 Samples

- *Sample source* : The raw wastewater from a total of 23 plants were tested. Eighteen of these plants are situated in Europe with 15 of them located in France. Majority of samples tested were from Europe because the first 2 years of this study was done in Paris, France. Samples were taken from plants with varying characteristics that are currently being operated by Suez-Lyonnaise-des-Eaux. Samples from four South African plants, located in the KwaZulu-Natal, Durban region, were also tested. Time constraints of the project limited the number of South African treatment plants tested.

- Grab samples of activated sludge were removed from a sampling point near the exit of the biological reactors. Raw wastewater was sampled just after the screening stage but prior to the primary settling tank. The samples were collected prior to the internal loading stage. Appendix I lists the some of the characteristics of the plants tested.

3.1.2 Analysis

- *COD analysis* : Chemical oxygen demand (COD) analysis was done on 2.5 ml samples according to the closed reflux colorimetric method in Standard Methods (APHA, 1992). The concentrated raw wastewater samples were diluted 1 in 2 or 1 in 3 while sludge samples were diluted 1 in 10 for all tests.

- *COD analysis of sodium acetate* : Tri-hydrated sodium acetate was used as a synthetic substrate in NUR batch tests. Since all the organic substrates were represented in electron equivalents *i.e.* COD, it was necessary to do the same for sodium acetate. The theoretical oxygen demand (TOD) was found to be 0.47

x sodium acetate concentration (mg/l). Experimentally, the conversion factor for sodium acetate was found to be 0.43 (Naidoo, 1999). For these studies it was decided to use the theoretical value of 0.47 for all conversions of sodium acetate as COD since the COD test is considered to be 90 to 100 % accurate (APHA, 1992).

- *Nitrate, nitrite and ortho-phosphate analysis* : Nitrate (mg NO_3^--N/l) and nitrite (NO_2^--N/l) were analysed by the cadmium reduction method in an automatic continuous flow system (Skalar). Ortho-phosphate (mgP/l) was also done using the continuous flow system.

- *Total suspended solids (TSS) and volatile suspended solids (VSS)* : These were determined on 100 ml samples which were first centrifuged (14 000 g, 10 min at 4°C). The pellet was dried at 105°C in a glass crucible for 24 h and at 550°C for 2 h (APHA, 1992).

- *Volatile fatty acids analysis* : The volatile fatty acids were analyzed by gas chromatography by the technical staff at the CIRSEE-Suez-Lyonnaise-des-Eaux laboratory.

3.1.3 Preparation and determination of COD fractions

Raw wastewater can be divided into different components depending on the method of separation chosen. In this study, the organic carbon components of wastewater were separated by various physico-chemical methods such as settling, centrifugation, filtration and coagulation. Certain components such as the particulates and truly soluble constituent of wastewater were also calculated based on a combination of theoretical considerations and physico-chemical determinations. These were represented in terms of COD (mgO_2/l) and are given the abbreviation S- for wastewater and X- for sludge.

- *Non-settleable fraction (S-ns.)* : Raw wastewater was added to an Imhoff cone and allowed to stand for 2 h. The COD of the supernatant was then measured. This non-settleable fraction should not be confused with samples taken from the primary settling tanks for NUR tests. The sample was taken directly from the primary settling tanks of the treatment plant and not from the settling tests conducted in the laboratory and is accordingly referred to as primary settler effluent.

- *Centrifuged fraction (S-ce)* : Raw wastewater was centrifuged at 14 000 g for 10 mins at 4°C using 500 ml tubes and the supernatant was then used in the batch tests. Samples were also removed for COD determinations.

- *Filtered fraction (S-$f_{0.45}$))* : Raw wastewater samples were filtered through 0.45µm membrane filters (Sartorius) with a 25 ml syringe for COD determination (S-$f_{0.45}$). Gelman SuporCap 100 (0.45µm) filters were used with the aid of a pump to filter larger volumes which were used as substrates in NUR batch tests.

Non-coagulated fraction (S-co) : Coagulation is regarded as a highly efficient chemical separation technique. The supernatant after coagulation is referred to as the truly soluble fraction. The soluble fraction was determined by the Jar Test method using ferric chloride as the coagulant (Naidoo, 1999).

- *Soluble unbiodegradable fraction (S_I)* : Activated sludge samples taken from systems operating at SRT's > 10 d were filtered through 0.45 µm filters and the soluble component was classed as the soluble unbiodegradable fraction (Henze *et al.*, 1995; Ekama *et al.*, 1986). This fraction may also be referred to as the filtered soluble sludge fraction (X-f).

- *Particulate fraction (S-p and X-p)* : Particulate COD values were determined by difference as shown in equation (3-1a). In the case of wastewater it is the difference between the total raw wastewater concentration (as mgO_2/l) and the 0.45 µm filtered raw wastewater fraction, while for activated sludge samples it is the difference between the total sludge concentration (X_T) (mgO_2/l) and the filtered sludge fraction (X-f) (eqn 3-1b). The calculation of these values were necessary to determine the substrate to biomass (S/X) and COD/VSS ratios of sludge.

$$S\text{-}p = St - S\text{-}f_{0.45} \qquad (3\text{-}1a)$$

$$X\text{-}p = X_T - X\text{-}f \qquad (3\text{-}1b)$$

where St represents the total raw wastewater COD concentration.

Readily biodegradable fraction (Ss) : According to Mamais *et al.* (1993) this fraction may be determined physico-chemically by the difference between the truly soluble fraction (S-co) and the soluble unbiodegradable fraction (S_I).

3.2. NUR TEST PROTOCOL AND INTERPRETATION

The basic set-up of this test is taken from the procedure provided by Ekama *et al.* (1986). However, some changes have been made to this procedure based on recent work by Sozen and Orhon (1996), and Sozen *et al.* (1998) (see **Chapter 2**). The raw data for all the NUR tests referred to in this report are listed in Naidoo (1999).

3.2.1 Batch test set-up

Denitrification kinetics were conducted in batch reactors which were continuously stirred and temperature controlled at 20°C (Figure 3-1 and 3-2). The total reactor volume was 2 l with a working volume of 1.4 to 1.6 l. Nitrogen (N_2) gas was used to maintain an oxygen-free environment. At the start of the experiment, N_2 was bubbled through the liquid to remove trace amounts of oxygen (O_2) (Figure 3-1 ; No.1). During sampling, nitrogen gas was passed over the liquid to minimise foaming, pH increase and to prevent oxygen introduction (Figure 3-1 ; No.2). Each reactor contained a gas outlet port which passed through a water trap to avoid pressure increase due to nitrogen and carbon dioxide (CO_2) production. The contents of the batch reactor were mixed throughout the duration of the test in order to ensure homogeneity (Figure 3-1 ; No.7). The duration of the tests was between 4 to 6 h but initial tests were run for longer periods (8 h).

3.2.1.1 pH

During denitrification the pH increases (Figure 3-3 ; (B)). This results in nitrite accumulation. Thus, controlling the pH at 7.5 is important for optimizing the NUR procedure. The pH was regulated at 7.5 ± 0.1 with 1M hydrochloric acid and 0.75 M sodium hydroxide (Figure 3-3 ; (A)).

3.2.1.2 Redox

Redox was found to be as a monitoring tool to determine oxygen ingression or nitrate depletion ((A) in Figure 3-4). A typical redox curve consisted of an initial rapid drop in redox, followed by a stable redox profile. A further rapid drop in redox represents the complete utilization of nitrate in the reactor and the onset of anaerobiosis ((B) in Figure 3-4).

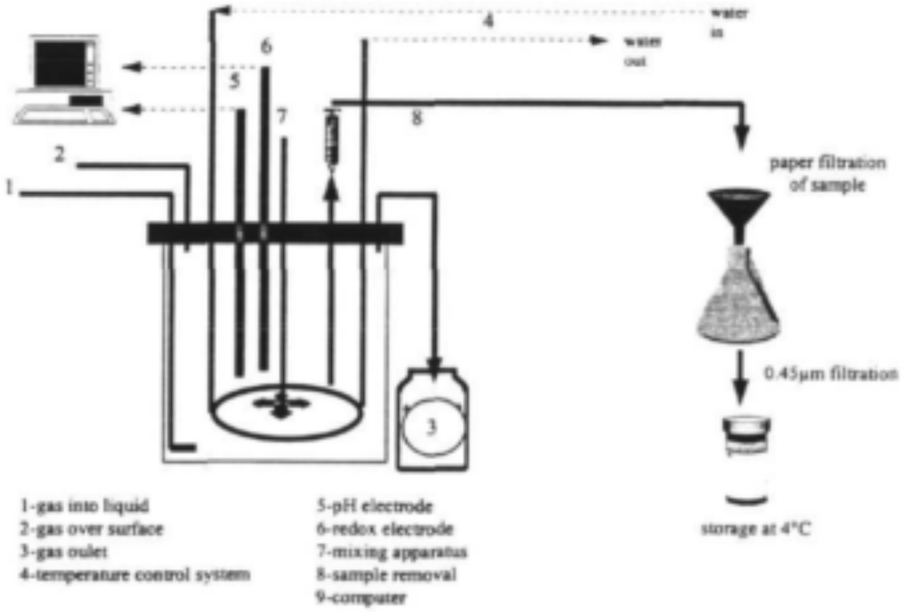
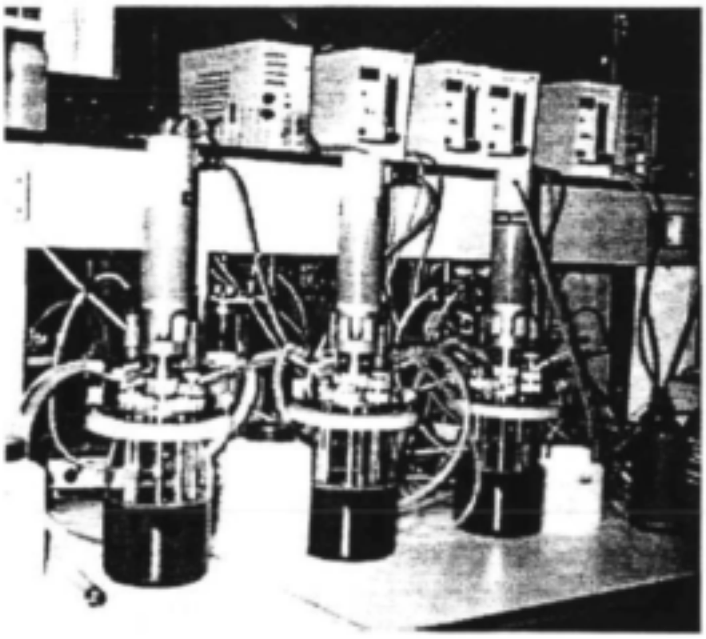


Figure 3-1: Illustration of batch experimental apparatus used for nitrate-N utilization rate tests.



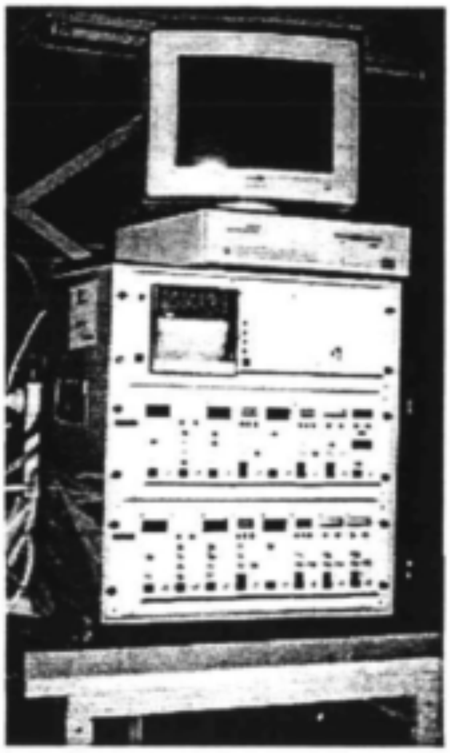


Figure 3-2: Photographs of batch reactors and data capture set-up for denitrification tests.

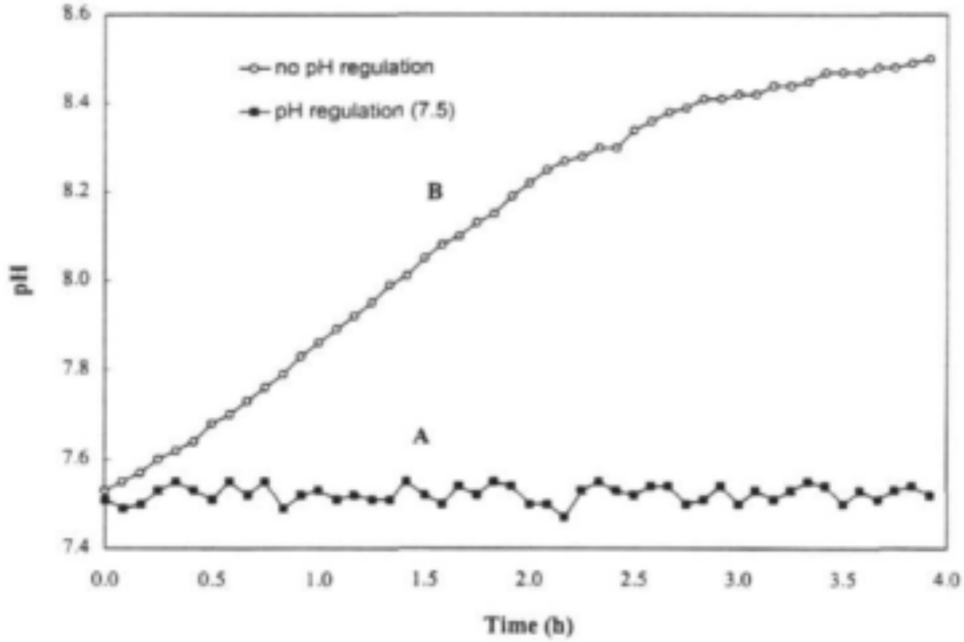


Figure 3-3: Examples of pH curves regulated at pH 7.5 ± 0.1 (A) and unregulated (B).

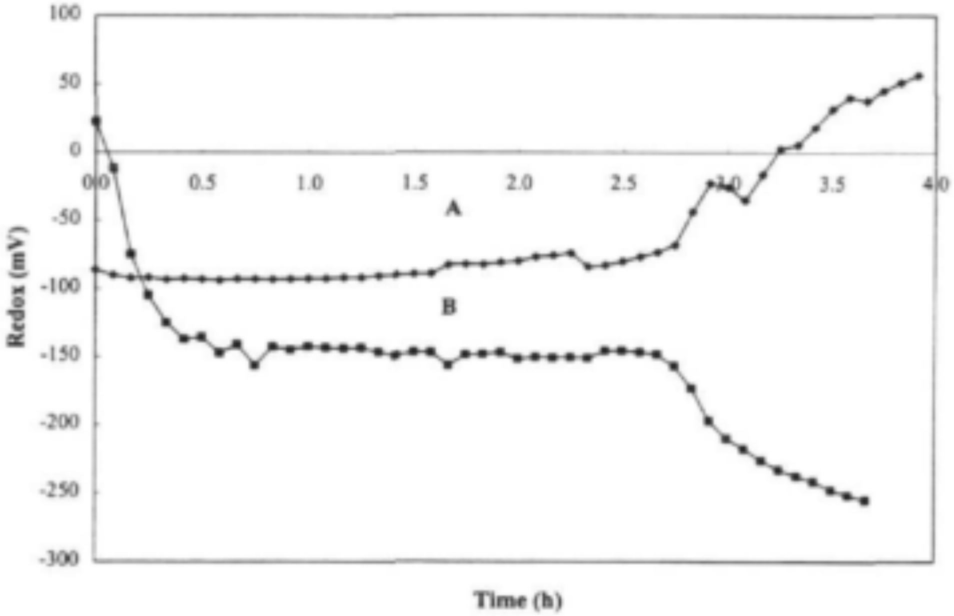


Figure 3-4 : Redox curves : (A) showing non-ideal anoxic conditions due to O_2 ingression and (B) showing a typical redox profile with complete nitrate utilization.

3.2.1.3 Choice of S/X ratio

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

- ***The S/X ratio for acetate*** : Four S/X ratios, 0.02 ; 0.05 ; 0.1 and 0.2 (mgCOD/mgCOD) were tested for acetate. A S/X ratio of 0.02 was found to reveal two phases for a 4 h batch test while S/X ratios, 0.05 to 0.2 revealed a single phase. The denitrification profiles for tests done at S/X ratios, 0.05 and 0.2 (mgCOD/mgCOD) are plotted in Figure 3-5. The S/X ratio, 0.02 (mgCOD/mgCOD) was chosen for acetate since the 2 phase profile provides a second slower rate which allows for the calculation of the amount of acetate (as COD) consumed during denitrification *i.e* the acetate mass balance ((A) in Figure 3-5). The calculation method for the acetate consumed (*i.e.* COD mass balance) is presented in section 3.2.6.

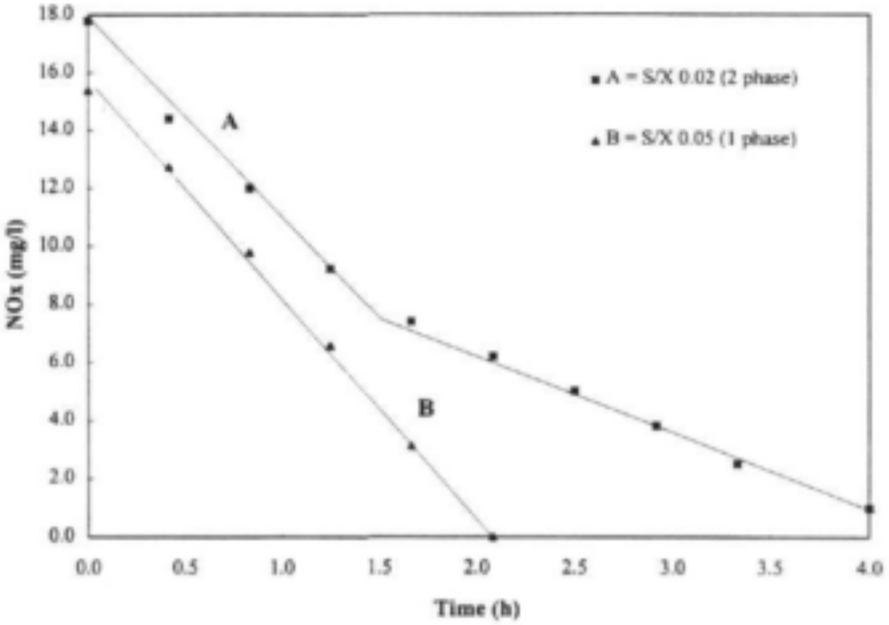


Figure 3-5 : Different S/X ratios (0.02 and 0.05 mgCOD/mgCOD) for acetate showing 1 (B) and 2 (A) phase profiles during the NUR test.

- ***The S/X ratio for raw wastewater*** : Initially the S/X ratios for raw wastewater were based on the total COD concentration (St). This proved to be unfavourable since highly concentrated wastewaters with low soluble COD (*i.e.* COD after filtration, S-$f_{0.45}$) concentrations gave unsuitable curves *i.e.* flat curves with indistinguishable breaks. Therefore, it was decided to base the substrate to biomass ratios on the soluble

COD values. S-$f_{0.45}$/X ratios > 0.02 mgCOD/mgCOD (*i.e.* with a S-$f_{0.45}$ COD concentration > 50 mgCOD/l) but less than 0.1 mgCOD/mgCOD gave more suitable curves. Occasionally, however, a situation may arise where even though the S/X ratio is correct, the NUR profile will be unsuitable due to the biodegradability of the soluble compounds.

Thus, by knowing the concentration required in the batch reactor, it is possible to calculate the volume of raw wastewater required by using equation 3-2, below.

$$V_{ww} = \frac{C_R \times V_T}{C_M} \tag{3-2}$$

V_{ww} = volume of wastewater that is required to avoid RBCOD limitation (l)

C_R = refers to the concentration of soluble COD required (*i.e* > 50 mgCOD/l)

V_t = total working volume of the reactor (l)

C_M = measured S-$f_{0.45}$ COD concentration (mgCOD/l)

3.2.2 *Examples of NOx profiles*

This section shows the typical NOx profiles obtained for NUR tests carried out with different substrates. Some atypical observations are also discussed.

- ***Endogenous denitrification profiles*** : A single linear phase was observed from batch tests with only sludge *i.e.* no exogenous substrate was added (Figure 3-6). The slope in this example is 1.3 mgNOx/l/h and the specific denitrification rate is 1.0 mgNOx/gVSS.h. In this case the bacteria use the substrates provided by endogenous respiration and could also use the slowly biodegradable COD attached to the sludge.

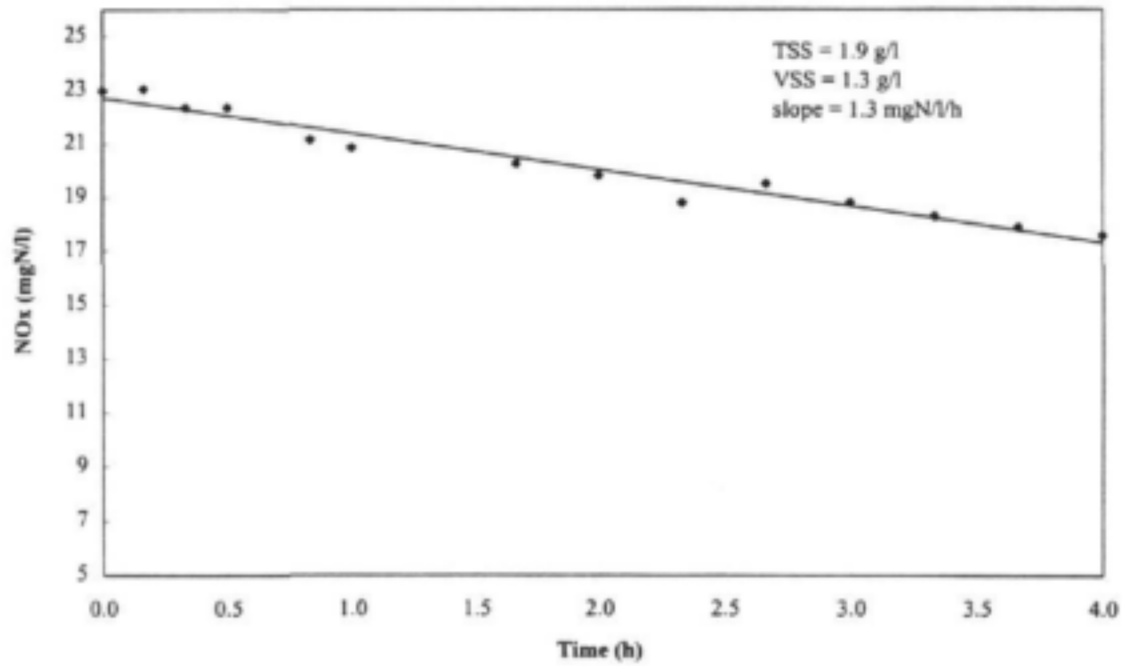


Figure 3-6 : An example of a typical endogenous denitrification profile (Boran WTP 25/02/97).

- ***Raw wastewater*** : Two types of profiles were observed for raw wastewater during the 6 h batch test (Figure 3-7). Some tests with raw wastewater revealed 2 phases while others produced 3 phases. The first phase in both curves (A) and (B) is due to the utilization of the RBCOD fraction of wastewater. For profile (A) the second phase is due to the utilization of SBCOD from the influent as well as endogenous respiration products. Phase 2 of profile B is thought to reflect the utilization of readily hydrolyzable COD of the influent (Figure 3-7). Phase 3 of (B) is considered to be due to the utilization of SBCOD of the influent wastewater as well as endogenous respiration products.

Sometimes, however, atypical NOx vs time profiles were observed revealing 4 phases (Figure 3-8). Since the first phase was extremely rapid and of short duration (10 to 30 min) it was decided to combine the first two phases of these curves as that occuring from the utilization of readily biodegradable COD. In this case, phase 3 and 4 are associated with readily hydrolyzable COD and SBCOD (biomass and influent) utilization, respectively.

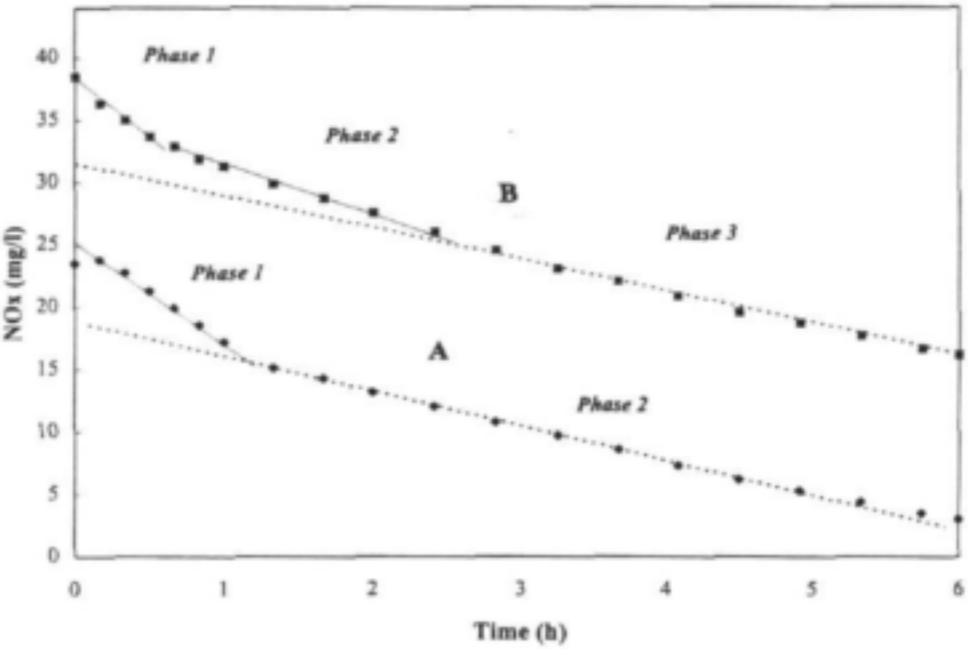


Figure 3-7: Examples of typical NUR tests with raw wastewater as substrate (A and B : wastewater and sludge from Crespiéres and Gouvieux WTP, respectively).

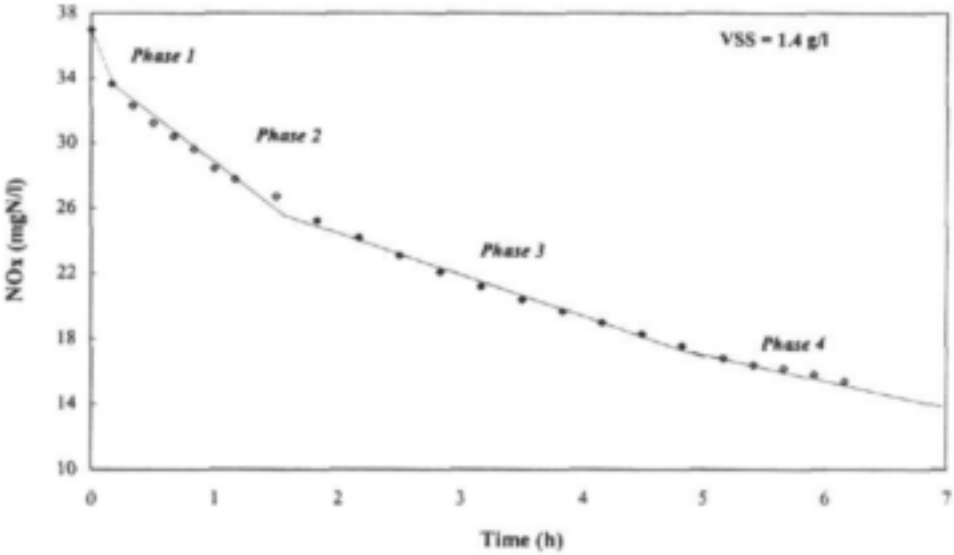


Figure 3-8 : An example of an atypical denitrification profile with wastewater (centrifuged) and sludge samples from Rostock WTP (17/03/97).

- *Acetate* : Based on the S/X ratio (0.02 mgCOD/mgCOD) adopted, 2 phases were expected for acetate-fed reactors. However, as was the case with raw wastewater, both 2- and 3-phase profiles were observed. For 2-phase profiles, the first phase is due to acetate utilization while phase 2 is due to the utilization of endogenous substrates provided for by bacterial death and lysis as well as SBCOD that may be attached to the biomass or released internally by the bacteria e.g. storage compounds. Three-phase profiles, however, presents a more complicated scenario. Phases 1 and 3 may be explained in terms of the utilization of acetate, and endogenous substrate and SBCOD, respectively (Figure 3-9). Phase 2 however, may be hypothesized to be due to one of 2 factors : i) the utilization of pre-existing storage compounds during denitrification, or, ii) the utilization of stored compounds that had been formed from the acetate added to the reactor. This reaction is therefore thought to be triggered by the presence of acetate and possibly readily biodegradable COD. This aspect will be discussed more comprehensively in **Chapter 4**.

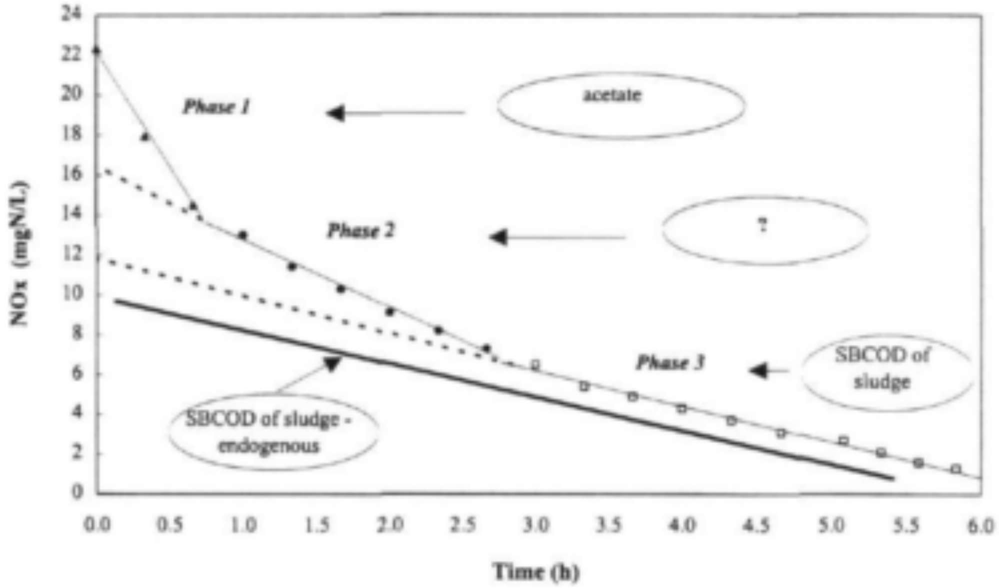


Figure 3-9 : NUR profiles for acetate-fed reactors revealing 3 phases (taken from tests with Rostock samples, 17/03/97).

3.2.3 Calculation of NOx

When substrate concentrations (COD and NO_3^-) are not limiting, denitrification follows zero order reaction kinetics. Approximately 20 mgN/l was added into the reactors at the start of the batch tests. Since nitrites are sometimes detected it is necessary to take these concentrations into consideration.

For biodegradable COD : $NOx = NO_3^-N + 0.6 \times NO_2^-N$ (3-3)

Equation 3-3 expresses NOx in terms of an electron balance. This aspect was discussed in **Chapter 2**.

3.2.4 Calculation of maximum specific denitrification rates

The NOx (as N) concentration is used for calculations based on the assumption that no NO or N_2O intermediates are accumulated. The specific denitrification rates can be calculated from the slope of the linear parts of the N utilization (NUR) curve (Figure 3-10 ; equation 3-4), using the VSS concentration (g/l) as the reference for the biomass concentration *i.e.* the specific denitrification rate is given by the slope of the linear segment divided by the X_{VSS} concentration (equation 3-4). Since more than a single linear phase is observed the rates, k, are given the subscripts k_1 , k_2 and k_3 (Figure 3-10). Note k rates do not reflect the capital K used in activated sludge models. In this study the specific denitrification rate k, is linked to the phases observed in the NUR profile. The rates, k_1, k_2, and k_3 refers to phase 1, phase 2, and phase 3, respectively.

$$k' = slope' / [X_{VSS}] \qquad (3\text{-}4)$$

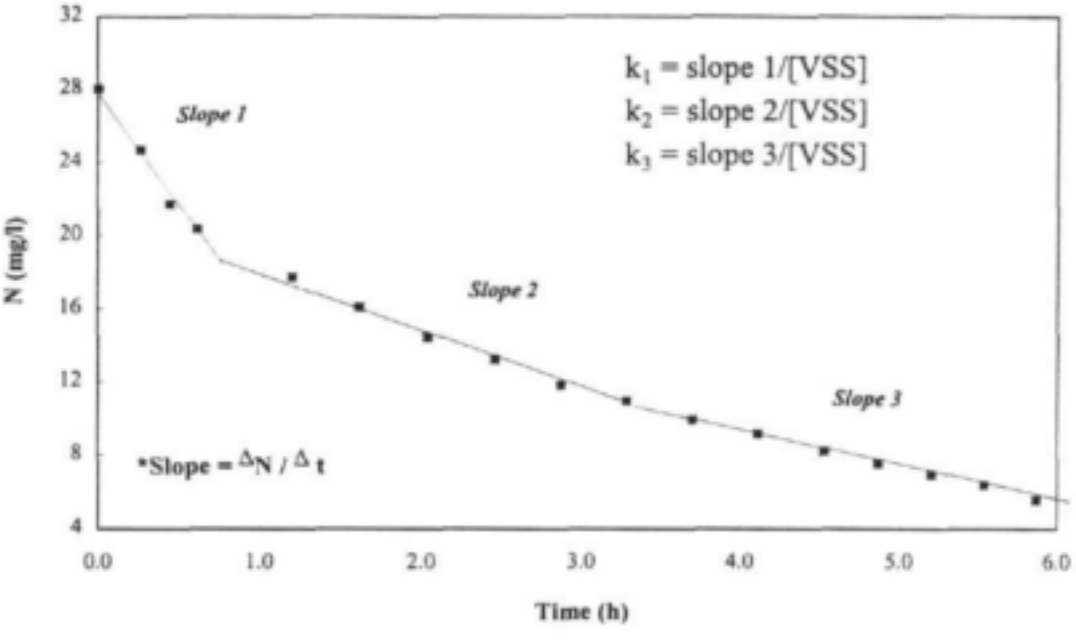


Figure 3-10 : Calculation of specific denitrification rates.

3.2.5 Calculation of RBCOD and RHCOD

With reference to the readily biodegradable fraction (RBCOD), this is calculated by determining the ΔNO_x 1 value. This is given by the difference between the initial NO_x-N concentration (A) and the extrapolated value

(B) drawn from the second phase *i.e.* ΔNO_x 1 = A-B (Figure 3-11)

A second biodegradable fraction, the readily hydrolyzable fraction can also be determined for kinetics revealing three phases. The second ΔNO_x (*i.e.* NO_x 2) can be determined by the difference between values determined by the extrapolation of phases 2 (B) and 3 (C) *i.e.* ΔNO_x 2 = B-C (Figure 3-11). In this case, it is assumed that the second phase is due to the utilization of readily hydrolyzable COD since the phase is of short duration (2-3 h) and the fraction of COD calculated is significantly smaller than that cited in the literature for SBCOD (*i.e* 30 to 60 % of the total COD concentration).

These ΔNO_x values, ΔNO_x 1 and ΔNO_x 2, can then be substituted in equation 3-5 to calculate the RBCOD and RHCOD, respectively. In this study for raw wastewater, the aerobic yield coefficient, 0.63 (mgCOD/mgCOD) was used.

$$\text{COD (mgCOD/l)} = [2.86/(1-Y_H)] \times \Delta NO_x \times (V_t/V_{ww}) \qquad (3\text{-}5)$$

V_{ww} volume of wastewater that is required to avoid RBCOD limitation (l)

V_t total working volume of the reactor (l)

Y_H is the yield coefficient (mgCOD/mgCOD)

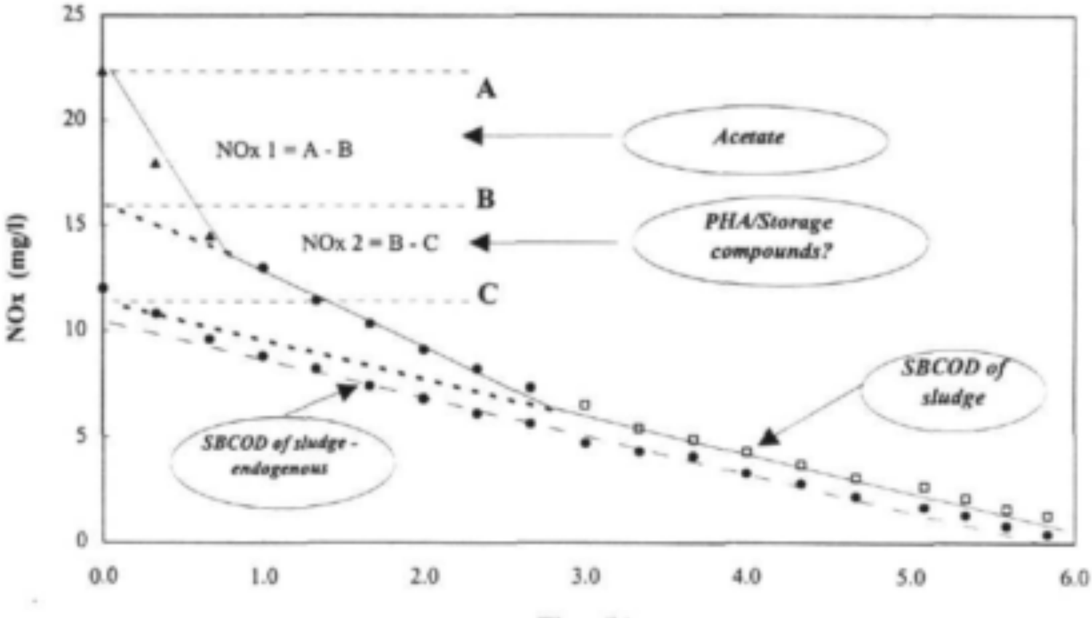


Figure 3-11 : Interpretation of 3-phase NUR profiles with raw wastewater as substrate.

3.2.6 Calculation acetate consumption

As discussed previously, acetate-fed reactors also revealed 2 or 3 phases. Phase 1 was linked to the utilization of acetate, phase 2 was hypothesised to be linked to the acetate that was added to the reactor and phase 3 was linked to the utilization of SBCOD attached to the sludge and endogenous respiration products released by the sludge. This is verified in endogenous denitrification tests using sludge as the organic carbon source *i.e.* no exogenous substrates were added (see D in Figure 3-12).

Since a known concentration of acetate was added, it was possible to calculate the amount of acetate (as COD) consumed to determine if all the acetate could be accounted for. Thus, ΔNO_x 1 and ΔNO_x 2 were calculated in the same manner as described for raw wastewater and the acetate consumed was determined by using equation 3-6. This is the same basic equation used for the calculation of RBCOD and RHCOD, except that the dilution factor was removed since the concentration of acetate (as COD) added to the reactor was known. In these studies, yield coefficients (Y_H), 0.5 and 0.63 (mgO_2/mgO_2), were used to calculate the acetate consumed. The acetate recovery or mass balance (%) can then be calculated by equation 3-7. For 3-phase profiles, acetate recovery 1, and acetate recovery 2 may be calculated based on ΔNO_x 1 and ΔNO_x 2, respectively (Figure 3-12).

$$\text{Acetate } (mgO_2/l) = [2.86/(1-Y_H)] \times \Delta NO_x \qquad (3\text{-}6)$$

$$\text{Acetate Recovery } (\%) = [\text{Acetate}_{calc}] / [\text{Acetate}_{added}] \times 100 \qquad (3\text{-}7)$$

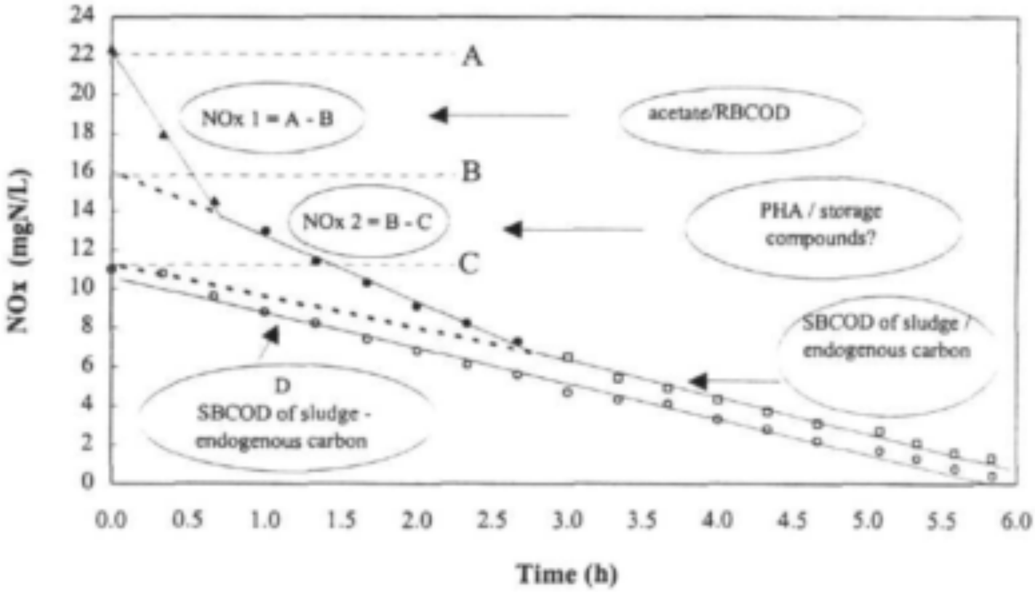


Figure 3-12 : A NOx-N-time profile with acetate revealing 3 phases of biodegradability and an endogenous curve (results from Rostock 17/03/97)

3.2.7 Reproducibility of data obtained from NUR tests

The reproducibility experiment was carried out to determine the experimental precision of the NUR batch test procedure for the determination of the :

1. biodegradable COD fractions, and
2. specific denitrification rates.

Three replicate batch tests were carried out under identical conditions with sludge and wastewater from Boran-sur-øise Wastewater Treatment Plant. NOx vs time profiles obtained in the 3 separate experiments conducted are presented in Figure 3-13. These curves showed that repeatability of the curves was good. Table 3-1 shows that the RBCOD and RHCOD fractions calculated from these tests were reproducible at 25 ± 1% and 11 ± 1%, respectively. The coefficient of variation (SD / mean) on the RBCOD and RHCOD in the reactors were found to be 5 % and 8 %, respectively. The denitrification rates, k_1, k_2, and k_3, were also found to be fairly repeatable with the coefficients of variation < 10% (Table 3-2). These results show that the RBCOD, RHCOD and denitrification rates can be ascertained from single batch tests with reasonable confidence in the method.

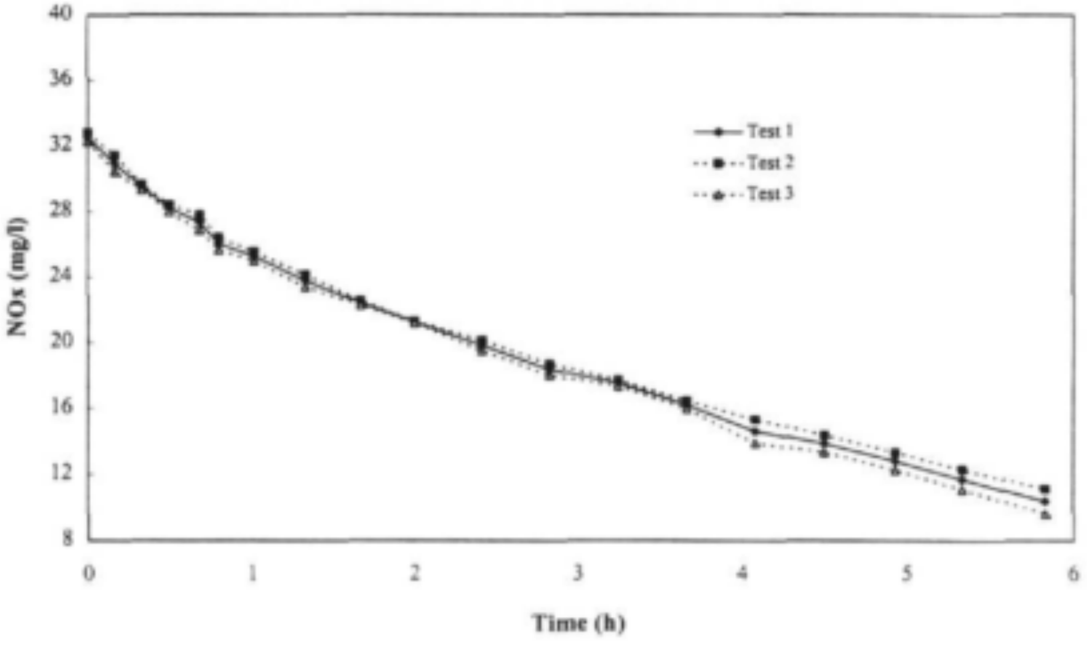


Figure 3-13 : Precision of 3 replicate batch NUR tests.

Table 3-1: Repeatability of RBCOD and RHCOD calculations from 3 replicate NUR batch tests.

		Test 1	Test 2	Test 3	mean	SD	CV (%)
RBCOD	mgCOD/l	257	253	231	247	11	5
	fraction of St (%)	26	25	23	25	1	5
RHCOD	mgCOD/l	100	119	101	107	9	8
	fraction of St (%)	10	12	10	11	1	8

The biodegradable fractions (RBCOD and RHCOD) are given as a % of the total raw wastewater concentration (St). (SD - standard deviation ; CV - coefficient of variation)

Table 3-2: Repeatability of denitrification rates (mgN/gVSS.h) from 3 replicate NUR batch tests.

Rates	Test 1	Test 2	Test 3	mean	SD	CV (%)
k1	6.1	7.1	7.4	6.9	0.6	8
k2	2.9	3.3	3.2	3.1	0.2	5
k3	2.4	2.6	2.6	2.5	0.1	4

(SD - standard deviation ; CV - coefficient of variation

Chapter Four

Acetate Utilization Under Anoxic Conditions

This chapter investigates the use of an experimental RBCOD substrate, acetate, in a denitrifying environment. Acetate was chosen as it is a simple, soluble compound which may be directly incorporated into the metabolic pathways via acetyl Co-A. Acetate, which is a readily biodegradable substrate, was tested with sludge from different sources as a reference for assessing the RBCOD determinations made from data obtained from NUR tests. The results from acetate fed reactors provide an important indicator of sludge activity and RBCOD utilization under anoxic conditions. *The objective of this study was to assess the efficiency and accuracy of the NUR method by using acetate as a reference substrate for the readily biodegradable COD component of raw wastewater.* The choice of EBPR plants, Compiègne and Samaritaine WTP, was based on performance (about 80% biological phosphorus removal). Thiverval WTP, while designed and operated to remove phosphorus biologically, removes only about 55 % of the phosphorus.

As discussed in Chapter 3 denitrification kinetics with acetate (approximately 50 mgCOD/l) revealed NOx time profiles with either two or three phases (see Figure 4-1). In those tests where only two phases were observed, the first phase was due to acetate utilization and the second phase was indicative of the utilization of slowly biodegradable substrates (see Figure 4-1). For the three phase NOx time profiles with acetate it was hypothesized that phase 1 was due to acetate utilization while phase 2 was due to the utilization of internally stored compounds. These storage products could arise from:

- the synthesis of storage products from acetate which are subsequently utilized internally (rapid synthesis / utilization reaction) when acetate becomes limiting (Figure 4-2). The capability to storage and utilize the storage compounds could be linked to bacterial composition and / or biomass history.

- the use of existing storage products whose utilization is triggered by the presence of acetate and/or the electron acceptor.

Since no exogenous substrates other than acetate were added to the batch reactors, phase 3 was considered to be due to the utilization of endogenous products released by the bacterial cells and / or SBCOD attached to the sludge (Figure 4-2).

The data from NUR tests with acetate was interpreted and discussed in several ways. Firstly, it was possible to indirectly calculate the yield coefficient by modifying equation 3-6 (**Chapter 3**) since the initial amount of acetate as COD was known. Secondly, one could assume that the yield coefficient for the different sludges was constant and thus, calculate the acetate consumed during denitrification. This was related to the initial amount of acetate added by determining the acetate recovery (i.e. mass balance %).

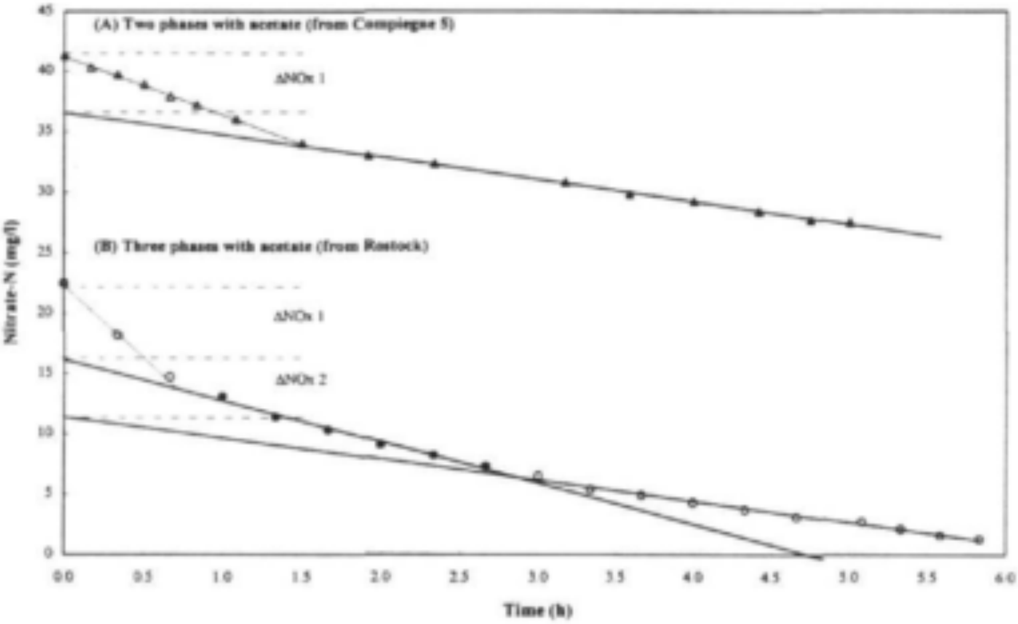


Figure 4-1: Typical Nitrate-N time profiles with acetate as the sole exogenous substrate source (T = 20°C, pH = 7.5).

Figure 4-2: Hypothesis of rapid synthesis of storage compounds from acetate and internal utilization of the stored products during denitrification

4.1. ESTIMATION OF YIELD COEFFICIENT

The yield coefficient can be estimated by modifying the equation 3-6 into equations 4-1a and 4-1b, where ΔNOx 1 or ΔNOx 2 refers to the amount of electron acceptor utilized and [Ace] refers to the concentration of acetate added at the start of the batch test. $Y_{HD}{}^{1}$ refers to the yield coefficient calculated from ΔNOx 1 data while $Y_{HD}{}^{1+2}$ refers to the yield coefficient from the sum of ΔNOx 1 and ΔNOx 2 data (see Figure 4-1). The latter was based on the assumption that phase 2 (rate 2) in a 3-phase acetate-NUR profile is due to the utilization of stored compounds that were produced from the acetate that had been added to the reactor.

$$Y_{HD}{}^{1} = 1 - [(2.86 \bullet \Delta NOx\ 1) / [ace]] \qquad (4\text{-}1a)$$

$$Y_{HD}{}^{1+2} = 1 - ([2.86 \bullet \Delta NOx\ 1+2] / [ace]) \qquad (4\text{-}1b)$$

The results obtained using equations 4-1 (a) and (b) are presented in Table 4-1. The frequency of distribution of the calculated yield coefficients ($Y_{HD}{}^{1}$ and $Y_{HD}{}^{1+2}$) are plotted in Figure 4-3. Majority of the $Y_{HD}{}^{1}$ values were between 0.60 and 0.79 (mgCOD/mgCOD) (Figure 4-3). The mean calculated $Y_{HD}{}^{1}$ value (based on ΔNOx 1) was found to be 0.69 mgCOD/mgCOD which is higher than the aerobic yield coefficient, 0.63 mgCOD/mgCOD. These results suggest that the denitrifiers use some of the acetate that is available for denitrification and rest is used to replenish the stored reserves or may be accumulated. The accumulation and storage process is considered to be rapid (Majone *et al*., 1999). This rapid accumulation or storage reaction is thought to be prevalent in biomass that has been growing under dynamic conditions. It is, however, also likely that the high yield coefficients calculated are due to the presence of polyphosphate accumulating organisms which take up and store some of the available acetate. This is supported by results obtained when both phase 1 and phase 2 are considered. The mean calculated $Y_{HD}{}^{1+2}$ (based on ΔNOx 1 + ΔNOx 2) value, was found to be 0.54 mgCOD/mgCOD which is lower than the aerobic yield coefficient, 0.63 (mgCOD/mgCOD). However, this value is closer to the anoxic yield coefficient values 0.50 and 0.54 mgCOD/mgCOD cited in the literature by Sperandio *et al*. (1997) and Sozen *et al*. (1998). The distribution frequency of these values varied from 0.50 to 0.69 mgCOD/mgCOD (Figure 4-3). The variation in the results suggested that the yield coefficient is not constant for all types of activated sludges or that the acetate was not utilized solely by denitrifiers. In other words, acetate utilization was influenced by other factors.

Table 4-1: The calculated yield coefficients for different sludges using acetate as an experimental readily biodegradable substrate (* - enhanced biological phosphorus removal plants ; N/A - not applicable).

Treatment Plant	Y_{HD}^{1}	Y_{HD}^{1+2}
Asnieres s/oise	0.72	N/A
Artemps-Seraucourt 1	0.62	0.44
Artemps-Seraucourt 2	0.69	0.57
Berwick	0.66	N/A
Boran 1	0.64	N/A
Boran 2	0.77	N/A
Boran 3	0.71	N/A
Boran 4	0.62	N/A
Boran 5	0.63	N/A
Boran 6	0.66	0.40
Boran 7	0.71	0.61
Boran 8	0.80	0.65
Boves	0.69	N/A
Brno	0.65	0.40
Compiègne 1*	0.80	N/A
Compiègne 2*	0.80	N/A
Compiègne 3*	0.73	N/A
Compiègne 4*	0.68	N/A
Compiègne 5*	0.73	N/A
Compiègne 6*	0.73	N/A
Creil 1	0.64	N/A
Creil 2	0.61	N/A
Crespières 2	0.69	N/A
Gouvieux	0.62	N/A
Laon	0.79	0.61
Morainvilliers	0.54	N/A
Orense	0.60	N/A
Rostock	0.62	0.39
Samaritaine 1*	0.66	N/A
Samaritaine 2*	0.78	0.55
Samaritaine 3*	0.78	0.57
Thiverval 1*	0.68	0.58
Thiverval 2*	0.72	0.60
Villers sous St. Leu	0.78	0.58
Average	0.69	0.54
Standard deviation	0.07	0.08

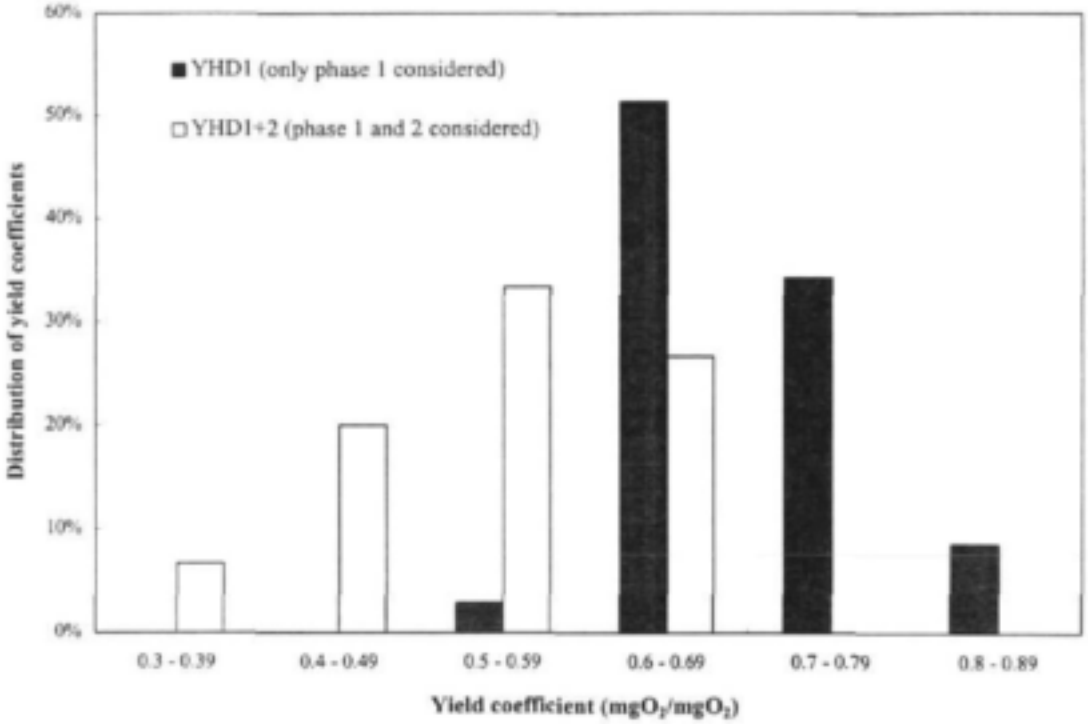


Figure 4-3:Frequency of distribution of the calculated yield coefficients with acetate as substrate.

4.2. ACETATE RECOVERY

As discussed in **Chapter 3**, since acetate was the sole readily biodegradable substrate added, the first ΔNOx (*i.e.* ΔNOx 1 - phase 1) was used to calculate the acetate recovery (percent acetate mass balance 1). However, in those cases where a second ΔNOx (*i.e.* ΔNOx 2 - phase 2) was determined it was used to calculate a second acetate recovery (percent acetate mass balance 2) since endogenous denitrification profiles revealed a single phase which implied that no additional COD contribution was made from the sludge apart from the SBCOD attached to the sludge or the endogenous respiration products released by the sludge.

Lower anoxic yield coefficients of about 0.5 mgCOD/mgCOD have been cited by Sozen *et al.* (1998) and Sperandio *et al.* (1997) for acetate. However, for these studies with acetate, a yield coefficient of 0.63 mgCOD/mgCOD, which is cited by Henze *et al.*, (1995) for anoxic reactions involving activated sludge, was used. This value is also fairly close to the yield coefficient of 0.66 mgCOD/mgCOD used in the modelling of wastewater processes.

These acetate recovery interpretations were made based on three assumptions :

- the NUR test is a reliable and accurate measure of the RBCOD concentration
- the yield coefficients, 0.63 mgCOD/mgCOD, used is correct and constant
- the interpretation of the NOx vs time curves is correct

Table 4-2 lists the acetate mass balances (recovery) for the different tests using a yield coefficient of 0.63 mgCOD/mgCOD. The percentage acetate recovery varied widely for the different types of activated sludges tested and the main observations are discussed below.

Table 4-2 :Acetate recovery 1 and 2 based on the use of constant yield coefficient, 0.63 (mgCOD /mgCOD).

		Concentration		$Y_{HD} = 0.63$		
Plant		**P**	**[Ace]**	**1 (%)**	**2 (%)**	**1+2 (%)**
Asnieres s/oise	11/9/97	4	50	74	0	-
Artemps	21/8/97	0	50	102	49	151
Artemps	22/8/97	1	50	83	33	117
Berwick	2/5/97	ND	50	92	0	-
Boran 1	22/10/96	ND	40	116	0	-
Boran 2	25/2/97	ND	70	97	0	-
Boran 3	1/4/97	ND	60	105	0	-
Boran 4	2/4/97	ND	70	106	0	-
Boran 5	3/6/97	0	50	104	0	-
Boran 6	9/6/97	0	50	99	0	-
Boran 7	11/6/97	0	50	90	72	162
Boran 8	17/7/97	3	50	79	27	106
Boran 9	31/7/97	7.5	50	53	40	93
Boran 10	22/8/97	3	50	61	0	-
Boran 11	29/8/97	4	50	78	0	-
Boves	4/9/97	3	50	84	0	-
Brno	1/6/97	ND	50	95	67	162
Compiègne 1*	3/6/97	7	50	53	0	-
Compiègne 2*	5/6/97	6.5	50	53	0	-
Compiègne 3*	9/6/97	6	50	73	0	-
Compiègne 4*	11/6/97	7	50	86	0	-
Compiègne 5*	28/8/97	6	50	73	0	-
Compiègne 6*	29/8/97	2	50	73	0	-
Creil 1	28/8/97	0	50	97	0	-
Creil 2	29/8/97	0	50	105	0	-
Crespières 2	21/8/97	2	50	84	0	-
Gouvieux	11/9/97	ND	50	102	0	-
Laon	7/8/97	4	50	56	48	104
Morainvilliers	26/2/97	ND	70	123	0	-
Orense	18/5/97	ND	50	108	0	-
Rostock	17/3/97	ND	50	102	61	163
Samaritaine 1*	23/4/97	ND	50	91	0	-
Samaritaine 2*	25/4/97	ND	50	60	61	121
Samaritaine 3*	28/4/97	ND	50	60	55	115
Thiverval 1*	17/7/97	2	50	85	29	114
Thiverval 2*	31/7/97	6.5	50	74	34	108
Villers	10/9/97	6	50	59	54	113

(P -ortho-phosphate as P ; Ace - acetate concentration added as COD ; ND - not determined ; * - Enhanced biological phosphorus removal plants)

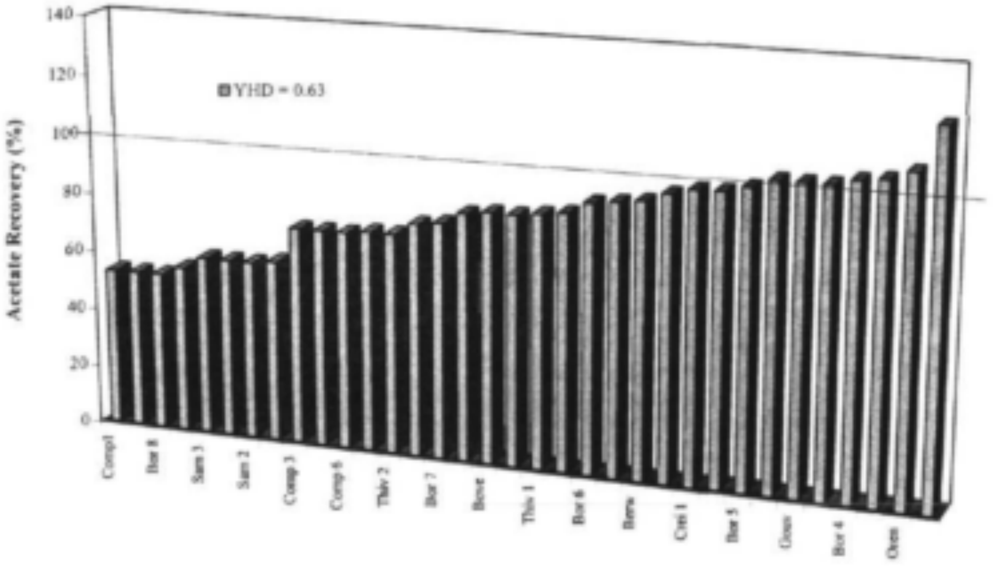


Figure 4-4: Acetate recovery based on ΔNOx 1 for Y_{HD} = 0.63 mgCOD/mgCOD.

4.2.1 Interpretation of data with constant Y_{HD} of 0.63mgCOD/mgCOD

With the use of the yield coefficient, 0.63 mgCOD/mgCOD, 4 trends were observed :

- 100 % acetate mass balance (ΔNOx 1)
- < 100 % acetate mass balance (ΔNOx 1)
- Sum of acetate recovery ≅ 100 % (ΔNOx 1 + ΔNOx 2)
- Sum of acetate recovery 1 and 2 > 100 % (ΔNOx 1 and ΔNOx 2)

1) 100 % recovery based on ΔNOx 1

A mass balance of 100 % was calculated for Artemps 1, Berwick, Boran (2, 3, 4, 5, 6), Brno, Creil (1, 2), Gouvieux, Orense and Rostock using ΔNOx 1 (Table 4-2 ; Figure 4-4). These results suggested that the activated sludge samples from these plants contained a higher proportion of denitrifiers and a smaller or insignificant proportion of polyphosphate accumulating organisms (PAO's). These PAO's were unable to compete with the denitrifiers for the available acetate. This inability to take up substrate for storage could also be explained in terms of *biomass history*. Majone *et al.* (1996) reported that the bacterial response (*i.e* utilization/growth, accumulation or storage) may be due to the microbial composition as well as the physiological state of the bacteria. The latter is influenced by the operating (dynamic) conditions imposed on the process.

2) < 100 % recovery based on ΔNOx 1

Kinetics with sludge from Boves (P release), Artemps 2 (P release), Asnieres (P release), Boran (7, 8, 9, 10, 11 - P release), Compiégne (1, 2, 3, 4, 5, 6 - P release), Samaritaine (2, 3 - P not determined), Thiverval (1, 2 - P release), and Villers (P release) resulted in a < 100 % acetate recovery (Table 4-2 ; Figure 4-4). In these cases, except for Samaritaine where ortho-phosphate (as P) was not determined, the activity of denitrifying polyphosphate accumulating organisms (DPAO's) and/or PAO's could account for the loss of acetate from the denitrification reaction. The P release observed for Boves, Asnieres and Boran was not expected since these are not phosphorus removal plants *i.e.* these are non-EBPR plants. Thus, it would appear that even in non-EBPR systems, a proportion of PAO's were present which were able to compete with the denitrifiers for the available acetate or that the physiological state of the biomass resulted in an accumulation or storage response.

3) Sum of acetate recovery ≅ 100% recovery based on ΔNOx 1 + ΔNOx 2

For those kinetics which produced three phases, acetate recovery 2 was calculated as a fraction of the acetate added to determine if a mass balance of 100 % was possible by taking the sum of acetate recovery 1 and 2. The sum of the acetate mass balances 1 and 2 was found to be approximately 100 % for Boran (8, 9), Laon, and Thiverval 2 (Table 4-2). These results seem to suggest that acetate may sometimes be diverted through three pathways, one for energy with the use of nitrates as electron acceptor, another for growth and the third is the production of storage compounds. Hence, there was < 100 % recovery with ΔNOx 1 but a 100 % mass balance when the sum of ΔNOx 1 and ΔNOx 2 was considered. However, the 100 % recovery with ΔNOx 1 and ΔNOx 2 suggests that the bacteria use storage products during denitrification. It was postulated that these storage products could arise from the synthesis of storage products from the acetate added to the reactor which are subsequently re-used when the acetate added becomes limiting (rapid synthesis / utilization reaction). This is substantiated with the observation from NUR profiles of a short *intermediate* phase which follows the first rapid phase. This *intermediate* phase appears to be similar to the *tailing phenomenon* described for OUR tests done with acetate (Majone *et al.*, 1999). Simultaneous denitrification and P release/uptake was observed in several of these tests (Figure 4-5). Since it is known that polyphosphate accumulating bacteria release storage compounds for use with the electron acceptor O_2 under aerobic conditions, it would seem likely that in the presence of the electron acceptor NO_3^- under anoxic conditions the same could apply. This is possible when denitrifying polyphosphate accumulating organisms are present (Meinhold *et al.*, 1999).

4) Sum of acetate recovery 1 and 2 > 100 % based on ΔNOx 1 and ΔNOx 2

The sum of recoveries 1 and 2 for Artemps (1, 2), Boran 7, Brno, Rostock and Samaritaine (2, 3), Thiverval 1 and Villers were greater than 100 % (Figure 4-6). For several of these tests about 100 % of the acetate added could be accounted for in phase 1 which suggests that the biodegradable COD that was calculated in phase 2 could be due to the utilization of existing storage compounds present in the heterotrophic biomass (Figure 4-6). One of the factors which could have led to these observations is the organic loading rate of the plant from which the samples were taken. Unfortunately the organic loading rate data were not available at the time of this report.

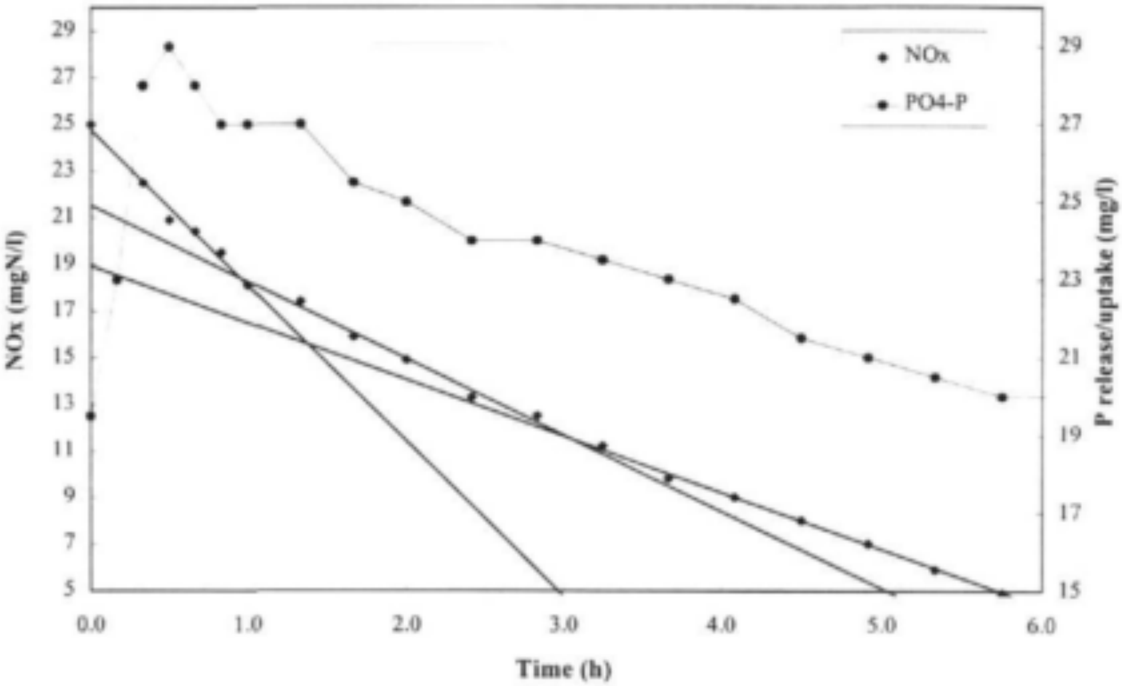


Figure 4-5: Typical example of ortho-phosphate (as P) release and uptake with simultaneous denitrification (T = 20°C; pH=7.5).

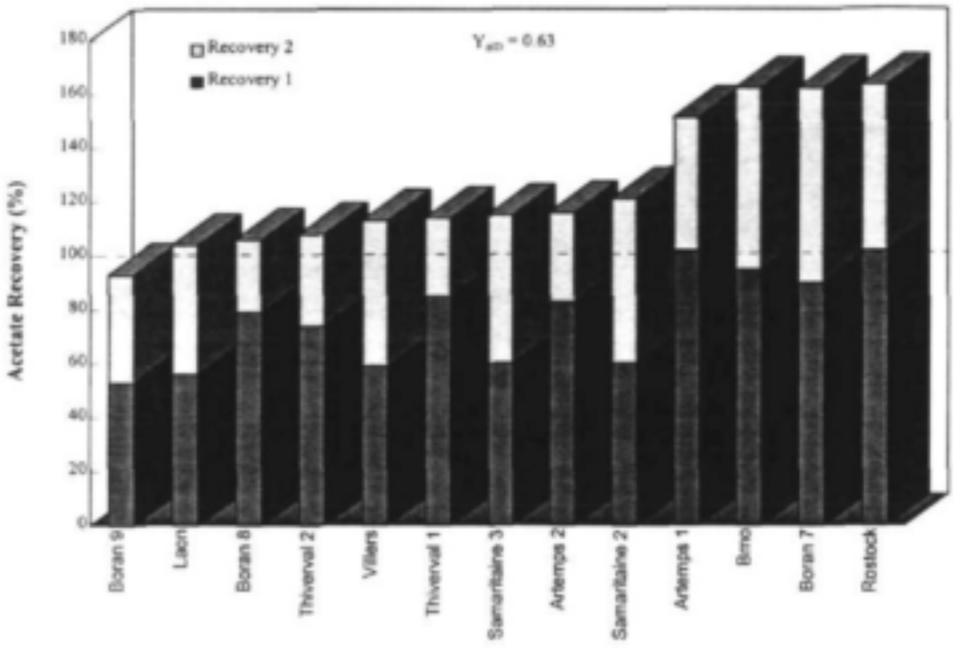


Figure 4-6 : Acetate recovery 1 and 2 with yield coefficient of 0.63 (mgCOD/mgCOD).

Based on these observations it was possible to interpret acetate utilization under anoxic conditions in the following way (see Figure 4-7):

i.) Acetate was used exclusively for denitrification by denitrifiers *i.e.* where acetate mass balances were 100 % (1 in Figure 4-7). In this case, the sludge contained a significant proportion of denitrifiers and little or no polyphosphate accumulating organisms or that the bacteria did not require storage compounds.

ii.) In cases where acetate recovery was < 100 %, it could be interpreted acetate was used for denitrification and for the production of storage products like polyhydroxyalkanoates (PHA's). It is possible that these bacteria do not contain sufficient storage material and therefore, the acetate that is taken up is not utilized during denitrification but stored for future use (1, 2, and 4 in Figure 4-7). In addition to the denitrifying bacteria these sludge samples probably contained a significant proportion of polyphosphate accumulating organisms but no denitrifying polyphosphate accumulating organisms.

iii.) Results also suggest that acetate is used for denitrification and for polyhydroxyalkanoate production. These stored (polyhydroxyalkanoate) compounds are subsequently re-utilized during denitrification. The utilization of the stored compounds in the second denitrification phase is supported by the P uptake observed after the P release and the presence of a short intermediate phase (Figure 4-5) (see step 1, 2 and 3, and 4 and 5 - Figure 4-7). This is supported by results which show that the sum of the two mass balances is approximately 100 %. Thus, these sludge samples contained denitrifiers and denitrifying polyphosphate accumulating organisms which competed for the available acetate but subsequently re-used the stored compounds.

iv.) Some results suggest that all the acetate was used for denitrification. However, the bacteria also used existing storage compounds. Therefore, the sum of recovery 1 and 2 is greater than 100 %. This scenario could be due to the presence of denitrifying polyphosphate accumulating organisms which already have sufficient internal storage compounds. Therefore, when an electron acceptor becomes available these storage compounds are utilized.

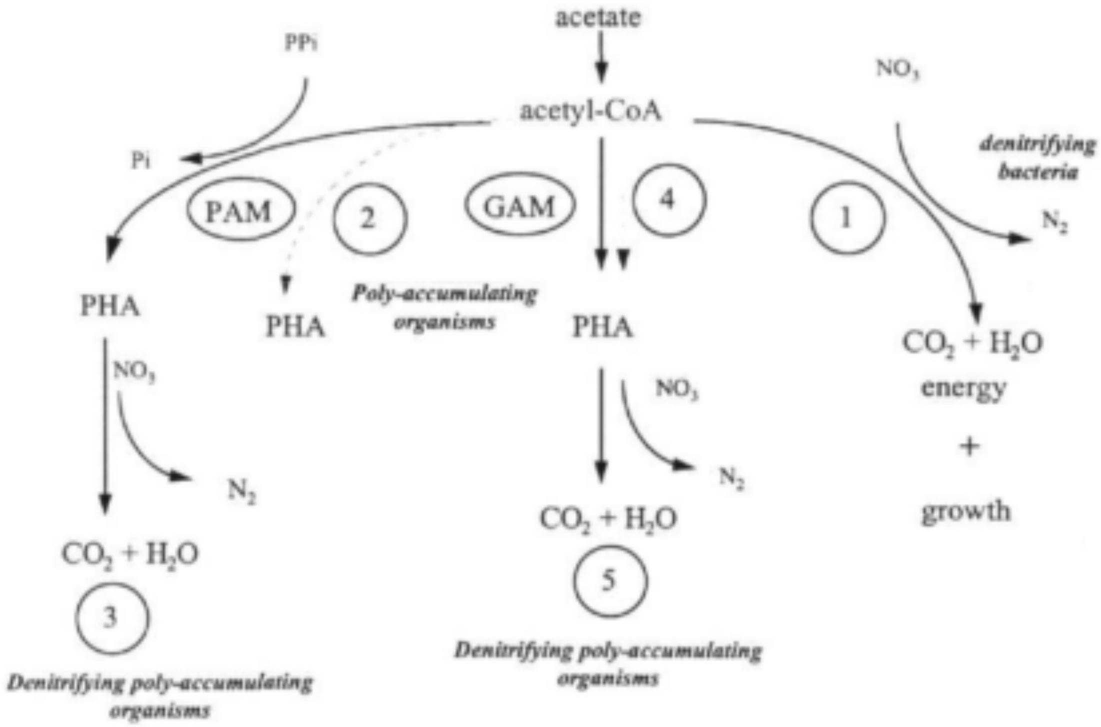


Figure 4-7: Pathways of acetate utilization that may be possible in an anoxic system containing polyphosphate accumulating organisms, denitrifying PAO's, and denitrifiers.

4.2.2 COD and P release correlations

Since the concentration of acetate added to each reactor was known, the COD taken up by polyphosphate accumulating organisms could be found by the difference between the COD added and the COD recovered (i.e. COD consumed during denitrification) (Table 4-3). Consequently, the ratio of COD taken up to phosphorus (P) ratios were calculated. By plotting sequestered COD vs P release curves, a weak correlation of COD_{seq} = 2.9 x P release; r = 0.67) was found between P release and COD consumed by PAO's which highlights the variability of theses ratios. The average COD/P ratio was found to be 3.5 ± 1.5 which correlates to the values 2 to 5 reported by Wentzel *et al.* (1986) (Table 6-3). Most of the COD/P ratios were between 2 and 4 (Figure 4-8).

Results also showed that the COD/P ratios were variable for individual plants. For example, Boran, a non-EBPR plant, gave COD/P ratios which ranged from 3.1 to 6.3 (mgCOD/mgP). In addition, Compiegne, an EBPR plant, gave COD/P ratios ranging from 1.1 to 7.0 (mgCOD/mgP). These are significantly different values for the same sludges. The pH could not be cited as a possible reason for the variability since the pH of the batch reactors were controlled at 7.5. The internal P content is cited as a factor which could contribute to this variability (Shuler and Jenkins, 1997). The variation in COD/P ratios could be linked to the energy source, polyphosphate and glycogen, used to drive the reaction. If more glycogen is expended for substrate uptake and conversion, then the amount of P released will be less while the amount of substrate taken up will remain the same. Thus, the COD/P ratio will decrease. If polyphosphates are mostly used to drive the substrate removal reaction then the COD/P ratio will increase. Therefore, glycogen or polyphosphate limitation may play a significant part in the amount of P released per mg COD taken up. The appearance of phosphorus in the bulk liquid is as a result of the degradation of the internal reserves of polyphosphates to provide the energy necessary for the production of polyhydroxyalkanoates. However, the dependency of polyP as an energy source can vary due to the balance between production and consumption of energy in the cell (Mino *et al.*, 1998 ; Brdjanovic *et al.*, 1998b). In addition, the indirect measurement of COD uptake could also explain the variation in COD/P ratios.

Table 4-3: Comparison of P release and acetate (mgO_2/l)recovery data using Y_{HD} = 0.63 (mgCOD/mgCOD).

	P release (mgP/l)	**COD consumed (mgCOD/l)**	**COD/P (mgCOD/ mgP)**
Asnieres s/oise	4.0	13	3.25
Boran 2	3.0	19	6.33
Boran 3	4.0	11	2.75
Boran 7	3.0	10	3.33
Boran 8	7.5	23	3.07
Boves	3.0	8	2.67
Compiègne 1*	7.0	24	3.57
Compiègne 2*	6.5	24	3.69
Compiègne 3*	6.0	14	2.33
Compiègne 4*	7.0	7	1.14
Compiègne 5*	6.0	13	2.17
Compiegne 6*	2.0	14	7.00
Crespieres 2	2.0	8	4.00
Laon	4.0	22	5.25
Thiverval 1*	2.0	7	3.50
Thiverval 2*	6.5	13	2.00
Villers	6.0	20	3.50

* Enhanced biological phosphorus removal (EBPR) plants

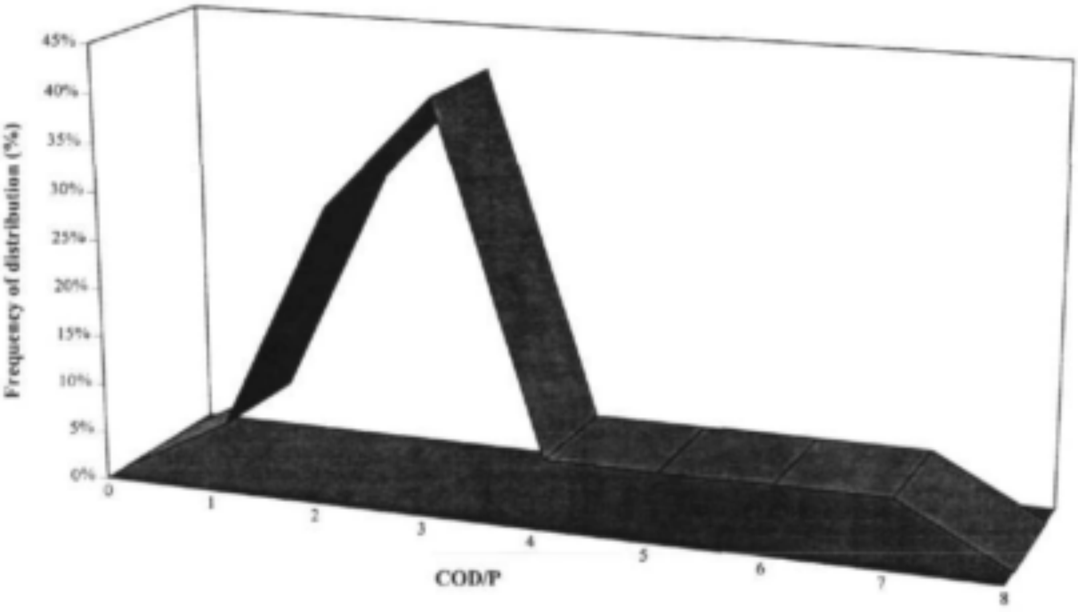


Figure 4-8: Frequency (%) of the COD/P ratios (n=17).

4.3. SUMMARY

This chapter contains important observations as they provide some idea of how the sludge may react to the acetate and hence, RBCOD in the wastewater. Thus, there are three possible interpretations of the acetate results.

- NUR tests which showed < 100 % acetate show that there will probably be an underestimation of RBCOD values if the NUR test is done with the enhanced biological phosphorus removal sludge. Thus, acetate (or RBCOD) may not always be used exclusively for denitrification during anoxic conditions.

- NUR tests which showed 100 % recovery when acetate mass balances 1 and 2 were added, suggest that the acetate that is stored can be re-utilized when acetate in the bulk liquid becomes limiting. However, the internal utilization of the stored compounds may be dependent on the internal concentration of the storage compounds found in bacterial cells and on the physiological state of the biomass used for the tests.

- Those NUR tests which showed > 100 % acetate recovery, suggest that some of the COD calculated by the NUR method may come from the storage products of the biomass. Thus, in some cases there may be a slight overestimation in RBCOD concentrations.

Chapter Five

Wastewater and Sludge Characterization

Wastewater characterization is an important first step when evaluating the operation and efficiency of existing plants. It also provides useful information for the construction and operation of future plants. In addition, it accumulates useful input data for modelling studies which can then be used to simulate best and worst case scenarios with regard to biological processes. The objectives of this part of study were to:

- Characterize the wastewater by a biological method, the NUR batch test.
- Characterize the wastewater by physical and chemical methods such as settling, centrifugation, filtration, and coagulation.
- Characterize the specific denitrification rate constants of wastewater and acetate.

The wastewater characteristics presented here have been determined from several different wastewater treatment plants in Europe and South Africa, with the majority of the wastewaters characterized from France.

5.1. BIOLOGICAL CHARACTERISTICS

The use of NUR tests allowed for the observation and monitoring of the biokinetic response of the denitrifying bacteria to the presence of available organic carbon in the raw wastewater samples tested. This allowed for the determination of a biodegradable component of raw wastewater. It was also possible in certain tests to calculate a second biodegradable component of raw wastewater. This second biodegradable COD fraction will be discussed in more detail in sub-section 5.1.2.

5.1.1 The RBCOD fraction

Table 5-1 lists the results obtained from NUR tests done on different wastewaters. The raw data for the NUR tests are reported in Naidoo (1999). In several of the tests the raw wastewater was separated by settling, centrifugation, filtration or coagulation and the *soluble* component was used as the substrate. The RBCOD fraction derived from NOx-N vs time profiles varied between 7 and 25 % with an average of 13 % (Table 5-1). Frequency distribution curves of the RBCOD concentrations showed that most of the samples contained between 40 to 120 mgCOD/l of readily biodegradable organic matter (Figure 5-1).

These readily biodegradable values (n = 40) were represented as a fraction of the total COD and divided into three groups *i.e.*

- **Group 1** : < 10 % of total raw wastewater COD concentration (St)
- **Group 2** : Between 10 to 20 % of total wastewater COD
- **Group 3** : Between 20 to 30 % of total COD of raw wastewater

The distribution of RBCOD fractions were plotted in Figure 5-2. Less than 5 % of the 40 samples tested contained RBCOD fractions greater than 20 % of the total COD. This suggests that most wastewaters do not contain a significant readily biodegradable COD fraction. In fact, a fairly high number (30 %) of raw wastewater samples contained RBCOD fractions which were less than 10 % of the total COD concentration. In such systems the dominant rate limiting factor of the denitrification or phosphorus removal process will be the type of organic carbon available (Henze, 1994). Majority of the samples (65 %) tested gave values between 10 and 20 % (of total COD). This compares well with the values of 10 to 20 % of total COD cited by Henze *et al*., (1995). It is important to note that 35 % of the samples did not fall within this range. This significant variability highlights the need to characterize the wastewaters of different plants independently for use in simulation studies. This variability in wastewater RBCOD fractions could be due to a number of factors such as type of sewer system, climatic conditions of the region, and dietary habits of the community from which the treatment plant receives its wastewater.

A RBCOD fraction of < 10 % of the total COD was calculated for 11 of the samples tested. A possible reason for these low RBCOD values could be the loss of RBCOD to polyphosphate accumulating organisms which sequester RBCOD for the production of storage compounds with concommittant P release. This could not be verified for the first three plants since phosphorus analysis was not conducted (Table 5-1) and no P release was observed for tests done on samples from Asnieres, Northerns, Kwa-Mashu, and Southerns WTP. However, P release of 2 and 3 mgP/l was observed for Boves and Villers, respectively, suggesting polyphosphate accumulating organism activity. Using the COD/P ratio of 2 and 5 mgCOD/mgP, the amount of RBCOD lost to polyphosphate bacteria was estimated to be between 1 and 2 % of the total COD which is small. Thus, these plants contain raw wastewater with a low RBCOD content.

The phosphorus release patterns for the French Wastewater Treatment Plants differed from Darvil WTP, the one South African enhanced biological phosphorus removal (EBPR) plant that was tested which showed P removal capabilities. For all the French EBPR plants sampled, simultaneous denitrification and phosphorus release was observed for the NUR tests (see Figure 4-4). However, for Darvil WTP the P release was sequential *i.e.* P was released only after all the nitrates-N had been consumed. It is possible that the French Treatment Plants are operated and configured in such a way as to promote the growth of denitrifying polyphophate accumulating organisms while the South African plant, Darvil has a significantly higher proportion of polyphosphate accumulating organisms which do not compete with the denitrifiers for available RBCOD under anoxic conditions. It is also possible that operating conditions such as loading rates and feeding regimes may be responsible for the culturing of different microorganisms which show different P release patterns. Majone *et al.* (1996) reported that biomass fed intermittently were more likely to accumulate and store substrate as a form of competitive or survival mechanism brought about by dynamic conditions such as concentration gradients. Thus, a storage response may not be completely due to the presence of polyphosphate accumulating organisms but may be brought about by ordinary heterotrophs as well.

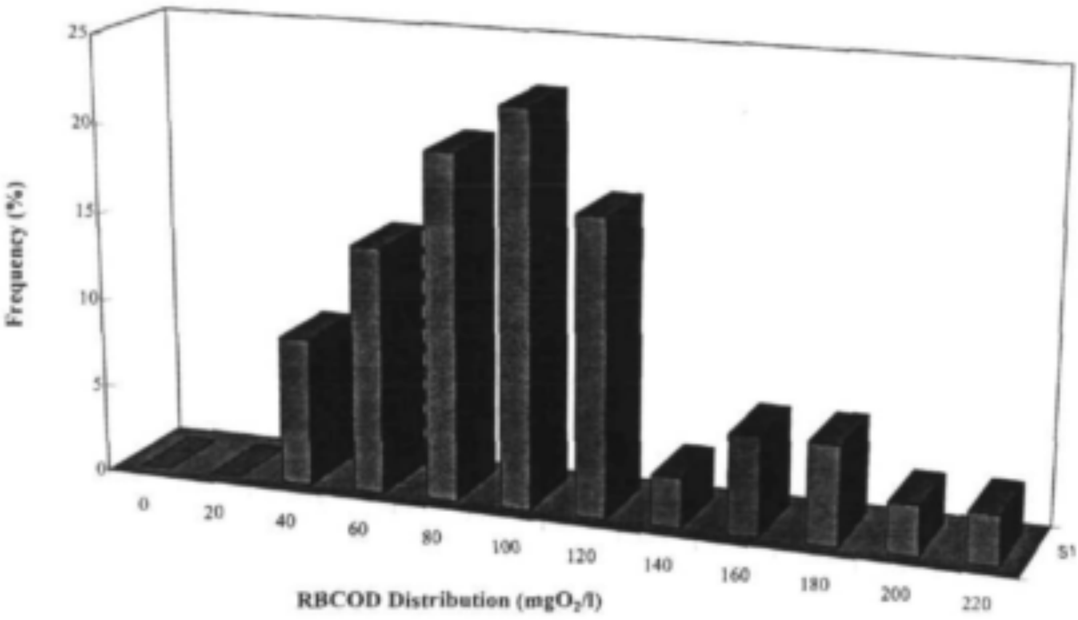


Figure 5-1: Frequency of distribution of the RBCOD concentrations from different raw wastewater samples (n = 40).

Table 5-1: Characterization of the readily biodegradable and readily hydrolyzable COD components of different wastewaters using the NUR method.

		St	RBCOD (1)		RHCOD (2)		1+2
Plant	Substrate	mgCOD/l	%	mgCOD/l	%	mgCOD/l	%
Crespieres 24/02/97	ww-cent	549	9	51	–	–	–
	ww-coag		8	44	–	–	–
Morainvilliers 24/02/97	ww-cent	891	7	65	15	137	22
Morainvilliers 26/02/97	ww-cent	344	17	58	–	–	–
	ww-coag		18	64	–	–	–
Boran 25/02/97	ww-cent	707	13	93	–	–	–
	ww-coag		12	84	–	–	–
Plaisir	ww-cent	691	16	108	–	–	–
	ww-coag		13	89	–	–	–
Rostock	ww-cent	953	17	161	18	176	35
	ww-filt		19	186	15	140	34
	ww-coag		11	105	12	112	23
Orense	ww	407	19	79	22	88	41
	ww-coag		7	29	26	108	33
Brno	ww	250	13	34	24	59	37
	ww-coag		12	31	32	79	34
Samaritaine^{BP} 23/04/97	ww	900	9	79	–	–	–
	ww-non-set.		9	80	–	–	–
Samaritaine^{BP} 25/04/97	ww	750	19	146	10	72	29
	ww-non-set.		17	125	11	83	28
Samaritaine^{BP} 28/04/97	ww	720	11	86	28	200	39
	ww-non-set.		15	110	26	189	41
Laon	ww	652	15	98	12	78	27
Artemps	ww	980	15	108	17	108	32
Creil	ww	853	20	145	–	–	–
Boves	ww	813	18	148	–	–	–
Villers	ww	923	9	80	13	120	22
Asnières	ww	1183	9	95	13	154	22
Gouvieux	ww	817	13	90	14	106	24
Compiègne^{BP} 3/06/97	ww	783	22	172	–	–	–
Compiègne^{BP} 5/06/97	ww	787	13	102	18	142	31
Compiègne^{BP} 9/06/97	ww	883	11	97	–	–	–
Compiègne^{BP} 11/06/97	ww	817	25	204	–	–	–
Compiègne^{BP} 28/08/97	ww	1257	11	75	–	–	–
Thiverval^{BP} 17/07/97	ww	437	11	48	14	55	25
Thiverval^{BP} 31/07/97	ww	977	12	119	5	52	17
Darvil *^{BP}	ww	958	14	135	14	138	28
Kwa-Mashu *	ww	869	8	71	12	105	20
Northerns *	ww	704	7	48	13	88	20
Southerns *	ww	624	7	42	7	41	14

(* - South African treatment plants; ww - raw wastewater; ww-non-set. - non-settleable component after 2 h settling test; ww-cent - centrifuged component; ww-filt - filtered (0.45 µm) component; ww-coag - non-coagulated component; BP - biological phosphorus removal plant)

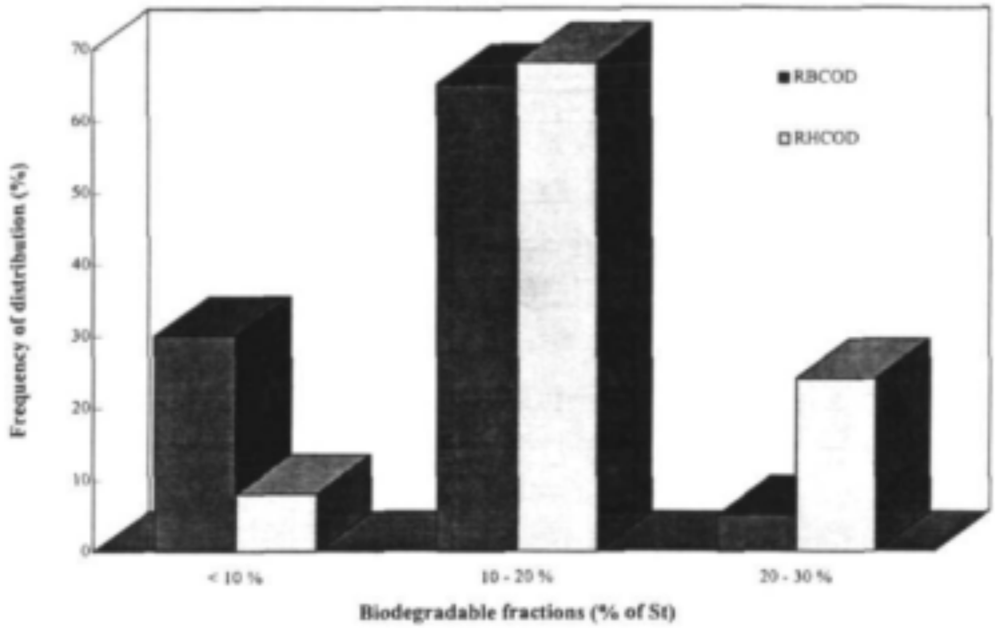


Figure 5-2: Distribution of the RBCOD (n=40) and 'intermediate' (n=24) fractions of raw wastewater measured by NUR tests.

5.1.2 The 'intermediate' fraction

As with the acetate fed tests, some of the NUR batch tests revealed 3 distinctive phases. This made it possible to calculate a second biodegradable COD fraction for 24 of the 40 samples tested. The second biodegradable COD fraction varied significantly between 5 and 30 % with an average of 16 % (Table 5-1). The biodegradable COD concentration, related to phase 2 in NOx(t) profiles, was found to range between 60 to 200 mg/l (Figure 5-3). A distribution diagram, Figure 5-4, shows that most of the values fell between 10 to 20 % of the total COD. Only 5 % of the samples (n = 24) had less than 10 % of biodegradable COD. However, about 29 % of the samples tested gave biodegradable COD values greater than 20 % of the total COD.

This *intermediate* phase which allowed for the calculation of the second biodegradable fraction can be explained in the following ways:

1. It could form part of the slowly biodegradable COD of the influent wastewater since most of these values do not compare well to the SBCOD fraction which is considered to make up about 30 to 60 % of the total COD concentration of wastewater (Henze *et al*., 1995). Since this second phase lasted for about 2 to 3 h and the biodegradable COD fractions calculated from the NUR tests were far lower than expected, this fraction could be classed as a readily hydrolyzable fraction (RHCOD) of the slowly biodegradable group of compounds found in raw wastewater.

2. It could also be that this second biodegradable COD fraction was part of the RBCOD and the intermediate phase was in fact a *residual phase* of phase 1. Thus, the RBCOD values and the second biodegradable COD values were added and grouped into 4 categories : < 10 % ; 10 to 20 % ; 20 to 30 % and > 30 % (Figure 5-4). The majority of the values were > 20 % while only 10 % of the values were between 10 and 20 % of the total COD, the expected range for RBCOD. This suggests that either French wastewaters have unusually high RBCOD concentrations, or that the NUR test measures an intermediate fraction of the raw wastewater *i.e.* the readily hydrolyzable fraction. The suggestion that the second phase is a residual phase of phase 1 is supported by the observations made with the acetate fed reactors (see Chapter 4) where an intermediate phase was also observed and the results from acetate mass balances suggested that phase 2 may also be linked to acetate utilization. Similarly, it could be postulated that some of the RBCOD fed to the biomass was used directly for energy and growth while some of the RBCOD was accumulated or stored. These accumulated and stored compounds become available to the bacteria once the RBCOD concentration becomes limiting. This re-use of accumulated or stored compounds is supported by work done by Majone *et al.* (1999) where a 'tailing phenomenon' was described for OUR tests done with acetate. This 'tailing phenomenon' was linked to a storage response because of the high observed yields.

The second explanation for the observation of the intermediate (residual) phase seems more likely. However, it is also probable that the second biodegradable fraction could not be calculated for all the kinetics because either the duration of the second phase may have been longer than the 6 hour test or the COD fraction causing phase 2 may have been too small to detect. However, these results do suggest that the NUR method may be able to show an intermediate fraction which could be the readily hydrolyzable fraction of SBCOD or a storage fraction of the RBCOD component of wastewater.

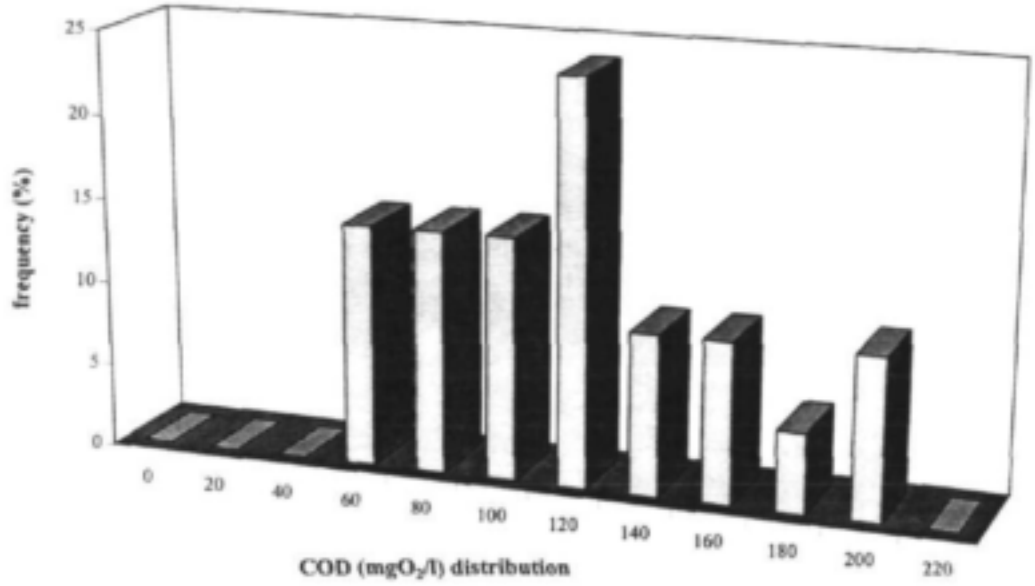


Figure 5-3: Frequency of distribution of the RHCOD concentrations from municipal wastewater (n = 24).

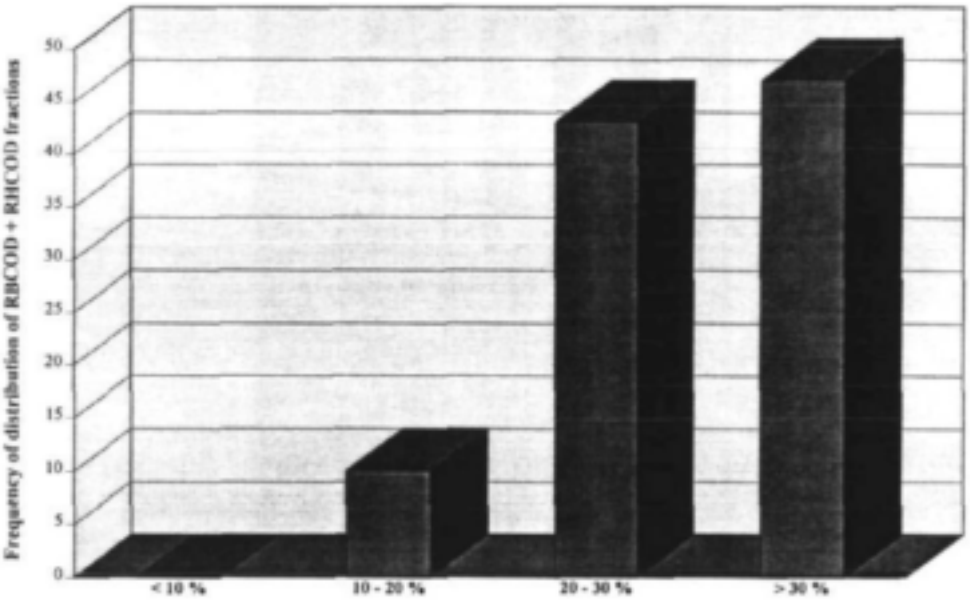


Figure 5-4: Distribution of sum of RBCOD and RHCOD fractions of raw wastewater samples.

5.2. PHYSICO-CHEMICAL CHARACTERISTICS OF WASTEWATERS

Raw wastewater was fractionated by settling (2 h) (S-ns), centrifugation (S-ce), filtration (S-$f_{0.45}$) and coagulation (S-co) (see **Chapter 3**, 3.1.3). All concentrations are given as COD (mgCOD/l) while the fractions are given as a percentage of the total COD concentration. Table 5-2 shows the characteristics of the different wastewaters.

The distribution of the raw wastewater COD concentrations and fractions (%) are plotted in Figure 5-5 and Figure 5-6. The total COD concentration varied significantly with maximum and minimum values of 1157 and 176 mgCOD/l, respectively. However, majority of the concentrations were found to be between between 700 and 1000 mgCOD/l (Figure 5-5). Plotting of the distribution frequencies for the coagulated (S-co), filtered (S-$f_{0.45}$), centrifuged (S-ce), and non-settleable (S-ns.) fractions of wastewater showed that the concentration of these fractions were approximately 100 to 200 mg/l, 200 to 300 mg/l, 200 to 400 mg/l, and 400 to 500 mg/l, respectively. Similarly, Figure 5-6 showed that the average coagulated, filtered (0.45 µm), centrifuged, and non-settleable *soluble* fractions were found to be 26 ± 8; 34 ± 10; 38 ± 15; and 58 ± 17 % of the total COD concentration, respectively. These results and trends were expected since coagulation was considered to be the most efficient of the methods tested for the separation of the soluble and particulate components of wastewater. Coagulation was followed by filtration (0.45 µm), and centrifugation, with settling being the least effect of the methods used. It is important to bear in mind that the results obtained are not only dependent on the separation process but also on the protocol used for the separation technique. For example, the results obtained for samples that had been separated by coagulation and filtration (0.45 µm) would have been more comparable if the final step in the coagulation protocol, *i.e.* filtration, had been removed. Similarly, the soluble fractions measured after filtration or centrifugation would have been less comparable if a lower centrifuge speed had been used.

The results from the 4 South African plants showed that the total COD concentrations were fairly concentrated ranging between 624 and 957 mgCOD/l (Table 1). The non-settleable fraction was found to be about 50 % of the total wastewater. The filtered and coagulated COD concentration for the South African wastewaters ranged between 248 to 268 mgCOD/l, and 139 to 241 mgCOD/l, respectively. The average filtered and coagulated fractions were found to be 34 and 26 % of the total COD concentration (Table 5-3). The results obtained from the 4 South African samples were less variable than the European ones. The limited variability of the different fractions determined from South African wastewater samples was partly due to the limited number of samples tested. Another factor which probably influenced the results was the fact that the samples were collected from treatment plants with similar characteristics e.g. plant capacity 100 000 to 300 000 population equivalents and the samples were taken from plants connected to separate sewers (Table 5-2).

Table 5-2: Characterization of municipal wastewater by physico-chemical methods (mgCOD/l).

	St	S-ns.		S-ce		S-$f_{0.45}$		S-co	
Plant	mg/l	%	Mg/l	%	mg/l	%	mg/l	%	mg/l
Crespieres 26/02/97	176	n.d.	n.d.	49	86	31	54	33	58
Brno	250	n.d.	n.d.	n.d.	n.d.	40	100	32	80
Morainvilliers 26/02/97	344	n.d.	n.d.	49	168	48	165	41	141
Orense	407	n.d.	n.d.	n.d.	n.d.	32	130	17	70
Thiverval 17/07/97	437	60	264	n.d.	n.d.	32	142	29	127
Crespieres 24/02/97	549	n.d.	n.d.	57	313	39	214	31	170
Thiverval 23/07/97	627	48	300	24	153	25	158	24	153
Laon	652	n.d.	n.d.	n.d.	n.d.	41	267	27	176
Boran 29/08/97	670	n.d.	n.d.	12	80	12	40	6	18
Plaisir	691	n.d.	n.d.	32	221	30	207	25	173
Boran 25/02/97	707	n.d.	n.d.	65	459	57	403	50	353
Samaritaine 28/04/97	720	63	453	n.d.	n.d.	35	252	26	187
Samaritaine 25/04/97	750	76	567	n.d.	n.d.	42	315	28	210
Boran 22/08/97	753	n.d.	n.d.	41	308	41	n.d.	n.d.	n.d.
Compiègne 3/06/97	783	76	596	n.d.	n.d.	35	274	n.d.	n.d.
Compiègne 5/06/97	787	42	329	n.d.	n.d.	29	229	23	180
Boves	813	n.d.	n.d.	42	341	40	325	31	252
Gouvieux	817	n.d.	n.d.	42	343	36	294	27	221
Compiègne 11/06/97	817	68	553	n.d.	n.d.	35	283	25	208
Creil	853	n.d.	n.d.	44	375	45	384	28	239
Compiègne 9/06/97	883	61	536	n.d.	n.d.	29	256	22	191
Morainvilliers 24/02/97	891	n.d.	n.d.	30	267	26	232	20	178
Samaritaine 23/04/97	900	69	620	n.d.	n.d.	40	360	24	216
Berwick	913	n.d.	n.d.	n.d.	n.d.	51	466	41	374
Villers sous St. Leu	923	n.d.	n.d.	43	397	41	378	31	286
Rostock	953	n.d.	n.d.	30	286	27	257	24	229
Thiverval 31/07/97	977	22	213	16	152	15	143	13	128
Artemps-Seraucourt	980	n.d.	n.d.	n.d.	n.d.	42	412	32	314
Asnières	1183	n.d.	n.d.	16	189	15	177	12	142
Compiègne 28/08/97	1257	n.d.	n.d.	31	390	29	364	24	302
Darvil *	957	50	482	n.d.	n.d.	28	268	25	241
Kwa-Mashu *	869	51	447	n.d.	n.d.	29	251	26	224
Northerns *	704	56	396	n.d.	n.d.	36	252	29	208
Southerns *	624	57	356	n.d.	n.d.	39	248	22	139

(* - South African treatment plants; n.d. - not determined)

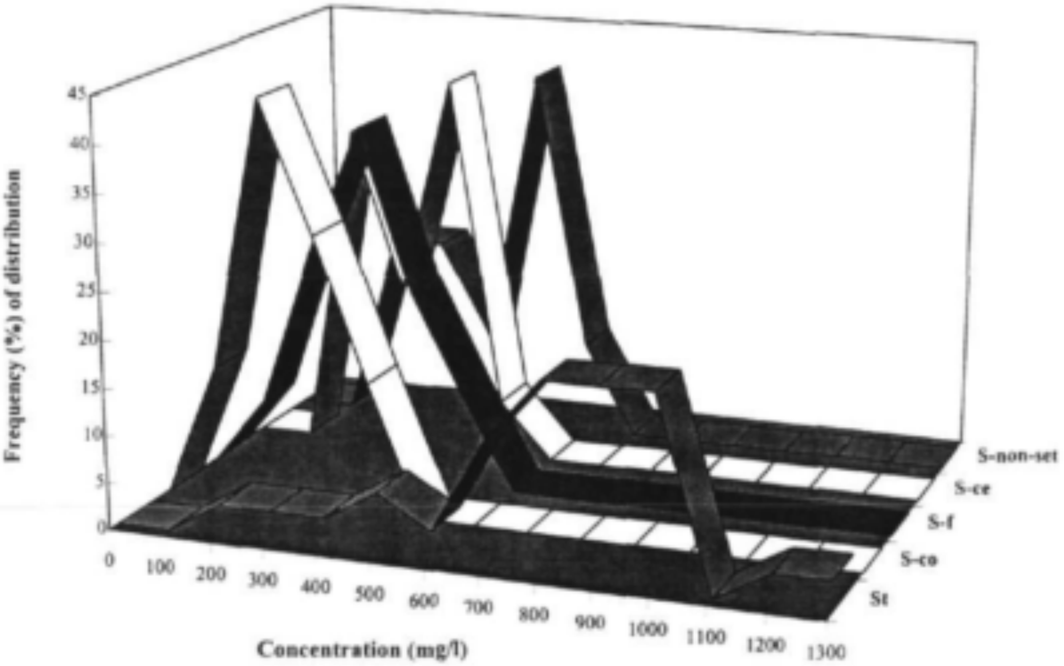


Figure 5-5: Distribution frequencies of the raw wastewater and the non-settleable, centrifuged, filtered (0.45 μm), and coagulated concentrations.

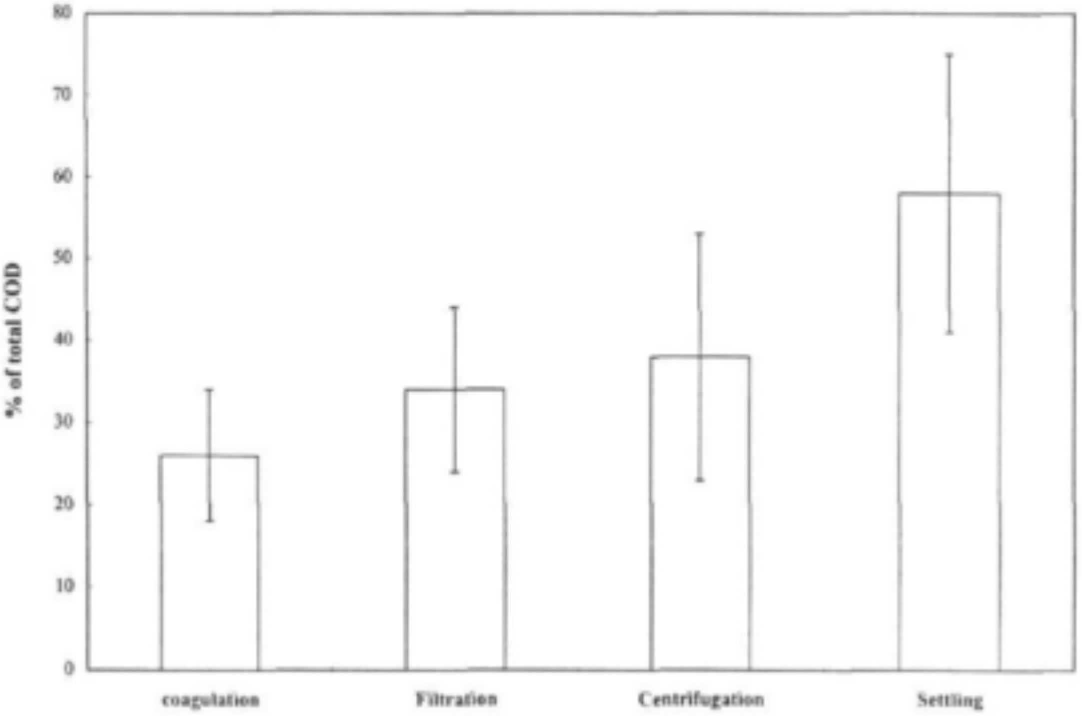


Figure 5-6: Distribution of the 'soluble' fractions of raw wastewater which were determined after coagulation, filtration (0.45 μm), centrifugation and settling tests.

Fairly good linear relationships were found between the total COD concentration and the filtered and coagulated fractions. The 0.45 µm filtered (S-f) and coagulated (S-co) fraction were found to be 33 and 25 % of the total COD concentration, respectively (Figure 5-7). Good correlations were found between the soluble fractions, S-ce, and S-f and S-co. Figure 5-8 shows that the coagulation and filtration method includes about 28 and 9 % less solids/colloids, respectively, than centrifugation. Thus, centrifugation and filtration were fairly similar in terms of threshold limits (Figure 5-8). In addition, a good correlation was also found between the S-f and the S-co fraction. The coagulated fraction was found to be approximately 76 % of the filtered fraction *i.e.* the coagulant takes out about 24 % more of the colloids that pass through a 0.45 µm filter (Figure 5-9).

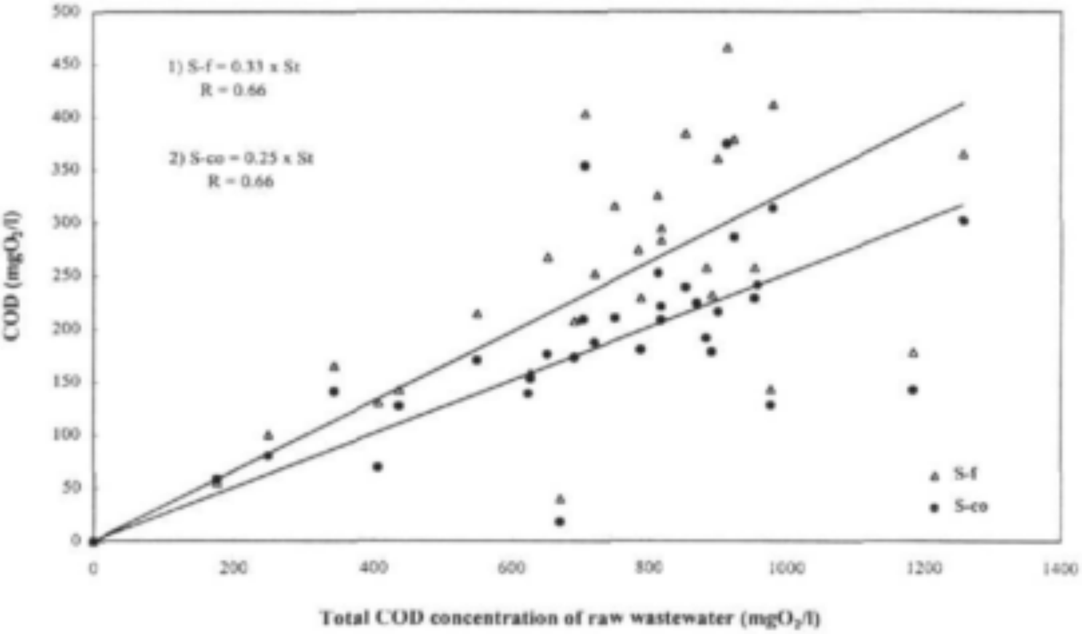


Figure 5-7: Relationship between total COD, and filtered (0.45 µm), and coagulated raw wastewater 'soluble' COD concentrations (n = 28).

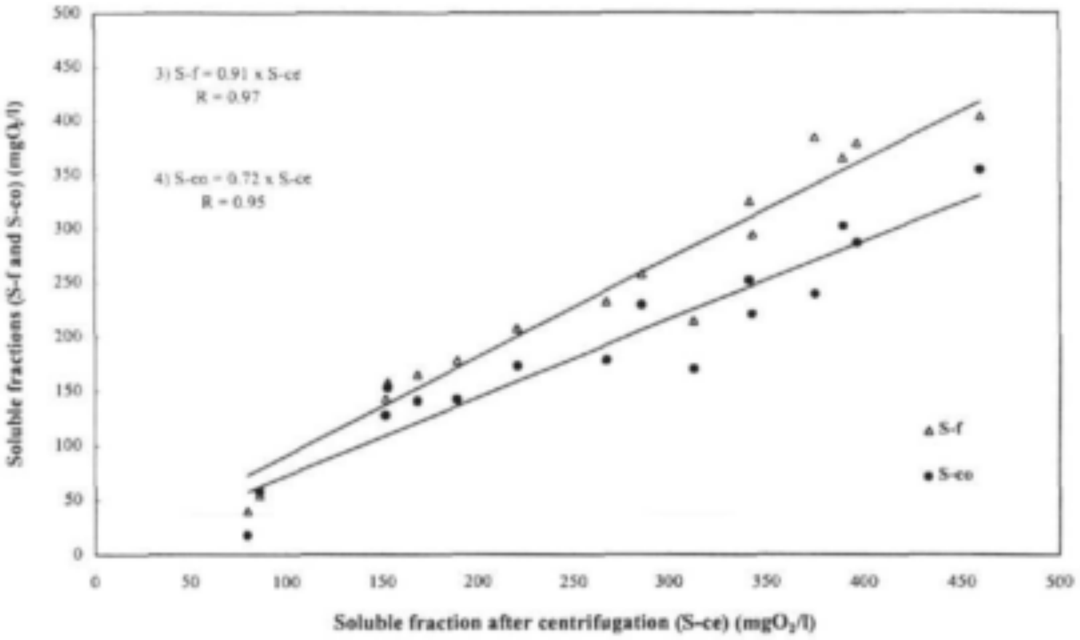


Figure 5-8: Relationships between the 'soluble' concentrations (derived by centrifugation, filtration or coagulation) (n = 17).

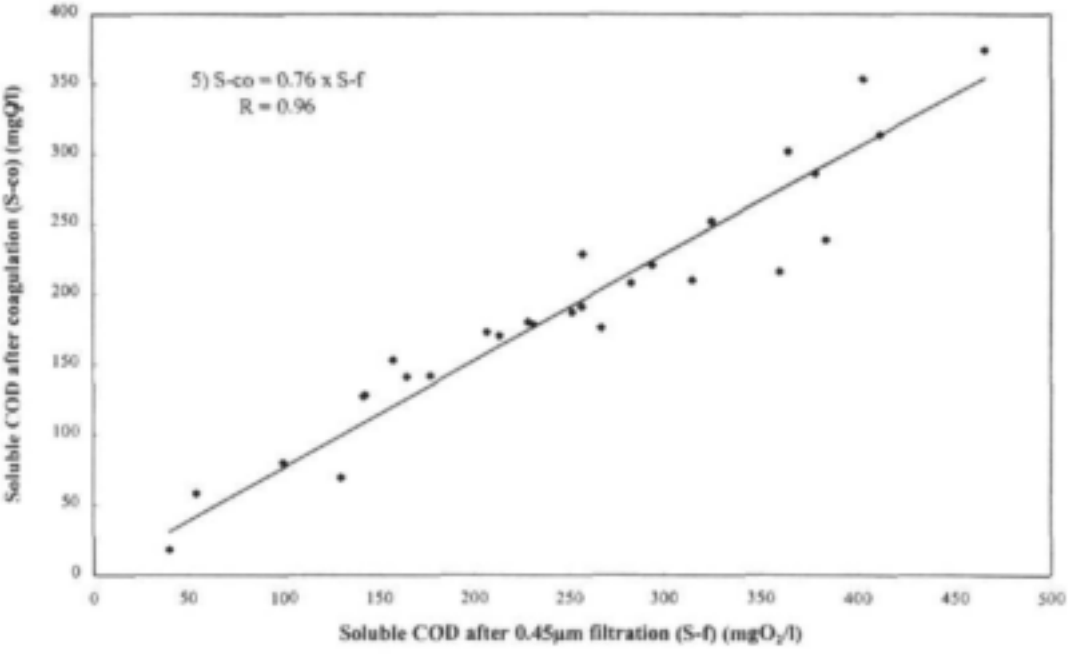


Figure 5-9: Correlation between the filtered and coagulated raw wastewater 'soluble' COD concentrations (n = 28).

5.3. COMPARISON BETWEEN EUROPEAN AND SOUTH AFRICAN WASTEWATER CHARACTERISTICS

The physico-chemical and biological characteristics obtained for European (n = 36) and South African (n = 4) plants were found to be fairly comparable (Table 5-3).

- The average total COD concentrations of the European and South African municipal wastewaters were found to be 749 ± 247 mg/l and 788 ± 155 mg/l, respectively.

- The average readily biodegradable (Ss) fraction which was calculated by difference between the coagulated soluble fraction (S-co) and the inert soluble fraction (Si) were comparable which suggests that the wastewaters from Europe and South Africa could have a similar RBCOD content. These results were compared with the average RBCOD and RHCOD values obtained from NUR tests. The RBCOD values from the NUR tests for both European and South African wastewaters were found to be lower than the soluble physico-chemically determined readily biodegradable COD fraction obtained from the coagulation tests.

- However, the sum of the average RBCOD and RHCOD fractions (*i.e.* 27 %) obtained with European wastewaters were highly comparable to the average Ss fraction (*i.e.* 27 %). The sum of the average RBCOD and RHCOD fractions (*i.e.* 26 %) for the South African wastewaters was slightly lower than the Ss value (*i.e.* 21 %) obtained after coagulation tests (Table 5-3).

Based on the limited comparative results, the South African wastewaters appear to have similar characteristics to the European wastewaters.

Table 5-3 : Comparison of physico-chemical and biological characteristics of some European and South African municipal wastewaters.

Average values	**Europe (n = 36)**	**South Africa (n = 4)**
Total COD	749 ± 247 mg/l	788 ± 151 mg/l
S-ce	37 ± 15 %	42 ± 4 %
S-f	34 ± 10 %	33 ± 5 %
Ss	27 ± 9 %	26 ± 3 %
RBCOD	12 ± 5 %	9 ± 3 %
RHCOD	15 ± 7 %	12 ± 3 %

5.4. DENITRIFICATION RATES

Denitrification batch kinetics generally produced two or three rates which depended on the substrate used, the biomass activity and the test conditions. The results from these NUR tests were combined and evaluated in this section. The rates obtained in this study are expressed as a function of volatile suspended solids concentration and not on the active biomass fraction, and therefore, are not applicable to activated sludge models.

5.4.1 Maximum specific denitrification rates

Table 5-3 shows the range of maximum denitrification rates calculated with either wastewater or acetate. The range for the two substrate, acetate and raw wastewater, was fairly similar. The variation in the individual k_1 values, 2.6 to 9.3 mgN/gVSS.h and 2.6 to 8.3 mgN/gVSS.h, with acetate and wastewater, respectively, highlights the influence of plant operating conditions such as solids retention time, feeding regimes, and type of substrate made available to the bacteria in the influent raw wastewater.

Several tests produced atypical denitrification rates. The maximum specific denitrification rate with sludge from Brno was considered to be far higher (k_1 = 14.2 mgN/gVSS.h) than the other tests conducted with acetate. It appears that this sludge is highly acclimatized to acetate as a substrate. Atypical observations were also made with tests conducted with raw wastewater samples from Rostock, Orense, and Berwick (Table 5-3). Rostock and Orense produced high first rates >13 mgN/gVSS.h. These rates were of short duration (20-30 min). In addition, Rostock revealed 4 phases as opposed to the 3 that were normally observed in the 6 h test (see Figure 3-8). Brno, Rostock and Orense are all European wastewater treatment plants which were located outside France and the wastewater samples had to be stored during transit to the laboratory. Therefore, one factor which could explain these observations is the storage of these samples which was for about 3 to 5 d. It is possible that during transport and storage (*i.e.* dynamic conditions), the bacteria accumulated and/or stored organic compounds that were taken up from the bulk liquid. When conditions became favourable there was a rapid uptake of nitrates from the bulk liquid. Hence, the high denitrification rates. Grau *et al.* (1982) and Daigger and Grady (1982) reported that both accumulation and storage are rapid responses which may be brought about by dynamic conditions such as starvation. Majone *et al.* (1996) hypothesized that in high starvation (low OUR) conditions accumulation is dominant while at low starvation (high OUR) conditions storage is dominant since the latter is more energy consuming.

Berwick was also considered as an atypical case since only a single phase was observed even though the *soluble* fraction ($S\text{-}f_{0.45}$) was not limiting at 374 mgCOD/l (41 % of the total COD). The specific denitrification rate obtained with raw wastewater from Berwick WTP was 1.6 mgN/gVSS.h, which is low (Table 5-3). It is likely that the industrial wastewater received from the beverage industries (orange and whisky) may have had an inhibitory effect on the bacteria or that the wastewater consisted of only slowly biodegradable COD. However, high maximum denitrification rates with acetate ($k_{1\ acetate}$ = 4.5 mgN/gVSS.h) as substrate showed that the low rates were not due to low bacterial activities. Thus, it appears that the raw wastewater from Berwick contains limiting concentrations of RBCOD but a significant concentration of slowly biodegradable substrates which are used at a low rate.

Table 5-4: Range of maximum specific denitrification rates (mgN/gVSS.h) and atypical maximum specific denitrification rates.

Substrate	Range	Atypical rates
acetate	2.6 - 9.3	Brno (14.2)
wastewater	2.6 - 8.3	Rostock (13-18)
		Orense (21)
		Berwick (1.6)

5.4.2 Distribution frequency of denitrification rates

5.4.2.1 Rates obtained with RBCOD and acetate utilization

The majority of the sludges tested produced k_1 values between 4 and 5 mgN/gVSS.h, and 4 and 6 mgN/gVSS.h for acetate and wastewater, respectively (Figure 5-10). The distribution range was wider for wastewater than for acetate. This was expected since acetate is a single simple compound and the rates obtained would be largely due to sludge activity and biomass history whereas municipal wastewater is more complex. However, the maximum specific rate obtained with raw wastewater is controlled to a large extent by both the characteristics of the sludge and the composition of the raw wastewater sample being tested. Since raw wastewater is composed of different compounds of varying biodegradability, a wider range of specific denitrification rates with a greater overlap between the second and third rates observable in the NUR test would be expected.

Determination of the frequency of distribution of the maximum specific denitrification rates from tests with acetate and wastewater showed that about 84 % of the maximum specific denitrification rates were between 3 and 6 mgN/gVSS.h. Only 6 % of the samples were found to have maximum specific denitrification rates less than 3 mgN/gVSS.h, while 10 % of the maximum denitrification rates were greater than 6 mgN/gVSS.h.

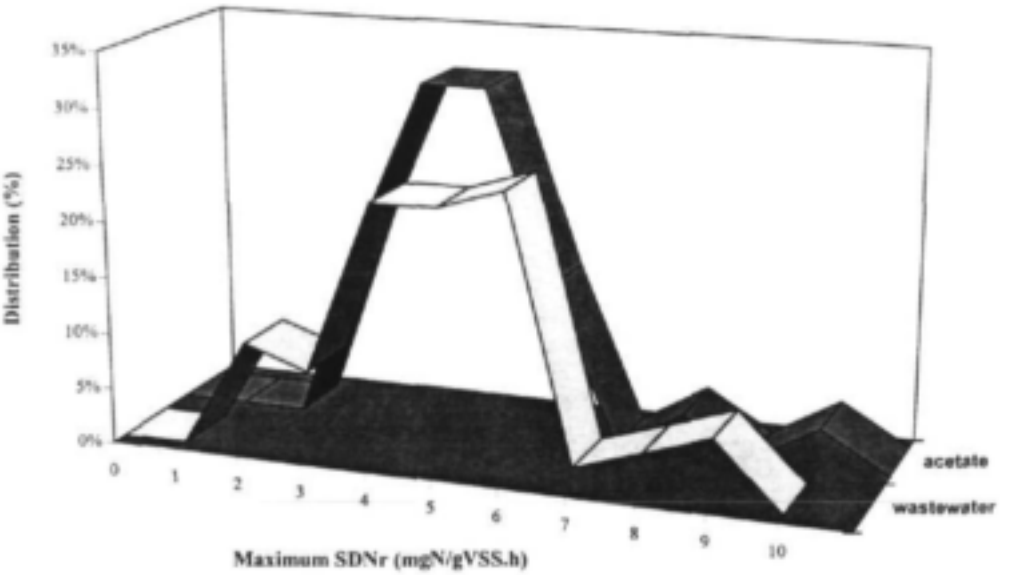


Figure 5-10: Frequency of distribution of the maximum denitrification rates (mgN/gVSS.h) for reactors fed with either acetate or raw wastewater.

5.4.2.2 Range of second rates (k_2) and third rates (k_3) obtainable with acetate or raw wastewater

The second rate or the second phase in the NOx-N profile was considered to be due to the readily hydrolyzable fraction of wastewater or the utilization of stored compounds. About 78 % of the rates calculated were found to lie in the range 2 to 3 mgN/gVSS.h (Figure 5-11). Less than 10 % of the samples gave values below 2 mgN/gVSS.h and only 12 % were above 3 mgN/gVSS.h. These results suggest that the intermediate rate lies between 2 and 3 mgN/gVSS.h. The third rate was considered to be due to the slowly biodegradable fraction and endogenous products and most of the rates (68 %) were found to be less than 1.5 mgN/gVSS.h (Figure 5-12). It is also possible that there may be an overlapping of hydrolytic rates where phase 2 (k_2) hydrolytic products may still be exerting its influence on k_3 values. The variation and range suggests that the slowly hydrolyzable and slowly biodegradable components of the wastewater samples are complex and variable in composition and concentration. Therefore, there was an overlap between rate 2 (k_2) and rate 3 (k_3).

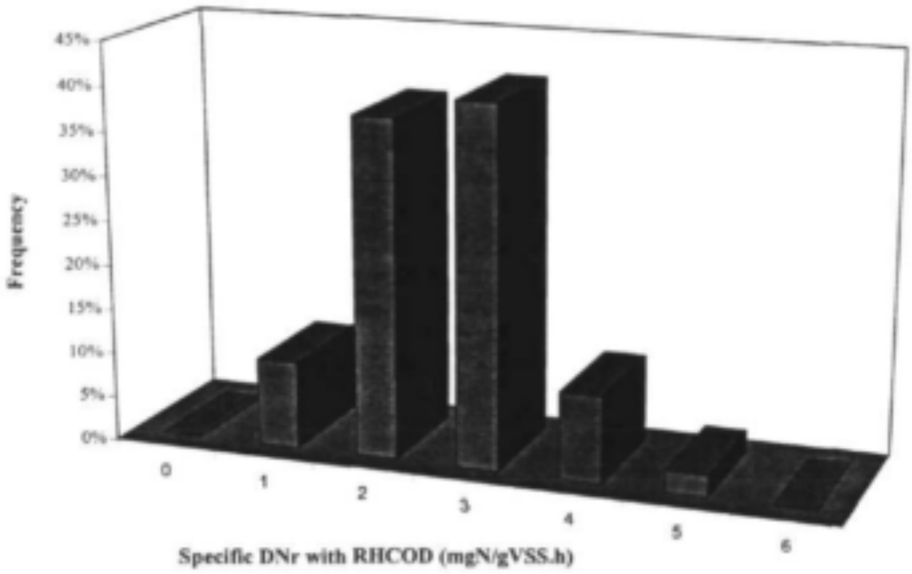


Figure 5-11: Distribution of second denitrification rates (k_2) for all samples (i.e. tests done with either acetate or wastewater) (n = 85).

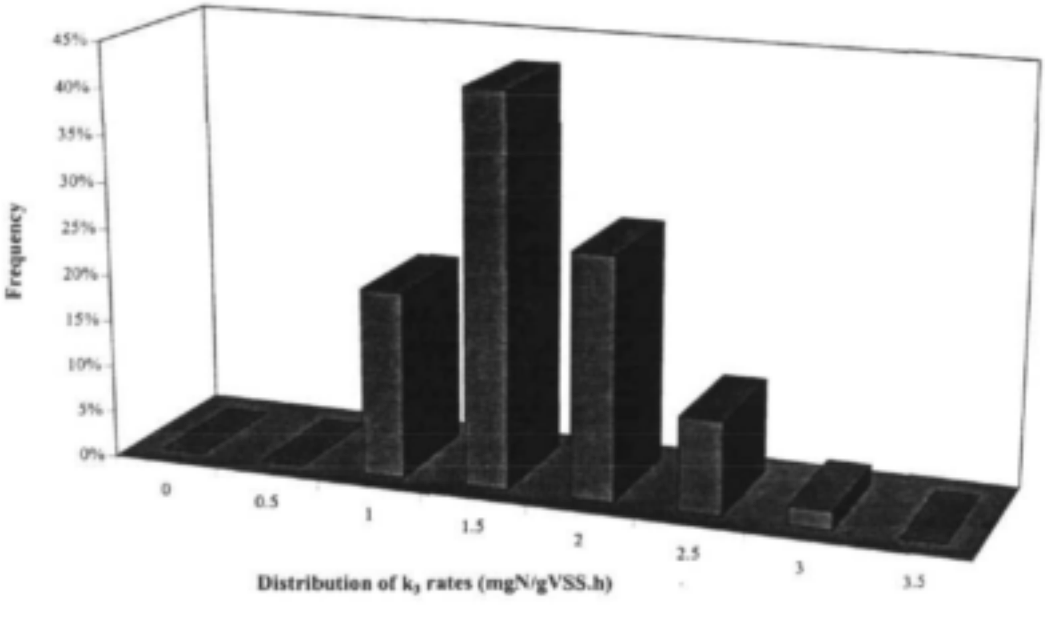


Figure 5-12: Frequency of distribution of the third denitrification rates (k_3) obtained from denitrification kinetics with acetate or raw wastewater (n = 50).

5.4.3 Acetate as a reference for rates obtainable with RBCOD?

A preliminary study was done with 4 different concentrations of acetate viz. : 40, 100. 200 and 400 mg/l as COD. The results showed that an increase in acetate concentration from 40 mg/l to 400 mg/l did not result in an increase in the specific denitrification rates. It was therefore, decided that for all the future NUR tests the use of approximately 50 mg/l acetate as COD was sufficient to give the maximum specific denitrification rate.

A plot of the ratio of k_1 of wastewater to k_1 of acetate was made (Figure 5-13) to determine if the maximum specific denitrification rates with acetate were comparable to the maximum specific denitrification rates with raw wastewater. About 21 % (n = 6) of the tests produced comparable rates for raw wastewater and acetate. In these cases, acetate could be used to mimic the maximum specific denitrification rate constants of raw wastewater. It also suggested that these sludge samples were exposed to substrates similar to acetate. Approximately 43 % of the tests produced a ratio greater than 1 which shows that these bacteria use other compounds which give higher denitrification rates. Several of the tests (n = 10) produced ratios that were lower than 1 suggesting that the acetate fraction was not high in these wastewaters. These results show that a single simple compound like acetate cannot be used as an efficient substitute for RBCOD which is more complex in composition.

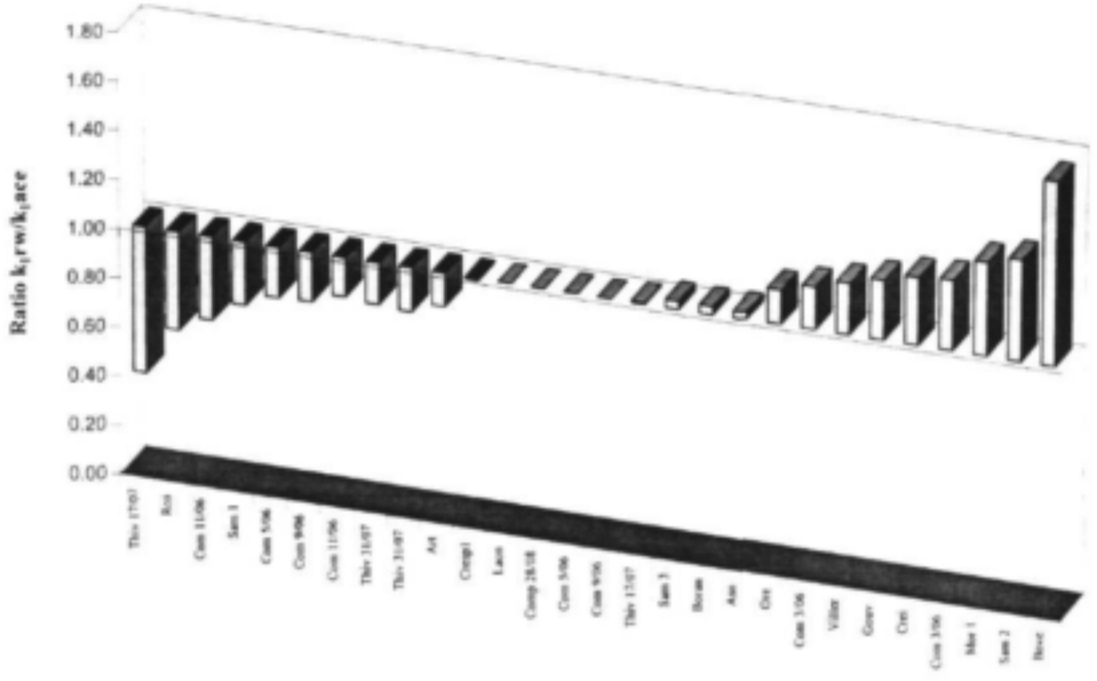


Figure 5-13: Ratio of the maximum denitrification rates with municipal wastewater ($k_{1,rw}$) and acetate ($k_{1,ace}$) for the samples used for NUR tests.

5.4.4 Relationships between the different denitrification rates

Fairly weak correlation's were found between rate 1, and rates 2 and 3 (see Figure 5-14 and Figure 5-15). Since hydrolysis is considered to be the rate limiting reaction, it can be said that the rate of hydrolysis of intermediate biodegradable COD compounds *i.e.* the readily hydrolyzable or storage fraction is approximately 44 % of the rate of utilization of RBCOD. Furthermore, the rate of hydrolysis of the endogenous products is 28 % of the rate of utilization of RBCOD i.e. rate 3 is ca. 0.3 of rate 1. Clayton et al. (1991) found the endogenous denitrification rate (K_3) to be 0.10 of rate 1. This suggests that rate 3 in these 6 h NUR batch tests is not the true endogenous rate. Figure 5-16 shows that the endogenous denitrification rate is about two-thirds the rate of hydrolysis of the readily hydrolyzable or storage fraction. These results could be used to estimate the slower denitrification rates (k_2 and k_3) in a particular system for a particular type of wastewater.

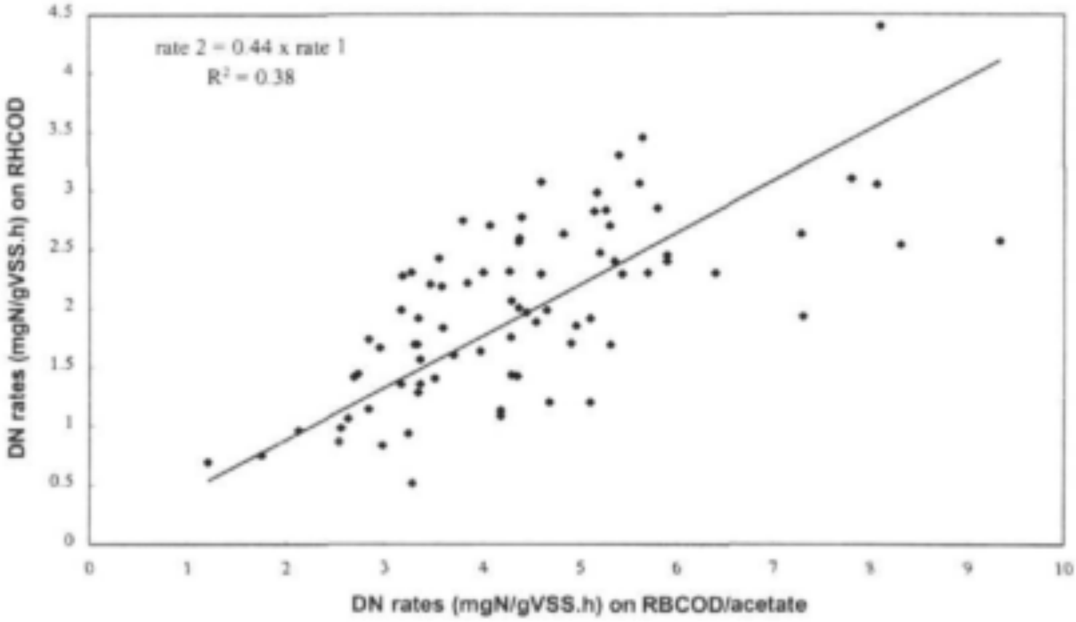


Figure 5-14: Correlation between rate 1 (RBCOD and acetate) and rate 2 (RHCOD) obtained from tests with raw wastewater or acetate (DN -denitrification).

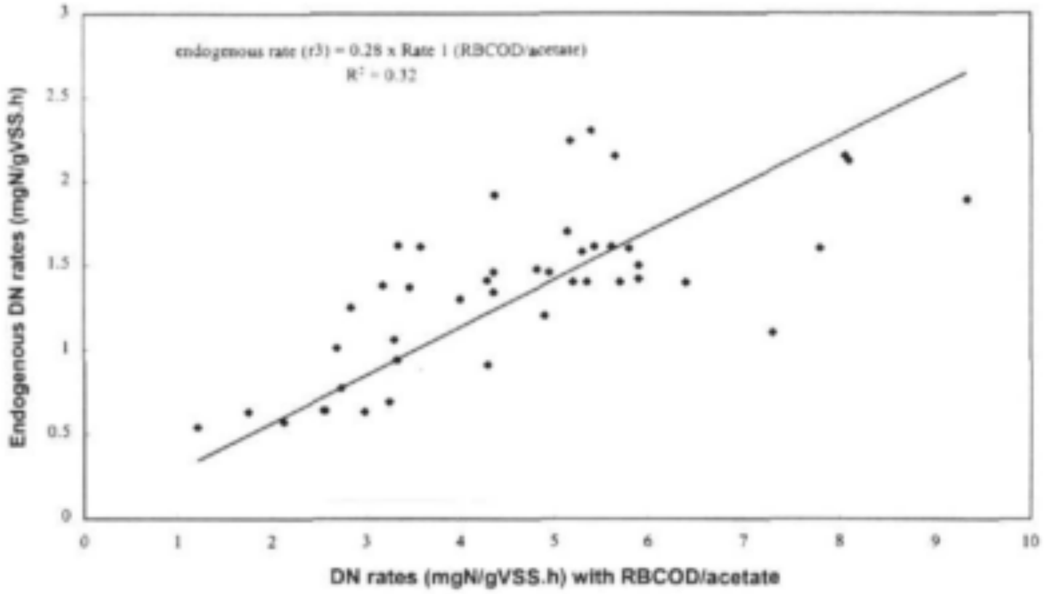


Figure 5-15: Correlation between rate 1 (RBCOD) and rate 3 (SBCOD and endogenous substrate) obtained from tests with raw wastewater or acetate (DN - denitrification)

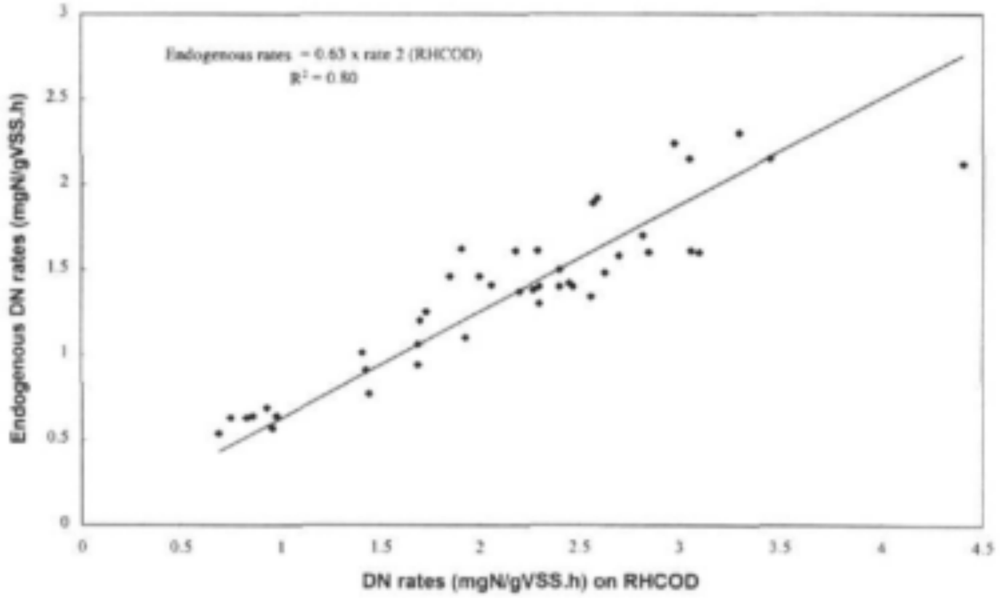


Figure 5-16: Correlation between rate 2 (RHCOD) and rate 3 (SBCOD and endogenous substrate) (DN - denitrification).

5.5. SUMMARY

- The RBCOD fraction determined from different wastewater samples varied between 7 and 25 % which highlighted the need to characterize wastewaters independently to obtain accurate input data for simulation studies.

- A second biodegradable fraction varied between 5 and 30 %. This intermediate fraction could have arisen from the utilization of a readily hydrolyzable component of SBCOD of wastewater or from the utilization of storage compounds that had been produced from the influent RBCOD fraction.

- Several correlation's (strong and weak) were made between the total COD and soluble fractions. Most of the raw wastewaters tested were fairly concentrated with total COD concentrations ranging between 700 and 1000 mgCOD/l.

- Fairly good linear relationships were found between the total COD and the filtered and coagulated COD fractions. The filtered and coagulated COD fractions were found to be 33 and 25 % of the total COD. Correlation plots also showed that centrifugation may include between 28 and 9 % more colloids / solids than coagulation and filtration (0.45 μm).

- The maximum denitrification rates (k_1) were found to be variable but within the range 3 to 6 mgN/gVSS.h. This variability was influenced by the wastewater composition and sludge activity. The second and third rates were less variable at 2 to 3 mgN/gVSS.h and 1.5 mgN/gVSS.h, respectively. The fairly high rate 3 value suggests that this is not the true endogenous denitrification rate. The latter two rates highlighted the difference in the hydrolysis rates for different slowly biodegradable COD substrates.

- The rate of hydrolysis of the SBCOD was found to be about 37 % slower than the rate of utilization of the readily hydrolyzable fraction of SBCOD or internal storage compounds. The rate of utilization of the *RHCOD* or *storage* fraction was found to be about 44 % of that of RBCOD utilization.

Chapter Six

Factors Influencing Wastewater Characteristics

This chapter discusses experiments aimed to understand the factors which may contribute to inaccuracies or variations in wastewater characteristics determined by the NUR method. These factors include the influence of polyphosphate accumulating organisms on wastewater characterization, the influence of storage on wastewater samples prior to the NUR tests, and the impact of acclimatization of activated sludge on the NUR data derived or calculated.

6.1. WASTEWATER CHARACTERIZATION WITH BIOLOGICAL PHOSPHORUS REMOVAL AND DENITRIFYING SLUDGE

One of the main factors which influences the characterization of readily biodegradable COD in anoxic systems is the presence of polyphosphate accumulating organisms (PAO's) and denitrifying polyphosphate accumulating organisms (DPAO's) which have the propensity to take up readily biodegradable COD (*i.e.* S_A fraction) with concomitant phosphorus release. In enhanced biological phosphorus removal (EBPR) systems this problem is increased due to the presence of higher numbers of PAO's which can influence biological wastewater characterization. In this investigation the activated sludges obtained from EBPR plants were referred to as bioP sludge while the samples from non-EBPR or denitrification (DN) plants are referred to as non-bioP sludge. Thus, the objective of this study was to determine the impact of the bioP sludge activity on wastewater characterization.

Two EBPR plants, Compiègne and Thiverval, were selected to study the impact that polyphosphate accumulating bacteria have on RBCOD characterization. Compiègne is a performance based plant (ca. 80 % P removal) while Thiverval is design based (ca. 55 % removal of P). Sludge from Boran WTP was used as a non-bioP sludge sample for both sets of NUR tests. The total COD concentration of wastewater was less variable for Compiègne than Thiverval (Table 6-1). The total COD concentration for Thiverval (31/07/97) was approximately two times the COD concentration measured for the first test conducted on 17/07/97. Since Thiverval is a smaller plant it was likely that it was susceptible to small perturbations in the network whereas the high capacity of Compiègne was able to buffer any changes (**Appendix I**).

Table 6-1: Characterization of municipal wastewater from the bioP plants, Compiègne and Thiverval.

		St	S-co	RBCOD				RHCOD			
	Date	**mg/l**	**%**	**mg/ l**		**%**		**Mg/ l**		**%**	
Compiègne 1 (M)	3/06	783	n.d.	145^{a}	172^{b}	18^{a}	22^{b}	n.o	_	n.o	_
2 (W)	5/06	787	23	70 ^{a}	104 ^{b}	9^{a}	13^{b}	94^{a}	142^{b}	12^{a}	18^{b}
3 (Su)	9/06	883	22	67^{a}	101^{b}	8^{a}	11^{b}	n.o	_	n.o	_
4 (Tu)	11/06	817	25	146^{a}	204^{b}	18^{a}	25^{b}	n.o	_	n.o	_
Thiverval 1 (W)	17/07	437	29	45^{a}	49^{b}	10^{a}	11^{b}	59^{a}	87^{b}	13^{a}	19^{b}
2 (W)	31/07	977	13	111^{a}.	119^{b}	8^{a}	12^{b}	87^{a}	52^{b}	9^{a}	5^{b}

(^{a} - bioP sludge; ^{b} - non-bioP sludge, St – total COD concentration, M - Monday, Tu - Tuesday, W - Wednesday, Su - Sunday)

6.1.1 RBCOD fraction

Figure 6-1 clearly showed that the NUR tests done with non-bio P sludge resulted in higher RBCOD values than the bioP sludge. Thus, the activity of polyphosphate accumulating organisms in the sludge from Compiègne and Thiverval resulted in an underestimation of RBCOD values. The difference between the RBCOD values derived using bioP (Compiègne) and non-bioP (Boran) sludge was considered as the RBCOD fraction lost to PAO activity under anoxic conditions. The RBCOD fraction of wastewater from Compiègne was found to be 8 to 18 % and 11 to 25 % of total COD with sludge from Compiègne and Boran, respectively (Table 6-1 ; Figure 6-1). This suggests that approximately 4 to 7 % of the RBCOD fraction of raw wastewater may be taken up by polyphosphate accumulating organisms when Compiègne sludge is used to conduct the NUR batch tests.

This trend was also observed with tests conducted at Thiverval, where the difference in RBCOD between the two sludges ranged between 1 and 4 % (Table 6-1 ; Figure 6-1). One of the reasons for the lower RBCOD loss was that the acetate-like fraction made available to the bioP bacteria was smaller for Thiverval 1 samples. This was confirmed with volatile fatty acid analysis of the raw wastewater sample which showed less than 10 mg/l of acetate as COD (Table 6-2). In all of the above tests, except Thiverval 1, phosphorus release and uptake was observed for the bioP sludge. Similarly, no change in the P concentration was observed for the on-bioP sludges, except for the final test (31/07/97) with wastewater and sludge from Thiverval and Boran WTP.

Phosphorus release of 1.5 mgP/l was observed for Boran for the test conducted on the 31/07/97. Sludge samples from Boran had previously not shown P release even in the presence of high concentrations of acetate. Enquiry's into the plant operation revealed that there was a malfunction in the process control system which is required for switching the aeration on and off when nitrates are absent. Thus, it is possible that the creation of anaerobic conditions and the presence of RBCOD from the influent would have created conditions that were ideal for the enrichment of polyphosphate accumulating organisms. Hence, there was a shift in population dynamics.

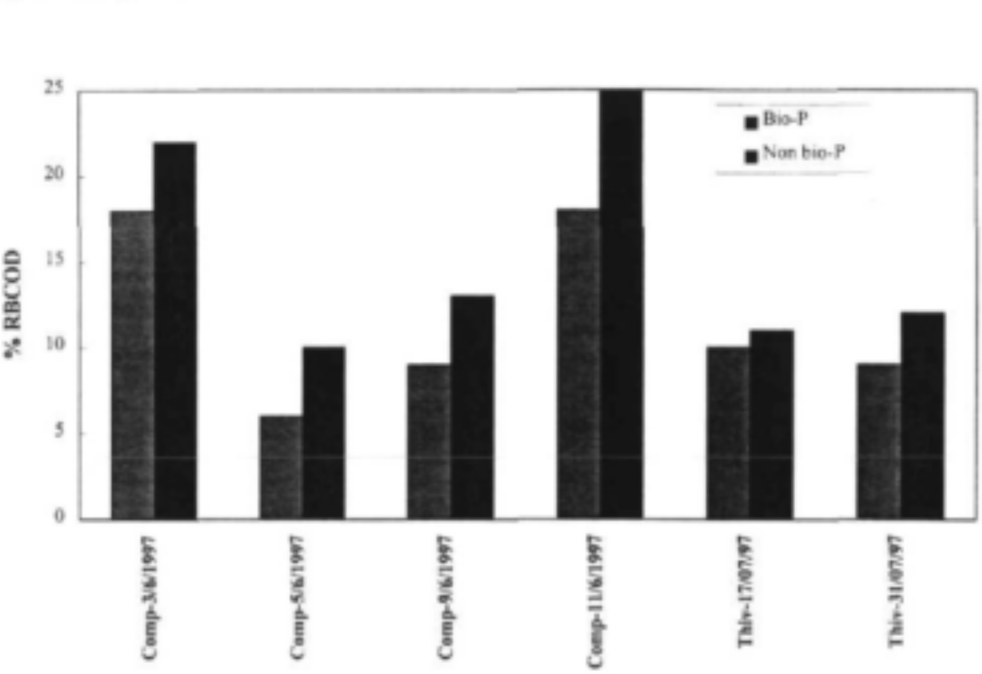


Figure 6-1: Comparison of RBCOD fractions calculated using bioP and non-bioP sludge with the NUR method.

6.1.2 Correction of the RBCOD lost with P and acetate data

It has been documented that polyphosphate accumulating organisms are only capable of using short chain fatty acids (VFA's) (Wentzel *et al.*, 1992). Therefore, the difference between the RBCOD values derived by bioP and non-bioP sludge were compared to the acetate and VFA concentrations (acetate and propionate as COD) measured for the influent raw wastewater by gas chromatography. The acetate fraction was found to be fairly comparable to the RBCOD fraction lost (Table 6-2). The VFA fraction (*i.e.* acetate and propionate) was less comparable. These preliminary results show that determination of the acetate fraction could be used to correct the underestimated RBCOD fraction when a bioP sludge is used to characterize the wastewater by the NUR test. Further tests will need to be done to verify if this is the trend for all NUR tests conducted with EBPR sludges.

Table 6-2: The wastewater fraction utilized by P removal organisms for P release under denitrifying conditions (% of total COD).

	RBCOD Fraction lost to P release (%)		**Measured acetate concentration (%)**		**Measured VFA concentration (%)**	
Kinetic/Date	%	mgCOD/l	%	mgCOD/l	%	MgCOD/l
1 - Com - 3/06/97	4	27	6	47	9	70
2 - Com - 5/06/97	4	34	5	39	5	39
3- Com - 9/06/97	3	34	3	26	3	26
4- Com - 11/06/97	7	58	5	41	7	57
5- Thiv - 17/17/97*	1	4	< 2	< 10	< 2	< 10
6 - Thiv - 31/17/97	4	48	4	39	4	39

(Com - Compiègne; Thiv - Thiverval; P - phosphorus as P; VFA - volatile fatty acid; * - not considered)

6.1.3 Relationship between RBCOD uptake and P release by PAO's

It is assumed that the RBCOD that is underestimated when EBPR sludge is used, is equivalent to the concentration of RBCOD sequestered by the polyphosphate accumulating organisms that are present in the EBPR sludge. By using the measured phosphorus release values and then converting the RBCOD fraction lost to the polyphosphate accumulating organisms into COD (mg/l), the COD/P (mgCOD/mgP) ratios were calculated (Table 6-3). The COD/P ratios varied between 1.4 to 4.1 with an average of 3.0. This compares well with the values, 2 to 5, cited by Mostert *et al.*, 1988 and Wentzel *et al.*, 1985. The phosphorus release and COD uptake results derived from the kinetics with acetate (see **Chapter 4**) as well as in this study were combined and plotted in Figure 6-2. Figure 6-2 shows that the correlation coefficient (R=0.83) is improved by the addition of data from this study. However, the variation is still large.

This variability in the COD/P ratios could be due to the difference in the internal phosphorus content of the bacterial cells which is known to influence the acetate/VFA uptake (Schuler and Jenkins, 1997). The pH value is also known to influence the ratio between VFA uptake and P release (Van Loosdrecht *et al.*, 1997b). However, this factor would not have influenced the ratio as the pH was controlled at 7.5 for all NUR tests. The variability of the COD/P ratios can also vary depending on the substrate. For example, Wentzel *et al.* (1985) cited ratios of 2 to 5 for acetate while Mostert *et al.* (1988) reported similar ratios for acetate but ratios of 3 to 14 for propionate and butyrate. Therefore, the use of the COD/P ratio would not be an ideal method to estimate the amount of COD taken up by polyphosphate accumulating organism activity due to the variability of the ratio.

Table 6-3: Comparison of average COD/P ratios with data collected from kinetics with wastewater from Compiègne WTP (COD in mg/l)

Kinetic	Date	P release	RBCOD lost	COD/P
1 - Compiègne	3/06/97	12	31	2.6
2 - Compiègne	5/06/97	9	31	3.4
3 - Compiègne	9/06/97	18	62	3.4
4 - Compiègne	11/06/97	18	25	1.4
5 - Thiverval	31/17/97	7	29	4.1
			Average	3.0

(P - phosphorus as P).

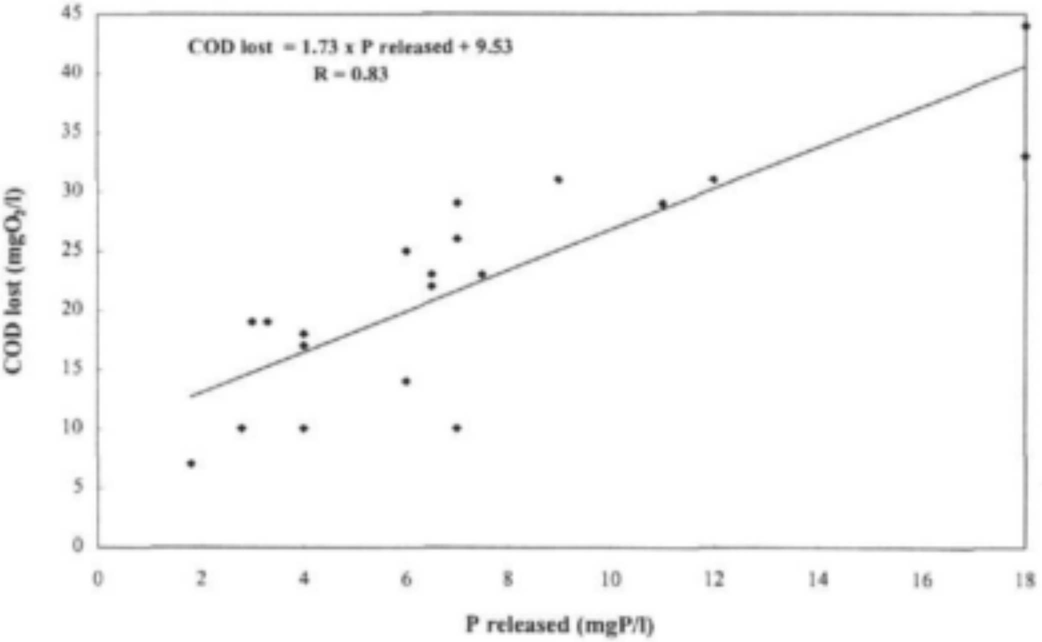


Figure 6-2 : Relationship between COD uptake and P release by polyphosphate accumulating organisms.

6.1.4 Impact of RBCOD loss in a denitrification process

In order to assess the influence of the 4 to 7 % loss in RBCOD due to bioP sludge activity, a simulation study was done using the IAWQ Activated Sludge Model I. The conditions of the simulation test are listed in Table 6-4. This simulation study was conducted to investigate how changes in the RBCOD concentration can affect denitrification and thus, the final effluent quality. It should be noted, however, that the organic carbon substrate available for denitrification in EBPR systems consists largely of slowly biodegradable COD. The simulation was done using a nitrification/denitrification system with 100 % nitrification.

Table 6-4: Conditions of simulation study using the IAWQ Activated Sludge model I for a nitrification/denitrification system with 100 % nitrification.

	Conditions
Reactor volume	9 m^3
Flow rate	0.5 m^3/h
Air on/off	1.5 h
HRT	18 h (0.75d)
SRT	7.5 d

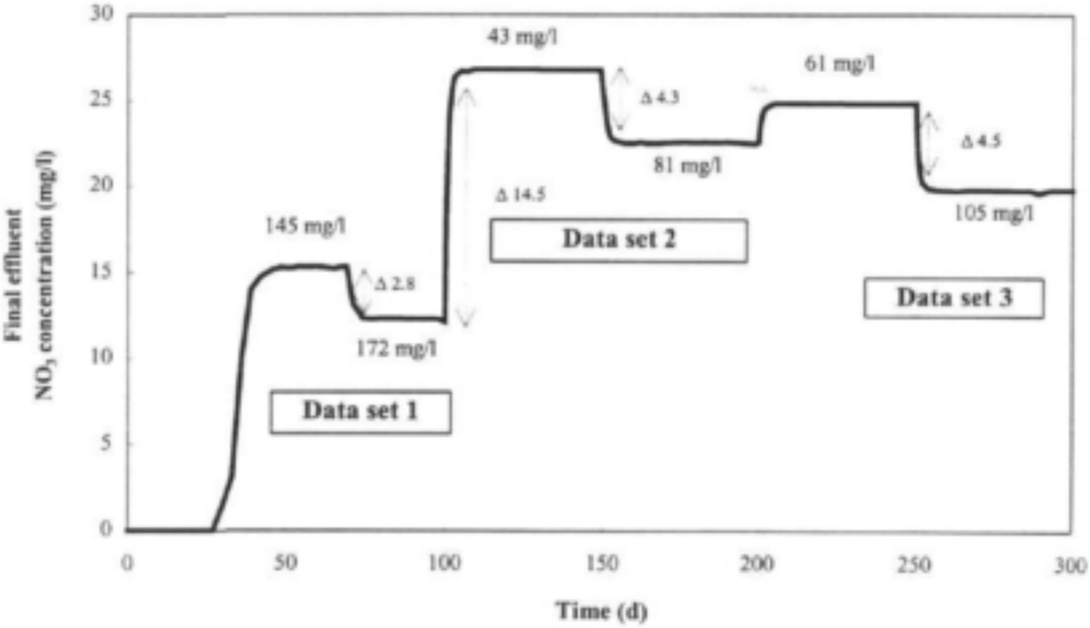


Figure 6-3: Impact of RBCOD variations on final effluent quality using RBCOD data derived from NUR tests with either bioP or non-bioP sludge (IAWQ Activated Sludge Model I).

The results from the simulation test are shown in Figure 6-3. The simulation shows how the variation in the RBCOD concentration of the influent raw wastewater can also severely impact on the final effluent NO_3-N concentration. For example, in data set 1 the RBCOD concentration made available to the denitrifying biomass was 145 mg/l (27 mg/l was lost to PAO's) but in data set 2 only 43 mg/l of RBCOD (38 mg/l was lost to PAO's) was available. This change was not due to a change in the PAO fraction or the total wastewater concentration, but was due to a significant variation (*i.e.* decrease) in the influent RBCOD concentration. Consequently, the final effluent NO_3-N concentration increased from about 15 mg/l to about 25 mg/l (Figure 6-3). Therefore, a wastewater treatment process must be operated in a manner which considers these variations in the influent RBCOD concentration without compromising the final effluent quality. Based on the simulation studies, the amount of NO_3^--N denitrified was determined. The results show that approximately 0.1 mg NO_3^--N was denitrified per mg of readily biodegradable COD consumed (Table 6-5).

Table 6-5: The concentration of nitrogen denitrified with the available RBCOD in a nitrification/denitrifcation system.

		Amount of N denitrified		
Sludge Type	**RBCOD conc.**	**Data set 1**	**Data set 2**	**Data set 3**
BioP	145	15.9		
non-bioP	172	18.7		
BioP	43		4.6	
non-bioP	81		8.9	
BioP	61			6.8
non-bioP	105			11.3
ΔN (mg/l)		2.8	4.3	4.5
ΔRBCOD (mg/l)		27	38	44
mgN/mgRBCOD		0.10	0.11	0.10

6.2. THE INFLUENCE OF STORAGE TIME ON WASTEWATER SAMPLES

Ideally, NUR batch tests for the purposes of wastewater characterization should be carried out as soon as possible after sampling. However, not all treatment plants are equipped with or situated near laboratories. In such cases, storage of wastewater and sludge for experimental purposes is necessary.

In this study, some of the samples were stored for 17 to 24 h at 4°C until the tests could be performed at the CIRSEE laboratory. In addition, samples that were collected from wastewater treatment plants that were located far from the CIRSEE laboratory then the samples had to be stored for longer periods (*i.e.* 2 to 3 days). Therefore, three main factors were investigated : (1) change in COD measurements, (2) change in the biodegradable fraction, and (3) the impact of storage on denitrifying activity.

Raw wastewater, primary settler effluent and sludge (mixed liquor suspended solids) samples were collected from Evry WTP (24/11/97). Three sets of NUR experiments were done on day 0 (0 h) (24/11/97), day 1 (after 24 h storage) (25/11/97), and day 4 (after 72 h storage) (27/11/97). Each set consisted of 3 reactors operating under identical conditions but with different types of substrate. The substrates tested were raw wastewater (i.e. after screening stage but before primary settler) and the primary settler effluent. The third reactor monitored endogenous denitrification *i.e.* sludge without any substrate addition

6.2.1 Chemical analysis of sludge and wastewater samples

As discussed earlier, two types of raw wastewater samples were collected from Evry wastewater treatment plant for these tests. Accordingly Table 6-6 provides the results from COD analysis conducted on both raw and primary settler effluent samples on day 1 (t0 h), day 2 (t24 h), and day 4 (t72 h). The COD analysis is divided into total COD (St), and COD after settling (2 h) (S-ns), centrifugation (S-ce), filtration (S-$f_{0.45}$) and coagulation (S-co).

The COD measurements showed that the raw wastewater and the primary settler effluent samples did not undergo any significant modifications during storage at 4 °C for 24 and 72 h. It would appear that storage resulted in no significant change in the wastewater quality. This is probably due to the fact that COD measurements give a representation of the global change in the wastewater rather than the change in the biodegradability of the fractions. It is nevertheless a useful first step when assessing wastewater changes.

Table 6-6: Wastewater characteristics after storage.

	raw wastewater			primary settler effluent		
Parameters	**t0 (h)**	**t24 (h)**	**t72 (h)**	**t0 (h)**	**t24 (h)**	**t72 (h)**
Total COD (St)	660	684	684	329	333	320
Non-settleable COD (S-ns)	377	406	383	320	329	304
Centrifuged COD (S-ce)	208	211	195	201	189	182
Filtered COD (S-$f_{0.45}$)	211	262	214	205	198	195
Non-coagulated COD (S-co)	137	144	153	122	109	118

6.2.2 Determination of COD contribution from sludge

Monitoring of endogenous denitrification profiles and rates were important as it allowed for the observation of any biological changes in sludge during storage (0 to 72 h) *i.e.* COD contribution from the sludge which may arise from the utilization of storage compounds. Figure 6-4 shows the denitrification profiles determined from tests done on day 0, 1, and 4. No nitrite accumulation was observed for any of these (Naidoo, 1999). Tests conducted with sludge samples on day 0 and 1 produced similar results *i.e.* a single phase and the same rate (1.0 mgN/gVSS.h) which suggested that sludge stored up to 24 h did not change sludge activity or characteristics (Table 6-7 and Figure 6-4). The day 4 test, however, revealed two phases, a short first phase and a slower second phase with a rate (k_2) that was half that of k_1. The second denitrification rate (k_2 = 1.1 mgN/gVSS.h) for t = 72 h samples was similar to the rates determined for samples stored for 0 h and 24 h (k_1 = 1.0 mgN/gVSS.h). These results suggest that phase 1 was probably linked to the use of internally stored compounds while phase 2 was due to the utilization of endogenous products and SBCOD attached to the sludge.

Extrapolation of the second phase and determination of the ΔNOx value for the tests conducted with 72 h stored sludge samples, allowed for the calculation of the biodegradable COD fraction which was responsible for the first rate of 2.2 mgN/gVSS.h (Figure 6-4). A COD concentration of 16 mgCOD/l was calculated. Therefore, it is probable that the 72 h sludge contributed about 16 mg/l of organic carbon to the biodegradable COD fractions calculated from raw wastewater and primary settler effluent samples that were calculated on day 4. Therefore, it is probable that for tests done with 72 h sludge samples, approximately 16 mgCOD/l can be due to the use of internally stored compounds.

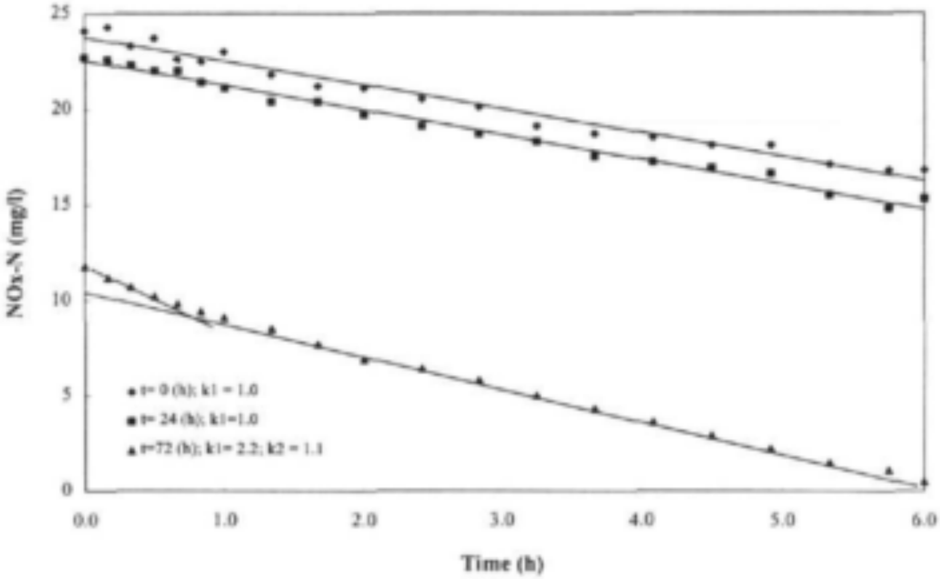


Figure 6-4: Comparison of endogenous denitrification rates after 0, 24 and 72 h storage of sludge samples at 4 °C.

Table 6-7: Endogenous denitrification rates of sludge stored for 0 h, 24 h and 72 h at 4 °C.

	Denitrification rates after		
	t0 (h)	**t24 (h)**	**t72 (h)**
k_1 (mgN/gVSS.h)	-	-	2.2
k_2 (mgN/gVSS.h)	1.0	1.0	1.1

6.2.3 Biodegradable COD concentrations

Biological organic carbon fractions were determined by the NUR method for wastewater samples that had been stored for 0 h, 24 h and 72 h (Table 6-8). Tests conducted with raw wastewater samples that had been stored for 0 and 24 h revealed 2 phases while tests with samples stored for 72 h revealed 3 phases. However, three phases were revealed for all the NUR tests with primary settler effluent samples.

Table 6-8 shows the readily biodegradable COD and readily hydrolyzable COD fractions calculated from tests with raw wastewater and primary settler effluent samples from Evry WTP. The fractions are represented as a percentage of the total COD concentration of raw wastewater on the day of the tests (see Table 6-6). The biodegradable COD concentrations that were calculated for t0 (h) to t72 (h) samples were comparable *i.e.* there was no change in the RBCOD fraction during storage (Table 6-8). For tests with raw wastewater, no readily hydrolyzable COD (RHCOD) fraction could be calculated for 0 h and 24 h samples. However, a RHCOD fraction of 11 % (79 mg/l) was calculated for the 72 h samples. This fraction which was only observed for the 72 h samples, could be due to the hydrolysis of SBCOD in the sludge (attached and endogenous) and / or hydrolysis of the slowly biodegradable COD in the wastewater sample. From NUR tests done with only sludge samples, approximately 16 mgCOD/l was from the use of internally stored compounds. This would account for only 2 % of 11 % of the COD that was calculated from the NUR test. Therefore, the observation of the second biodegradable COD fraction on day 4 was not completely due to the sludge. These results suggest that longer storage periods (> 24 h at 4 °C) does promote some hydrolysis. Thus, NUR tests done with raw wastewater samples stored for 72 h could reveal an *apparent* readily hydrolyzable COD fraction which would not have been observed with fresh wastewater and sludge samples.

Table 6-8: Comparison of RBCOD and RHCOD values after 0, 24, and 72 h of storage using raw wastewater and primary settler effluent samples (% of total COD).

	Raw wastewater				**Settler effluent samples**			
	RBCOD		**RHCOD**		**RBCOD**		**RHCOD**	
	mg/l	%	mg/l	%	mg/l	%	mg/l	%
0 h	71	11	–	–	54	8	72	13
24 h	77	11	–	–	65	9	75	11
72 h	75	9	79*	11*	64	9	73*	11*

* - note that the 16mg/l (i.e. 2 %) of COD calculated from tests conducted with sludge samples only have been removed from the 72 h biodegradable COD values.

Similarly, the tests with primary settler effluent showed no significant changes in RBCOD content during storage (Table 6-8). Furthermore, it was observed that the tests with the primary settler effluent samples consistently produced lower RBCOD values than the tests with raw wastewater. This suggests the biological activity in the primary settler at Evry WTP may have resulted in a slight reduction in the RBCOD fraction. A second biodegradable COD fraction, the RHCOD fraction, was revealed in all three of the tests conducted with the wastewater samples taken from the primary settler from Evry wastewater treatment plant. It is probable that the retention of the raw wastewater in the primary settling tank enhances biological hydrolysis of the SBCOD fraction, thereby creating an intermediate biodegradable group which is rapidly hydrolyzable, *i.e* RHCOD, which was observable in the 6 hour NUR batch test.

6.2.4 *Denitrification rates*

The first rates ranged between 4.7 and 7.2 mgN/gVSS.h (Table 6-9). No clear trend could be observed for the maximum specific denitrification rates for the studies with raw wastewater or primary settler effluent samples. However, the tests with the primary settler effluent did produce slightly higher specific denitrification rates. The second and third rates were found to be less variable and ranged between 2.0 to 2.5 and 1.3 to 2.0 mgN/gVSS.h, respectively. The third rates observed for both substrates (1.3 to 2.0 mgN/gVSS.h) were higher than the endogenous denitrification rates of about 1.0 mgN/gVSS.h (30 to 100 % difference between K_3 of raw wastewater and K_1 of sludge)(see Table 6-7 and 6-9). These results suggest that the final rate observed for the NUR tests fed with wastewater was due to the utilization of slowly biodegradable COD of the substrate added and was not due solely to the utilization of endogenous products released by the biomass.

Table 6-9: Specific denitrification rates (mgN/gVSS.h) obtained from tests with stored samples collected from Evry WTP (24/11/97).

		Storage period		
Substrate	**rate**	**t0 (h)**	**t24 (h)**	**t72 (h)**
raw wastewater	k_1	-5.3	-4.7	-5.1
	k_2	-	-	-2.3
	k_3	-2.0	-1.9	-1.5
Primary settler	k_1	-5.0	-7.2	-6.6
Effluent samples	k_2	-2.1	-2.0	-2.5
	k_3	-1.4	-1.3	-1.3

6.3. THE IMPACT OF SLUDGE ACCLIMATIZATION ON WASTEWATER CHARACTERIZATION

The use of an *external and unacclimatized* sludge *i.e.* sludge sampled from a different source to that of the wastewater sample may be used for certain NUR tests. This may become necessary when denitrifying sludge is absent or there are operational problems at wastewater treatment plants. This may also be the case when characterizing wastewater from enhanced biological phosphorus removal (EBPR) plants is necessary. Sludge from EBPR plants contain higher proportions of polyphosphate accumulating organisms (PAO's). Thus, the aims of this experiment were to determine if raw wastewater could be characterized (using the NUR method) with an unacclimatized activated sludge (foreign biomass) sample and if denitrification rates are influenced by the origin of the sludge or the origin of the raw wastewater.

Four comparative tests were made between acclimatized and unacclimatized sludge. The total wastewater concentration for the different wastewater samples varied between 700 and 1132 mgO_2/l (Table 6-10). The significant difference in the total COD concentrations for Boran WTP samples could be due to fact that Boran is a small treatment plant and therefore, unable to buffer any small perturbations in the COD load. The soluble fractions measured after filtration (S-$f_{0.45}$) and coagulation (S-co) varied between 41 to 53 %, and 41 to 45 %, respectively. Wastewater from Boran (22/08/97) was tested with sludges from Artemps-Seraucourt WTP and Crespiéres WTP. The wastewaters from Boran and Gouvieux which were sampled on the 19/11/97 were interchanged and tested with sludge from Gouvieux and Boran, respectively.

Table 6- 10: COD characterization of raw wastewater sampled from Boran and Gouvieux WTP for acclimatization tests (n.d. - not determined).

		St	**S-$f_{0.45}$**		**S-co**	
Date	**Substrate source**	mg/l	mg/l	%	mg/l	%
22/08/97	**Boran**	753	309	41	n.d.	n.d.
19/11/97	**Boran**	1132	553	49	467	41
19/11/97	**Gouvieux**	700	374	53	317	45

6.3.1 Comparison of biodegradable fractions using acclimatized and unacclimatized sludge

Of the four comparisons made with acclimatized and unacclimatized sludges, three of the tests (tests 1, 3, and 4) were found to be comparable (Table 6-11). The results of test 2 with Boran and Crespiéres, however, were not comparable. In this case, tests done with the acclimatized sludge, Boran, produced 9 % RBCOD and 25 % RHCOD. However, batch tests with the unacclimatized sludge from Crespiéres and raw wastewater from Boran produced a RBCOD fraction of 23 %, which was more than 2 times greater than the RBCOD fraction calculated with the acclimatized sludge of Boran. In addition, only one biodegradable fraction could be measured for the tests using the unacclimatized sludge of Crespiéres. It would appear that the unacclimatized Crespiéres sludge was capable of using the readily biodegradable COD and some of the readily hydrolyzable COD rapidly but not all of it. Thus, the measured readily biodegradable COD appears to be a combination of the RBCOD and some of the RHCOD present in the raw wastewater sampled from Boran.

Table 6-11: Comparison of RBCOD/RHCOD values calculated for wastewater sampled from Boran and Gouvieux using acclimatized and unacclimatized sludges.

			% RBCOD		**% RHCOD**	
Test	**Substrate**	**Sludge source**	mg/l	%	mg/l	%
1-(22/08/97)	**Boran**	**Boran** ^{a}	72	9	189	25
		Artemps-Seraucourt ^{ua}	82	11	177	24
2-(22/08/97)	**Boran**	**Boran** ^{a}	72	9	189	25
		Crespiéres ^{ua}	**176**	**23**	n.o.	
3-(19/11/97)	**Boran**	**Boran** ^{a}	246	22	n.o.	
		Gouvieux ^{ua}	256	23	n.o.	
4-(19/11/97)	**Gouvieux**	**Gouvieux** ^{a}	196	28	n.o.	
		Boran ^{ua}	217	31	n.o.	

(n.o - not observable, ^{a} – acclimatized sludge, ^{ua} – unacclimatized sludge)

6.3.2 Denitrification rates

The rates obtained with the different sludge samples were variable. The maximum (k_1), second (k_2) and third (k_3) rates varied between 5.8 to 3.2, 2.5 to 1.5 and 0.8 to 0.5 mgN/gVSS.h, respectively (Table 6-12). The ratios of the different rates of the acclimatized to the unacclimatized sludges were plotted in Figure 6-5. The results showed that none of the unacclimatized sludges produced rates that was comparable to the acclimatized sludges. In addition, some of the unacclimatized sludges *eg.* Artemps-Seraucourt and Boran produced higher rates than the acclimatized sludges (Table 6-12). This suggests that even though the wastewater quality may influences the rates to a certain extent, the magnitude of the rates are largely due to sludge characterization which are brought upon by plant operating conditions such as solids retention time, loading rates, and feeding regimes (continuous or intermittent). In addition, comparative tests between Boran (22/08/97) and Artemps (22/08/97) showed that although the rates (k_1 and k_2) measured for the two sludges were significantly different, the biodegradable fractions were comparable (see Table 6-11 and Table 6-12).

Table 6-12 : Comparison of denitrification rates for acclimatized and unacclimatized sludges.

Date	**substrate source**	**sludge source**	**k_1**	**k_2**	**k_3**
1-22/08/07	Boran	Boran	-3.2	-1.7	-0.8
1-22/08/97	Boran	Artemps-Seraucourt	**-11.2**	**-4.1**	**0.5**
2-22/08/97	Boran	Crespieres	-3.9	-1.5	-
3-19/11/97	Boran	Boran	-5.8	-2.5	-
3-19/11/97	Boran	Gouvieux	-3.4	-1.7	-
4-19/11/97	Gouvieux	Gouvieux	-3.3	-1.7	-
4-19/11/97	Gouvieux	Boran	-3.7	-2.4	-

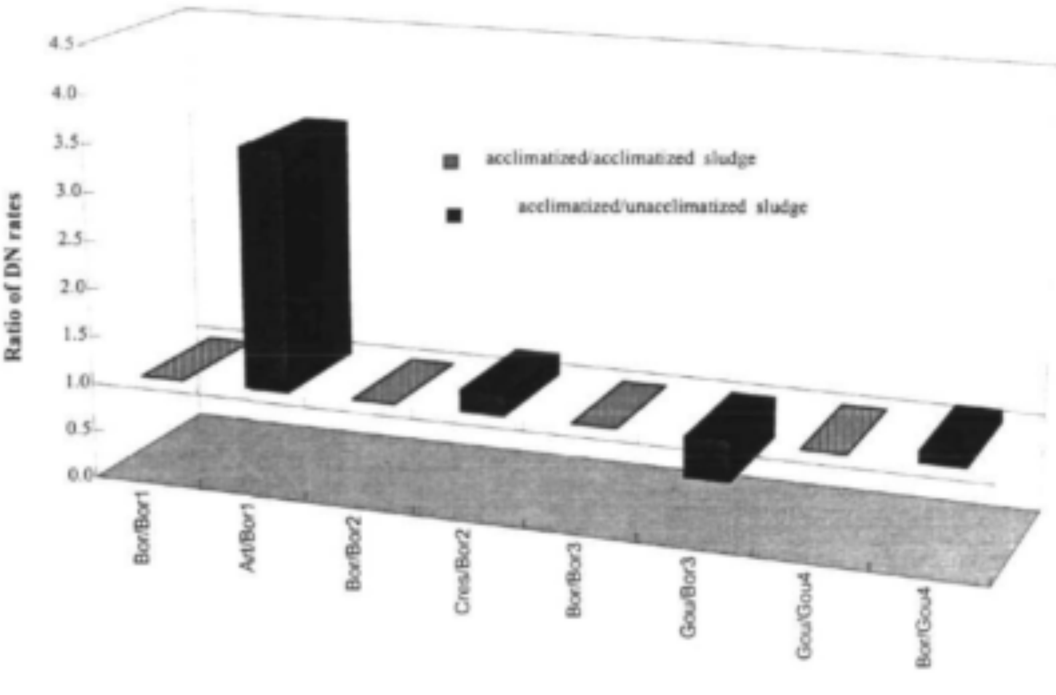


Figure 6-5: Ratios of denitrification rates of acclimatized to unacclimatized sludges (Bor -Boran, Art - Artemps-Seraucourt, Cres - Crespiéres, Gou - Gouvieux).

6.4. SUMMARY

- Tests with Compiegne (a bio P plant) and Thiverval (bioP) wastewater clearly showed that the RBCOD fraction was underestimated when a bioP sludge was used. This was assumed to be due to the activity of polyphosphate accumulating organisms. This RBCOD fraction, which was taken up by polyphosphate accumulating organisms, was found to be approximately 4 to 7% of the influent raw wastewater (9 to 33 % of RBCOD).

- The RBCOD lost to polyphosphate accumulating organisms was found to be fairly comparable to the acetate fraction of the influent raw wastewater sample. Thus, the fraction lost to PAO's can be roughly accounted for when conducting NUR tests with bioP sludge by adding the measured influent acetate concentration to the RBCOD concentration.

- A weak correlation was found for COD uptake and P release. However, the COD/P ratio was variable and therefore, would not accurately account for the RBCOD loss in bioP sludge. These tests confirmed the need to measure phosphorus during the denitrification batch test.

- Furthermore, a simulation of a nitrification/denitrification process with complete nitrification showed that approximately 0.10 mg NO_3^--N was denitrified per mg of RBCOD consumed. Therefore, approximately 2.3 mg of RBCOD is required to remove 1 mg of NO_3^--N.

- The storage of wastewater samples up to 72 h at 4 °C was shown to have no major effect on the determination of the RBCOD fraction *i.e.* the RBCOD fraction did not change during storage. However, longer storage periods may pose problems.

- The results obtained from the storage experiment highlighted the advantage of a primary settler at the treatment plant since the tests with 0 h and 24 h samples from the primary settler revealed 2 biodegradable fractions in the 6 h tests as opposed to the single biodegradable fraction for raw wastewater samples that had been stored for the same time period.

- Most of the tests showed that the readily biodegradable COD and readily hydrolyzable COD fractions determined with acclimatized and unacclimatized sludges were comparable. However, there was an exception, *e.g.* the unacclimatized sludge of Crespiéres gave RBCOD values that compared poorly to the results obtained with the acclimatized sludge of Boran. Therefore, this suggests that not all the sludges are compatible with different wastewaters.

Chapter Seven

Conclusions and Recommendations

One of the aspects of this study was to assess the experimental approach adopted for the realization of the nitrate-N utilization rate (NUR) batch tests. This was dependent on the analysis and interpretation of the NUR profiles. Two types of profiles were observed for raw wastewater and acetate fed reactors during the 6 h batch test.

- Some tests with raw wastewater revealed 2 phases. The first phase was due to the utilization of the readily biodegradable COD of raw wastewater while the latter phase was due to the utilization of slowly biodegradable COD present in the influent raw wastewater and sludge.

- In the case of 3-phase NUR profiles, phase 1 was once again attributed to readily biodegradable COD consumption. However, phase 2 was thought to reflect the utilization of an intermediate fraction which could either be classed as readily hydrolyzable fraction (RHCOD) present in the influent raw wastewater or a storage fraction which is linked to the rapid uptake of RBCOD. The final phase (phase 3) was due to a combination of slowly biodegradable and endogenous substrate utilization.

- Similarly, for acetate fed reactors, the 2 phase profile was linked to acetate utilization (phase 1) and slowly biodegradable and endogenous substrate utilization (phase 2).

- Three-phase NUR profiles, however, present a more complicated scenario for acetate fed reactors. Phases 1 and 3 may be explained in terms of the utilization of acetate and SBCOD / endogenous substrates from the sludge, respectively. Phase 2 however, was hypothesized to be due to one of two mechanisms:

    i) the utilization of internally stored compounds present in the original mixed liquor seed, or,

    ii) a rapid accumulation / utilization reaction whereby some of the exogenous acetate was rapidly accumulated and / or converted to storage compounds by ordinary heterotrophs or denitrifying polyphosphate accumulating organisms. The accumulated or stored compounds are subsequently utilized during denitrification. This second mechanism was supported by

NUR tests done on only sludge which revealed a single phase. These observations suggested that phase 2 was acetate-linked. This reaction could, therefore, be triggered by the presence of acetate but only in some cases.

From NUR tests done with acetate (ca. 50 mgCOD/l) as the sole exogenous substrate source, it was possible to calculate COD mass balances. The mass balance results obtained for acetate-fed NUR tests were used to formulate several conclusions on acetate utilization under anoxic conditions.

- Firstly, it was concluded that for some of the wastewaters and sludges tested, acetate was used exclusively for denitrification by denitrifiers *i.e.* where acetate mass balances were 100 %. In this case, the sludge contains a significant proportion of denitrifiers and little or no polyphosphate accumulating organisms or that the physiological state of the biomass is such that rapid accumulation or storage is not required. This was the case for samples taken from Artemps, Boran (tests 1 to 6), Creil, Gouvieux, Orense and Rostock wastewater treatment plants.

- Secondly, in the cases, e.g. Compiegne, Asnieres, Boran 10 and 11, Boves and Crespieres, where the acetate mass balances were found to be < 100 %, it can be concluded that the acetate could have been used for denitrification as well as for the production of storage products like polyhydroxyalkanaotes. It is possible that some of the bacteria found in the mixed liquor sequester the acetate in order to replenish the reserves of storage compounds. These sludge samples probably contained denitrifying bacteria as well as a significant proportion of polyphosphate accumulating organisms. These observations imply that the RBCOD fraction of wastewater samples characterized by the NUR test may be slightly underestimated.

- Thirdly, tests done with sludge from Laon, Thiverval and Villers, showed acetate mass balances were 100 % when the 2 biodegradable fractions from the NUR test were calculated. These results suggest that acetate could be used for denitrification and for polyhydroxyalkanoate synthesis by denitrifying polyphosphate accumulating organisms. The acetate that the DPAO's take up is re-utilized during denitrification. This utilization in the second denitrification phase is supported by the observation of phosphorus uptake. Thus, these sludge samples contain denitrifiers and denitrifying polyphosphate accumulating organisms which compete for the available acetate. A similar scenario could be possible for raw wastewater samples.

- Finally, in cases where the acetate mass balances were > 100 %, it was concluded that all the acetate was used for denitrification and the excess COD that was calculated was as a result of the use of existing storage compounds. These results suggest that in some cases, the NUR tests may overestimate the RBCOD content of the influent wastewater.

These are important observations as they show that RBCOD compounds, particularly the acetate fraction was not always used exclusively for denitrification purposes under anoxic conditions but could be used for accumulation or storage. These interpretations will need to be considered when determining the RBCOD fractions of wastewater calculated using this biological wastewater characterization method.

One of the objectives of this study was to characterize the wastewater by physical and chemical methods. The total COD concentrations from the various wastewater samples tested varied between 176 mgCOD/l to 1157 mgCOD/l. Majority of the samples fell within the 700 to 1000 mgCOD/l range. In addition, raw wastewater was fractionated by settling (2h) (S-non-set), centrifugation (S-ce), filtration (S-$f_{0.45}$) and coagulation (S-co). Several correlations (strong and weak) were made between the total COD and soluble fractions. The filtered (0.45 µm) and coagulated fractions were found to be approximately 33 and 25 % of the total COD, respectively. Studies also showed that filtration (0.45 µm) and coagulation included about 9 and 28 % less colloids (solids) than centrifugation.

The use of NUR tests allowed for the observation and monitoring of the biokinetic response of the denitrifying bacteria in the presence of available organic carbon in the raw wastewater samples tested. This allowed for the determination of the biodegradable component of raw wastewater.

- The RBCOD fraction determined from different wastewater samples varied between 7 and 25 % which highlighted the need to characterize raw wastewater samples independently to obtain accurate input data for simulation studies. However, majority of the results fell with the 10 to 20 % of the total COD group, which was expected.

- It was also possible in certain tests to calculate a second biodegradable component of raw wastewater. The intermediate fraction was found to vary between 5 and 30 % of the total COD concentration. This second biodegradable was considered to be part of the readily hydrolyzable component of wastewater or the storage fraction based on two hypotheses.

    i) Since this second phase lasted for about 2 to 3 h and the biodegradable COD fractions calculated from the NUR tests were far lower than expected, this fraction was classed as a readily hydrolyzable fraction (RHCOD) of the SBCOD found in raw wastewater. The slowly biodegradable COD fraction of raw wastewater is reported to comprise 30 to 60 % of the total COD. However, these results do suggest that the NUR method may be able to differentiate between an intermediate readily hydrolyzable fraction and the slowly hydrolyzable fraction for some wastewater samples. While the NUR test is able to calculate part of the SBCOD, in the form of the readily hydrolyzable fraction, it is, however, unable to measure the whole SBCOD component of raw wastewater.

ii) It could also be that this second biodegradable COD fraction was part of the RBCOD and the intermediate phase was in fact a *residual phase* of phase 1. Combination of phase 1 and phase 2 values showed that the majority of the RBCOD were > 20 % while only 10 % of the values were between 10 and 20 % of the total COD, the expected range for RBCOD. This suggests that either French wastewater samples have unusually high RBCOD concentrations, or that the NUR test measures an intermediate fraction of the raw wastewater *i.e.* the readily hydrolyzable fraction. The suggestion that phase 2 is a residual phase of phase 1 is supported by the observations made with the acetate fed reactors where an intermediate phase was also observed. It was, therefore, postulated that some of the RBCOD fed to the biomass was used directly for energy and growth while some of the RBCOD was accumulated or stored. These accumulated and stored compounds become available to the bacteria once the RBCOD concentration becomes limiting.

Denitrification batch kinetics generally produced two or three rates which depended on the substrate used, the biomass activity and the test conditions. While these rates cannot be used in the existing activated sludge models since they are a function of the total VSS concentration and not the active biomass concentration, they do provide a useful indication of the types of substrates that are being used by the denitrifying bacteria.

- The maximum denitrification rates (k_1) were found to be variable but within the range 3 to 6 mgN/gVSS.h.

- The second rate (k_2) or the second phase in the NOx-N profile was considered to be due to the readily hydrolyzable fraction of wastewater or sludge. About 78 % of the rates calculated were found to lie in the range 2 to 3 mgN/gVSS.h. Less than 10 % of the samples gave values below 2 mgN/gVSS.h and only 12 % were above 3 mgN/gVSS.h. These results suggest that the intermediate rate lies between 2 and 3 mgN/gVSS.h.

- The third rate (k_3) was considered to be due to the slowly biodegradable fraction and endogenous products. Most of the k_3 rates (68 %) were found to be less than 1.5 mgN/gVSS.h. However, a significant number were found to be between 2 and 3 mgN/gVSS.h. In this case it is also possible that the utilization of hydrolytic products of phase 2 (k_2) may still be exerting its influence on k_3 values. The variation and range suggests that the SBCOD components of the wastewater samples are complex and variable in composition and concentration.

In order to better understand the anoxic process and wastewater characterization several secondary experiments were done to investigate the influence of various factors on the accuracy of the results. The studies done with acetate showed that the results obtained by the NUR method may be influenced by the presence of polyphosphate accumulating organisms or denitrifying polyphosphate accumulating organisms. Therefore, tests were done with sludge obtained from the enhanced biological phosphorus removal plants (bio-P), Compiègne and Thiverval.

- The results clearly showed that the readily biodegradable COD fraction was underestimated by about 4 to 5 % (of the total COD) when a bio-P sludge was used.

- Furthermore, the RBCOD lost to polyphosphate accumulating organisms was found to be fairly comparable to the acetate fraction. The COD/P ratio was found to be variable and therefore, will not provide an accurate estimation of the RBCOD underestimation. These tests confirmed the need to measure phosphorus during the NUR batch test.

Several of the wastewater and sludge samples had to be stored prior to the NUR test. It was, therefore, necessary to do a preliminary study to determine the effect of storage at 4°C on the biodegradable fractions found in wastewater. Samples were collected from Evry Wastewater Treatment Plant and stored for 0, 24, and 72 h prior to the NUR test.

- The storage of raw wastewater samples up to 72 h at 4 °C did not change the readily biodegradable COD fraction which was found to comprise about 9 % of the total COD.

- However, the raw wastewater samples that had been stored for 72 h did reveal a second biodegradable fraction, a readily hydrolyzable fraction, which suggests that longer storage periods ($\geq$ 72 h) could foster hydrolysis even at 4 °C or that the longer starvation periods triggers the utilization of existing storage products.

- This experiment showed that the accuracy and reliability of the data from NUR tests was not compromised for samples stored up to 24 h.

Tests were also done to determine if the use of an unacclimatized sludge would yield inaccurate biodegradable COD results. Most of the tests showed that the biodegradable COD fractions determined with acclimatized and unacclimatized sludges were comparable with one exception, Crespiéres. Biodegradable COD results obtained with the unacclimatized sludge of Crespiéres and the acclimatized sludge of Boran were incomparable. Tests with the acclimatized sludge of Boran revealed 2 biodegradable fractions while tests with the unacclimatized sludge of Crespiéres revealed a single fraction. Therefore, it would appear that not all the sludges are compatible with different wastewaters.

The NUR method was an effective tool for the characterization of municipal wastewaters but for on-site characterization it remains a tedious and time-consuming method. The major disadvantage lies in the off-line procedure which requires liquid samples to be taken at specific time intervals in order to follow NOx utilization. This characterization method should gain more use once an effective electrode or method is found which is able to follow the kinetics automatically. In addition, the NUR method showed that the characterization was necessary since there was a significant variation in the RBCOD content of wastewaters. Since this project analyzed several wastewater samples from plants of similar and differing characteristics, the biodegradable fractions (RBCOD and RHCOD) data presented here can be used to do sensitivity analysis using the IAWQ Activated Sludge Models I and II to assess process efficiency and operation. Unfortunately, the rate constants determined cannot be used in these models as they are a function of the total VSS concentration and not the active biomass concentration.

The NUR method also allowed for the calculation of a second biodegradable fraction. The source of the second biodegradable fraction needs to be validated since it will have a major impact on the way current respirometric techniques are used as a characterization tool. In addition, the validation of the rapid accumulation / storage hypothesis will influence the way wastewater processes are modelled in the future.

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APPENDIX I

Description and Characteristics of Wastewater Treatment Plants

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Samples from a total of 23 different plants were tested, 15 of which were French wastewater treatment plants (WTP) and four were from various European countries (Britain, Germany, Spain and the Czech Republic). Four South African plants which are located in the KwaZulu-Natal, Durban region were also tested.

Table I-1 lists the different plants, their capacity, the type of wastewater treated, the distribution system, the presence or absence of primary settlers and the biological processes employed. The majority of the plants sampled treat only municipal wastewater. However, some WTP's do treat small proportions of industrial waste.

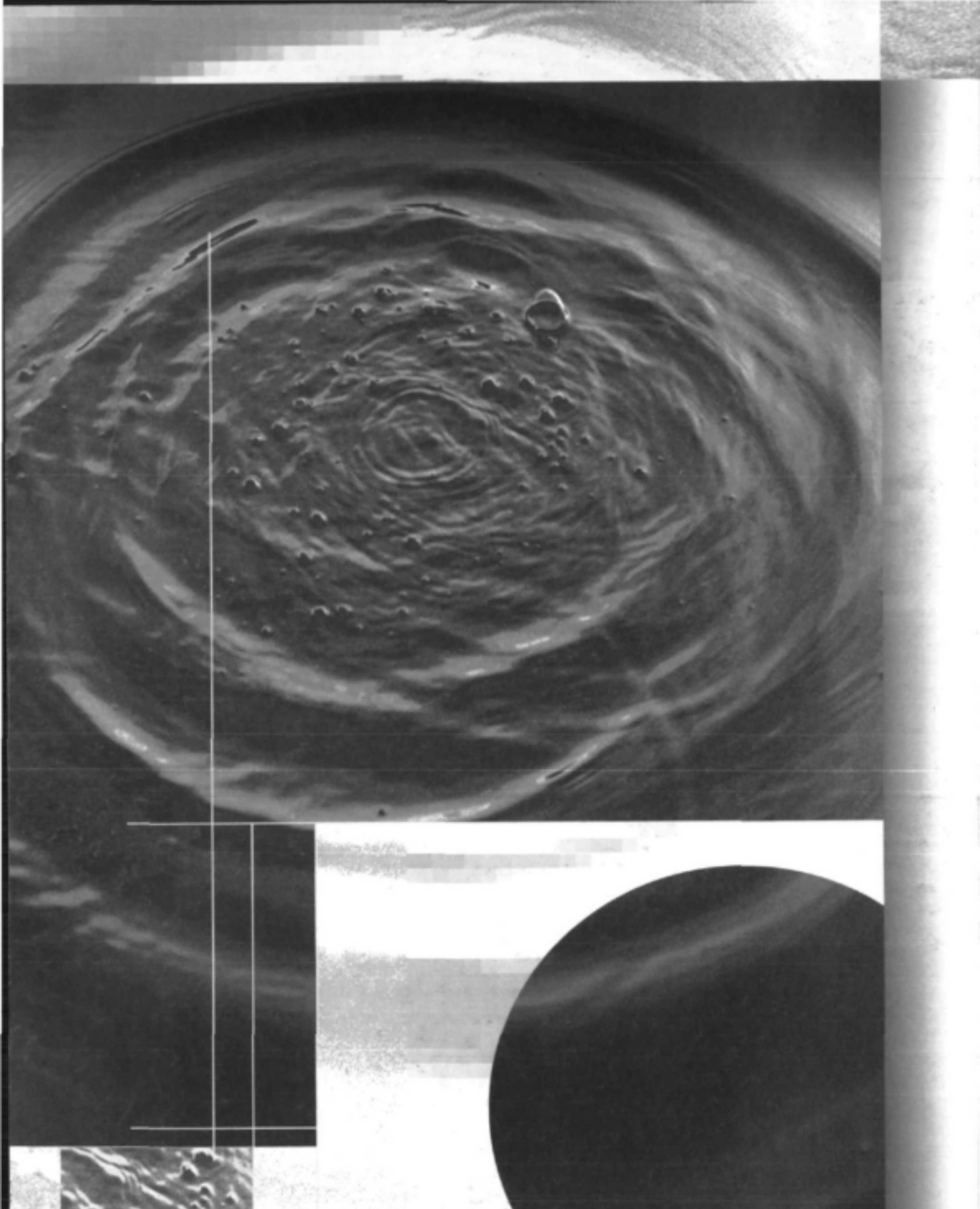
Table I-1 : Description of the different wastewater treatment plants.

Plant	**Country**	**Capacity (p.e)**	**Type of wastewater**	**Distribution system**	**Primary settler**	**Biological process**
Asnieres-sur-oise	France	42 000	Municipal	separate	+	carbon and ammonia removal
Artemps-Seraucourt	France	1 500	Municipal	separate	-	carbon removal
Berwick	England	13 390	Municipal and effluent from beverage industry (orange juice and whisky)	pseudo-separate	+	carbon removal
Boran-sur-oise	France	3 000	Municipal	separate	-	carbon and nitrogen removal
Boves	France	2 500	Municipal	combined	-	carbon and ammonia removal
Brno	Czech Republic	600 000	70 % municipal and 30 % industrial effluent (unidentified)	combined	+	carbon removal
Compiegne	France	220 000	Municipal with some effluent from industries	combined	-	carbon, nitrogen and phosphorus removal
Creil	France	110 000	Municipal and some industrial effluent	pseudo-separate	+	carbon and nitrogen removal
Crespiéres	France	1 500	Municipal	combined	-	carbon and ammonia removal
Darvil	South Africa	300 000	Municipal and some industrial effluent	separate	+	carbon, nitrogen and phophorus removal
Evry	France	250 000	Municipal	seeparate	+	carbon and nitrogen removal
Gouvieux	France	10 000	Municipal	combined	-	carbon and nitrogen removal
Kwa-Mashu	South Africa	220 000	Municipal	separate	+	carbon and nitrogen removal
Laon	France	40 000	Municipal	combined	+	carbon and nitrogen removal
Morainvilliers	France	13 000	Municipal	1/3separate; 2/3 combined	-	carbon and nitrogen removal
Northerns	South Africa	100 000	Municipal and ca. 15 % industrial effluent	separate	+	carbon and nitrogen removal
Orense	Spain	170 000	Municipal and a low percentage of abbatoir wastewater in the mornings	combined	+	carbon removal (nitrogen removal occurs but not controlled)

Table cont......

Plaisir	France	42 000	Municipal	separate	+	Carbon removal
Rostock	Germany	170 000	Municipal	pseudo-separate	+	carbon and nitrogen removal
Samaritaine	France	40 000	Municipal	separate	-	carbon , nitrogen and phosphorus removal
Southerns	South Africa	160 000	Municipal and industrial (but separate treatment)	separate	+	carbon and nitrogen removal
Thiverval-Grignon	France	12 000	Municipal	separate	-	carbon and nitrogen removal (P-chemical removal
Villers sous St. Leu	France	500	Municipal	combined	-	carbon and phosphorus removal

p.e. - population equivalent, + present, - absent, PMB - Pietermaritzburg, DBN - Durban.



Water Research Commission

PO Box 824, Pretoria, 0001, South Africa

Tel: +27 12 330 0340, Fax: +27 12 331 2565

Web: http://www:wrc.org.za

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