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**SUBSTRATE STORAGE PHENOMENA
IN THE MODELING OF
ACTIVATED SLUDGE SYSTEMS**

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**AKTİF ÇAMUR SİSTEMLERİNİN
MODELLENMESİNDE
SÜBSTRAT DEPOLAMA KAVRAMI**

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PREFACE

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LIST OF SYMBOLS

b_A	: Endogenous Respiration Rate of Autotrophs [T^{-1}]
$b_{A,NO}$	: Anoxic Autotrophic Decay Rate [T^{-1}]
b_H	: Endogenous Respiration Rate of Heterotrophs [T^{-1}]
$b_{H,NO}$	: Anoxic Endogenous Decay Rate [T^{-1}]
b_{STO}	: Endogenous Respiration Rate of Storage Products [T^{-1}]
$b_{STO,NO}$	: Anoxic Decay Rate of Stored Polymers [T^{-1}]
C_{SI}	: Initial Total Biodegradable COD Concentration [$M \text{ COD. L}^{-3}$]
C_S	: Total Biodegradable COD Concentration [$M \text{ COD. L}^{-3}$]
f_I	: The Fraction of the Particulate Metabolic Products
f_{SI}	: The Fraction of Soluble Inert COD Generated in Biomass Decay
i_{NBM}	: Nitrogen Content of Biomass
i_{NSI}	: Nitrogen Content of Soluble Inert Substrate
i_{NSS}	: Nitrogen Content of Readily Biodegradable Substrate
i_{NXI}	: Nitrogen Content of Particulate Inert Substrate
i_{NXS}	: Nitrogen Content of Slowly Biodegradable Substrate
i_{TSBM}	: TSS to COD Ratio for Biomass
i_{TSSTO}	: TSS to COD Ratio for Storage Polymer Based on PHB
i_{TSXI}	: TSS to COD Ratio for Particulate Inert COD
i_{TSXS}	: TSS to COD Ratio for Slowly Biodegradable COD
$K_{A,HCO}$	: Bicarbonate Saturation Constant of Autotrophic Biomass [$M.L^{-3}$]
$K_{A,NH}$	: Half Saturation Constant for Ammonia [$M.L^{-3}$]
$K_{A,O}$	: Half Saturation Constant of Oxygen for Autotrophs [$M.L^{-3}$]
K_{HCO}	: Bicarbonate Saturation Constant of Heterotrophic Biomass [$M.L^{-3}$]
K_O	: Half Saturation Constant of Oxygen [$M.L^{-3}$]
K_{NH}	: Ammonium Saturation as Nutrient [$M \text{ N.L}^{-3}$]
K_{NO}	: Half Saturation Constant for Nitrate Concentration [$M.L^{-3}$]
K_S	: Half Saturation Constant of Substrate [$M.L^{-3}$]
K_{STO}	: Half Saturation Constant of Storage [$M \text{ COD.}(M \text{ COD}^{-1})$]
k_{STO}	: Maximum Rate of Storage [$M \text{ COD.}(M \text{ CellCOD.T})^{-1}$]
K_X	: Half Saturation Constant for the Hydrolysis Rate
k_H	: Hydrolysis Rate [T^{-1}]
N_D	: True Denitrification Potential
N_{DP}	: Denitrification Potential
N_{OX}	: Nitrification Potential
N_X	: Amount of Ammonia Consumption
NUR	: Nitrogen Utilization Rate [$M.L^{-3}.T^{-1}$]
OUR	: Oxygen Utilization Rate [$M.L^{-3}.T^{-1}$]
OUR_{dec}	: Oxygen Utilization Rate for Endogenous Decay [$M.L^{-3}.T^{-1}$]
OUR_{gro}	: Oxygen Utilization Rate for Growth [$M.L^{-3}.T^{-1}$]
$OUR_{respXsto}$	: Oxygen Utilization Rate for Respiration of X_{STO} [$M.L^{-3}.T^{-1}$]
OUR_{sto}	: Oxygen Utilization Rate for Storage [$M.L^{-3}.T^{-1}$]
OR_H	: Carbonaceous Oxygen Requirement

OR_T	: Total Oxygen Requirement
P_{XA}	: Daily Sludge Production of Autotrophic Biomass [$M.T^{-1}$]
P_{XH}	: Daily Sludge Production of Heterotrophic Biomass [$M.T^{-1}$]
P_{XI}	: Daily Sludge Production of Particulate Inert COD Fraction [$M.T^{-1}$]
P_{XS}	: Daily Sludge Production of Slowly Biodegradable COD [$M.T^{-1}$]
P_{XSTO}	: Daily Sludge Production of Storage Polymers [$M.T^{-1}$]
P_{XT}	: Total Daily Sludge Production [$M.T^{-1}$]
Q	: Wastewater Flow Rate [$L^3.T^{-1}$]
Q_W	: Sludge Waste Flow Rate [$L^3.T^{-1}$]
Q_R	: Sludge Recycle Flow Rate [$L^3.T^{-1}$]
Q_{RI}	: Internal Recycle Flow Rate [$L^3.T^{-1}$]
Q_{RS}	: Sludge Recirculation Flow Rate [$L^3.T^{-1}$]
R	: Recycle Ratio
S_{HCO1}	: Influent Bicarbonate Concentration [$mole.L^{-3}$]
S_{HCO}	: Bicarbonate Concentration [$mole.L^{-3}$]
S_{II}	: Influent Soluble Inert COD Concentration [$M.COD.L^{-3}$]
S_I	: Soluble Inert COD Concentration [$M.COD.L^{-3}$]
S_{NH1}	: Influent Ammonia Concentration [$M.N.L^{-3}$]
S_{NH}	: Ammonia Concentration [$M.N.L^{-3}$]
S_{NO1}	: Influent Nitrate Concentration [$M.N.L^{-3}$]
S_{NO}	: Nitrate Concentration [$M.N.L^{-3}$]
S_{O1}	: Influent Dissolved Oxygen Concentration [$M.O_2.L^{-3}$]
S_O	: Dissolved Oxygen concentration [$M.O_2.L^{-3}$]
S_{SI}	: Influent Readily Biodegradable COD Concentration [$M.COD.L^{-3}$]
S_S	: Readily biodegradable COD concentration [$M.COD.L^{-3}$]
V	: Reactor Volume [L^3]
V_A	: Aerobic Reactor Volume [L^3]
V_D	: Anoxic Reactor Volume [L^3]
X_{A1}	: Influent Autotrophic Biomass Concentration [$M.COD.L^{-3}$]
X_A	: Autotrophic Biomass Concentration [$M.COD.L^{-3}$]
X_{H1}	: Influent Heterotrophic Biomass Concentration [$M.COD.L^{-3}$]
X_H	: Heterotrophic Biomass Concentration [$M.COD.L^{-3}$]
X_{I1}	: Influent Particulate Inert COD Concentration [$M.COD.L^{-3}$]
X_I	: Particulate Inert COD Concentration [$M.COD.L^{-3}$]
X_{S1}	: Influent Slowly Biodegradable COD Concentration [$M.COD.L^{-3}$]
X_S	: Slowly Biodegradable COD Concentration [$M.COD.L^{-3}$]
X_{STO1}	: Influent Storage Polymer Concentration [$M.COD.L^{-3}$]
X_{STO}	: Storage Polymer Concentration [$M.COD.L^{-3}$]
X_T	: Total Particulate COD Concentration [$M.COD.L^{-3}$]
X_{TS}	: Particulate Solids Concentration [$M.COD.L^{-3}$]
X_R	: Recycled Sludge Concentration [$M.COD.L^{-3}$]
Y_A	: Autotrophic Growth Yield [$M.COD.(M.COD)^{-1}$]
Y_H	: Heterotrophic Growth Yield [$M.COD.(M.COD)^{-1}$]
Y_{H1}	: Heterotrophic Yield Coefficient for Direct Growth [$M.COD.(M.COD)^{-1}$]
Y_{H2}	: Heterotrophic Yield Coefficient for Growth on Stored Polymers [$M.COD.(M.COD)^{-1}$]
$Y_{H,NO}$	: Anoxic Heterotrophic Growth Yield [$M.COD.(M.COD)^{-1}$]
Y_{NH}	: Net Heterotrophic Growth Yield [$M.COD.(M.COD)^{-1}$]
Y_{NSTO}	: Net Storage Yield [$M.COD.(M.COD)^{-1}$]

Y_{STO}	: Storage Yield [M COD.(M COD) ⁻¹]
$Y_{STO,NO}$	: Anoxic Storage Yield [M COD.(M COD) ⁻¹]
μ_A	: Maximum Specific Growth Rate for Autotrophs [T ⁻¹]
μ_H	: Maximum Specific Growth Rate for Heterotrophs [T ⁻¹]
μ_{HI}	: Maximum Specific Growth Rate for Direct Growth [T ⁻¹]
μ_{H2}	: Maximum Specific Growth Rate for Secondary Growth [T ⁻¹]
θ_X	: Sludge Age [T]
θ_h	: Hydraulic Retention Time [T]
θ_{hD}	: Anoxic Hydraulic Retention Time [T]
ΔO_{STO}	: Amount of Oxygen Utilized for Storage [ML ⁻¹]
ΔS_S	: Amount of Substrate Utilized for Storage [ML ⁻¹]
η_{NO}	: Anoxic Correction Factor for Storage and Heterotrophic Growth
η_{bH}	: Anoxic Correction Factor for Endogenous Decay of Heterotrophs
η_{bSTO}	: Anoxic Correction Factor for Endogenous Decay of Stored Polymers
η_{YSTO}	: Anoxic Correction Factor for Storage Yield
η_{YH}	: Anoxic Correction Factor for Heterotrophic Growth Yield
η_{bA}	: Anoxic Correction Factor for Endogenous Decay of Autotrophs

SUBSTRATE STORAGE PHENOMENA IN THE MODELING OF ACTIVATED SLUDGE SYSTEMS

SUMMARY

Substrate storage under dynamic conditions is recently regarded as a significant process for activated sludge systems. Substrate concentration gradients are always present in wastewater treatment plants, which are caused by dynamic conditions due to changes in wastewater composition, treatment plant scheme, reactor hydraulics and variations in the modes of operation of treatment plants. The dynamic conditions and substrate gradients convey activated sludge cultures to develop a storage response when external substrate is present in the system. Activated sludge culture adapts to the dynamic conditions by storing the substrate when available and surviving on the stored substrate when external substrate is not present.

Substrate storage is incorporated into activated sludge modeling with Activated Sludge Model No. 3 (ASM3) and with biochemical models for pure substrates. ASM3 has been proposed for activated sludge systems both for aerobic and anoxic conditions. Introducing storage phenomena has also introduced a number of stoichiometric and kinetic coefficients making the model rather complicated with many degrees of freedom. Although some default values have been given in the model, calibration in terms of kinetic and stoichiometric parameters is still needed for various applications. A part of this study aimed to reduce the complexity brought by the numerous parameters in ASM3 by providing a systematic approach for the estimation of the essential kinetic and stoichiometric parameters.

In this context, ASM3 was investigated in detail for organic carbon and nitrogen removal. The conventional activated sludge system for carbon removal and the conventional pre-denitrifying single sludge system were considered in terms of process stoichiometry, according to ASM3. It is necessary to obtain the steady state solutions of the mass balances based on the model, in order to describe the fate of each parameter in the system. The mass balance equations constructed using ASM3 involved the input, the output and the generation of the parameter under concern, without any accumulation in the system.

An experimental procedure was developed for the respirometric determination of bacterial storage yield as defined in the Activated Sludge Model No. 3. The proposed approach is based on the oxygen utilization rate (OUR) profile obtained from a batch test. The procedure defines the graphical method to calculate the amount of oxygen associated with substrate storage. Model simulation was used to evaluate the procedure for different initial experimental conditions. The procedure was used to determine the storage yield, Y_{STO} , associated with acetate, glucose and domestic sewage, together with mixtures of acetate/glucose and acetate/domestic sewage at different initial F/M ratios. Y_{STO} was calculated as 0.78 gCOD/gCOD for acetate, 0.87 gCOD/gCOD for glucose and 0.96 gCOD/gCOD for domestic sewage. The Y_{STO} of substrate mixtures was found to reflect the characteristics of the dominant fraction in the mixture.

The effect of substrate composition and specifically culture composition on the observed respirometric responses under anoxic conditions were also examined. In this context different mixtures of readily biodegradable substrates have been investigated. The results provided examples and data on the experimental assessment of storage yield for different substrates and heterotrophic growth yield based on nitrogen utilization rate tests. In the tests conducted with biomass acclimatized to a 4-compound substrate mixture of acetate, propionate, ethanol and glucose, the observed anoxic storage yields were assessed as 0.70 gCOD/gCOD when fed with the same mixture and 0.71 gCOD/gCOD when fed only with acetate and propionate. However for another culture enriched with acetate-propionate mixture, the observed storage yields were estimated as 0.61 gCOD/gCOD when fed with the 4-compound mixture and 0.71 gCOD/gCOD when fed with acetate and propionate. This result has been evaluated as a possible consequence of culture adaptation. The anoxic growth yields in the tests were calculated to be equivalent to an average of 0.64 gCOD/gCOD. The study could serve as a new perspective for the experimental determination of model parameters for the design of activated sludge systems with different substrate compositions.

Activated Sludge Model No.1 (ASM1) has been used extensively for the design and simulation of biological treatment systems. Batch respirometric experiments have been described in the model for the determination of model coefficients and respirometric studies have been proved to be useful in kinetic parameter estimation and wastewater characterization for ASM1. Activated Sludge Model No. 3 (ASM3) has also introduced a number of kinetic and stoichiometric coefficients with the new processes defined in the model, also suggesting some default values in the model. Recent studies on the application of ASM3 are limited to special cases in terms of parameter determination. Proper calibration of ASM3 parameters can be a difficult task without involving respirometric procedures as experimental tools. Respirometric batch tests were conveniently used in this study in order to estimate ASM3 parameters and the main kinetic and stoichiometric model coefficients were successfully and uniquely determined for aerobic and anoxic conditions for acetate.

In order to have better predictions for real case applications and batch tests for model parameter determination ASM3 was modified in terms of storage and growth process descriptions. The results have provided strong indication that there was a need for considering direct growth on primary substrate as a significant biological mechanism. This study provides the structural framework for the proposed modified version of Activated Sludge Model No.3 (ASM3), where direct heterotrophic growth on readily biodegradable substrate is included as a new process and provision is made so that growth on internal storage compounds is started sequentially, after the depletion of the external primary substrate pool.

The study also explored the conversion processes of hydrolysable substrates by activated sludge in the context of activated sludge modeling. Experimental data were collected from a sequencing batch reactor and from batch tests using activated sludge acclimated to native potato starch. Parallel batch tests were run with particulate native potato starch, soluble starch, maltose and glucose for comparative evaluation. The fate of organic carbon in the reactor was followed directly by measuring poly-glucose and oxygen uptake. Results indicated that adsorption was the dominant mechanism for starch removal with subsequent enzymatic hydrolysis inside the flocs. The role of bulk liquid enzyme activity was minimal. Starch was observed to hydrolyze to maltose rather than glucose. The behavior of native potato starch and

soluble starch was quite similar to maltose in terms of poly-glucose formation and oxygen uptake. Glucose exhibited a significantly different removal and storage pattern. ASM3 could be adopted for an adequate description of the process stoichiometry and kinetics associated with the utilization of hydrolysable substrates, but improving ASM3 with a change in the model structure allowing simultaneous growth and storage on soluble substrate was necessary. The study also showed that differentiation of readily biodegradable and slowly biodegradable COD should better be based on the kinetics of their utilization rather than simple physical characterization.

This study also aims to investigate and present appropriate modeling techniques for slowly biodegradable COD fraction with simultaneous storage and growth concept, using particulate native potato starch and soluble starch as model substrates. Three different models have been investigated in terms of oxygen uptake rate (OUR), substrate disappearance, storage polymer generation and consumption. The evaluation of the results has shown that it is extremely important to use the appropriate stoichiometric relationships for different models. Better predictions were achieved when hydrolysis and primary growth processes are taken into account in the models. Comparing the degradation of two different types of hydrolysable substrate (particulate native potato starch and soluble starch), it can be stated that although the substrates were hydrolyzed with similar rates, primary and secondary growth processes on soluble starch were more efficient, with higher yields, due to the more easily utilizable products of soluble starch, both in terms of extra-cellular hydrolysis and the hydrolysis of stored poly-glucose.

It has been pointed out that it was extremely important to use the appropriate stoichiometric relationships for different models. Therefore, any selected model should be investigated in detail, concerning the assumptions involved and the correct stoichiometry being carefully determined. The selected values of the stoichiometric parameters should be determined according to the experimental data obtained for the three major system components, namely substrate, storage polymers, and oxygen.

AKTİF ÇAMUR SİSTEMLERİNİN MODELLENMESİNDE SUBSTRAT DEPOLAMA KAVRAMI

ÖZET

Son yıllarda aktif çamur sistemlerinde substrat depolama kavramı önemli bir proses olarak kabul edilmektedir. Atıksu kompozisyonunda ve arıtma tesisi yapısında oluşan, reaktör hidroliği ve arıtma tesislerinin işletme biçimlerinde ortaya çıkan değişiklikler nedeniyle oluşan dinamik koşullar sonucu, atıksu arıtma tesislerinde her zaman substrat konsantrasyon gradyanları görülmektedir. Bu dinamik koşullar ve substrat gradyanları aktif çamur kültürlerini, substratı depolama tepkisi geliştirmeye yönlendirmektedir. Aktif çamur kültürü, ortamda bulunan substratı depolayıp substrat tükendiğinde ise depolanan substrat üzerinden çoğalarak, dinamik koşullara adapte olmaktadır.

Substrat depolama kavramı, aktif çamur modellemesine, Aktif Çamur Modeli No.3 (ASM3) ve saf substratlar için önerilen biyokimyasal modellerle dahil edilmiştir. ASM3, aktif çamur sistemlerinde hem aerobik hem de anoksik koşullar için önerilmiştir. Depolama kavramının ortaya konması, modele çok fazla serbestlik derecesi sağlayarak, modeli daha karmaşık bir hale getiren pek çok kinetik ve stokiyometrik katsayının da dahil edilmesine yol açmıştır. Modelde bu katsayılar için bazı değerler önerilmekle birlikte çeşitli uygulamalar için modelin kinetik ve stokiyometrik katsayılar açısından kalibre edilmesi gerekmektedir. Bu çalışmanın bir bölümünde, ASM3 ile ortaya konan pek çok parametre ile oluşan bu karmaşıklık azaltmak amacıyla, öncelikli kinetik ve stokiyometrik parametrelerin belirlenmesi için sistematik bir yaklaşım ortaya konmaktadır.

Bu çerçevede, ASM3 organik karbon ve azot giderimi için detaylı olarak incelenmiştir. Karbon gideren klasik aktif çamur sistemi ve tek çamurlu klasik pre-denitrifikasyon sistemi, ASM3 uyarınca proses stokiyometrilere açısından ele alınmıştır. Sistemdeki her parametrenin durumunu tanımlamak için, modele dayalı kütle dengelerinin kararlı hal çözümlerini elde etmek gerekmektedir. ASM3 kullanılarak elde edilen kütle dengesi denklemleri, sistemde akümüülasyon olmadan girdi, çıktı ve ilgili parametre için oluşum veya tüketim ifadelerini içermektedir.

Bu çalışmada, Aktif Çamur Modeli No.3'te (ASM3) yer alan en önemli parametrelerden biri olan depolama dönüşüm oranının (Y_{STO}) belirlenmesi için deneysel bir yaklaşım önerilmektedir. Önerilen yaklaşım kesikli deneylerle elde edilen oksijen tüketim hızına (OTH) dayanmaktadır. Yöntem substrat depolaması için kullanılan oksijen miktarının hesaplanması için grafiksel bir metot tanımlar. Yöntemin değerlendirilmesi için farklı deneysel başlangıç koşullarının kullanıldığı model simülasyonlarından yararlanılmıştır. Önerilen deneysel yöntemi denemek üzere asetat, glikoz ve evsel atıksuyun ve bunların karışımlarının kullanıldığı respirometrik ölçümler gerçekleştirilmiştir. Farklı F/M oranlarında yürütülen deneyler ile depolama dönüşüm oranları, asetat için 0.78 gKOİ/gKOİ, glikoz için 0.87 gKOİ/gKOİ ve evsel atıksu için 0.96 gKOİ/gKOİ olarak belirlenmiştir. Substrat

karışımları için elde edilen Y_{STO} değerlerinin karışımda daha yoğun olan substratın özelliklerini yansıttığı ortaya konmuştur.

Çalışmada ayrıca, substrat kompozisyonunun ve özellikle kültür kompozisyonunun anoksik koşullar altında gözlenen respirometrik tepkiler üzerindeki etkileri ele alınmaktadır. Bu çerçevede, farklı kolay ayrışan substratların farklı karışımları incelenmiştir. Elde edilen sonuçlar, farklı substratlar için depolama ve heterotrofik çoğalma dönüşüm oranlarının azot tüketim hızı testleri ile belirlenmesi için gerekli verileri ortaya koymaktadır. Asetat, propiyonat, etanol ve glikozdan oluşan 4 bileşenli karışıma aklime edilmiş biyokütle ile yürütülen deneylerde gözlenen depolama dönüşüm oranı aynı substrat karışımı için 0.70 gKOİ/gKOİ, sadece asetat ve propiyonat beslendiğinde 0.71 gKOİ/gKOİ olarak elde edilmiştir. Ancak, asetat-propiyonat karışımı ile geliştirilmiş diğer bir kültür için, gözlenen depolama dönüşüm oranı belirtilen 4 bileşenli karışım ile beslendiğinde 0.61 gKOİ/gKOİ, asetat-propiyonat karışımı ile beslendiğinde ise 0.71 gKOİ/gKOİ olarak belirlenmiştir. Bu sonuç kültür adaptasyonunun bir sonucu olarak değerlendirilmiştir. Bu testlerde elde edilen anoksik çoğalma dönüşüm oranlarının ortalaması 0.64 gKOİ/gKOİ olarak hesaplanmıştır. Bu çalışma, aktif çamur sistemlerinin tasarımında kullanılan model parametrelerinin farklı substrat kompozisyonları söz konusu olduğunda, deneysel olarak belirlenmesi için yeni bir perspektif ortaya koymaktadır.

Aktif Çamur Modeli No.1 (ASM1) biyolojik arıtma sistemlerinin tasarımı ve simülasyonu için geniş kullanım alanı bulmuştur. Model katsayılarının belirlenmesi için kesikli respirometrik deneyler tanımlanmış ve respirometrik çalışmalar, kinetik parametrelerin hesaplanması ile ASM1 atıksu karakterizasyonu için son derece yararlı olmuştur. Aktif Çamur Modeli No.3, (ASM3) ile yeni tanımlanan proseslerde, kinetik ve stokiyometrik pek çok yeni katsayı içermektedir. ASM3'ün uygulamaları konusunda son dönemde gerçekleştirilen çalışmalar, parametrelerin belirlenmesi açısından özel durumlarla sınırlı kalmıştır. Deneysel bir araç olarak respirometrik yöntemlerin kullanılmadığı durumlarda ASM3 parametrelerinin uygun kalibrasyonu son derece güç olabilmektedir. ASM3 parametrelerini hesaplamak için respirometrik kesikli deneyler son derece uygundur ve bu çalışmada modele ait önemli kinetik ve stokiyometrik katsayılar, asetat için aerobik ve anoksik koşullarda başarılı bir biçimde belirlenmiştir.

Bu çalışma çerçevesinde, gerçek durum uygulamaları ve model parametrelerinin belirlenmesi için gerçekleştirilen kesikli deneyleri daha iyi değerlendirebilmek için, ASM3'te yer alan depolama ve çoğalma proseslerinin tanımları modifiye edilmiştir. Sonuçlar, birincil substrat üzerinden gerçekleşen çoğalmanın etkili biyolojik bir mekanizma olarak değerlendirilmesi gerektiğini ortaya koymuştur. Bu çalışmada, Aktif Çamur Modeli No.3 (ASM3) için önerilen modifikasyon için gerekli yapısal çerçeve belirlenmiştir. Bu modifikasyonda, kolay ayrışan substrat üzerinde oluşan doğrudan heterotrofik çoğalma, yeni bir proses olarak modele eklenmiş ve depolama ürünleri üzerinden gerçekleşen çoğalma prosesinin, ancak birincil substrat havuzu tükendiğinde devreye girmesi sağlanmıştır.

Aktif çamur modellemesinde hidroliz olabilen substratların ayrışma prosesleri de bu çalışma kapsamında araştırılmıştır. Deneysel veriler, yerel patates nişastası ile aklime edilmiş aktif çamur kullanılarak yürütülen kesikli deneyler ve işletilen ardışık kesikli reaktörden elde edilmiştir. Kıyaslamalı değerlendirme için, yerel patates nişastası, çözünür nişasta, maltoz ve glikoz ile paralel deneyler gerçekleştirilmiştir. Reaktördeki organik karbon giderimi, poli-glikoz ve oksijen tüketiminin doğrudan

ölçülmesi ile izlenmiştir. Sonuçlar, nişasta gideriminde etkin mekanizmanın adsorpsiyon olduğunu ve bunu izleyen hidrolizin flokların içinde gerçekleştiğini göstermiştir. Sıvı fazda gerçekleşen enzim aktivitesi çok düşük orandadır. Nişastanın glikoz yerine maltoza hidroliz olduğu gözlenmiştir. Poli-glikoz oluşumu ve oksijen tüketimi açısından yerel patates nişastası ve çözünen nişastanın, maltoz ile büyük benzerlik gösterdiği belirlenmiştir. Glikoz oldukça farklı bir giderim ve depolama davranışı göstermiştir. ASM3, hidroliz olabilen substratlar için uygun stokiometrik ve kinetik proses tanımları ortaya koyacak şekilde adapte edilebilmektedir ancak ASM3 model yapısını çözünmüş substratlar üzerinden gerçekleşen simültane çoğalma ve depolama kavramını içerecek şekilde değiştirmek gerekmektedir. Çalışmada ayrıca kolay ve yavaş ayrışan KOİ bileşenleri arasındaki ayrımın basit fiziksel özellikler yerine giderim kinetiklerinin incelenmesi ile belirlenmesinin daha doğru olacağı ortaya konmuştur.

Bu çalışma ayrıca, partiküler yerel patates nişastası ve çözünen nişastanın model substratlar olarak kullanılması ile, yavaş ayrışan KOİ bileşeni için simültane çoğalma ve depolama kavramını da içeren uygun modelleme tekniklerini incelemeyi ve ortaya koymayı da amaçlamıştır. Oksijen tüketim hızı (OTH), substrat giderimi ve depolama ürünleri oluşumu ve tüketimini esas alan üç farklı model incelenmiştir. Modellerde, hidroliz ve simültane depolama ve çoğalma prosesleri dikkate alındığında daha iyi simülasyonlar elde edilmiştir. Hidroliz olabilen iki farklı substratın (yerel patates nişastası ve çözünen nişasta) ayrışmaları kıyaslandığında, substratların benzer hızlarla hidroliz olmalarına rağmen, çözünen nişasta ile gerçekleşen birincil ve ikincil çoğalmanın, çözünen nişastanın hücre dışı hidrolizi ve bu nişastadan depolanan poli-glikozun hidrolizi sonucu oluşan ürünlerin kolay tüketilmesi sebebiyle, daha verimli oldukları ve daha yüksek dönüşüm oranları ile gerçekleştikleri belirlenmiştir.

Farklı modeller için uygun stokiometrinin kullanılması gerekliliği ortaya konmuştur. Bu yüzden, seçilen herhangi bir modelin detaylı bir şekilde, model varsayımlarını da dikkate alarak, incelenmesi ve doğru stokiometrinin belirlenmesi gerekmektedir. Stokiometrik parametreler için seçilen değerler, mutlaka substrat, depolama polimerleri ve oksijen için, yani üç ana sistem bileşeni için elde edilen deneysel veriler kullanılarak belirlenmelidir.

1. INTRODUCTION

1.1 Significance of the Subject

Recent studies of activated sludge systems have pointed out the significance of substrate storage under dynamic conditions. Substrate concentration gradients always present in wastewater treatment plants, convey activated sludge cultures to develop a storage response when external substrate is present in the system. Dynamic conditions are caused by changes in wastewater composition, treatment plant scheme, reactor hydraulics and variations in the modes of operation of treatment plants. Activated sludge culture adapts to the dynamic conditions by storing the substrate when available and surviving on the stored substrate when external substrate is not present.

Although substrate storage response was already a known phenomena, its significance in the modeling and design of activated sludge systems has recently been recognized. Substrate storage is incorporated into activated sludge modeling with Activated Sludge Model No. 3 (ASM3) and biochemical models for pure substrates. With the introduction of storage process, activated sludge models have gained a new perspective and biochemical transformations inside the biomass have been taken into account together with the already considered extracellular processes. Describing the mechanisms taking place inside the cells is not straight forward and thus modeling approaches are rather complicated and therefore need simplifying assumptions. The verification of the models with substrate storage is only possible if these assumptions are correct. Extensive research and refinement of the model is necessary for the appropriate integration of substrate storage in activated sludge modeling.

This study presents a comprehensive survey on the estimation of ASM3 with experimental assessment techniques for significant parameters of the model, simultaneous substrate storage and growth concept for further improvement of the model and on the modeling approaches for storage when complex, slowly

biodegradable substrates are concerned. The results of this study and the survey conducted in this manner, would contribute at a high extend to the modeling efforts of activated sludge systems with substrate storage.

1.2 Aim and Scope

The aim of this study is to evaluate the incorporation of substrate storage in the modeling of activated sludge systems. ASM3 is the first attempt to model substrate storage, but the identification of the model parameters and model verification is necessary.

ASM3 has been studied in detail in terms of stoichiometric evaluation of conventional activated sludge systems for carbon and nitrogen removal. The steady state solutions of the mass balances involved in the design of conventional carbon and nitrogen removal systems were achieved. As the model was investigated, it has been observed that the most important parameter is the storage yield and its experimental determination was necessary. Respirometry has been used for the experimental assessment of the storage yield for ASM3 both for aerobic and anoxic conditions.

Modeling studies were conducted for the verification of ASM3 for aerobic and anoxic experimental conditions. The results of ASM3 model calibration have shown that the physical definition of readily biodegradable substrate in ASM3 and the assumption of substrate consumption occurring only through storage need to be modified. The modification of the model was suggested considering direct growth on primary substrate, in order to describe the actual case in a more realistic way.

Substrate storage phenomena in activated sludge modeling, is further investigated with experimental studies conducted with complex, hydrolysable substrates. The studies involved a detailed investigation of the hydrolysis process for particulate and soluble substrates. The concept of simultaneous storage and growth has been applied for the modeling of activated sludge systems with complex substrates.

1.2.1 Substrate storage phenomena

Activated sludge models provide a reliable basis for the design and operation of activated sludge models. The aim is to identify a rational mechanistic description of the process in order to define and predict system performance. These models offer the basic relationships between process components, kinetics and stoichiometric characteristics and basic system parameters.

Development of mathematical modeling for the design and operation of an activated sludge systems achieving simultaneous carbon and nitrogen removal has lead to the evolution of Activated Sludge Model No.1, ASM1 (**Henze *et al.*, 1987**).

The ability of microorganisms to accumulate internal storage polymers is well documented (**Chudoba *et al.*, 1973**; **Van den Eijnde *et al.*, 1984**). Studies conducted on both pure and mixed activated sludge cultures provided substantial proof on the ability of microorganisms to convert substrate into internal storage products under dynamic conditions (**van Loosdrecht *et al.*, 1997**). The growth response of heterotrophic microorganisms was observed to take place mainly at the expense of stored products (**Majone *et al.*, 1999**). Substrate storage was also recognized as a key process for biological phosphorus removal (**Wentzel *et al.*, 1986**; **Mino *et al.*, 1987**). It was recently introduced within Activated Sludge Model No.3 (ASM3); a comprehensive new modeling approach for activated sludge cultures, as the first step for the utilization of readily biodegradable substrate (**Gujer *et al.*, 2000**).

The metabolic model of PHB storage, suggests that acetate is taken up and converted to acetyl-CoA, which is used to produce biomass and for PHB synthesis. Acetyl-CoA is also used as the energy source. When all the acetate is taken up, stored PHB is hydrolyzed to generate acetyl-CoA under famine conditions. The described glycogen metabolism, implies that glucose is taken up and Glucose-6-phosphate (G6P) is produced, which is then converted into glycogen and used for biomass production. G6P is also used for catabolic reactions consuming oxygen. After the depletion of primary substrate, glycogen is used for the synthesis of G6P.

1.2.2 Modeling substrate storage

The components and processes of ASM3 are similar to ASM1. However, ASM3 includes nitrogen and alkalinity limitations for the growth of organisms, which were not taken into account in ASM1. Biodegradable soluble and particulate organic nitrogen model components in ASM1 have been eliminated in the new model. Thus, the deficiency of ASM1 for the proper description of ammonification kinetics was defeated by assuming a constant nitrogen to COD ratio.

The stoichiometry and kinetics of biological storage have been defined in ASM3, but the experimental procedures for the determination of the coefficients associated with the process kinetics have not been standardized yet. The new model, ASM3 (Gujer *et al.*, 2000), involves entire conversion of readily biodegradable substrate into storage products prior to growth on stored polymers. The model describes storage, microbial growth and endogenous decay as energy consuming processes with different rates of electron acceptor utilization. It should be noted that the merit of the new models mainly depends on the accuracy and reliability of the information they reflect on the biochemical mechanisms involved. This information must be experimentally determined. Respirometric methods have served extensively in the experimental assessment of kinetic and stoichiometric coefficients associated with ASM1 (Ekama *et al.*, 1986; Spanjers and Vanrolleghem, 1995; Sözen *et al.*, 1998). ASM3 introduced, together with the concept of biochemical storage, a new set of kinetic and stoichiometric coefficients, totally different from ASM1, with practically no experimental information on applicable values. Only some default levels were suggested with the model.

The storage yield Y_{STO} , is one of the most important parameters of the model, since it represents the stoichiometric amount of the substrate converted into storage products, which are then utilized for growth. Although certain complicated techniques were suggested for its assessment (Goel *et al.*, 1998; Dircks *et al.*, 1999), very little experimental data of practical value for activated sludge systems is so far available.

The information on the magnitude of the storage yield was mostly derived from pure culture studies: The storage yield for *Paracoccus pantotrophus* fed with acetate has been experimentally determined by van Aalst-van Leeuwen *et al.* (1997). For activated sludge cultures fed with pure substrates, Beun *et al.* (2000a) reported the

value of storage yield for acetate under aerobic and anoxic conditions (Beun *et al.*, 2000b). Goel *et al.* (1999) have assumed a storage yield for glucose, considering that the formation of glycogen from glucose requires less energy as compared to PHB accumulation from acetate. Dircks *et al.* (2001) experimentally found the storage yield of glucose. ASM3 suggested the default values of Y_{STO} under aerobic and anoxic conditions for domestic sewage.

Recent studies on the application of ASM3 have been limited to special cases in terms of parameter estimation (Koch *et al.*, 2000). The trend however is to use respirometric procedures for the assessment of ASM3 parameters. This approach has been successfully applied for the determination of the storage yield coefficient, Y_{STO} under aerobic conditions (Karahan *et al.*, 2002a).

It is also important to determine the difference in the rate of major biochemical processes described in ASM3 under aerobic and anoxic conditions. ASM3 interprets this difference using a single correction factor, η_D . This study illustrates the merit of respirometry in the experimental evaluation of ASM3 under aerobic and anoxic conditions.

Several studies have been conducted on the modeling of substrate storage under aerobic conditions, since the introduction of the process in activated sludge modeling with Activated Sludge Model No.3 (ASM3) by Gujer *et al.* (2000). The studies have mostly been performed with synthetic substrates (Beun *et al.*, 2000a; Dircks *et al.*, 2001; Karahan *et al.*, 2002b), however, the application of the model for domestic sewage (Dircks *et al.*, 1999; Koch *et al.*, 2000; Karahan *et al.*, 2002a) and for industrial wastewaters (Dizdaroglu-Risvanoglu *et al.*, 2004) have been limited.

1.2.3. Simultaneous Storage and Growth Concept Considering Hydrolysis

Storage of excess substrate available under feast conditions allows microorganisms capable of substrate storage to survive on the accumulated substrate reserves when no external substrate is present (famine conditions). These microorganisms have the benefit of a more balanced growth and advantage for competition (van Loosdrecht *et al.*, 1997).

Recognition of substrate fractions with substantially different biodegradation rates may be regarded as one of the most significant milestones in the modeling of activated sludge. This approach differentiates readily biodegradable substrate from its slowly biodegradable counterpart, which represents the major fraction of the complex organics in domestic sewage and industrial wastewaters (**Dold *et al.*, 1980; Henze *et al.*, 1987; Orhon and Ubay Cokgör, 1997; Orhon *et al.*, 2002**).

Slowly biodegradable substrate, as defined for wastewaters, involves a large spectrum of compounds with different nature and size. In activated sludge models, their biodegradation is conveniently defined by means of a hydrolysis mechanism converting them into simpler readily biodegradable compounds. While this approach provides a useful tool for the interpretation of system behavior and performance, it does not elucidate the true mechanism of the utilization of complex organics. This subject has been an attractive area of experimental research, and starch is selected to represent the slowly biodegradable substrate in the majority of the experiments: **San Pedro *et al.* (1994)** stated that starch, like larger complex organics, was degraded slowly because it hydrolyzed through extracellular enzyme activity after its adsorption onto biomass. Hydrolysis was defined as the rate limiting step of starch utilization. Biomass was found to exert no significant effect on the hydrolysis rate. Based on similar experiments, **Goel *et al.* (1998a)** suggested that the hydrolytic enzymes are found to be released into the bulk in pure cultures whereas the enzyme activity was found to be mainly associated with the cell surfaces in activated sludge.

One of the major concerns in the modeling of activated sludge is the fate of the hydrolysis products. Activated Sludge Model No.1, (ASM1), is structured upon the concept that simpler compounds resulting from hydrolysis are solely utilized for heterotrophic growth (**Henze *et al.*, 1987**). Activated Sludge Model No.3, (ASM3), involves complete storage of substrate prior to growth. Consequently, there is less emphasis on the extracellular hydrolysis of complex organic matter because a significant part of the 'slow oxygen uptake rate' is due to growth on stored substrate (**Gujer *et al.*, 2000**), whereas in ASM1 this is solely due to growth on hydrolyzed substrate. Experiments indicate however that the extent of storage and the level of internal hydrolysis depend much on operating characteristics and feast (presence of external substrate) and famine (absence of external substrate) conditions created within the system operation (**Majone *et al.*, 1996**).

Recent modeling efforts attempt to bring clarification to simultaneous substrate storage and growth (Krishna and van Loosdrecht, 1999; Beun *et al.*, 2000a; Karahan *et al.*, 2003).

Basic stoichiometry is important both for ASM1 and ASM3; correct assessment of the corresponding yield coefficients (heterotrophic yield, Y_H and storage yield, Y_{STO}) is required for an acceptable interpretation of the experimental results. This interpretation also depends on the way in which different COD fractions are defined in the models: ASM3 assumes that the readily biodegradable fraction is the soluble portion of the biodegradable COD after filtration through 0.45 μm membrane filter, while ASM1 postulates that only a part of the soluble portion, which can be quantified by means of respirometry, is readily biodegradable.

It has been stated in several studies that the assumptions, which ASM3 has been based on about substrate storage are inappropriate for the definition of the real case, since substrate storage process only serves as a tool to maximize substrate uptake and simultaneous growth on the readily biodegradable substrate should also be incorporated in the model (Krishna and van Loosdrecht, 1999; Karahan *et al.*, 2003). Biochemical models on pure substrates have also pointed out that there is substantial evidence that substrate consumption is progressed by simultaneous growth and storage on the primary substrate both for pure (van Aalst-van Leuwen *et al.*, 1997) and for mixed cultures (Beun *et al.*, 2000a; Dircks *et al.*, 2001).

Two types of starch have been selected to predict the biochemical transformations for hydrolysable substrates in this study, namely Native Potato Starch (NPS) and Soluble Starch (SolS). NPS can be characterized by 64% particulate and 36% soluble COD. SolS is on the other hand 97% soluble and has only 3% particulate COD fraction after filtration through 0.45 μm pore size membrane. The difference in the solubility characteristics of the two types of starch is due to the difference in their molecular structure. Starch, in general is composed of two different kinds of molecules: a linear molecule with 1,4- α -D glucose linkages, called amylose and a branched molecule with both 1,4 and 1,6- α -D glucose linkages, amylopectin. The ratio of amylose and amylopectin molecules that starch is composed of plays an important role in determining the unique physical properties of each type of starch.

Hydrolysis of starch involves the breakage of the linkages between the glucose units, either enzymatically or by means of a strong acid. Since amylose and amylopectin have a number of sites for hydrolysis, there is nearly an infinite number of combinations of hydrolysis products, such as; glucose, maltose, isomaltose, etc. Some hydrolysis reactions are shown in Figure 1.1. The investigation of starch hydrolysis have also shown that starch is a polysaccharide which is degraded to maltose, malto-tri-ose, and/or other two-three unit sugars by extracellular hydrolysis (Ubukata, 1999, Mino *et al.*, 1995, Karahan *et al.*, 2005a).

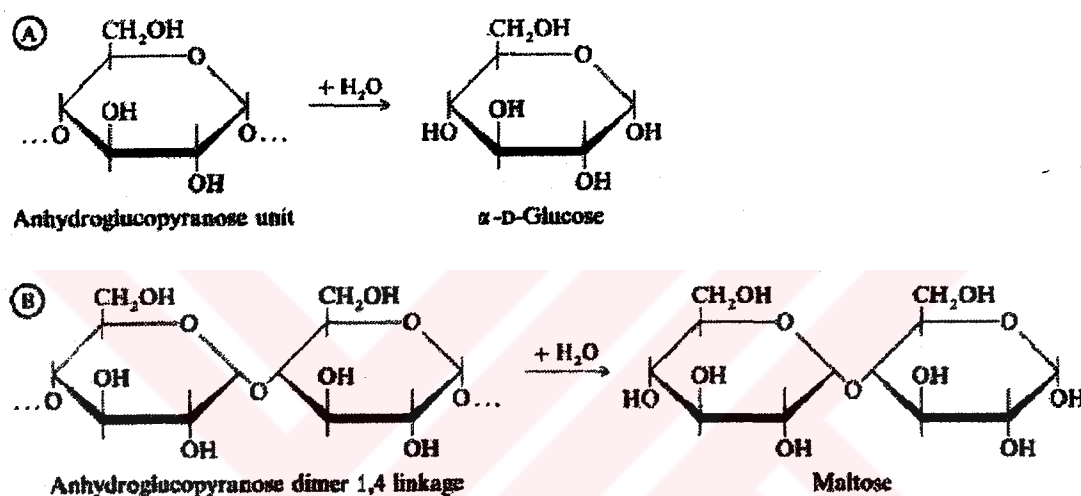


Figure 1.1: Hydrolysis reaction examples for starch

Starch has been used as the model substrate in order to investigate the fate of slowly biodegradable substrate in many studies (Mino *et al.*, 1995; Goel *et al.*, 1998a). Being slowly biodegradable in terms of microbial utilization, starch first has to go through extracellular hydrolysis prior to its consumption by biomass (Figure 1.2). Recent studies have also shown that starch rapidly disappears from the bulk liquid and is adsorbed on the biomass before being hydrolyzed (Karahana *et al.*, 2005a).

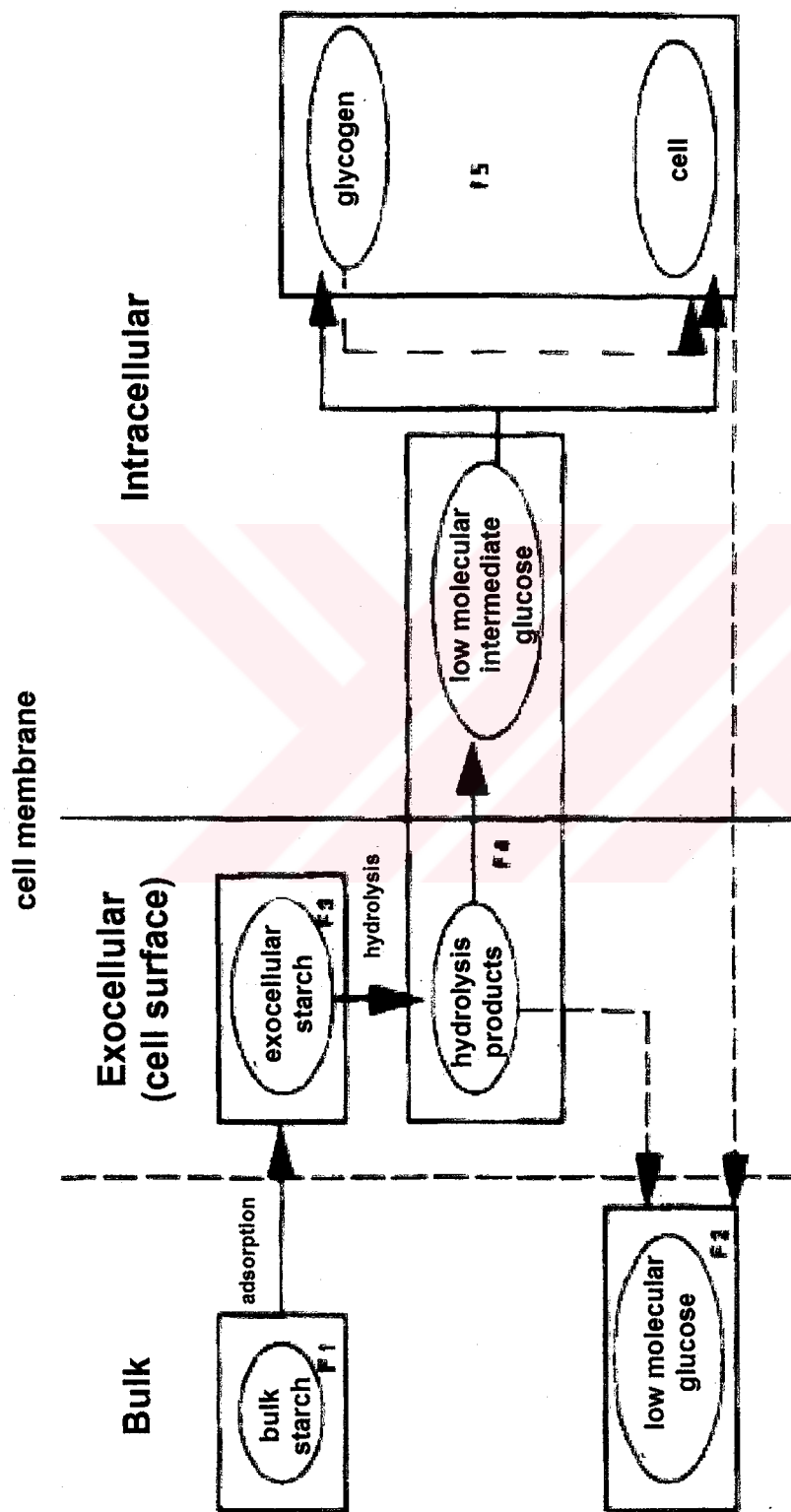


Figure 1.2: Starch removal mechanism in activated sludge systems (San Pedro *et al.*, 1994)

2. EVALUATION OF ASM3 STOICHIOMETRY FOR ORGANIC CARBON AND NITROGEN REMOVAL

2.1 ASM3 Stoichiometry for Organic Carbon Removal

Activated Sludge Model No.3 (ASM3) has been developed for carbon and nitrogen removal, as an alternative to ASM1. The main concept in ASM3 is the additional substrate storage process, where all the soluble biodegradable substrate is first assumed to be consumed to produce storage polymers, which are then used for growth in the absence of external substrate. ASM3 also possesses the conceptual approach that the rates and yields under anoxic conditions would be lower than the rates and yields under aerobic conditions, including the endogenous decay process. The matrix given in Table 2.1 presents the full model of ASM3.

The conventional activated sludge systems for carbon removal are composed of an aerobic reactor and a clarifier. The aeration reactor is assumed to be ideal completely stirred tank and the clarifier is assumed to be a settler, where no biochemical transformation occurs.

The schematic representation of the conventional activated sludge system is given in Figure 2.1. The figure includes all the model components present in the wastewater fed to the system, the components in the reactors and the ones discharged from the system. Sludge waste is assumed to be performed with the removal of particulate components from the aerobic reactor. Although the particulate fractions are discharged from the bottom of the clarifier a fictive portion of the particulates dependent on the hydraulic retention time and the sludge age are assumed to be leaving the system in order to be accurate in the COD removal calculations.

Table 2.1: The Model: ASM3

COMPONENT PROCESS	S _O O ₂	S _I COD	S _S COD	S _{NH} N	S _{N2} N	S _{NO} N	S _{HCO} mole	X _I COD	X _S COD	X _H COD	X _{STO} COD	X _A COD	X _{TS} TSS	RATE
Hydrolysis		f _{SI}	1-f _{SI}	i _{NSI}			$\frac{i_{NSI}}{14}$		-1				-i _{TSXS}	$k_H \frac{X_S/X_H}{K_X + X_S/X_H} X_H$
Heterotrophic Organisms, Denitrification														
Aerobic Storage of COD	-(1-Y _{STO,O2})		-1	i _{NSS}			$\frac{i_{NSS}}{14}$				Y _{STO,O2}		i _{TSSTO} ·Y _{STO,O2}	$k_{STO} \frac{S_O}{K_O + S_O} \frac{S_S}{K_S + S_S} X_H$
Anoxic Storage of COD			-1	i _{NSS}	$\frac{(1-Y_{STO,NO})}{2.86}$	$\frac{(1-Y_{STO,NO})}{2.86}$	$\frac{(1-Y_{STO,NO})}{14 \cdot 2.86} + \frac{i_{NSS}}{14}$				Y _{STO,NO}		i _{TSSTO} ·Y _{STO,NO}	$k_{STO} \eta_{NO} \frac{K_O}{K_O + S_O} \frac{S_{NO}}{K_{NO} + S_{NO}} \frac{S_S}{K_S + S_S} X_H$
Aerobic Growth	$\frac{(1-Y_{H,O2})}{Y_{H,O2}}$			-i _{NBM}			$-\frac{i_{NBM}}{14}$			1	-1/Y _{H,O2}		$i_{TSBM} - \frac{i_{TSSTO}}{Y_{H,O2}}$	$\mu_H \frac{S_O}{K_O + S_O} \frac{S_{NH}}{K_{NH} + S_{NH}} \frac{S_{HCO}}{K_{HCO} + S_{HCO}} \frac{X_{STO}/X_H}{K_{STO} + X_{STO}/X_H} X_H$
Anoxic Growth (Denitrification)				-i _{NBM}	$\frac{(1-Y_{H,NO})}{2.86 \cdot Y_{H,NO}}$	$\frac{(1-Y_{H,NO})}{2.86 \cdot Y_{H,NO}}$	$\frac{(1-Y_{H,NO})}{14 \cdot 2.86 \cdot Y_{H,NO}} - \frac{i_{NBM}}{14}$			1	-1/Y _{H,NO}		$i_{TSBM} - \frac{i_{TSSTO}}{Y_{H,NO}}$	$\mu_H \eta_{NO} \frac{K_O}{K_O + S_O} \frac{S_{NO}}{K_{NO} + S_{NO}} \frac{S_{NH}}{K_{NH} + S_{NH}} \frac{S_{HCO}}{K_{HCO} + S_{HCO}} \frac{X_{STO}/X_H}{K_{STO} + X_{STO}/X_H} X_H$
Aerobic Endogenous Respiration	-(1-f _I)			i _{NBM} -f _I ·i _{NXI}			$\frac{i_{NBM} - f_I \cdot i_{NXI}}{14}$	f _I		-1			$i_{TSBM} + f_I i_{TSXI}$	$b_{H,O2} \frac{S_O}{K_O + S_O} X_H$
Anoxic Endogenous Respiration				i _{NBM} -f _I ·i _{NXI}	$\frac{(1-f_I)}{2.86}$	$-\frac{(1-f_I)}{2.86}$	$\frac{(1-f_I)}{14 \cdot 2.86} + \frac{i_{NBM} - f_I \cdot i_{NXI}}{14}$	f _I		-1			$i_{TSBM} + f_I i_{TSXI}$	$b_{H,NO} \frac{K_O}{K_O + S_O} \frac{S_{NO}}{K_{NO} + S_{NO}} X_H$
Aerobic Respiration of X _{STO}	-1										-1		-i _{TSSTO}	$b_{STO,O2} \frac{S_O}{K_O + S_O} X_{STO}$
Anoxic Respiration of X _{STO}					1/2.86	-1/2.86	1/(14·2.86)				-1		-i _{TSSTO}	$b_{STO,NO} \frac{K_O}{K_O + S_O} \frac{S_{NO}}{K_{NO} + S_{NO}} X_{STO}$
Autotrophic Organisms, Nitrification														
Nitrification	$\frac{(4.57 - Y_A)}{Y_A}$			-(1/Y _A +i _{NBM})		1/Y _A	$-\frac{2/Y_A + i_{NBM}}{14}$					1	i _{TSBM}	$\mu_A \frac{S_O}{K_{A,O} + S_O} \frac{S_{NH}}{K_{A,NH} + S_{NH}} \frac{S_{HCO}}{K_{A,HCO} + S_{HCO}} X_A$
Aerobic Endogenous Respiration	-(1-f _I)			i _{NBM} -f _I ·i _{NXI}			$\frac{i_{NBM} - f_I \cdot i_{NXI}}{14}$	f _I				-1	$i_{TSBM} + f_I i_{TSXI}$	$b_{A,O2} \frac{S_O}{K_O + S_O} X_A$
Anoxic Endogenous Respiration				i _{NBM} -f _I ·i _{NXI}	$\frac{(1-f_I)}{2.86}$	$-\frac{(1-f_I)}{2.86}$	$\frac{(1-f_I)}{14 \cdot 2.86} + \frac{i_{NBM} - f_I \cdot i_{NXI}}{14}$	f _I				-1	$i_{TSBM} + f_I i_{TSXI}$	$b_{A,NO} \frac{K_O}{K_O + S_O} \frac{S_{NO}}{K_{NO} + S_{NO}} X_A$
Composition Matrix														
Conservatives														
COD g COD	-1	1	1		-1.71	-4.57		1	1	1	1	1		
Nitrogen g N		i _{NSI}	i _{NSS}	1	1	1		i _{NXI}	i _{NXS}	i _{NBM}			i _{NBM}	
Ionic Charge Mole +				1/14		-1/14	-1							
Observables														
TSS g TSS								i _{TSXI}	i _{TSXS}	i _{TSBM}	i _{TSSTO}	i _{TSBM}		

2.1.1 Mass balances and steady state solutions

It is necessary to obtain the steady state solutions of the mass balances based on the model, in order to describe the fate of each parameter in the system. The mass balance equations constructed using ASM3 involve the input, the output and the generation of the parameter under concern, without any accumulation in the system.

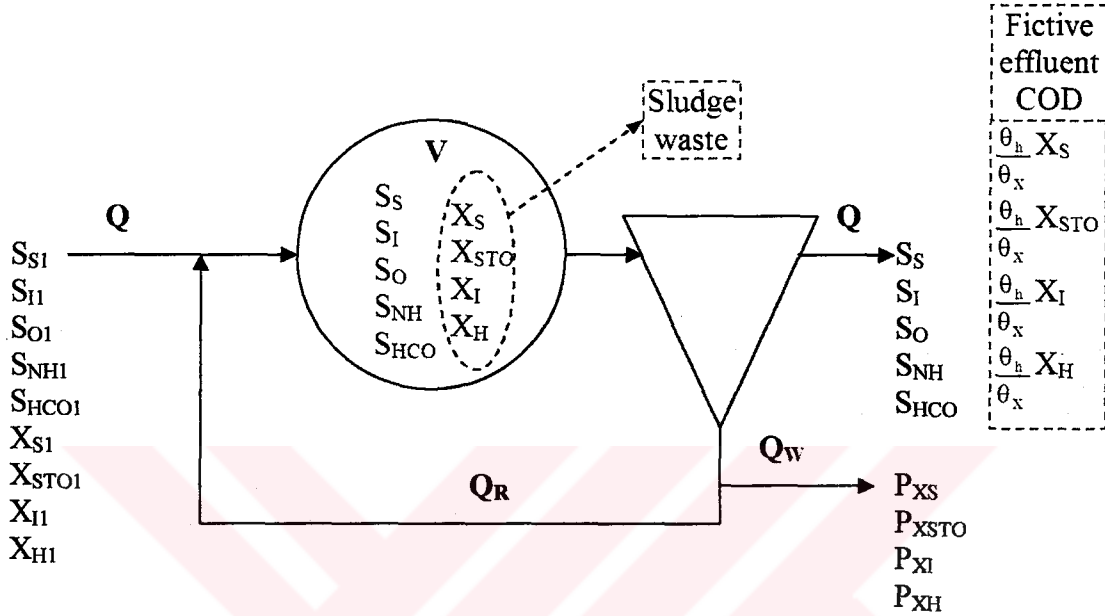


Figure 2.1: Schematic representation of conventional activated sludge system for carbon removal with ASM3 components

The heterotrophic biomass, X_h , is one of the key components in the system. Heterotrophic biomass enters the system with influent, X_{hi} , and leaves the system with sludge waste. X_h is also generated as a result of growth process and reduced by endogenous decay. The mass balance on the heterotrophic biomass is given in equation 2.1.

$$Q \cdot X_{hi} - Q_w \cdot X_h + V \cdot \mu_h \cdot X_h - V \cdot b_h \cdot X_h = 0 \quad (2.1)$$

The heterotrophic biomass concentration in the influent is generally very low and therefore X_{hi} is assumed to be zero. In this case equation 2.1 can be simplified as in equation 2.2, generating a new definition for the sludge age of the system.

$$\frac{1}{\theta_x} = \mu_h - b_h \quad (2.2)$$

The sludge age is traditionally defined as the volume of the aeration tank divided by the daily sludge waste, based on system operating conditions, as given below.

$$\theta_x = \frac{V}{Q_w} \quad (2.3)$$

Equation 2.2 can be extended by incorporating the rate expression for heterotrophic growth as:

$$\frac{1}{\theta_x} + b_H = \mu_H \cdot \frac{(X_{STO} / X_H)}{K_{STO} + (X_{STO} / X_H)} \quad (2.4)$$

Equation 2.4 can be rearranged as:

$$\frac{\mu_H \cdot \theta_x}{(1 + b_H \cdot \theta_x)} = \frac{K_{STO} \cdot X_H + X_{STO}}{X_{STO}} \quad (2.5)$$

Expression 2.5 can be solved for X_H and the heterotrophic biomass concentration is expressed as given in equation 2.6. As described by the expression the heterotrophic biomass concentration in the system is dependent on the storage polymer concentration, X_{STO} ; in the system.

$$X_H = \frac{\mu_H \cdot \theta_x - (1 + b_H \cdot \theta_x)}{K_{STO} \cdot (1 + b_H \cdot \theta_x)} \cdot X_{STO} \quad (2.6)$$

The readily biodegradable substrate, S_s , comes into the system with the influent, S_{S1} , and runs off the system with effluent discharged. Readily biodegradable COD is consumed in the system by the storage process as given in the mass balance below:

$$Q \cdot S_{S1} - Q \cdot S_s - V \cdot k_{STO} \cdot \frac{S_s}{K_s + S_s} \cdot X_H = 0 \quad (2.7)$$

Readily biodegradable substrate consumed in the system can be defined as:

$$\Delta S_s = S_{S1} - S_s \quad (2.8)$$

The amount of substrate consumed is equal to the amount of substrate stored in the system according to the descriptions in ASM3 and the mass balance in equation 2.7 is used to account for the substrate consumption with equation 2.9.

$$Q \cdot \Delta S_s = V \cdot k_{STO} \cdot \frac{S_s}{K_s + S_s} \cdot X_H \quad (2.9)$$

The storage polymers, X_{STO} , is also a key component in the system, since it appears in both storage and growth process. Storage products may enter the system with influent as X_{STO1} , but at very low concentrations and this particulate fraction leaves the system with sludge waste. X_{STO} is generated in the system by the storage process, consumed by heterotrophic growth and is also degraded by endogenous decay of storage polymers. The mass balance on X_{STO} is given in equation 2.10.

$$Q \cdot X_{STO1} - Q_w \cdot X_{STO} + V \cdot Y_{STO} \cdot k_{STO} \cdot \frac{S_s}{K_s + S_s} \cdot X_H - V \cdot \frac{\mu_H}{Y_H} X_H - V \cdot b_{STO} \cdot X_{STO} = 0 \quad (2.10)$$

The influent storage products concentration can be neglected as selecting X_{STO1} as zero and using the equation from the mass balance of S_s (2.9), the expression is rearranged as follows:

$$-Q_w \cdot X_{STO} + Q \cdot Y_{STO} \cdot \Delta S_s - V \cdot \frac{\mu_H}{Y_H} X_H - V \cdot b_{STO} \cdot X_{STO} = 0 \quad (2.11)$$

Equation 2.11 can be solved for heterotrophic biomass and the biomass concentration in the system, X_H , can be described as in equation 2.12.

$$X_H = \frac{Y_H}{(1 + b_H \cdot \theta_X)} \left[Y_{STO} \cdot \Delta S_s - (1 + b_{STO} \cdot \theta_X) \cdot \frac{\theta_h}{\theta_X} \cdot X_{STO} \right] \cdot \frac{\theta_X}{\theta_h} \quad (2.12)$$

Combining equations 2.6 and 2.12 and arranging the expressions as given below, the description of storage products concentration, X_{STO} , in the system is obtained with expression 2.16.

$$\frac{\mu_H \theta_X - (1 + b_H \theta_X)}{K_{STO} (1 + b_H \theta_X)} X_{STO} = \frac{Y_H}{(1 + b_H \cdot \theta_X)} \left[Y_{STO} \cdot \Delta S_s - (1 + b_{STO} \cdot \theta_X) \cdot \frac{\theta_h}{\theta_X} \cdot X_{STO} \right] \cdot \frac{\theta_X}{\theta_h} \quad (2.13)$$

$$\frac{\mu_H \theta_X - (1 + b_H \theta_X)}{K_{STO} Y_H} X_{STO} + (1 + b_{STO} \cdot \theta_X) \cdot X_{STO} = \frac{\theta_X}{\theta_h} Y_{STO} \cdot \Delta S_s \quad (2.14)$$

$$\frac{\mu_H \theta_X - (1 + b_H \theta_X) + K_{STO} Y_H (1 + b_{STO} \cdot \theta_X)}{K_{STO} Y_H} \cdot X_{STO} = \frac{\theta_X}{\theta_h} Y_{STO} \cdot \Delta S_s \quad (2.15)$$

$$X_{STO} = \frac{K_{STO} Y_H}{\mu_H \theta_X - (1 + b_H \theta_X) + K_{STO} Y_H (1 + b_{STO} \cdot \theta_X)} Y_{STO} \cdot \Delta S_s \frac{\theta_X}{\theta_h} \quad (2.16)$$

A stoichiometric expression can be defined as given in equation 2.17 in order to simplify the defined formulae.

$$A(\theta_X) = \frac{K_{STO} Y_H}{\mu_H \theta_X - (1 + b_H \theta_X) + K_{STO} Y_H (1 + b_{STO} \cdot \theta_X)} \quad (2.17)$$

The expression for the heterotrophic biomass given in equation 2.6 can be rearranged as;

$$X_H = [1 - (1 + b_{STO} \cdot \theta_X) \cdot A(\theta_X)] \frac{Y_H}{(1 + b_H \cdot \theta_X)} Y_{STO} \cdot \Delta S_s \cdot \frac{\theta_X}{\theta_h} \quad (2.18)$$

where, another stoichiometric expression is defined as:

$$B(\theta_X, \theta_h) = [1 - (1 + b_{STO} \cdot \theta_X) \cdot A(\theta_X)] \frac{Y_H}{(1 + b_H \cdot \theta_X)} Y_{STO} \cdot \frac{\theta_X}{\theta_h} \quad (2.19)$$

The expression for the effluent COD given in equation 2.9 can be rearranged as given in equation 2.21, using the definition of hydraulic retention time θ_h given in equation 2.20.

$$\theta_h = \frac{V}{Q} \quad (2.20)$$

$$\Delta S_s = \theta_h \cdot k_{STO} \cdot \frac{S_s}{K_s + S_s} \cdot X_H \quad (2.21)$$

Using the simplified expression for the heterotrophic biomass concentration, expression 2.22, the effluent readily biodegradable COD concentration is estimated with the definition given in equation 2.23.

$$X_H = B(\theta_x, \theta_h) \cdot \Delta S_s \quad (2.22)$$

$$S_s = \frac{K_s}{\theta_h k_{STO} B(\theta_x, \theta_h) - 1} \quad (2.23)$$

Substituting the value of B, S_s can be expressed as follows:

$$S_s = \frac{K_s (1 + b_H \cdot \theta_x)}{k_{STO} \cdot Y_H \cdot Y_{STO} \cdot \theta_x \cdot [1 - (1 + b_{STO} \cdot \theta_x) \cdot A(\theta_x)] - (1 + b_H \cdot \theta_x)} \quad (2.24)$$

Hydrolysis of the slowly biodegradable COD fraction, X_s , is defined with the non-linear rate expression as given in Table 2.1. This expression may be simplified using first order kinetics with respect to X_s concentration and the mass balance equation is then expressed as given below:

$$Q \cdot X_{s1} - Q_w \cdot X_s - V \cdot k_H \cdot X_s = 0 \quad (2.25)$$

The slowly biodegradable COD concentration in the system is then described by equation 2.26.

$$X_s = \frac{\frac{\theta_x}{\theta_h} X_{s1}}{1 + k_H \theta_x} \quad (2.26)$$

2.1.2 COD removal and sludge production

The total COD removal in the system is defined by the sum of removal of readily and slowly biodegradable COD. Since the slowly biodegradable COD fraction has been assumed to be particulate the effluent slowly biodegradable COD concentration is expressed as the fictive fraction of X_s retained in the system.

$$\Delta C_s = C_{s1} - C_s = (S_{s1} + X_{s1}) - (S_s + \frac{\theta_h}{\theta_x} X_s) \quad (2.27)$$

Substituting the expression obtained for X_s in equation 2.26 the total COD removal in the system is given as in equation 2.28.

$$\Delta C_s = (S_{s1} + X_{s1}) - (S_s + \frac{X_{s1}}{1 + k_H \theta_x}) \quad (2.28)$$

The daily sludge production of heterotrophic biomass would yield P_{XH} if only readily biodegradable COD removal, namely ΔS_s , is concerned, as given below:

$$P_{XH} = Q \cdot \frac{Y_H}{(1 + b_H \cdot \theta_X)} \left[Y_{STO} \cdot \Delta S_s - (1 + b_{STO} \cdot \theta_X) \cdot \frac{\theta_h}{\theta_X} \cdot X_{STO} \right] \quad (2.29)$$

It is possible to arrange the above equation in terms of total COD removal, ΔC_s and using the net heterotrophic growth yield as given in equation 2.30. The expression for P_{XH} is obtained as given in equation 2.31.

$$Y_{NH} = \frac{Y_H}{(1 + b_H \cdot \theta_X)} \quad (2.30)$$

$$P_{XH} = Q \cdot Y_{NH} \left[Y_{STO} \cdot \Delta C_s - (1 + b_{STO} \cdot \theta_X) \cdot \frac{\theta_h}{\theta_X} \cdot X_{STO} \right] \quad (2.31)$$

A factor, f , is defined as the fraction of the storage products which are not consumed for heterotrophic growth but either retained in the system due to the operating conditions or degraded by endogenous respiration, as in equations 2.32 and 2.33.

$$f = \frac{(1 + b_{STO} \cdot \theta_X) \cdot \frac{\theta_h}{\theta_X} \cdot X_{STO}}{Y_{STO} \cdot \Delta C_s} = \frac{K_{STO} \cdot Y_H \cdot (1 + b_{STO} \cdot \theta_X)}{\mu_H \theta_X - (1 + b_H \theta_X) + K_{STO} Y_H (1 + b_{STO} \cdot \theta_X)} \quad (2.32)$$

$$f = (1 + b_{STO} \cdot \theta_X) \cdot A(\theta_X) \quad (2.33)$$

The sludge production is then given with expression 2.34. The expression suggests that the daily net amount of heterotrophic biomass generated in the system, is described as the fraction of stored polymers which are produced as a fraction of the total COD removed in the system.

$$P_{XH} = Q \cdot Y_{NH} \cdot (1 - f) \cdot Y_{STO} \cdot \Delta C_s \quad (2.34)$$

The sludge production of storage products is given as:

$$P_{XSTO} = Q \cdot \frac{K_{STO} \cdot Y_H}{\mu_H \theta_X - (1 + b_H \theta_X) + K_{STO} Y_H (1 + b_{STO} \cdot \theta_X)} \cdot Y_{STO} \cdot \Delta C_s \quad (2.35)$$

Rearranging equation 2.35 and substituting the variables $A(\theta_x)$ and f , defined previously, the daily sludge production of X_{STO} is described by equation 2.36.

$$P_{XSTO} = Q \cdot A(\theta_x) \cdot Y_{STO} \cdot \Delta C_s = Q \cdot f \cdot \frac{Y_{STO}}{(1 + b_{STO} \theta_x)} \cdot \Delta C_s \quad (2.36)$$

The net yield of storage products, Y_{NSTO} is defined as follows:

$$Y_{NSTO} = \frac{Y_{STO}}{(1 + b_{STO} \cdot \theta_x)} \quad (2.37)$$

The net daily sludge production of storage products in the system, P_{XSTO} , is obtained with equation 2.38.

$$P_{XSTO} = Q \cdot f \cdot Y_{NSTO} \cdot \Delta C_s \quad (2.38)$$

The net daily sludge production of slowly biodegradable COD fraction in the system, P_{XS} , is obtained with equation 2.39.

$$P_{XS} = Q \cdot \frac{X_{SI}}{(1 + k_H \theta_x)} \quad (2.39)$$

The inert particulate COD in the reactor is defined as the sum of influent particulate inert COD and the particulate microbial products of endogenous respiration as shown below:

$$X_I = X_{II} \frac{\theta_x}{\theta_h} + f_I \cdot b_H \cdot X_H \cdot \theta_x \quad (2.40)$$

The sludge production of the particulate inerts is given by equation 2.41:

$$P_{XI} = Q \cdot X_{II} + V \cdot f_I \cdot b_H \cdot X_H \quad (2.41)$$

The total sludge production of the system is given as the sum of the individual fractions is given by equation 2.42.

$$P_X = P_{XH} + P_{XSTO} + P_{XS} + P_{XI} = \frac{V \cdot X_H}{\theta_x} + \frac{V \cdot X_{STO}}{\theta_x} + \frac{V \cdot X_S}{\theta_x} + \frac{V \cdot X_I}{\theta_x} \quad (2.42)$$

2.2 ASM3 Stoichiometry for Nitrogen Removal

2.2.1 The conventional pre-denitrification system

The conventional single sludge pre-denitrification system is composed of an anoxic reactor followed by an aerobic reactor and a clarifier. The reactors are regarded as ideal completely stirred tank reactors. It is assumed that there is no biochemical reaction taking place in the clarifier, but it only serves as a settler for particulate fractions.

The schematic representation of the pre-denitrification system used for stoichiometric evaluation is given in Figure 2.2. The figure includes all the model components present in the wastewater fed to the system, the components in the reactors and the ones discharged from the system according to ASM3. Sludge waste is assumed to be performed with the removal of particulate components from the aerobic reactor. Although the particulate fractions are discharged from the bottom of the clarifier a fictive portion of the particulates dependent on the hydraulic retention time and the sludge age are assumed to be leaving the system in order to be accurate in the COD removal calculations. The internal link from the aerobic reactor to the anoxic volume carries all the soluble and particulate fractions similar to the return sludge from the clarifier.

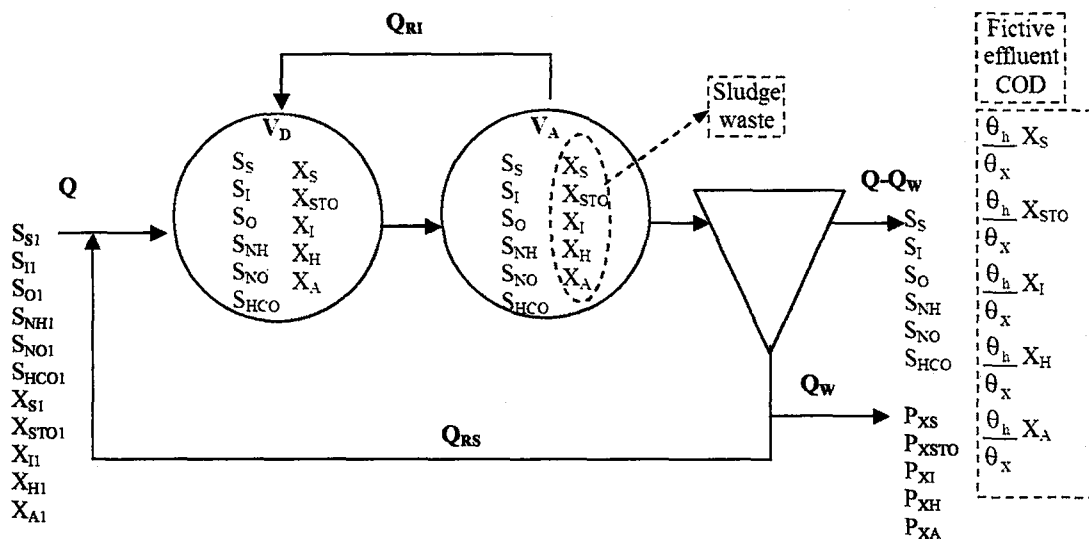


Figure 2.2: Schematic representation of conventional activated sludge system with pre-denitrification for nitrogen removal

2.2.2 Mass balances and steady state solutions

It is necessary to obtain the steady state solutions of the mass balances based on the model, in order to describe the fate of each parameter in the pre-denitrification system.

The mass balance on the heterotrophic biomass, X_H , is given in equation 2.43 as:

$$QX_{HI} - Q_W X_H + V_D \eta_{NO} \cdot \mu_H \cdot X_H + V_A \mu_H \cdot X_H - V_D b_{H,NO} \cdot X_H - V_A b_H \cdot X_H = 0 \quad (2.43)$$

The equation is simplified with the generally acceptable assumption that the heterotrophic biomass in the influent is negligible and the sludge retention time, θ_x , is defined as the total volume of the system, V , divided by the flow rate of sludge waste, Q_W (Equation 2.44).

$$\frac{1}{\theta_x} + (V_D/V) \cdot b_{H,NO} + (1 - (V_D/V)) \cdot b_H = \{ (V_D/V) \eta_{NO} + [1 - (V_D/V)] \} \cdot \mu_H \quad (2.44)$$

For further ease of use, three new variables are defined as; the anoxic correction factor for endogenous decay of heterotrophs, η_{b_H} , the fictive heterotrophic decay coefficient, b'_H and the fictive heterotrophic growth rate, μ'_H and their formulations are given in equations 2.45, 2.46 and 2.47, respectively.

$$\eta_{b_H} = \frac{b_{H,NO}}{b_H} \quad (2.45)$$

$$b'_H = b_H \{ 1 - (V_D/V) [1 - \eta_{b_H}] \} \quad (2.46)$$

$$\mu'_H = \mu_H \{ 1 - (V_D/V) [1 - \eta_{NO}] \} \quad (2.47)$$

Using these new variables equation 2.44 can be cut down to equation 2.48.

$$\frac{1}{\theta_x} = \mu'_H - b'_H \quad (2.48)$$

Rearranging equation 2.48 and solving for X_H results in equation 2.49; describing the heterotrophic biomass concentration in the system, which is not only dependent on the stoichiometry and kinetics of the model together with the system parameters, such as the flow rates and volumes, but also on the concentration of the storage products, X_{STO} .

$$X_H = \frac{\mu_H' \cdot \theta_X - (1 + b_H' \cdot \theta_X)}{K_{STO} \cdot (1 + b_H' \cdot \theta_X)} \cdot X_{STO} \quad (2.49)$$

The mass balance on the readily biodegradable substrate, S_S , can be written as given in equation 2.50.

$$Q \cdot S_{S1} - Q \cdot S_S - V_D \cdot k_{STO} \cdot \eta_{NO} \cdot \frac{S_S}{K_S + S_S} \cdot X_H - V_A \cdot k_{STO} \cdot \frac{S_S}{K_S + S_S} \cdot X_H = 0 \quad (2.50)$$

The difference in the influent and effluent concentrations of readily biodegradable substrate is described as the amount of readily biodegradable substrate removed in the system, as given in equation 2.51.

$$Q \cdot \Delta S_S = V \cdot \{1 - (V_D/V)[1 - \eta_{NO}]\} \cdot k_{STO} \cdot \frac{S_S}{K_S + S_S} \cdot X_H \quad (2.51)$$

Stored polymers, although incorporated in the biomass are considered as a different component, namely X_{STO} . Storage process, growth on stored polymers and the decay of X_{STO} run during both anoxic and aerobic phases and thus the mass balance on X_{STO} through the system can be described by equation 2.52.

$$Q \cdot X_{STO1} - Q_W \cdot X_{STO} + V_A Y_{STO} k_{STO} \frac{S_S}{K_S + S_S} X_H + V_D Y_{STO,NO} k_{STO} \eta_{NO} \frac{S_S}{K_S + S_S} X_H - V_A \frac{\mu_H}{Y_H} X_H - V_D \frac{\mu_H}{Y_{H,NO}} \eta_{NO} X_H - V_A b_{STO} X_{STO} - V_D b_{STO,NO} X_{STO} = 0 \quad (2.52)$$

The equation is handled easier with new definitions for $\eta_{b_{STO}}$, $\eta_{Y_{STO}}$, η_{Y_H} and b'_{STO} as given through the equations 2.53-2.56.

$$\eta_{b_{STO}} = \frac{b_{STO,NO}}{b_{STO}} \quad (2.53)$$

$$\eta_{Y_{STO}} = \frac{Y_{STO,NO}}{Y_{STO}} \quad (2.54)$$

$$\eta_{Y_H} = \frac{Y_{H,NO}}{Y_H} \quad (2.55)$$

$$b'_{STO} = b_{STO} \left\{ 1 - (V_D/V) [1 - \eta_{b_{STO}}] \right\} \quad (2.56)$$

If the storage products in the influent, X_{STOI} , are assumed to have a negligible concentration the mass balance equation may be simplified as below:

$$\left(\frac{1}{\theta_X} + b'_{STO} \right) X_{STO} = \left\{ \left\{ 1 - (V_D/V) [1 - \eta_{Y_{STO}} \eta_{NO}] \right\} Y_{STO} k_{STO} \frac{S_s}{K_s + S_s} - \left\{ 1 - (V_D/V) \left[1 - \frac{\eta_{NO}}{\eta_{Y_H}} \right] \right\} \frac{\mu_H}{Y_H} \right\} X_H \quad (2.57)$$

Using equation 2.48 and the mass balance of S_s , namely equation 2.51, equation 2.58 is obtained.

$$\left(\frac{1}{\theta_X} + b'_{STO} \right) X_{STO} = \frac{1}{\theta_h} \frac{\left\{ 1 - (V_D/V) [1 - \eta_{Y_{STO}} \eta_{NO}] \right\} Y_{STO} \Delta S_s - \left\{ 1 - (V_D/V) [1 - (\eta_{NO}/\eta_{Y_H})] \right\} (1 + b'_H \theta_X) \frac{\mu_H}{Y_H} X_H}{\left\{ 1 - (V_D/V) [1 - \eta_{NO}] \right\}} \quad (2.58)$$

Further simplification is possible using the following definitions of a, b, and c for the anoxic volume corrections:

$$a = \left\{ 1 - (V_D/V) \left[1 - \eta_{Y_{STO}} \eta_{NO} \right] \right\} \quad (2.59)$$

$$b = \left\{ 1 - (V_D/V) \left[1 - \frac{\eta_{NO}}{\eta_{Y_H}} \right] \right\} \quad (2.60)$$

$$c = \left\{ 1 - (V_D/V) \left[1 - \eta_{NO} \right] \right\} \quad (2.61)$$

The concentration of storage products, X_{STO} , in the system can be calculated with equation 2.62.

$$X_{STO} = \frac{a \cdot K_{STO} Y_H}{b \cdot [\mu'_H \theta_X - (1 + b'_H \theta_X)] + c \cdot K_{STO} Y_H (1 + b'_{STO} \cdot \theta_X)} Y_{STO} \cdot \Delta S_s \frac{\theta_X}{\theta_h} \quad (2.62)$$

Inserting this expression into equation 2.49, the heterotrophic biomass in the system can be calculated by equation 2.63.

$$X_H = \frac{Y_H}{(1 + b'_H \cdot \theta_X)} \left[\frac{a}{b} Y_{STO} \cdot \Delta S_s - \frac{c}{b} (1 + b'_{STO} \cdot \theta_X) \cdot \frac{\theta_h}{\theta_X} \cdot X_{STO} \right] \cdot \frac{\theta_X}{\theta_h} \quad (2.63)$$

Two stoichiometric expressions can be defined as functions of the sludge age, the flow rate and the volume fractions as given below:

$$A'(\theta_X) = \frac{a \cdot K_{STO} Y_H}{b \cdot [\mu'_H \theta_X - (1 + b'_H \theta_X)] + c \cdot K_{STO} Y_H (1 + b'_{STO} \cdot \theta_X)} \quad (2.64)$$

$$B'(\theta_X, \theta_h) = \left[\frac{a}{b} - \frac{c}{b} (1 + b'_{STO} \cdot \theta_X) \cdot A'(\theta_X) \right] \frac{Y_H}{(1 + b'_H \cdot \theta_X)} Y_{STO} \cdot \frac{\theta_X}{\theta_h} \quad (2.65)$$

The expression for the heterotrophic biomass given in equation 2.63 can be rearranged as:

$$X_H = B'(\theta_X, \theta_h) \cdot \Delta S_s \quad (2.66)$$

Equation 2.66 enables the calculation of heterotrophic biomass present in the system based on the sludge age and the hydraulic retention time of the system and anoxic, aerobic volume fractions, using the kinetic and stoichiometric coefficients defined by the model. The expression is arranged in such a way that X_H can be calculated only

using the amount of readily biodegradable COD consumed in the system and the expression does not involve the X_{STO} parameter. Using this expression obtained the effluent readily biodegradable COD, S_s , given in equation 2.51 can be rearranged as:

$$S_s = \frac{K_s}{\theta_h \cdot a \cdot k_{STO} \cdot B'(\theta_x, \theta_h) - 1} \quad (2.67)$$

Substituting the value of B' , S_s can be calculated independently as given in equation 2.68.

$$S_s = \frac{K_s(1 + b'_H \cdot \theta_x)}{a \cdot k_{STO} \cdot Y_H \cdot Y_{STO} \cdot \theta_x \cdot \left[\frac{a}{b} - \frac{c}{b} (1 + b'_{STO} \cdot \theta_x) \cdot A'(\theta_x) \right] - (1 + b'_H \cdot \theta_x)} \quad (2.68)$$

In order to obtain the steady state value of the slowly biodegradable COD fraction, X_s , the non-linear rate expression for hydrolysis is simplified using first order kinetics with respect to X_s concentration and the mass balance equation is then expressed as given below:

$$Q \cdot X_{s1} - Q_w \cdot X_s - V \cdot k_H \cdot X_s = 0 \quad (2.69)$$

The slowly biodegradable COD concentration, X_s , in the system can be estimated as given in equation 2.70.

$$X_s = \frac{\frac{\theta_x}{\theta_h} X_{s1}}{1 + k_H \theta_x} \quad (2.70)$$

Hydrolysis of X_s in the anoxic volume can be expressed by the following mass balance over the anoxic compartment;

$$Q \cdot X_{s1} - Q \cdot (1 + R) \cdot X_{s2} + Q \cdot R \cdot X_s - V_D \cdot k_H \cdot X_{s2} = 0 \quad (2.71)$$

where, X_{s2} is the concentration of slowly biodegradable COD in the effluent from the anoxic reactor and, R , is the return sludge ratio, which is the sum of internal recycle ratio and the sludge recirculation ratio ($R_I + R_S$). The portion of slowly biodegradable COD leaving the anoxic volume, X_{s2} , is given as:

$$X_{s2} = \frac{X_{s1} + R \cdot X_s}{(1 + R) + k_H \theta_{hd}} \quad (2.72)$$

where, the hydraulic retention time for denitrifying volume is defined as in equation 2.73.

$$\theta_{hd} = \frac{V_D}{Q} \quad (2.73)$$

2.2.3 COD and nitrogen removal

The total COD removal in the system is defined by the removal of readily and slowly biodegradable COD. Since the slowly biodegradable COD fraction has been assumed to be particulate the effluent slowly biodegradable COD concentration is expressed as the fictive fraction of X_s retained in the system.

$$\Delta C_{Se} = C_{s1} - C_s = (S_{s1} + X_{s1}) - (S_s + \frac{\theta_h}{\theta_x} X_s) \quad (2.74)$$

$$\Delta C_{Se} = (S_{s1} + X_{s1}) - (S_s + \frac{X_{s1}}{1 + k_H \theta_x}) \quad (2.75)$$

However, the conversion of the biodegradable in the system is lower than the fictively defined value above. The overall degraded COD can be expressed as below:

$$\Delta C_s = (S_{s1} + X_{s1}) - (S_s + X_s) = (S_{s1} + X_{s1}) - (S_s + \frac{\frac{\theta_x}{\theta_h} X_{s1}}{1 + k_H \theta_x}) \quad (2.76)$$

The degradation of biodegradable COD in the anoxic volume can be estimated with equation 2.77, using the assumption that the entire readily biodegradable COD fraction is depleted in the denitrifying zone.

$$\Delta C_{s2} = (S_{s1} + X_{s1}) - (S_s + X_{s2}) = (S_{s1} + X_{s1}) - (S_s + \frac{X_{s1} + R \cdot X_s}{(1 + R) + k_H \theta_{hd}}) \quad (2.77)$$

The mass balance for the autotrophic biomass is given in equation 2.78.

$$Q \cdot X_{A1} - Q_W \cdot X_A + V_A \mu_A \frac{S_{NH}}{K_{A,NH} + S_{NH}} X_A - V_A b_A X_A - V_D b_{A,NO} X_A = 0 \quad (2.78)$$

The initial autotrophic biomass in the effluent is assumed to be negligible and the coefficients η_{b_A} , b'_A and μ'_A are defined as below;

$$\eta_{b_A} = \frac{b_{A,NO}}{b_A} \quad (2.79)$$

$$b'_A = b_A \left\{ 1 - (V_D/V) [1 - \eta_{b_A}] \right\} \quad (2.80)$$

$$\mu'_A = \mu_A [1 - (V_D/V)] \quad (2.81)$$

Equation 2.78 can be arranged and solved for the ammonia concentration in the effluent, S_{NH} as described in equation 2.82.

$$S_{NH} = \frac{K_{A,NH} \cdot (1 + b'_A \cdot \theta_X)}{\mu'_A \cdot \theta_X - (1 + b'_A \cdot \theta_X)} \quad (2.82)$$

Ammonia is used for biomass and microbial products build up in the system while it is produced by the degradation of readily and slowly biodegradable COD, apart from nitrification. The fraction of ammonia produced and consumed in the system is defined as N_X and is given by the following expression:

$$QN_X = i_{NBM}Q_W X_H + i_{NBM}Q_W X_A + i_{NXI}Q_W X_I + i_{NSI}Q\Delta S_I - i_{NXS}(QX_{S1} - Q_W X_S) - i_{NSS}Q\Delta S_S \quad (2.83)$$

The above equation can be rearranged and the ammonia consumption of autotrophic biomass can be neglected as given below:

$$N_X = [i_{NBM}X_H - i_{NXS}X_S - i_{NXI}X_I](\theta_H/\theta_X) - [i_{NXS}X_{S1} + i_{NSS}\Delta S_S + i_{NSI}\Delta S_I] \quad (2.84)$$

The nitrification potential of the system is defined as N_{OX} and can be expressed by the following mass balance:

$$N_{OX} = S_{NH1} - S_{NH} - N_X \quad (2.85)$$

Autotrophic biomass can be calculated using the nitrification potential as given in equations 2.86-2.88.

$$QN_{OX} = V_A \frac{\mu_A}{Y_A} \cdot \frac{S_{NH}}{K_{A,NH} + S_{NH}} X_A \quad (2.86)$$

$$\frac{1}{[1 - (V_D/V)] \theta_h} N_{OX} = \frac{(1 + b'_A \theta_X)}{\theta_X Y_A} X_A \quad (2.87)$$

$$X_A = N_{OX} \frac{Y_A}{(1 + b'_A \theta_X)} \frac{\theta_X}{[1 - (V_D/V)] \theta_h} \quad (2.88)$$

The denitrification potential, N_{DP} , of the system is defined as the nitrate requirement, which can be calculated as the electron acceptor demand in the anoxic zone as given in equation 2.89.

$$N_{DP} = \frac{V_D}{Q} \left\{ \left(\frac{1 - Y_{STO,NO}}{2.86} \right) \cdot k_{STO} \eta_{NO} \frac{S_s}{K_s + S_s} X_H + \left(\frac{1 - Y_{H,NO}}{2.86 \cdot Y_{H,NO}} \right) \cdot \mu_H \eta_{NO} \frac{X_{STO}/X_H}{K_{STO} + X_{STO}/X_H} X_H \right. \\ \left. + \left(\frac{1 - f_I}{2.86} \right) \cdot b_{H,NO} X_H + \left(\frac{1}{2.86} \right) \cdot b_{STO,NO} X_{STO} \right\} \quad (2.89)$$

However, this kinetic expression of denitrification potential gives a dynamic definition of the concept. It is required to estimate the nitrate requirement in the anoxic zone, in terms of substrate utilized, in order to solve for the steady state case. The denitrification potential can also be calculated using process stoichiometry, with the assumption that all the readily biodegradable COD, S_s , is utilized in the anoxic reactor and overall conversion of S_s into storage products and further to heterotrophic biomass is completed in the denitrifying zone. The biodegradable substrate consumed in the anoxic volume is given in equation 2.77 and the nitrate requirements of specific processes can be defined as given in equations 2.90-2.93.

$$\Delta S_{NO,sto} = \frac{(1 - Y_{STO,NO})}{2.86} \cdot \Delta C_{S2} \quad (\text{anoxic storage}) \quad (2.90)$$

$$\Delta S_{NO,gro} = \frac{(1 - Y_{STO,NO} \cdot Y_{H,NO})}{2.86} \cdot \Delta C_{S2} \quad (\text{anoxic growth}) \quad (2.91)$$

$$\Delta S_{NO,end} = \frac{(1 - f_1)}{2.86} \cdot b_{H,NO} \cdot B'(\theta_X, \theta_h) \cdot \Delta C_{S2} \quad (\text{anoxic endogenous decay}) \quad (2.92)$$

$$\Delta S_{NO,resp} = \frac{1}{2.86} \cdot b_{STO,NO} \cdot A'(\theta_X) \cdot Y_{STO,NO} \cdot \frac{\theta_X}{\theta_h} \cdot \Delta C_{S2} \quad (\text{anoxic respiration of } X_{STO}) \quad (2.93)$$

The nitrate utilization due to the anoxic endogenous decay of autotrophs is negligible, since the amount of autotrophic biomass in pre-denitrification systems is relatively small.

The denitrification potential of the system can then be expressed as the sum of the nitrate requirements of the four major processes taking place in the anoxic volume as given in the below expression. This approximation enables the estimation of, N_{DP} solely based on the amount of biodegradable substrate utilized in the denitrifying zone.

$$N_{DP} = \frac{1}{2.86} \cdot \left\{ \begin{aligned} & (1 - Y_{STO,NO}) + (1 - Y_{STO,NO} \cdot Y_{H,NO}) + \left[(1 - f_1) \cdot B'(\theta_X, \theta_h) \cdot b_{H,NO} \right] \\ & + \left[A'(\theta_X) \cdot b_{STO,NO} \cdot Y_{STO,NO} \cdot \frac{\theta_X}{\theta_h} \right] \end{aligned} \right\} \quad (2.94)$$

The mass balance equation of nitrate in the system is given by equation 2.95.

$$S_{NOI} - S_{NO} + N_{OX} - N_{DP} = 0 \quad (2.95)$$

The denitrification potential of the system, however, is reduced by the dissolved oxygen carried to the anoxic volume with the recycle. Assuming that equivalent amounts of electron acceptors would be used under aerobic and anoxic conditions, the denitrification potential will be reduced as described by equation 2.96.

$$N_D = N_{DP} - R \frac{S_0}{2.86} \quad (2.96)$$

Therefore the correct nitrate concentration in the effluent is given by equation 2.97.

$$S_{NO} = S_{NOI} + N_{OX} - N_D \quad (2.97)$$

2.2.4 Sludge production

The total sludge production of the system is given as the sum of the individual fractions of heterotrophic biomass, X_H , storage polymers, X_{STO} , slowly biodegradable COD fraction retained in the system, X_S , and the particulate inert fraction, X_I , as follows:

$$P_X = P_{XH} + P_{XSTO} + P_{XS} + P_{XI} = \frac{V \cdot X_H}{\theta_X} + \frac{V \cdot X_{STO}}{\theta_X} + \frac{V \cdot X_S}{\theta_X} + \frac{V \cdot X_I}{\theta_X} \quad (2.98)$$

Sludge production of heterotrophic biomass can be estimated as in equation 2.99:

$$P_{XH} = Q \cdot \frac{Y_H}{(1 + b_H \cdot \theta_X)} \left[Y_{STO} \cdot \Delta S_S - (1 + b_{STO} \cdot \theta_X) \cdot \frac{\theta_h}{\theta_X} \cdot X_{STO} \right] \quad (2.99)$$

The net heterotrophic yield, Y_{NH} , is defined as given in equation 2.100:

$$Y_{NH} = \frac{Y_H}{(1 + b_H \cdot \theta_X)} \quad (2.100)$$

The sludge production of heterotrophic biomass can be defined as:

$$P_{XH} = Q \cdot Y_{NH} \left[Y_{STO} \cdot \Delta C_S - (1 + b_{STO} \cdot \theta_X) \cdot \frac{\theta_h}{\theta_X} \cdot X_{STO} \right] \quad (2.101)$$

If a factor of f is defined as the amount of storage products, which are not utilized for heterotrophic growth, as given in equation 2.102 and 2.103:

$$f = \frac{(1 + b_{STO} \cdot \theta_X) \cdot \frac{\theta_h}{\theta_X} \cdot X_{STO}}{Y_{STO} \cdot \Delta C_s} = \frac{K_{STO} \cdot Y_H \cdot (1 + b_{STO} \cdot \theta_X)}{\mu_H \theta_X - (1 + b_H \theta_X) + K_{STO} Y_H (1 + b_{STO} \cdot \theta_X)} \quad (2.102)$$

$$f = (1 + b_{STO} \cdot \theta_X) \cdot A(\theta_X) \quad (2.103)$$

then, the heterotrophic sludge production can then be calculated as in equation 2.104.

$$P_{XH} = Q \cdot Y_{NH} \cdot (1 - f) \cdot Y_{STO} \cdot \Delta C_s \quad (2.104)$$

The sludge production of storage products is given as:

$$P_{XSTO} = Q \cdot \frac{K_{STO} \cdot Y_H}{\mu_H \theta_X - (1 + b_H \theta_X) + K_{STO} Y_H (1 + b_{STO} \cdot \theta_X)} \cdot Y_{STO} \cdot \Delta C_s \quad (2.105)$$

The net yield of storage products, Y_{NSTO} , is defined as in equation 2.106:

$$Y_{NSTO} = \frac{Y_{STO}}{(1 + b_{STO} \cdot \theta_X)} \quad (2.106)$$

The sludge production of the stored material described in equation 2.105 can be calculated using equation 2.106, as below:

$$P_{XSTO} = Q \cdot f \cdot Y_{NSTO} \cdot \Delta C_s \quad (2.107)$$

The amount of slowly biodegradable COD, which is assumed to be particulate, discarded from the system is defined as given in equation 2.108:

$$P_{XS} = Q \cdot \frac{X_{SI}}{(1 + k_H \theta_X)} \quad (2.108)$$

The inert particulate COD in the reactor is defined as the sum of influent particulate inert COD and the particulate microbial products of endogenous respiration as:

$$X_I = X_{II} \frac{\theta_X}{\theta_h} + f_i \cdot b_H \cdot X_H \cdot \theta_X \quad (2.109)$$

The sludge production of the particulate inerts is given as:

$$P_{XI} = Q \cdot X_{II} + V \cdot f_i \cdot b_H \cdot X_H \quad (2.110)$$

2.2.5 Oxygen requirement in the system

The oxygen requirement of the conventional pre-denitrification system, OR_T , can be calculated as the sum of the oxygen requirement of heterotrophic biomass and oxygen utilized for ammonia oxidation. However, the oxygen equivalent of the amount of nitrate utilized in the system should be discarded from the carbonaceous oxygen demand as in equation 2.111.

$$OR_T = OR_H + 4.57 \cdot Q \cdot N_{OX} - 2.86 \cdot Q \cdot N_{DP} \quad (2.111)$$

The heterotrophic oxygen demand, OR_H , can be estimated as the difference between the biodegradable COD removed and the sludge produced in the system, as described in equation 2.112:

$$OR_H = Q \cdot (C_{SI} - C_S) - (P_{XH} + P_{XSTO} + P_{XI} - Q \cdot X_{II}) \quad (2.112)$$

2.2.6 Design algorithm

A conventional pre-denitrification system can be designed according to ASM3, using the algorithm given below:

1. The values of stoichiometric and kinetic coefficients of the model, influent properties and the design criteria should be determined.
2. The sludge age, θ_X should be selected.
3. S_{NH} should be calculated and compared with the design criteria for effluent ammonia concentration. If the ammonia concentration, S_{NH} exceeds the effluent criteria, then the sludge age should be reselected as in step 2.
4. The hydraulic retention time, θ_h , and volume ratio, V_D/V should be selected.
5. Effluent COD fractions, namely, readily biodegradable COD, S_S , slowly biodegradable COD, X_S and the total COD of the effluent, C_S , should be calculated and compared with the design criteria. If effluent COD fractions exceed the effluent concentration criteria, then the hydraulic retention time and anoxic/aerobic volume fractions should be reselected as in step 4.
6. N_X and N_{OX} should be calculated.
7. The return sludge ratio, R , should be selected.

8. N_{DP} and S_{NO} should be calculated and compared with the design criteria. If S_{NO} exceeds the nitrate effluent concentration given in the design criteria, then the hydraulic retention time and anoxic/aerobic volume ratio should be reselected as in step 4.

9. The return sludge ratio, R , should be calculated as given in equation 2.113.

$$R = \frac{N_{OX} - S_{NO}}{S_{NO}} \quad (2.113)$$

10. The selected and calculated return sludge ratio, R should be compared and the iteration between steps 7 and 10 should be done until the difference in the selected and calculated return sludge ratio is within the acceptable limits.

11. P_{XT} and X_T should be calculated as in equation 2.114 and compared with the design criteria depending on the sludge settling requirements. If the concentration of particulates in the system, X_T , exceeds the limits set by the sludge settling quality, then the hydraulic retention time and anoxic/aerobic volume ratio should be reselected as in step 4.

$$X_T = \frac{P_{XT} \cdot \theta_x}{V} \quad (2.114)$$

12. The oxygen requirement, OR_T should be calculated.

13. The residual alkalinity, S_{HCO} should be calculated.

14. The return sludge concentration, X_R , should be select and R_s and R_l should be calculated, using equation 2.115.

$$R_s = \frac{1 - \theta_h / \theta_x}{X_R / X_T - 1} \quad (2.115)$$

The above procedure allows the calculation of all the design parameters of a conventional single sludge pre-denitrification system using ASM3.

3. RESPIROMETRIC ASSESSMENT OF SUBSTRATE STORAGE YIELD FOR ASM3

3.1 Experimental Assessment of Bacterial Storage Yield[†]

Mechanistic understanding of the behavior of activated sludge cultures under different conditions exhibited a significant evolution in the last years. A major milestone in the evolution has been the introduction of Activated Sludge Model No.1 (ASM1), introducing a general standard, with defined model components and processes for the kinetic interpretation of oxygen and substrate utilization (Henze *et al.*, 1987). Continued scientific efforts improved the model, especially for nitrogen and phosphorus removal (Henze *et al.*, 1995; Gujer *et al.*, 2000).

The ability of microorganisms to accumulate internal storage polymers is well documented (Chudoba *et al.*, 1973; Van den Eijnde *et al.*, 1984) and recognized as the key process for biological phosphorus removal (Wentzel *et al.*, 1986; Mino *et al.*, 1987). A storage process was recently introduced in the modeling of carbon and nitrogen removal by activated sludge cultures; Activated Sludge Model No.3 (ASM3), as the initial step for the utilization of readily biodegradable substrate (Gujer *et al.*, 2000). The stoichiometry and kinetics of biological storage have been defined in ASM3, but the experimental procedures for the determination of the coefficients associated with the process kinetics have not been standardized yet.

The storage yield (Y_{STO}) is one of the most important parameters of the model, since it represents the stoichiometric amount of substrate converted into storage products, which are subsequently utilized for growth. The information on the magnitude of the storage yield was mostly derived from pure culture studies: The storage yield for *Paracoccus pantotrophus* fed with acetate has been experimentally determined as 0.648 C-mole/C-mole (0.73 mgCOD/mgCOD) by van Aalst-van Leeuwen *et al.* (1997). For activated sludge cultures fed with pure substrates, Beun *et al.* (2000a)

[†] Section 3.1 is a part of the paper entitled "Experimental assessment of bacterial storage yield" (2002) ASCE Journal of Environmental Engineering, 128(11), 1030-1035.

reported the value of storage yield for acetate as 0.69 mgCOD/mgCOD under aerobic conditions and 0.59 mgCOD/mgCOD under anoxic conditions (Beun *et al.*, 2000b). Goel *et al.* (1999) have assumed a Y_{STO} value of 0.9 mgCOD/mgCOD for glucose, considering that the formation of glycogen from glucose requires less energy as compared to PHB accumulation from acetate. Dircks *et al.* (2001) experimentally found 0.91 mgCOD/mgCOD for the storage yield of glucose. ASM3 suggested the default values of Y_{STO} as 0.85 mgCOD/mgCOD under aerobic and 0.80 mgCOD/mgCOD under anoxic conditions for domestic sewage.

This section presents an experimental procedure for the determination of the storage yield as defined in ASM3. The procedure is based on respirometry and does not involve measurement of storage products, as it will not always be possible or reliable to determine the amount of all the storage products when a complex substrate such as domestic sewage is concerned.

3.1.1 Conceptual basis

Batch respirometric tests performed on readily biodegradable substrates like acetate; result in two phases of respiration, as if a readily biodegradable substrate and a slowly biodegradable substrate are present in the system (Dircks *et al.*, 1999). ASM1 is unable to simulate this behavior which is due to the formation and subsequent growth on the stored polymer of the original readily biodegradable substrate. ASM3 incorporates the concept of COD fractionation with two major differences with respect to previous models. Firstly, readily biodegradable substrate, S_S is defined as the biodegradable fraction of soluble COD (Gujer *et al.*, 2000). However it is recommended to estimate the amount of S_S in wastewaters by respirometric tests to decrease model uncertainty in WWTP simulations (Koch *et al.*, 2000). Secondly, ASM3 also assumes that all biodegradable COD is converted into internal storage products and growth occurs only at the expense of storage products. Storage is a faster process compared to growth and may be identified as the dominant process in terms of electron acceptor utilization after adding a pulse of substrate in a batch test. The reaction kinetics and stoichiometry of ASM3 for organic carbon removal, simplified for soluble biodegradable COD as the sole substrate component, as evaluated in this study, is given in Table 3.1.

Table 3.1: Matrix Representation of Activated Sludge Model No.3 (ASM3)

COMPONENT PROCESS	S _O O ₂	S _S COD	X _I COD	X _H COD	X _{STO} COD	RATE
Storage of S _S	-(1-Y _{STO})	-1			Y _{STO}	$k_{STO} \frac{S_O}{K_O + S_O} \frac{S_S}{K_S + S_S} X_H$
Growth on X _{STO}	$-\frac{(1-Y_H)}{Y_H}$			1	-1/Y _H	$\mu_H \frac{S_O}{K_O + S_O} \frac{X_{STO}/X_H}{K_{STO} + X_{STO}/X_H} X_H$
Endogenous Respiration	-(1-f _i)		f _i	-1		$b_H \frac{S_O}{K_O + S_O} X_H$
Respiration of X _{STO}	-1				-1	$b_{STO} \frac{S_O}{K_O + S_O} X_{STO}$

The basic stoichiometry between the readily biodegradable COD (S_S) utilized, the storage products generated and the dissolved oxygen consumed under aerobic conditions may be schematized, as shown in Figure 3.1.

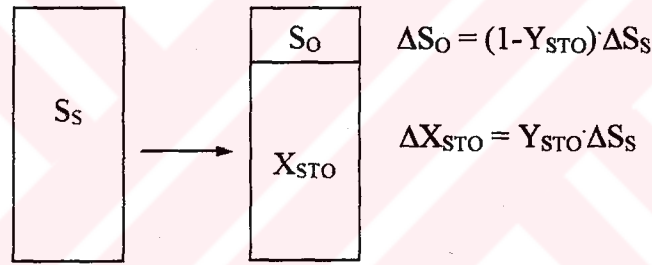


Figure 3.1: COD stoichiometry of aerobic storage

The mass balance illustrated in this figure leads to the following equation for the amount of oxygen utilized for storage:

$$\Delta O_{STO} = \Delta S_O = (1 - Y_{STO}) \Delta S_S \quad (3.1)$$

At the depletion of all initially available readily biodegradable COD, S_{SI}, the above expression can be manipulated to give the storage yield, Y_{STO}:

$$Y_{STO} = \left(1 - \frac{\Delta O_{STO}}{S_{SI}}\right) \quad (3.2)$$

3.1.2 Calculation of the storage yield

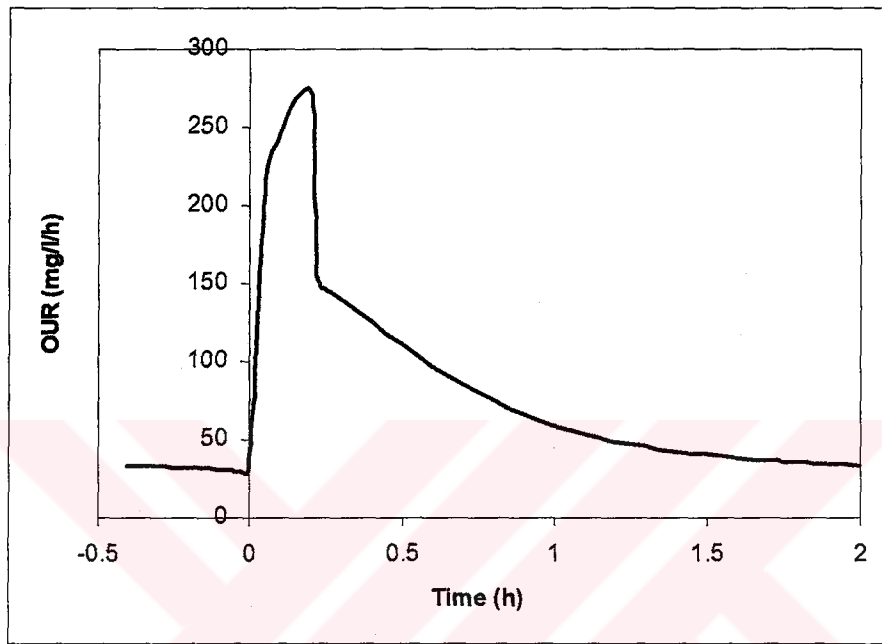
Expression 3.2 indicates that the amount of oxygen utilized for storage can be used as a convenient parameter for the calculation of the storage yield. In fact, Y_{STO} may be computed if the oxygen used for the storage of a known amount of readily biodegradable COD could be determined by means of respirometric measurements. Such measurements however only provide the total oxygen utilization rate (OUR) of the system and not the OUR specific for the process of interest alone. Thus, it is necessary to understand and interpret the components of a total OUR versus time curve associated with the utilization of a readily biodegradable substrate. A batch reactor is a perfect tool for this purpose as it exhibits all the transient OUR responses. The procedure only requires the OUR profile of a filtered substrate in an aerated batch reactor. The biodegradable fraction of the soluble/filtered substrate must also be determined through the experimental assessment of the soluble inert fraction using one of the experimental procedures consistent with ASM3 (Orhon *et al.*, 1999a; Wentzel *et al.*, 1999).

The OUR profile in an aerated batch reactor was simulated using AQUASIM[®] for the solution of the non-linear mass balance equations involved (Reichert *et al.*, 1998). Suggested values for the kinetic and stoichiometric coefficients in ASM3 were used in the simulation (Table 3.2).

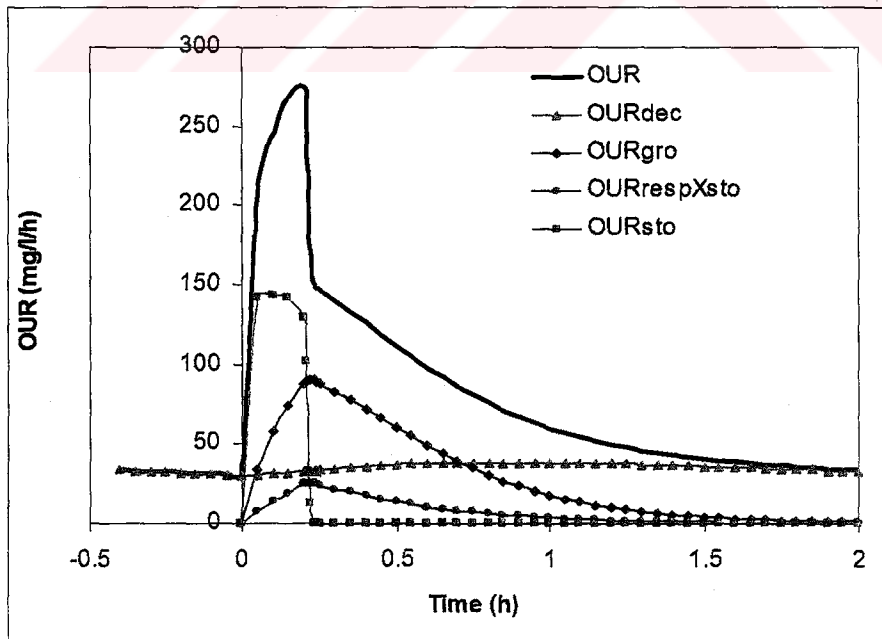
Table 3.2: ASM3 Default Values for 20°C

Model Coefficient	Default Value
k_{STO}	5 1/d
K_S	2 mg COD/l
Y_{STO}	0.85 gCOD/gCOD
μ_H	2 1/d
K_{STO}	1
Y_H	0.63 gCOD/gCOD
b_{STO}	0.2 1/d
b_H	0.2 1/d
f_I	0.2

Simulation was initiated without any initial substrate addition in order to determine the endogenous OUR level, assuming that the amount of storage products (X_{STO}) present in the cells is negligible. A pulse feed was given at the desired F/M ratio after this famine period. Figure 3.2(a) illustrates a simulated OUR profile obtained with an F/M ratio of 1.0 mgCOD/mg cellCOD, an initial active heterotrophic biomass concentration, X_H , of 200 mg/l COD and initial X_{STO} of zero.



(a)



(b)

Figure 3.2: (a) Standard OUR curve as obtained by ASM3 (F/M = 1 gCOD/g cellCOD), (b) OUR curve fractionated for each process

This OUR profile can be fractionated into its components as shown in Figure 3.2(b), for each oxygen consuming process described in ASM3, according to equations 3.3-3.6. This simulation exercise enables the calculation of the amount of oxygen utilized for each process for any definite time interval by simple integration techniques. In other words, the area under each curve gives the amount of oxygen used for a specific process. Thus, it is possible to calculate the area under the OUR curve associated for storage to get the amount of oxygen utilized for the storage of all the readily biodegradable COD initially present in the batch reactor.

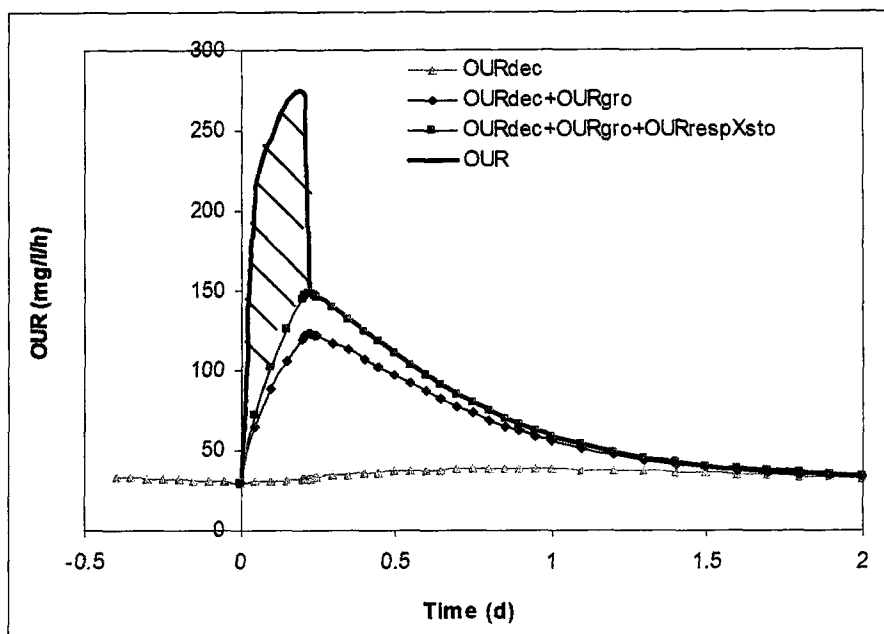
$$\text{OUR}_{\text{sto}} = (1 - Y_{\text{STO}}) \cdot k_{\text{STO}} \frac{S_{\text{O}}}{K_{\text{O}} + S_{\text{O}}} \frac{S_{\text{S}}}{K_{\text{S}} + S_{\text{S}}} X_{\text{H}} \quad (3.3)$$

$$\text{OUR}_{\text{gro}} = \frac{(1 - Y_{\text{H}})}{Y_{\text{H}}} \cdot \mu_{\text{H}} \frac{S_{\text{O}}}{K_{\text{O}} + S_{\text{O}}} \frac{X_{\text{STO}}/X_{\text{H}}}{K_{\text{STO}} + X_{\text{STO}}/X_{\text{H}}} X_{\text{H}} \quad (3.4)$$

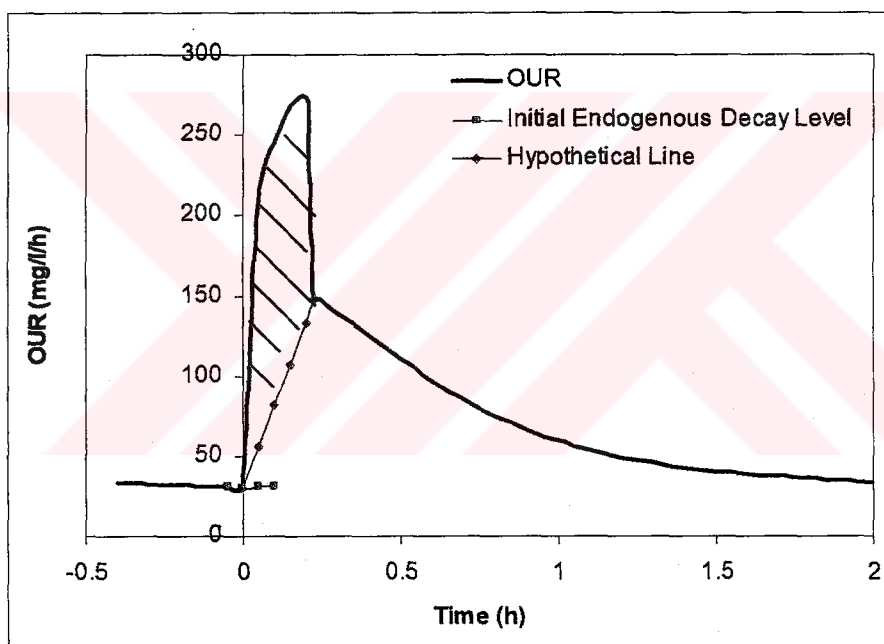
$$\text{OUR}_{\text{dec}} = (1 - f_{\text{I}}) \cdot b_{\text{H}} \frac{S_{\text{O}}}{K_{\text{O}} + S_{\text{O}}} X_{\text{H}} \quad (3.5)$$

$$\text{OUR}_{\text{respXsto}} = b_{\text{STO}} \frac{S_{\text{O}}}{K_{\text{O}} + S_{\text{O}}} X_{\text{STO}} \quad (3.6)$$

As indicated in Figure 3.3(a), it is also possible to obtain area representing storage graphically. A combined OUR curve is obtained through model simulation by the summation of OUR values of all three processes (equations 3.4, 3.5 and 3.6) except that of storage. The area between the overall OUR profile and the combined OUR curve excluding storage is the theoretical equivalent of $\Delta\text{O}_{\text{STO}}$. The proposed procedure for the graphical determination of the amount of oxygen used for storage, $\Delta\text{O}_{\text{STO}}$, without model simulation, involves drawing a line connecting the initial OUR level due to endogenous decay (the initial/average OUR_{dec} level), up to the break point of the overall OUR curve, which reflects the depletion of the initially available readily biodegradable COD and therefore, the end point of storage. The area above this line would directly yield $\Delta\text{O}_{\text{STO}}$ (Figure 3.3(b)).



(a)



(b)

Figure 3.3: (a) Area representing storage (hatched) obtained from model simulation, (b) estimated area (hatched) for the oxygen consumption of storage process as proposed

This procedure includes an approximation by the linear connection of two points under the OUR profile. A sensitivity evaluation was performed by comparing theoretical model estimations with the calculated Y_{STO} values based on the proposed method for a wide range of F/M ratios between 0.02 and 1.5 mgCOD/mg cellCOD. The results are outlined in Table 3.3.

Table 3.3: Calculations for the Verification of the Proposed Respirometric Method

	Model Initial Conditions			Model Estimations		Calculations Based on the Proposed Method		
	S_{Siini} (mg/l COD)	X_{Hiini} (mg/l COD)	Y_{STO} (COD/COD)	ΔO_{STO} (mg/l)	ΔO_{Total} (mg/l)	ΔO_{STO} (mg/l)	Y_{STO} (COD/COD)	% Error
0.02	4	200	0.85	0.14	53.72	0.10	0.974	14.58
	10	500	0.85	0.02	133.93	0.63	0.937	10.25
	20	1000	0.85	0.00	267.76	1.24	0.938	10.35
0.10	20	200	0.85	2.34	63.26	2.53	0.874	2.79
	50	500	0.85	6.14	158.40	6.66	0.867	1.97
	100	1000	0.85	12.93	317.41	14.02	0.860	1.16
0.50	100	200	0.85	14.32	111.76	16.42	0.836	1.68
	250	500	0.85	35.52	279.10	39.82	0.841	1.09
	500	1000	0.85	71.81	558.81	78.05	0.844	0.72
1.00	200	200	0.85	29.31	171.95	33.53	0.832	2.07
	500	500	0.85	73.10	429.65	82.04	0.836	1.66
	1000	1000	0.85	146.92	859.71	160.89	0.839	1.28
1.50	300	200	0.85	44.30	231.53	49.85	0.834	1.90
	750	500	0.85	110.72	578.73	122.52	0.837	1.57
	1500	1000	0.85	219.74	1155.15	242.63	0.838	1.38

Except for very low F/M ratios, corresponding to practically very low soluble biodegradable COD levels for batch tests, they indicate that the proposed procedure allows the experimental assessment of Y_{STO} with less than 2% error, excluding analytical errors associated with COD measurements. The amount of oxygen utilized for storage is defined by the following expression:

$$\frac{dS_O}{dt} = (1 - Y_{STO}) \cdot \frac{dS_S}{dt} \quad (3.7)$$

Therefore, it is only sensitive to the amount of S_S , which is, according to ASM3, measured by 0.45 μm membrane filter test. When OUR is described using the process kinetics as in Equation (3.3); the OUR response would be such that, although the form of the curve may differ with different coefficients, the area under it would be constant for the same amount of S_S . Therefore, for the proposed procedure, the sensitivity of the kinetic coefficients would be irrelevant, since the total oxygen uptake and the estimated amount of S_S are insensitive to the changes in the kinetic parameters of the model.

3.2 Respirometric Assessment of Storage Yield for Different Substrates[†]

Studies conducted on both pure and mixed activated sludge cultures provided substantial proof on the ability of microorganisms to convert substrate into internal storage products under dynamic conditions (van Loosdrecht *et al.*, 1997). The growth response of heterotrophic microorganisms was observed to take place mainly at the expense of stored products (Majone *et al.*, 1999).

The storage yield Y_{STO} , is one of the most important parameters of the model, since it represents the stoichiometric amount of the substrate converted into storage products, which are then utilized for growth. The assessment of storage yield is therefore crucial for the accurate estimation of the overall electron acceptor utilization and sludge production. Although certain complicated techniques were suggested for its assessment (Goel *et al.*, 1998b; Dircks *et al.*, 1999), very little experimental data of practical value for activated sludge systems is so far available.

This section presents the application of the experimental procedure given in Section 3.1. The proposed procedure was tested on acetate, glucose and domestic sewage, together with mixtures of acetate/glucose and acetate/domestic sewage. Relevant experimental data were generated at different initial F/M ratios, enabling the identification of Y_{STO} values related to tested substrates and substrate mixtures.

3.2.1 Materials and methods

Respirometric measurements were conducted for the experimental testing of the proposed procedure on different substrates. Acetate, glucose and domestic sewage together with combinations of glucose/acetate and domestic sewage/acetate at different ratios were used as substrate. For single substrates, parallel tests were performed to visualize the effect of the initial F/M ratio on the magnitude of the storage yield. The tests were carried out in completely mixed, aerated 2-3 l batch reactors, seeded with active biomass taken from fill and draw acclimation reactors operated at steady state. The reactors were initially operated with no substrate for the assessment of the OUR level associated with the endogenous phase. The initial active biomass concentration was estimated by model simulation on the basis of an endogenous decay rate, b_H of 0.2 1/d, the suggested value in ASM3. OUR

[†] Section 3.2 is a part of the paper entitled "Respirometric assessment of storage yield for different substrates" (2002) Water Science and Technology, 46(1-2), 345-352.

measurements were conducted with a Manotherm RA-1000 continuous respirometer with PC connection. In the experiments, pH was kept in the range of 7.0-8.0, suitable for biological activity. COD measurements were performed as described in the method ISO 6060 (1986). Domestic sewage used in the experiments was filtered through 0.45 μm cellulose acetate filters.

3.2.2 Experimental results

In the study, acetate was selected as the main compound for the testing of the proposed procedure. It is a well studied readily biodegradable substrate, known to be stored as polyhydroxybutyrate, (PHB), and recognized in biological phosphorus models as a significant model component. Glucose is also tested for the assessment of the corresponding storage yield, as it is known to trigger glycogen storage through a metabolic pathway completely different from that of PHB storage. It is commonly agreed that the majority of the readily biodegradable substrate mixture in wastewaters is likely to be stored as PHA and glycogen. Therefore, these two pure substrates were selected as the precursors of the two extreme cases for the evaluation of the storage yield. Filtered domestic sewage was also used in the OUR experiments to reflect a real case example of a more complex readily biodegradable source for the proposed experimental procedure.

3.2.2.1 Experiments on acetate and glucose

Acetate experiments: Experiments on acetate were conducted as eight parallel runs for different initial F/M ratios varying in the range of 0.09-3.65 gCOD/gcellCOD. Y_{STO} values obtained by means of the proposed procedure, together with related experimental data, are outlined in Table 3.4. The resulting average Y_{STO} could be calculated as 0.78 gCOD/gCOD, slightly lower than the suggested average value of 0.85 gCOD/gCOD in ASM3. This value is in agreement with the ones reported in the literature as 0.73 gCOD/gCOD (van Aalst-van Leeuwen *et al.*, 1997) and 0.69 (Beun *et al.*, 2000a). Y_{STO} was observed to change only within the narrow range of 0.75-0.82 gCOD/gCOD, leading to conclude that the F/M ratio did not have an appreciable effect on the magnitude of Y_{STO} . The F/M ratio only affected the shape of the OUR curve and the resulting ΔO_{STO} , as illustrated in Figure 3.4: An F/M ratio of 0.09 gCOD/gcellCOD, started with initial S_{SI} concentration of 93 mg/l COD, generated only $\Delta O_{\text{STO}} = 23.3$ mg/l and yielded a Y_{STO} of 0.75 gCOD/gCOD. ΔO_{STO}

was increased to 110.2 mg/l for $F/M = 0.87$ gCOD/gcellCOD and $S_{S1} = 548$ mg/l, resulting in a slightly higher Y_{STO} of 0.80 gCOD/gCOD.

Table 3.4: Experimental assessment of Y_{STO} for acetate

Set No.	Reactor Volume (l)	Biomass Concentration (mg COD/l)	Substrate Concentration (mg COD/l)	F/M Ratio (COD/COD)	Y_{STO}
Set 1	2	1000	93	0.09	0.75
Set 2	2	880	105	0.12	0.76
Set 3	2	1450	280	0.19	0.76
Set 4	2	870	274	0.31	0.80
Set 5	2	440	187	0.42	0.75
Set 6	2	230	164	0.71	0.77
Set 7	2	630	548	0.87	0.80
Set 8	2	300	1094	3.65	0.82
Average					0.78

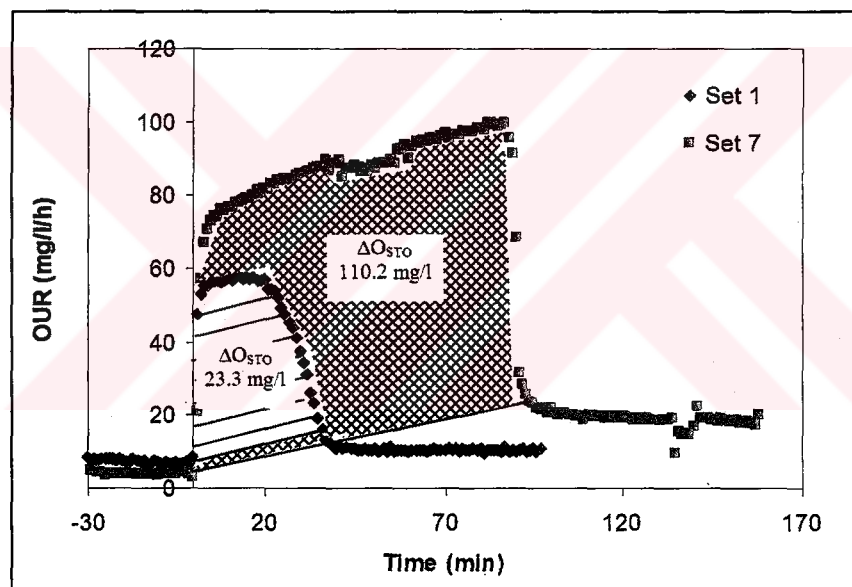


Figure 3.4: OUR profiles for acetate

The proposed procedure calculates Y_{STO} in accordance with ASM3, with the assumption that available readily biodegradable substrate is entirely converted into internal storage products. There is experimental evidence however for partial storage allowing for simultaneous growth both competing for the same pool of external substrate (Majone *et al.*, 1996; Beun *et al.*, 2000a; Dircks *et al.*, 2001). It could then be argued that limiting external substrate at low F/M ratios would allow simultaneous microbial growth, as in conventionally operated activated sludge systems, whereas a sudden increase of external substrate with high F/M ratios after a famine period would highlight internal storage as the dominant mechanism. Since

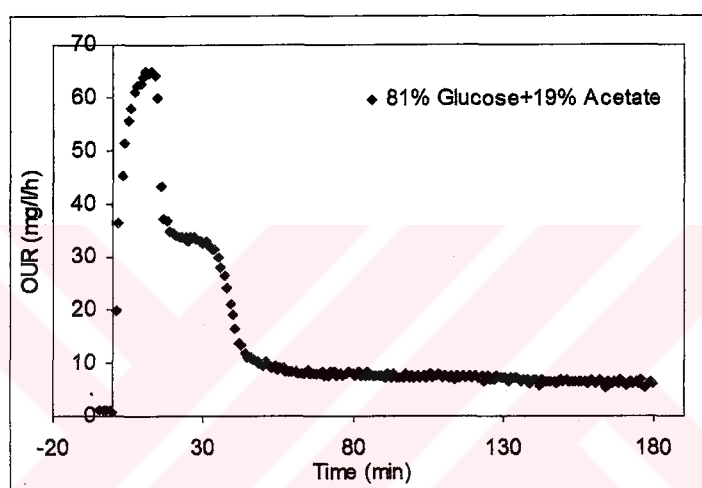
growth and storage are commonly defined by significantly different yield coefficients, the resulting weighted average yield value reflected by overall OUR measurements would exhibit a changing pattern parallel to adopted F/M values. The experimental results in this study provided a reliable indication that this was not the case for acetate utilization, which was best interpreted with total storage.

Glucose experiments: Two runs were conducted on glucose with initial F/M ratios of 0.05 and 0.78 gCOD/gcellCOD. As indicated in Table 3.5, they both yielded a Y_{STO} of 0.87 gCOD/gCOD, a value significantly higher as compared to acetate. This value agrees well with a Y_{STO} of 0.9 mgCOD/mgCOD assumed by **Goel *et al.* (1999)** and with the experimental results of **Dircks *et al.* (2001)** for glucose, with consideration that the formation of glycogen from glucose requires less energy as compared to PHB accumulation from acetate. It is stated that the maximum yield of glycogen from glucose is 46% greater than that of PHB from acetate due to the fact that storage of glycogen, spending 0.17 ATP/C-mole is energetically more efficient than storage of PHB which spends 0.25 mole ATP/C-mole (**Dircks *et al.*, 2001**).

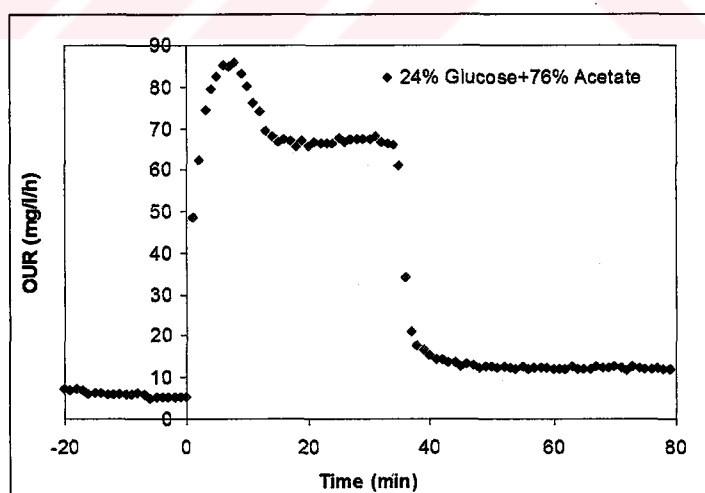
Experiments on glucose/acetate mixtures: After experiments on acetate and glucose, three additional runs were conducted using glucose/acetate mixtures. In these runs, the reactor was seeded by biomass previously acclimatized to the mixture. The initial substrate concentration was adjusted to 150-200 mg/l COD, with gradually decreasing the corresponding glucose fraction from 81% to 49 and 24%. As shown in Table 3.5, Y_{STO} was affected by the dominant substrate fraction in the mixture, exhibiting a parallel decrease from 0.85 gCOD/gCOD to 0.78 gCOD/gCOD. The OUR curves of two runs where glucose (81%) and acetate (76%) were adjusted as the major substrate fraction are given in Figure 3.5. It is interesting to note that the OUR profiles reveal two distinctly different rates, a faster utilization rate for glucose dominating the initial phase of the OUR curve and partially eclipsing a continuing slower process associated with the storage of acetate. This confirms similar findings reported by **Carta *et al.* (2001)**.

Table 3.5: Experimental assessment of Y_{STO} for glucose and glucose/acetate mixtures

Set No.	Reactor Volume (l)	Composition	Biomass Concentration (mg COD/l)	Substrate Concentration (mg COD/l)	F/M Ratio (COD/COD)	Y_{STO}
Set 1	2.5	Glucose	260	200	0.78	0.87
Set 2	2.5	Glucose	1000	50	0.05	0.87
Set 3	2.5	81% Glucose 19% Acetate	190	148	0.78	0.85
Set 4	2	49% Glucose 51% Acetate	950	205	0.22	0.82
Set 5	2	24% Glucose 76% Acetate	725	155	0.21	0.78



(a)



(b)

Figure 3.5: OUR profiles for glucose/acetate mixtures; (a) 81% glucose, (b) 24% glucose

3.2.2.2 Experiments on domestic sewage

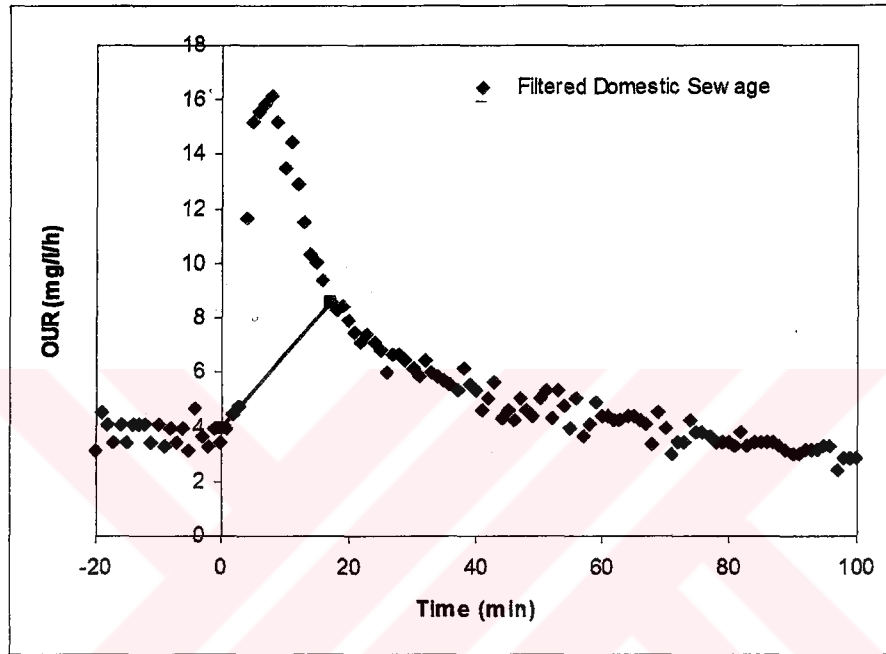
Four tests were conducted on domestic sewage at different F/M ratios varied in the range of 0.09-0.42 gCOD/gcellCOD, using the filtered portion of a daily composite sample collected from the inlet of the Ataköy plant, a small wastewater treatment facility in Istanbul treating only domestic sewage. The sample could be characterized by a total soluble COD of 100 mg/l and a soluble biodegradable fraction of 90 mg/l, based on a S_I/S_T ratio of 0.10 ascertained for the same sewage. As outlined in Table 3.6, a Y_{STO} of 0.96 gCOD/gCOD, a substantially higher value as compared to acetate and glucose, was practically applicable to all four tests. Table 3.6 also gives the results of three additional experiments carried out on domestic sewage/acetate mixtures. The results confirm the validity of Y_{STO} values individually calculated for domestic sewage and acetate, indicating a transient pattern reflecting the character of the dominant substrate fraction in the mixture (Figure 3.6).

Table 3.6: Experimental assessment of Y_{STO} for domestic sewage and domestic sewage/acetate mixtures

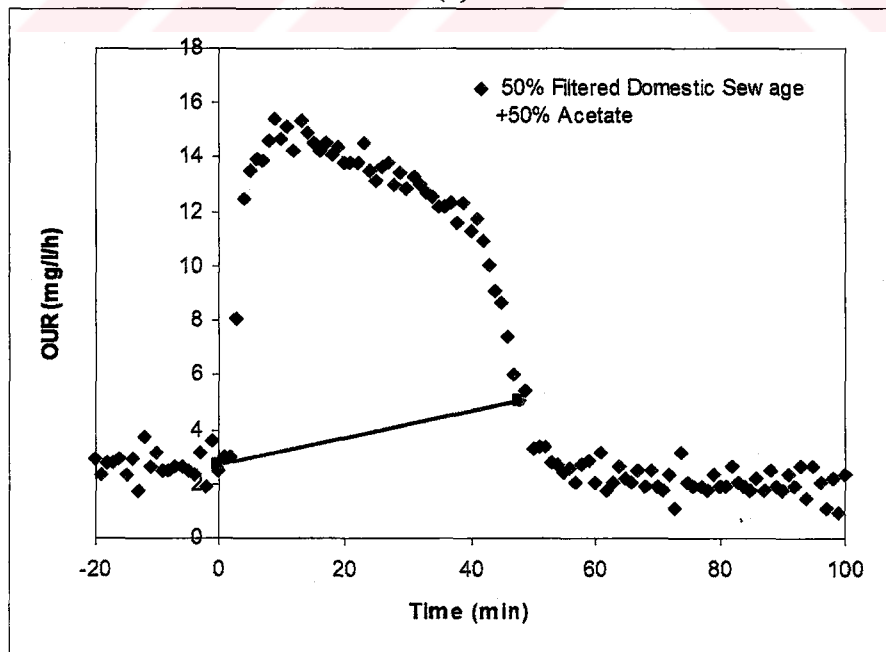
Set No.	Reactor Volume (l)	Composition	Biomass Concentration (mg COD/l)	Substrate Concentration (mg COD/l)	F/M Ratio (COD/COD)	Y_{STO}
Set 1	2.5	Domestic sewage	580	51	0.09	0.95
Set 2	3	Domestic sewage	265	50	0.19	0.97
Set 3	2.5	Domestic sewage	145	54	0.37	0.96
Set 4	2.5	Domestic sewage	120	51	0.42	0.96
Set 5	3	80% Domestic sewage+20% Acetic acid	80	45	0.56	0.90
Set 6	3	50% Domestic sewage+50% Acetic acid	180	50	0.28	0.87
Set 7	3	27% Domestic sewage+73% Acetic acid	160	50	0.31	0.82

The calculations were made with the assumption that the entire soluble biodegradable COD could be regarded as readily biodegradable substrate. This assumption, although valid for pure substrates, requires, as in this case, careful evaluation for wastewaters with more complex substrate compositions. If readily biodegradable substrate is actually less than what is indicated by the assumption introduced with ASM3, the corresponding oxygen consumption can only be interpreted with a superficially higher Y_{STO} . The proposed procedure may also be used to calculate S_{S1} for a generally adopted Y_{STO} value, as it defines a stoichiometric

procedure between the storage yield and the available readily biodegradable substrate. In this context, adoption of $Y_{STO} = 0.78 \text{ gCOD/gCOD}$, associated with acetate in this study, would yield $S_{S1} = 18 \text{ mg/l}$, corresponding to a S_S / S_T ratio of 0.18. Similarly, $S_{S1} = 29 \text{ mg/l}$, approximately 32% of the level that was previously assumed as readily biodegradable COD, would be obtained using the Y_{STO} value of 0.85 gCOD/gCOD suggested in ASM3.



(a)



(b)

Figure 3.6: OUR profiles for (a) domestic sewage (Set 2), (b) 50% domestic sewage and 50% acetate mixture (Set 6)

3.3 Effect of Substrate Composition on Storage under Anoxic Conditions[†]

Stringent effluent limitations for nitrogen necessitate an accurate interpretation of design and operation conditions of BNR systems. Reliable estimation of the electron acceptor demand is one of the key issues in determining the resulting nitrogen removal efficiency. Different process modifications of single sludge systems are envisaged for effective nitrogen removal. The nitrogen removal efficiency is strongly affected by the C/N ratio in the wastewater. In case of an insufficient C/N ratio, different additional carbon sources may be used to achieve the desired nutrient removal efficiency.

One of the additional carbon sources used for denitrification in recent studies is the fermentation products of primary settled domestic sewage, which embodies a high content of volatile fatty acids (VFAs), in the nature of readily biodegradable substrate, participating in the process at the highest rate. Since the behavior of the activated sludge differs with the substrate type, studies are concentrated on understanding of the performances by using the respirometric methods.

This section was undertaken with the main intention of defining the storage stoichiometry and kinetics, using the electron acceptor utilization rates on different carbon sources under anoxic conditions (nitrate utilization rate, NUR). The second and equally important objective of this study was to demonstrate the effect of biomass acclimation to different organic substrates. For this purpose two mixtures of readily biodegradable substrates were used to outline the differences observed in the respirometric response of different cultures under anoxic conditions.

3.3.1 Conceptual framework

The new model ASM3 was chosen to describe the stoichiometric and kinetic relationships of biochemical processes under anoxic conditions. A simplified matrix representation of ASM3 is shown in Table 3.7. As the table shows, the storage of organic substrate is an electron acceptor consuming process, which is identified with an anoxic storage yield of Y_{STOD} . The kinetic expression of the storage mechanism is defined by a Monod type rate expression, which involves the maximum storage rate

[†] Section 3.3 is a part of the paper entitled "Effect of substrate composition on storage under anoxic conditions" (2004) *Proceedings of IWA 4th World Water Conference*, 19-25 September 2004, Marrakech, Morocco.

constant k_{STO} , reduced by a factor η_D , when the electron acceptor is nitrate instead of oxygen. The factor 2.86 represents the oxygen equivalent of nitrate as electron acceptor.

Table 3.7: Simplified ASM3 matrix for anoxic processes

Component Process	S_s COD	S_{NO} N	X_p COD	X_H COD	X_{STO} COD	Process rate equation, ρ_j , all $\rho_j \geq 0$
Anoxic storage of COD	-1	$-\frac{1-Y_{STOD}}{2.86}$			Y_{STOD}	$k_{STO} \eta_D \frac{S_s}{K_s + S_s} X_H \frac{S_{NO}}{K_{NO} + S_{NO}}$
Anoxic growth		$-\frac{1-Y_{HD}}{2.86 Y_{HD}}$		1	$-\frac{1}{Y_{HD}}$	$\mu_H \eta_D \frac{X_{STO}/X_H}{K_{STO} + X_{STO}/X_H} X_H \frac{S_{NO}}{K_{NO} + S_{NO}}$
Anoxic endogenous respiration		$-\frac{1-f_t}{2.86}$	f_t	-1		$b_{HD} X_H \frac{S_{NO}}{K_{NO} + S_{NO}}$
Anoxic respiration of X_{STO}		$-\frac{1}{2.86}$			-1	$b_{STOD} X_{STO} \frac{S_{NO}}{K_{NO} + S_{NO}}$

The stoichiometry of the growth process occurred on stored polymers (X_{STO}) is defined by the anoxic growth yield Y_{HD} . The growth rate is expressed by a saturation type equation, involving the maximum specific growth rate of heterotrophs $\hat{\mu}_H$ and the half saturation constant K_{STO} . In the model two different endogenous respiration processes are defined for the decay of heterotrophic biomass (X_H) and the decay of stored products (X_{STO}), by first order process rates with respect to X_H and X_{STO} concentrations, respectively.

The processes identified in the ASM3 model can be observed in a sequence in respirometric batch tests as illustrated in Figure 3.7.

The NUR profile (S_{NO} curve) generally consists of 3 segments, the slopes of which represent 3 different rates of electron acceptor utilization, namely, r_1 , r_2 , and r_3 . In a batch anoxic reactor, the rate is affected by storage, growth and endogenous decay processes.

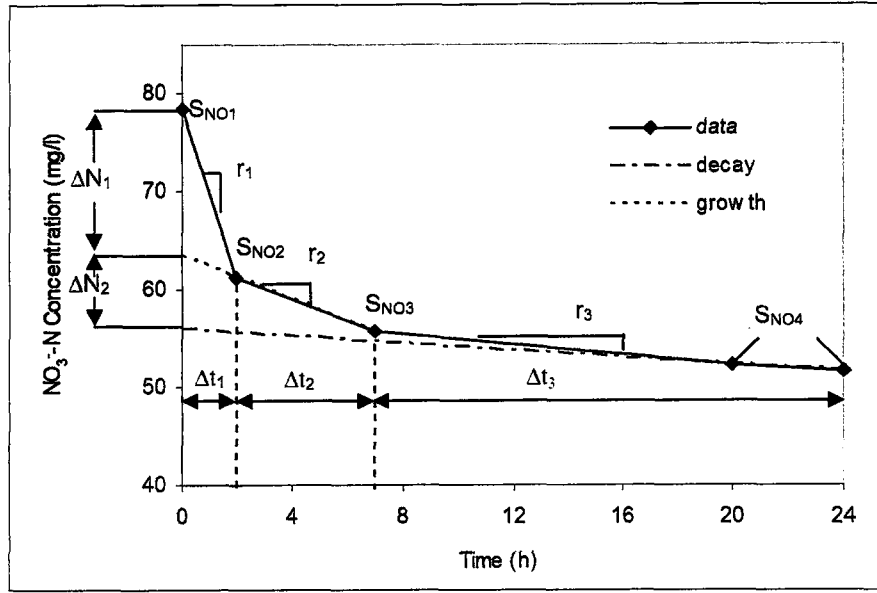


Figure 3.7: Graphical representation of a typical NUR profile

The high level of nitrate utilization in the first stage (r_1) is representative for mainly storage besides growth and decay. The second stage (r_2) is controlled by mainly growth and the third rate is attributed to endogenous decay processes (r_3).

$$r_1 = \frac{(S_{NO1} - S_{NO2})}{\Delta t_1} \quad (3.8)$$

$$r_2 = \frac{(S_{NO2} - S_{NO3})}{\Delta t_2} \quad (3.9)$$

$$r_3 = \frac{(S_{NO3} - S_{NO4})}{\Delta t_3} \quad (3.10)$$

For the determination of the utilized amount of oxidized nitrogen for storage (ΔN_1), the amount utilized in the first sequence is to be corrected for the interference of nitrate used for the growth and decay processes (Ubay Cokgör *et al.*, 1998).

Depending on process stoichiometry, given in Table 3.7, the corresponding yields for storage and growth can be calculated with the expressions given in equations 3.11 and 3.12, when the amount of readily biodegradable substrate, S_{S1} , consumed in the reactor is known.

$$Y_{STOD} = 1 - \frac{2.86 \cdot \Delta N_1}{S_{S1}} \quad (3.11)$$

For the calculation of the anoxic heterotrophic yield coefficient, Y_{HD} , the total amount of stored polymers, X_{STO} , has to be estimated as the fraction of initial readily biodegradable COD converted to storage products, as given below:

$$Y_{HD} = 1 - \frac{2.86 \cdot \Delta N_2}{Y_{STOD} \cdot S_{SI}} \quad (3.12)$$

In case of complex wastewater or substrate mixtures, the experimentally determined storage yield should be regarded as an overall observed yield, corresponding to the lumped yields of individual compounds. The amount of electron acceptor utilized for the storage of each substrate should be added in order to calculate the total nitrate consumption for storage:

$$\Delta N_1 = \frac{1}{2.86} \sum_{i=1}^k (1 - Y_{STOD_i}) \cdot S_{SI_i} \quad (3.13)$$

The analogy is not correct for the growth yield Y_H , since the growth occurs on the stored product X_{STO} and Y_H is to be considered as the same for the similar type of stored polymers.

3.3.2 Materials and methods

Two parallel sets of experiments were conducted with different enriched cultures. Respirometric batch tests for the measurement of nitrate utilization rate (NUR) were conducted under anoxic conditions. Nitrate and nitrite analyses were performed on filtered samples. Obtained results were recalculated and evaluated, considering the nitrite accumulation as described by Sözen and Orhon (1999).

3.3.2.1 Biomass and growth conditions

The biomass originating from a denitrifying wastewater treatment plant and two different cultures, namely culture A and culture B, were enriched in two parallel reactors. Reactors with a 1 l volume were operated in fill and draw mode in a sequence of aerobic and anoxic conditions, with a hydraulic retention time of 1 day and a sludge age of 7 days. Culture A has been acclimatized to a substrate mixture consisting of 50% acetate, 20% propionate, 10% ethanol and 20% glucose on COD basis. Culture B has been acclimatized to a substrate mixture of 50% acetate and

50% propionate. Synthetic wastewater was prepared with the above mentioned substrates and mineral medium with trace elements and phosphate buffer.

3.3.2.2 Batch experiments

Batch experiments were carried out to estimate the electron acceptor utilization rates on different carbon compounds under anoxic conditions. Batch experiments were conducted in 50 ml serum bottles, each bottle inoculated with 3 ml of biomass from culture A and culture B as identified in Table 3.8. The mixed liquor volume was adjusted to 40 ml and N₂ was flushed through the reactor to remove oxygen. In all sets C/N ratio was supplied as 3:1 to avoid nitrate limitation with a concentrated solution of nitrate (KNO₃). The reactors were maintained in suspension with continuous mixing.

Batch experiments with culture A were conducted in two parallel NUR tests as A1 and A2. In test A1, the mixture of acetate, propionate, ethanol and glucose were used, while the second test (A2) was conducted with acetate/propionate mixture. Analogous tests were also performed with culture B. The initial COD and nitrate contents in the reactors are also given in Table 2.

Ammonium (NH₄⁺-N), nitrate and nitrite (NO₂⁻-N and NO₃⁻-N) concentrations were measured by colorimetric methods as described by Schmidt and Bock (1997) and van de Graaf *et al.* (1996), respectively.

Table 3.8: Experimental set-up of batch tests

Culture	Set	Substrate mixture	COD	NO ₃ ⁻ -N
			(mg/l)	(mg/l)
A	A1	50% acetate+20% propionate+10% ethanol+20% glucose	160	65
	A2	50% acetate + 50% propionate	150	80
B	B1	50% acetate+20% propionate+10% ethanol+20% glucose	160	75
	B2	50% acetate + 50% propionate	150	90

3.3.3 Experimental results

The results obtained were evaluated on the basis of process stoichiometry and kinetics with the emphasis on the effect of substrate composition and biomass acclimation. Respirometric batch tests carried out with two different acclimatized biomass and two different substrate mixtures are illustrated in Figures 3.8 and 3.9. Three phases with descending slopes could be identified in all the experiments as shown in the figures. The different nitrate utilization rates were used for the assessment of storage, growth and decay parameters as described in the conceptual framework.

Results obtained from the experiment on culture A indicate that the storage process has been completed in 2 hours for both sets, A1 and A2. The kinetic structure of NUR experiments has been considered in terms of volumetric rates with the understanding that a direct comparison is not justifiable as the literature data are expressed on VSS basis. The rates in the first phase (r_1), representing mainly the storage rates, were estimated as 11 mg/l/h and 10 mg/l/h for sets A1 and A2 respectively.

The profiles showed that the microbial growth phase lasted 5-6 hours with a rate of 2 mg/l/h for both sets. The third rate associated with endogenous decay resulted in much lower rates of 0.25 mg/l/h and 0.20 mg/l/h for A1 and A2, indicating that the substrate available in the reactor has been depleted.

Besides the rate calculations, these experimental sets have also been used for the stoichiometric evaluation. The nitrate utilization rates (corrected for nitrite accumulation) were effectively used for the assessment of the storage yields by equation 3.11.

An observed storage yield of 0.70 gCOD/gCOD) was calculated for set A1 and 0.71 gCOD/gCOD was found for A2. Using the results of set A2, fed only with acetate and propionate, the anoxic storage yield of propionate was estimated as 0.77 gCOD/gCOD by equation 3.13, with the assumption of a Y_{STOD} of 0.66 gCOD/gCOD for acetate as determined by *Avcioglu et al. (2002)*. According to this assessment, the anoxic storage yield of ethanol was calculated as 0.68 gCOD/gCOD, using the experimental data for glucose as 0.90 gCOD/gCOD under aerobic conditions (*Karahan et al., 2002a*), reduced by an η factor of 0.85.

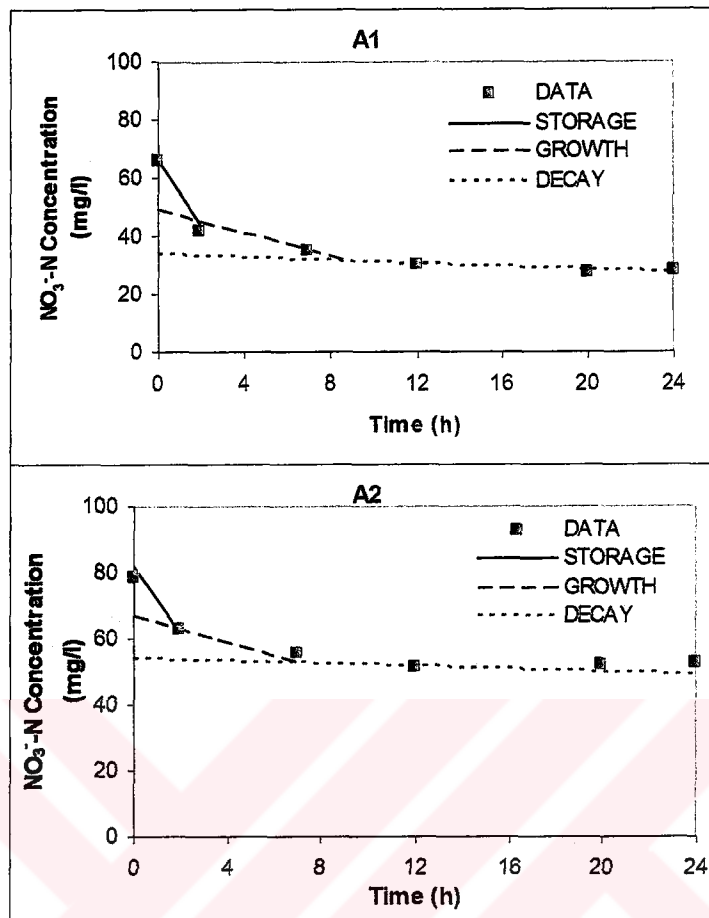


Figure 3.8: Experimental results of culture A

Another and equally important issue is the assessment of the growth yield. Anoxic yields obtained from the second phase of the NUR tests were 0.61 gCOD/gCOD and 0.65 gCOD/gCOD for A1 and A2, respectively. It must be noted that these values are much higher than 0.50 gCOD/gCOD, the theoretically calculated yield for domestic wastewater on the basis of energetic considerations (Orhon *et al.*, 1996).

The other group of experiments on culture B are illustrated in Figure 3.9. Same procedures were applied on the experimental data for the assessment of volumetric rates. The results reflected that r_1 and r_2 were the same for both B1 and B2. The first rate was assessed as 9 mg/l/h, whereas the rate of growth phase was calculated as 2 mg/l/h. A significant point of interest reflected by the experimental results was the observation in the endogenous decay phase exerting a utilization rate of 0.5 mg/l/h, twice higher than the rate obtained with culture A. This observation can only be explained by a possible interference of the utilization of nitrate due to extended growth.

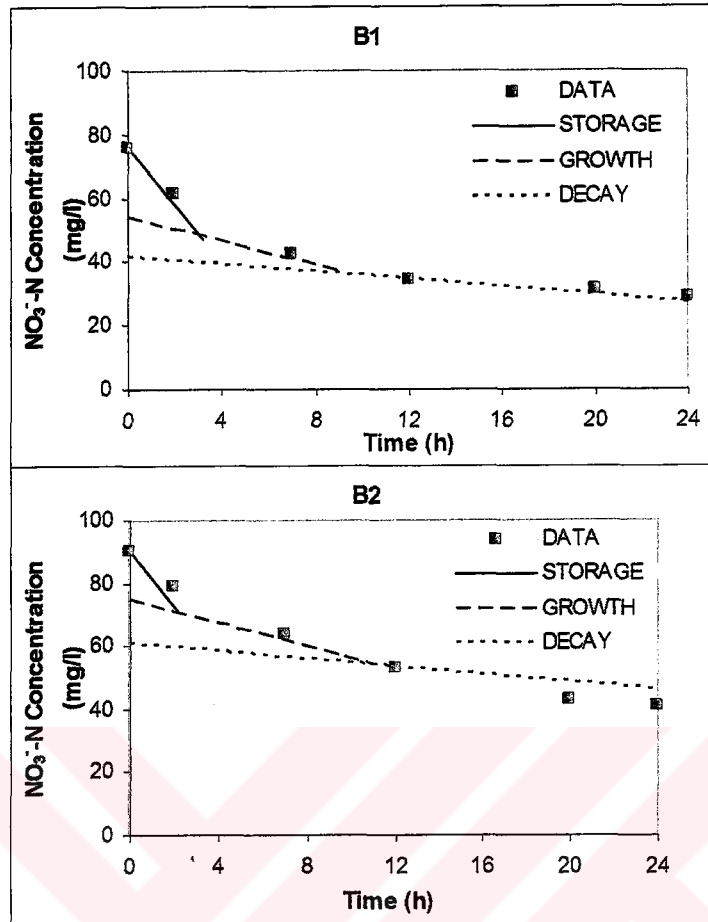


Figure 3.9: Experimental results of culture B

It has been observed that 58% of the maximum storage yields could be achieved for glucose and ethanol in system B1, when maximum Y_{STOD} for glucose is 0.77 gCOD/gCOD and maximum Y_{STOD} of ethanol is 0.68 gCOD/gCOD as found in system A1. This is probably due to insufficient adaptation, since the culture was not able to adjust its metabolism for maximum storage capacity in the period of the short test. At this point, due to the lack of optimization for storage of glucose and ethanol, the electron acceptor consumption was greater and thus, the process efficiency was lower than that of the acclimatized mixed culture A. On the other hand, evaluation of the tests on enriched culture B2 showed that the calculated Y_{STOD} for propionate was 0.76 gCOD/gCOD confirming the yield achieved in system A2.

The growth yields observed for culture B have been estimated to be the same for both sets B1 and B2, the Y_{HD} values being 0.64 gCOD/gCOD. This result can be interpreted as a consequence of the assumption of ASM3 stating that growth only occurs on stored products.

3.4 Results and Discussion

A procedure was proposed for the experimental assessment of the storage yield as defined in ASM3. The procedure makes use of the OUR curve generated by a soluble (filtered) substrate in an aerated batch reactor and enables graphical identification and calculation of the amount of oxygen consumed for storage. Biodegradable fraction of the soluble/filtered substrate must also be determined in a way that is consistent with ASM3. No model simulation or evaluation is required as the suggested procedure is based on simple stoichiometry between substrate utilized and dissolved oxygen consumed.

Model simulation indicated that the proposed procedure was quite consistent with model-input values, involving an error of less than 2%, aside from analytical errors associated with standard COD measurements, for tests to be conducted with F/M ratios over 0.1 gCOD/g cellCOD.

The proposed procedure was tested on acetate, glucose and domestic sewage, together with mixtures of acetate/glucose and acetate/domestic sewage. Relevant experimental data, generated at different initial F/M ratios, yielded an average Y_{STO} value of 0.78 gCOD/gCOD for acetate, 0.87 gCOD/gCOD for glucose and 0.96 gCOD/gCOD for domestic sewage. The high Y_{STO} level related to domestic sewage, consistently obtained for a wide range of initial F/M ratios, challenges the validity of the concept of S_S in ASM3, which is defined as the biodegradable fraction of the soluble substrate and tested in the study. The proposed procedure may also be used to calculate initial readily biodegradable COD concentration, S_{S1} , for a generally adopted Y_{STO} value, as it defines a stoichiometric procedure between the storage yield and the available readily biodegradable substrate.

The experiments conducted on substrate mixtures confirmed the validity of Y_{STO} values calculated for individual substrates, yielding a transient pattern reflecting the character of the dominant substrate fraction in the mixture. For glucose/acetate mixtures, they provided a clear indication of a faster storage rate for glucose as compared to acetate.

Nitrate utilization in batch tests were evaluated for the storage response under anoxic conditions. Nitrate utilization profiles provide a sensitive basis for both biochemical storage and heterotrophic growth stoichiometry and kinetics. Evaluation of batch

tests conducted showed that the observed storage yields for substrate mixtures were lumped yields and these experimental values should be investigated in detail to obtain individual anoxic storage yields of each compound. In this context, the anoxic storage yield of propionate was estimated as 0.77 gCOD/gCOD, with the assumption of an anoxic storage yield, Y_{STOD} , of 0.66 gCOD/gCOD for acetate and that of ethanol was calculated as 0.68 gCOD/gCOD, using the experimental data for glucose as 0.90 gCOD/gCOD under aerobic conditions, reduced by a factor of 0.85. The anoxic growth yields in the tests were calculated to be equivalent to an average Y_{HD} of 0.64 gCOD/gCOD.

The experiments conducted on cultures with different adaptation characteristics were evaluated for their storage response to different substrates under anoxic conditions. It has been observed that insufficient adaptation which does not allow the culture to adjust its metabolism for maximum storage capacity to a new substrate introduced to the system in the period of the short test, only 58% of the maximum storage yields could be achieved for glucose and ethanol in the system seeded with culture enriched with acetate and propionate. At this point, it can be concluded that when the substrate composition is changed under dynamic conditions, the electron acceptor consumption will be greater due to the lack of optimization of the microbial population for storage and thus, the process efficiency of the culture will be lower than that of the acclimatized mixed culture.

4. MODELING ACTIVATED SLUDGE SYSTEMS WITH SUBSTRATE STORAGE

4.1 Estimation of Stoichiometric and Kinetic Coefficients of ASM3 under Aerobic and Anoxic Conditions via Respirometry[†]

Activated sludge behavior is now studied using complex models involving a large array of kinetic and stoichiometric coefficients. Activated sludge Model No.1 (ASM1) should be regarded as the pioneering effort in this respect, providing a giant improvement in the mechanistic understanding of carbon and nitrogen removal (Henze *et al.*, 1987). It was soon modified for endogenous decay (Orhon and Artan, 1994). Recently, Activated Sludge Model No. 3 (ASM3) was proposed adopting endogenous decay and advocating biochemical storage as the primary mechanism of substrate utilization (Gujer *et al.*, 2000).

It should be noted that the merit of the new models mainly depends on the accuracy and reliability of the information they reflect on the biochemical mechanisms involved. This information must be experimentally determined. Respirometric methods have served extensively in the experimental assessment of kinetic and stoichiometric coefficients associated with ASM1 (Ekama *et al.*, 1986; Spanjers and Vanrolleghem, 1995; Sözen *et al.*, 1998).

ASM3, together with the concept of biochemical storage, introduced a new set of kinetic and stoichiometric coefficients, totally different from ASM1, with practically no experimental information on applicable values. Only some default levels were suggested with the model. Recent studies on the application of ASM3 have been limited to special cases in terms of parameter estimation (Koch *et al.*, 2000). The trend however is to use, as illustrated in this section, the same respirometric procedures for the assessment of ASM3 parameters. This approach has been

[†] Section 4.1 is a part of the paper entitled "Estimation of stoichiometric and kinetic coefficients of ASM3 under aerobic and anoxic conditions via respirometry" (2003) Water Science and Technology, 48(8), 185-194.

successfully applied for the determination of the storage yield coefficient, Y_{STO} under aerobic conditions (Karahan *et al.*, 2002a).

It is also important to determine the difference in the rate of major biochemical processes described in ASM3 under aerobic and anoxic conditions. ASM3 interprets this difference using a single correction factor, η_D . The major objective of this section was to illustrate the merit of respirometry in the experimental evaluation of ASM3 under aerobic and anoxic conditions. In this context, oxygen and nitrogen uptake rate measurements (OUR and NUR) were carried out in batch reactors for the estimation of significant kinetic and stoichiometric parameters involved in ASM3 structure.

4.1.1 Conceptual approach

A simplified matrix representation of ASM3 for aerobic and anoxic conditions is depicted in Table 4.1. As the table shows, storage is defined for both aerobic and anoxic conditions involving aerobic and anoxic stoichiometric coefficients, Y_{STO} and Y_{STOD} respectively. Growth rate under anoxic conditions is reduced by a correction factor, η_D . Endogenous respiration and respiration of storage products processes are also specified for aerobic and anoxic conditions.

Respirometric batch tests have been conveniently used to evaluate different activated sludge models and thus, respirometric responses obtained from batch tests can be evaluated to estimate ASM3 parameters, as shown in Figures 4.1(a) and (b).

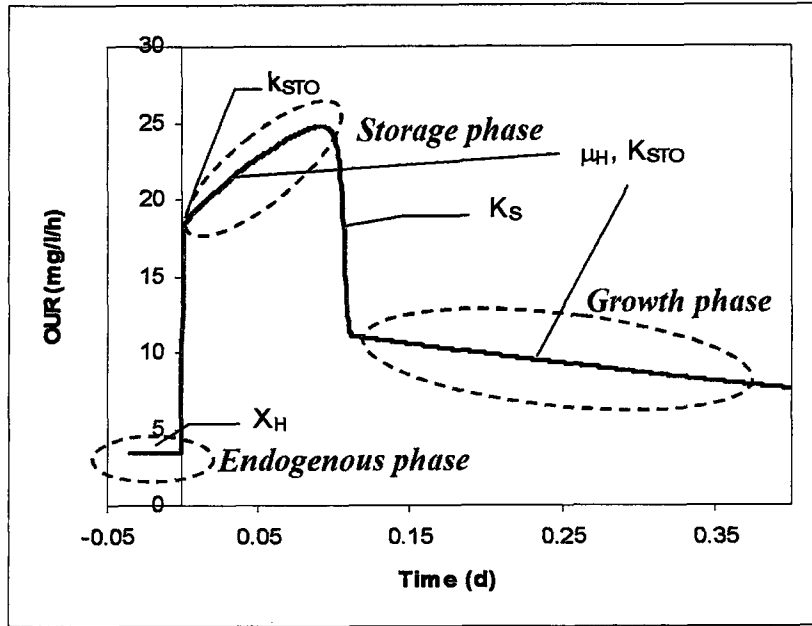
Figure 4.1(a) depicts the interpretation of an OUR curve according to ASM3. The mechanisms participating in oxygen utilization are storage, growth, endogenous decay and respiration of storage products. The aerobic rate expressions and the associated stoichiometric coefficients defined in the model are given in Table 4.1.

The initial phase of the curve is endogenous decay, where oxygen utilization is given by equation 4.1:

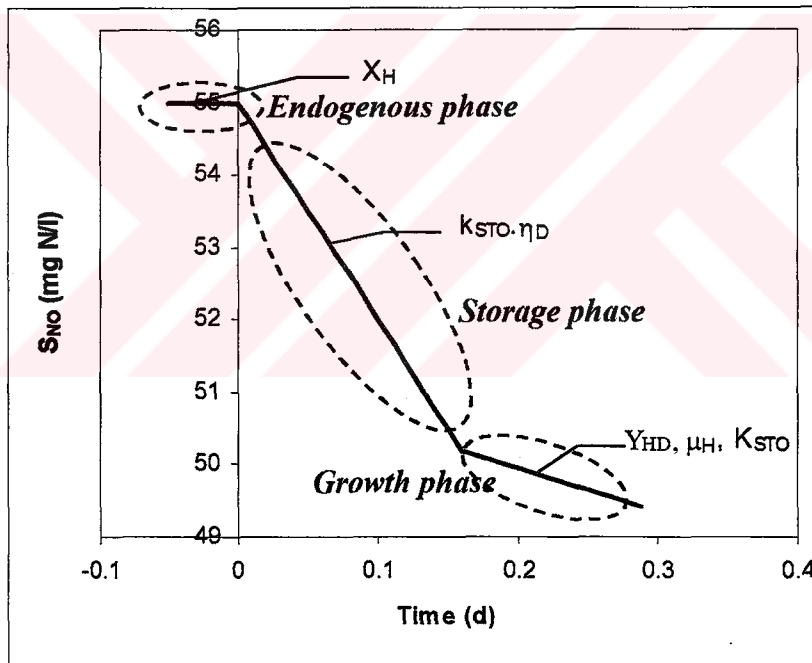
$$OUR_{Dec.phase} = \left(\frac{dS_O}{dt} \right)_{Dec.phase} = \left(\frac{dS_O}{dt} \right)_{Dec.} + \left(\frac{dS_O}{dt} \right)_{Resp.Xsto} \quad (4.1)$$

Table 4.1: A simplified ASM3 matrix for aerobic and anoxic processes

j ↓	Process	Component i →	1 S _s COD	2 S _o COD	3 S _{NO} N	4 X _P COD	5 X _H COD	6 X _{STO} COD	Process rate equation, ρ _j , all ρ _j ≥ 0
1	Aerobic storage of COD		-1	-(1 - Y _{STO})				Y _{STO}	$k_{STO} \frac{S_s}{K_s + S_s} X_H \frac{S_o}{K_o + S_o}$
2	Anoxic storage of COD		-1		$-\frac{1 - Y_{STOD}}{2.86}$			Y _{STOD}	$k_{STO} \eta_D \frac{S_s}{K_s + S_s} X_H \frac{S_{NO}}{K_{NO} + S_{NO}}$
3	Aerobic growth			$-\frac{1 - Y_H}{Y_H}$			1	$-\frac{1}{Y_H}$	$\mu_H \frac{X_{STO}/X_H}{K_{STO} + X_{STO}/X_H} X_H \frac{S_o}{K_o + S_o}$
4	Anoxic growth				$-\frac{1 - Y_{HD}}{2.86 Y_{HD}}$		1	$-\frac{1}{Y_{HD}}$	$\mu_H \eta_D \frac{X_{STO}/X_H}{K_{STO} + X_{STO}/X_H} X_H \frac{S_{NO}}{K_{NO} + S_{NO}}$
5	Aerobic endogenous respiration			-(1 - f _l)		f _l	-1		$b_H X_H \frac{S_o}{K_o + S_o}$
6	Anoxic endogenous respiration				$-\frac{1 - f_l}{2.86}$	f _l	-1		$b_{HD} X_H \frac{S_{NO}}{K_{NO} + S_{NO}}$
7	Aerobic respiration of X _{STO}			-1				-1	$b_{STO} X_{STO} \frac{S_o}{K_o + S_o}$
8	Anoxic respiration of X _{STO}				$-\frac{1}{2.86}$			-1	$b_{STOD} X_{STO} \frac{S_{NO}}{K_{NO} + S_{NO}}$



(a)



(b)

Figure 4.1: Estimated parameters of ASM3 using (a) aerobic, (b) anoxic respirometric curves

Assuming that the concentration of the storage products are much less than that of heterotrophic biomass, the first phase of the OUR curve can be successfully used to obtain an estimate value for active biomass concentration, X_H , for an accepted value of aerobic heterotrophic decay rate, b_H , and the inert biomass fraction, f_i , as shown in equation 4.2 (Marais and Ekama, 1976; Dold *et al.*, 1980; Dold and Marais, 1986):

$$OUR_{Dec.phase} = \left(\frac{dS_O}{dt} \right)_{Dec.phase} = (1 - f_1) \cdot b_H X_H \quad (4.2)$$

The second phase of the OUR curve is generated after the addition of exogenous substrate and is associated mainly with storage, where the other three processes are also active. The rate of storage, k_{STO} , can be estimated from the initial OUR value after substrate addition, since storage process has its maximum rate at this point and this OUR level is defined directly by $k_{STO} \cdot X_H$, provided that the stoichiometry of storage is well defined. Storage phase OUR response is defined by equation 4.3:

$$OUR_{Sto.phase} = \left(\frac{dS_O}{dt} \right)_{Sto.phase} = \left(\frac{dS_O}{dt} \right)_{Sto.} + \left(\frac{dS_O}{dt} \right)_{Gro.} + \left(\frac{dS_O}{dt} \right)_{Dec.} + \left(\frac{dS_O}{dt} \right)_{Resp.Xsto} \quad (4.3)$$

Equations 4.1 and 4.3 predict the difference between the OUR values of the two phases due to the oxygen utilization by storage and growth, however with the previously accepted assumption of relatively low concentration of storage products ($X_{STO} \ll X_H$) at the start of feeding ($t=0$), the contribution of growth process is negligible. The maximum rate of storage, k_{STO} , can be estimated by expression 4.4 when there is no substrate limitation. The value of storage yield can be determined by the estimation of amount of oxygen utilized for storage from experimental OUR data (Karahana *et al.*, 2002a).

$$\Delta OUR_{(t=0)} = \Delta \left(\frac{dS_O}{dt} \right)_{(t=0)} = (1 - Y_{STO}) \cdot k_{STO} \cdot X_H \quad (4.4)$$

The maximum heterotrophic growth rate, μ_H , can be estimated from the slope of the storage (second) phase of the OUR curve as shown in Figure 4.1(a), since the slope is determined by the growth process, and thus by μ_H and the half saturation coefficient for storage products, K_{STO} , when the growth yield, Y_H , is known. Although, substrate half saturation coefficient, K_S , also has considerable effect on this slope and the maximum attainable value of OUR, K_S can be directly and almost independently estimated by the sharpness and inclination of the drop in the OUR.

The growth (third) phase is governed by three processes, since storage process stops after the depletion of readily biodegradable substrate, as given in equation 4.5. The slope of the third phase, namely the growth phase is given by μ_H and K_{STO} couple, thus, the values of μ_H and K_{STO} can be estimated via model simulations by using both the storage (second) and growth (third) phases of the OUR curve.

$$OUR_{Gro,phase} = \left(\frac{dS_O}{dt} \right)_{Gro,phase} = \left(\frac{dS_O}{dt} \right)_{Gro.} + \left(\frac{dS_O}{dt} \right)_{Dec.} + \left(\frac{dS_O}{dt} \right)_{Resp.Xsto} \quad (4.5)$$

In the same manner, a nitrate utilization rate (NUR) profile can be evaluated according to ASM3 as shown in Figure 4.1(b). The rate for the first phase is associated with endogenous respiration and the respiration of storage products, where the latter may be neglected due to the comparably less amount of storage products. Thus, the active initial heterotrophic biomass concentration can be determined by:

$$NUR_{Dec,phase} = \left(\frac{dS_{NO}}{dt} \right)_{Dec.} = \frac{(1-f_I)}{2.86} b_{HD} X_H \quad (4.6)$$

Literature values can be assumed for the heterotrophic anoxic decay coefficient, b_{HD} and the fraction of inert endogenous matter, f_I (Gujer *et al.*, 2000).

The storage phase emerges after the addition of exogenous substrate. The rate of this phase is determined by the four anoxic processes namely, storage, growth, endogenous decay and respiration of storage products as follows:

$$NUR_{Sto,phase} = \left(\frac{dS_{NO}}{dt} \right)_{Sto,phase} = \left(\frac{dS_{NO}}{dt} \right)_{Sto.} + \left(\frac{dS_{NO}}{dt} \right)_{Gro.} + \left(\frac{dS_{NO}}{dt} \right)_{Dec.} + \left(\frac{dS_{NO}}{dt} \right)_{Resp.Xsto} \quad (4.7)$$

The third phase in Figure 4.1(b) is the growth phase and the total rate is due to the processes of growth, endogenous respiration and respiration of storage products:

$$NUR_{Gro,phase} = \left(\frac{dS_{NO}}{dt} \right)_{Gro,phase} = \left(\frac{dS_{NO}}{dt} \right)_{Gro.} + \left(\frac{dS_{NO}}{dt} \right)_{Dec.} + \left(\frac{dS_{NO}}{dt} \right)_{Resp.Xsto} \quad (4.8)$$

Thus, the difference between the rates of storage and the growth phases, denoted as ΔS_{NO} on the y-intercept as depicted in Figure 4.2, defines the amount of nitrate utilized for storage.

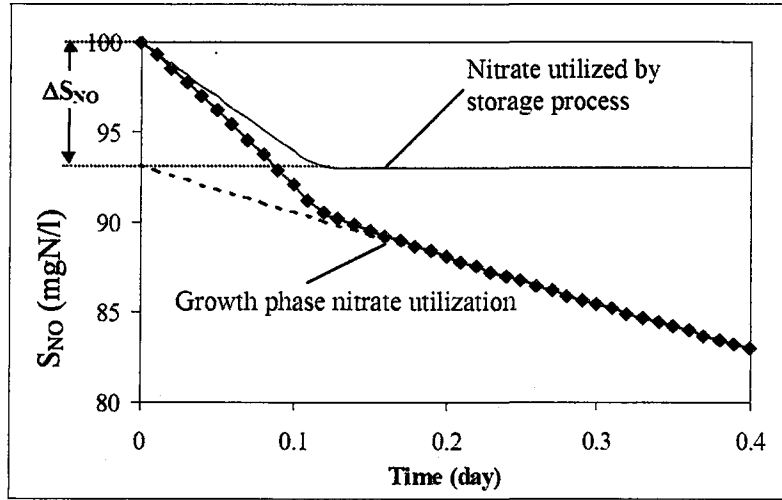


Figure 4.2: Graphical representation of the calculation of S_{NO} utilized for storage

The anoxic storage process shown in Table 4.1 can be expressed in terms of substrate utilization as follows:

$$\left(\frac{dS_{NO}}{dt} \right)_{Sto.} = \frac{(1 - Y_{STOD})}{2.86} \frac{dS_s}{dt} \quad (4.9)$$

Rearranging and integrating equation 4.9 with respect to time:

$$2.86 \Delta S_{NO} = (1 - Y_{STOD}) S_{SI} \quad (4.10)$$

Thus, Y_{STOD} can be calculated by for a known amount of S_s by equation 4.11:

$$Y_{STOD} = 1 - \frac{2.86 \Delta S_{NO}}{S_{SI}} \quad (4.11)$$

The rate expression for storage phase is governed by the storage rate and can be written as given in Table 4.1:

$$NUR_{Sto.phase} = \left(\frac{dS_{NO}}{dt} \right)_{Sto.} = \frac{(1 - Y_{STOD})}{2.86} k_{STO} \eta_D \frac{S_s}{K_s + S_s} X_H \quad (4.12)$$

The $S_s/(K_s+S_s)$ term can be neglected for a readily biodegradable substrate expected to have a low half saturation constant, K_s , value. Then, expression 4.12 can be reduced to:

$$NUR_{Sto,phase} = \left(\frac{dS_{NO}}{dt} \right)_{Sto.} = \frac{(1 - Y_{STOD})}{2.86} k_{STO} \eta_D X_H \quad (4.13)$$

Substituting the value of the calculated Y_{STOD} , it would be possible to calculate the product of the storage rate constant, k_{STO} and the anoxic reduction factor, η_D in equation 4.13.

Similar to the aerobic case, the anoxic heterotrophic yield coefficient, Y_{HD} , μ_H and K_{STO} can be estimated from the slope of the growth phase by the aid of model simulation. Substrate affinity coefficient, K_s can be adjusted by the inclination and the smoothness of the transition from the second phase to the third phase.

4.1.2 Materials and methods

Aerobic batch experiments: Aerated batch reactors of 2 l volume were used for the aerobic respirometric batch tests. The tests were carried out in completely mixed, aerated batch reactors, seeded with active biomass taken from fill and draw reactor of 5 l volume acclimated to acetate and operated at a sludge age of 20 days. The tests were started with the biomass seeding alone, for the assessment of the OUR level associated with the endogenous phase, for a minimum period of 45 minutes. Aerobic reactors were provided with buffer and mineral solutions. The final volume was made up with distilled water. Acetic acid solutions were prepared with 100% glacial acetic acid and were added on the biomass in the reactor to reach the final concentrations of 164, 187, 274 and 548 mg COD/l in order to attain F/M ratios of 0.22, 0.25, 0.29 and 0.64 mg COD/mg VSS, respectively. The OUR response was monitored for 2-3 hours with a Manotherm RA-1000 continuous respirometer with PC connection.

Anoxic batch experiments: Anoxic 1 l batch reactors were set up to monitor the nitrate utilization rate. Biomass was inoculated from fill and draw reactors acclimated to acetate, operated under 24 h aerobic/24 h anoxic conditions with a sludge age of 20 days. Nitrate was added externally in adequate amounts. The reactors were provided with buffer and mineral solutions. The final volume in the

reactors was adjusted to 1 l using tap water. Acetate was added as the external substrate. Initial concentrations of acetate in the anoxic reactors were 50, 100, 120 and 175 mg COD/l. The corresponding F/M values for acetate were between 0.14-0.16 mg COD/mg VSS. 100% glacial acetic acid was used as the acetate source.

The anoxic reactors were equipped with magnetic stirrers and rubber stoppers with piping for sampling, nitrogen supply and outflow. Nitrogen gas was continuously purged to avoid air intrusion. Samples were withdrawn from the reactors for every 5-10 minutes. Nitrite and nitrate-nitrogen analyses were performed on filtered samples, using a CHEMLAB[®] auto-analyzer operated in accordance with hydrazine reduction method as defined in **Standard Methods (1998)**.

In the experiments, pH was kept in the range of 7.0-8.0, suitable for biological activity. COD measurements were performed as described in the method **ISO 6060 (1986)**. Testing of the experimental data was performed by model simulation, using the AQUASIM[®] computer program developed by the Swiss Federal Institute for Environmental Science and Technology (**Reichert *et al.*, 1998**).

4.1.3 Results and discussion

4.1.3.1 Oxygen utilization

OUR measurements obtained for four sets of aerobic experiments were used to estimate the main stoichiometric and kinetic parameters of ASM3 as explained in the previous sections and the results are presented in Table 4.2. The endogenous phase OUR data was used to calculate the amount of active heterotrophic biomass, according to equation 4.2. The second phase, namely the storage phase, was used to estimate the storage yield according to a pre-defined procedure (**Karahan *et al.*, 2002b**) and the rate of storage, k_{STO} , was determined using equation 4.4 for different substrate concentrations and F/M ratios. The obtained storage rates (13 ± 2.60 1/d) were much higher than the default value of ASM3, however in agreement with that of **Krishna and van Loosdrecht (1999a)**, where k_{STO} for acetate was reported as 10 1/d.

Table 4.2: Aerobic kinetic and stoichiometric coefficients of ASM3 obtained for acetate

		Set 1	Set 2	Set 3	Set 4
S_{Si}	mgCOD/l	164	187	274	548
F/M	gCOD/gVSS	0.22	0.25	0.29	0.64
b_H	1/d	0.20	0.20	0.20	0.20
b_{STO}	1/d	0.20	0.20	0.20	0.20
f_I		0.20	0.20	0.20	0.20
K_S	mgCOD/l	3	2	3	2
K_{STO}		1	1	1	1
k_{STO}	1/d	16	12	10	14
μ_H (2 nd phase)	1/d	5	6	3	4
μ_H (3 rd phase)	1/d	2	2.5	1.6	2
Y_H	gCOD/gCOD	0.63	0.63	0.63	0.63
Y_{STO}	gCOD/gCOD	0.77	0.75	0.80	0.80

Model simulations were performed for the estimation of μ_H , K_{STO} and K_S with the growth yield, Y_H , assumed as the default value of ASM3 in order to decrease the degrees of freedom of the model. The maximum value of OUR and the inclination of the OUR drop at the end of the second phase was used to evaluate K_S value by model simulations. Model parameters like b_H , b_{STO} and f_I were assumed as the default values due to their relatively low sensitivities for batch respirometric tests.

The second and the third phases were modeled to calculate μ_H - K_{STO} couple and the best fits were obtained for K_{STO} default value of 1 gCOD/gCOD for all experimental runs. However, the simulation results of 4 sets of aerobic respirometric data indicated that, it was not quite possible to obtain ASM3 calibration using a single μ_H value. Two different μ_H values were used during simulation studies for fitting the first and second OUR plateau. The higher μ_H value fit the first plateau while the value of the second plateau was appreciably higher than the experimental plateau. In the same way, the lower μ_H value gave better fit to the second plateau value while the value of the first plateau stayed remarkably lower than the experimental data. Apparently two different growth rates were needed in order to simulate OUR data in the storage and growth phases as shown in Figure 4.3.

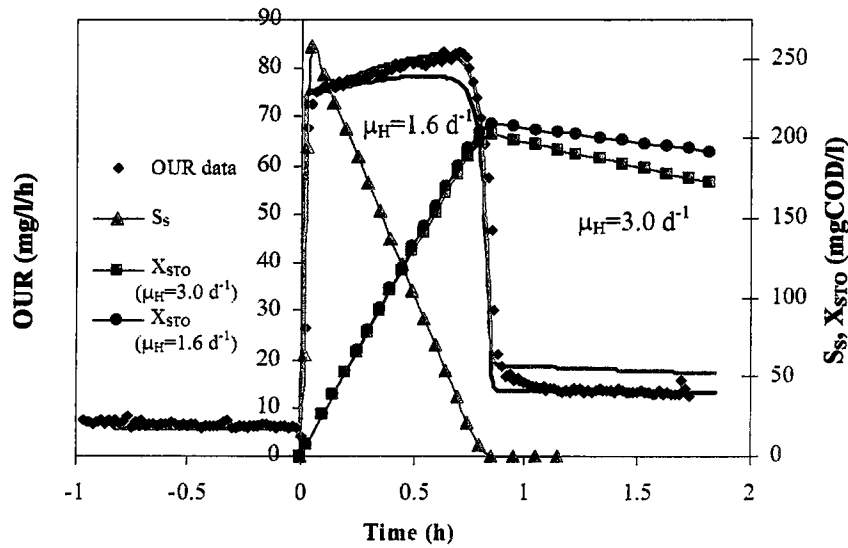


Figure 4.3: ASM3 simulation results for acetate aerobic set 3

4.1.3.2 Nitrate utilization

Nitrate utilization data obtained from four sets of anoxic runs were used as input for model simulation using the AQUASIM computer program modified for ASM3 structure. For modeling purposes, the initial active heterotrophic biomass concentration, X_H , was calculated from the aerobic endogenous decay phase data using expression 4.2.

The storage (second) phase emerged after the addition of the readily biodegradable substrate, S_S , namely acetate. The expression representing the storage mechanism (equation 4.13) involved three unknowns, namely the anoxic storage yield coefficient Y_{STOD} , storage rate constant, k_{STO} and anoxic reduction factor, η_D . Y_{STOD} could be calculated for pre-selected concentrations of S_{S1} (Table 4.3), by using equation 4.11. k_{STO} values were adopted as 13 1/d from the model output of aerobic experimental sets. Thus, η_D was left as the only unknown parameter in expression 4.13 and could be calculated for each run by using the experimental slope of the storage phase in the S_{NO} profile. The calculated η_D values were between 0.50 and 0.56, with an average of 0.53.

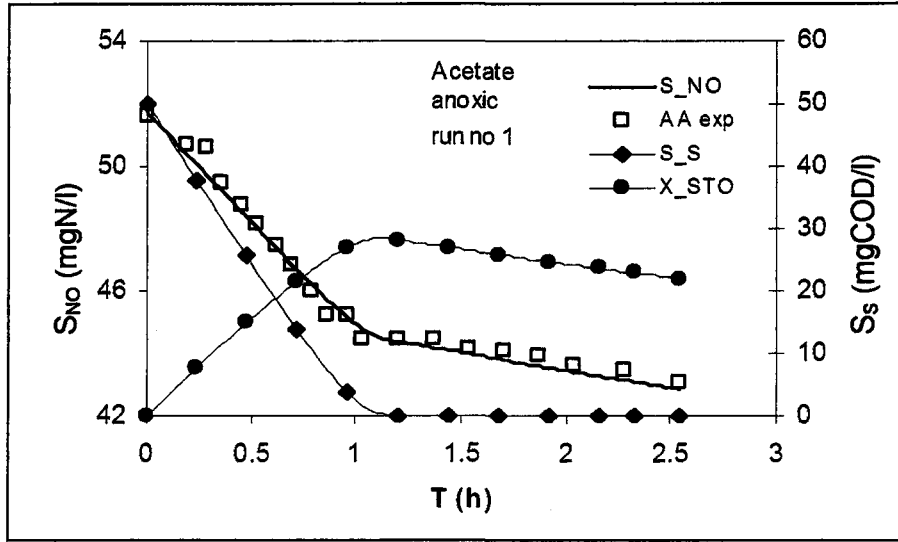
The rate of the third phase of the anoxic electron acceptor utilization profile was dependant on the growth of heterotrophic biomass on the stored substrate. As also shown in Figure 4.1(b), the major rate determining parameters in this phase were the anoxic heterotrophic yield coefficient, Y_{HD} , the heterotrophic maximum growth rate,

μ_H , and the saturation constant for storage products, K_{STO} . μ_H was adopted as 6 1/d. By substituting the calculated η_D and Y_{STOD} values together with the average k_{STO} value as the model inputs, by simulation, Y_{HD} was obtained with an average of 0.44 gCOD/gCOD. In contrast to the aerobic respirometric data, model calibration was achieved for the anoxic data for all sets by using a single μ_H value. The effect exerted by μ_H was more pronounced for the storage and growth phases of the OUR vs. time data but the same parameter exerted a significant effect only on the growth phase of the S_{NO} vs. time profile because although OUR is an obtainable parameter for each time unit, it is experimentally not possible to monitor NUR vs. time. Instead, only an average nitrate utilization rate can be calculated for each phase.

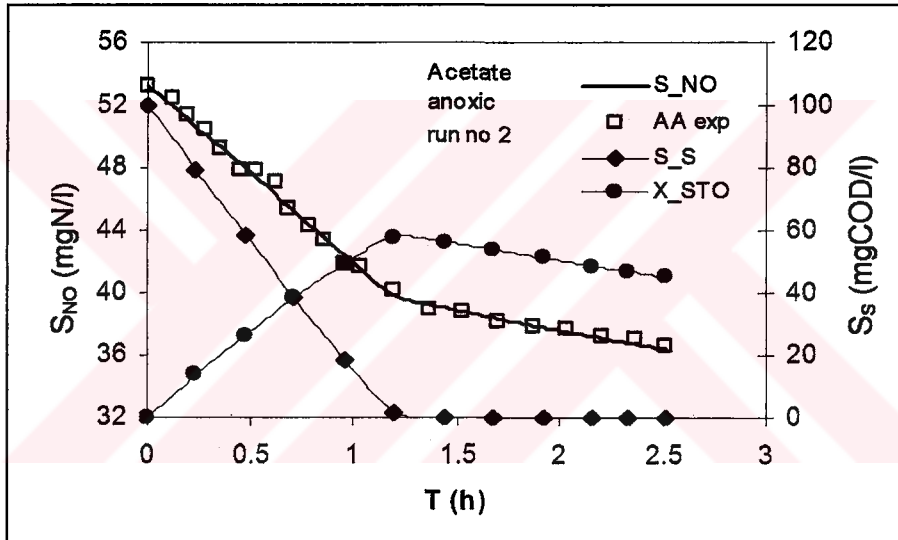
The default saturation constant for substrate, K_S value (2 mg COD/l) seemed to provide the right transition from the second to the third phase. The experimental data gave a good fit with the model output using suggested value of K_{STO} as in ASM3. The remaining kinetic coefficients namely, the anoxic respiration rate for storage products, b_{STOD} , and nitrate-nitrogen half saturation constant, K_{NO} did not exert significant effects on the S_{NO} profile and were assumed as in ASM3. Table 4.3 lists the values of the kinetic and stoichiometric coefficients adopted for model evaluation. Figures 4.4(a) and (b) show S_{NO} , S_S and X_{STO} vs. time profiles obtained by ASM3 simulation for acetate anoxic sets 1 and 2.

Table 4.3: Kinetic and stoichiometric coefficients used for anoxic acetate experiments

		Default	Set 1	Set 2	Set 3	Set 4
S_{Si}	mgCOD/l		50	100	120	175
F/M	gCOD/gVSS		0.14	0.16	0.13	0.14
b_{HD}	1/d	0.10	0.10	0.10	0.10	0.10
b_{STOD}	1/d	0.10	0.10	0.10	0.10	0.10
η_D		0.60	0.50	0.55	0.56	0.52
f_I		0.2	0.2	0.2	0.2	0.2
K_S	mg COD/l	2	2	2	2	2
K_{STO}	gCOD/gCOD	1	1	1	1	1
K_{NO}	mgNO ₃ -N/l	0.5	0.5	0.5	0.5	0.5
k_{STO}	1/d	5	13	13	13	13
μ_H	1/d	2	6	6	6	6
Y_{HD}	gCOD/gCOD	0.54	0.43	0.45	0.41	0.45
Y_{STOD}	gCOD/gCOD	0.80	0.64	0.67	0.68	0.64



(a)



(b)

Figure 4.4: ASM3 simulation results for acetate anoxic runs; (a) no 1, (b) no 2

The methodology involved the use of aerobic respirometric data for the estimation of aerobic coefficients by model simulation and substituting the obtained aerobic coefficients in the anoxic data to evaluate the anoxic parameters. By respirometric methods, it was possible to calculate the aerobic and anoxic storage yield coefficients independently. The average values of Y_{STOD} and Y_{STO} were 0.66 gCOD/gCOD and 0.78 gCOD/gCOD respectively, with a ratio of 0.85. The calculated η_D range was between 0.50 and 0.56 with an average of 0.53. This coefficient would reduce the aerobic storage rate of 13 1/d to approximately 7 1/d under anoxic conditions. The average aerobic heterotrophic growth rate was used in the anoxic simulations to estimate the anoxic heterotrophic growth yield, Y_{HD} . The model simulation outputs

yielded an average Y_{HD} value of 0.44 gCOD/gCOD, which is relatively lower than the suggested value of 0.54 in ASM3. The corresponding Y_{HD}/Y_H ratio was 0.70.

While storage kinetics and stoichiometry could very well be defined, the simulation results of 4 sets of aerobic respirometric data indicated that, it was not quite possible to obtain ASM3 calibration by using a single μ_H value. Apparently two different growth rates were needed in order to simulate OUR data in the storage and growth phases and this finding suggests that the concept of growth on storage products adopted in ASM3 needs to be re-evaluated. In this framework, modification of ASM3 considering simultaneous storage and direct growth on readily biodegradable substrate, followed by growth on stored products may be considered.

4.2 Modification of Activated Sludge Model No.3 Considering Direct Growth on Primary Substrate[†]

Substantial research has shown that storage mechanism plays an important role under dynamic substrate conditions experienced in treatment plants. Storage of excess substrate available under feast conditions allows microorganisms capable of substrate storage to survive on the accumulated substrate reserves when no external substrate is present (famine conditions). These microorganisms have the benefit of a more balanced growth and advantage for competition (van Loosdrecht *et al.*, 1997).

4.2.1 Conceptual approach

ASM3 assumes that storage of readily biodegradable substrate is the preliminary step before growth solely occurs on the stored products. This assumption of simultaneous storage and growth on the storage polymers is not valid mechanistically, since microorganisms utilize the stored polymers as carbon and energy source only after the depletion of the primary substrate. This is taken into account in metabolic models predicting that excess substrate is stored while the primary substrate, if present, is utilized for growth (van Aalst-van Leeuwen *et al.*, 1997; Dircks *et al.*, 2001). ASM3, as it is, can be used to describe the behavior of activated sludge systems for real cases (Koch *et al.*, 2000) but the description is not satisfactory for experimental data obtained for batch tests. It has been reported that a superficially high storage

[†] Section 4.2 is a part of the paper entitled "Modification of Activated Sludge Model No.3 Considering Direct Growth on Primary Substrate" (2003) Water Science and Technology, 47(11), 219-225.

yield (Y_{STO}) value of 0.96 gCOD/gCOD was obtained for filtered domestic sewage due to the definition of readily biodegradable COD and the total storage approach (Karahana *et al.*, 2002a). ASM3 also fails to simulate high rate of oxygen utilization in the feast phase and the lower rate in the famine phase due to the single growth process definition. Two different growth rates are necessary to describe the OUR response of a batch system. A more consistent description could be provided if growth in the feast phase is included in the model (Krishna and van Loosdrecht, 1999a) and thus, one of the main issues that need to be evaluated in conjunction with ASM3 is heterotrophic growth on primary substrate which occurs as a competing mechanism with storage. This mechanism could play an important role when different reactor and treatment plant configuration and flow regimes are considered or temperature varies (Krishna and van Loosdrecht, 1999b).

4.2.2 Model development

Storage of readily biodegradable COD by heterotrophs under aerobic conditions involves two different mechanisms; (i) storage of poly- β -hydroxybutyrate (PHB) when acetate is fed to the system, (ii) storage of glycogen when glucose is the carbon source. PHB metabolism has been described by van Aalst-van Leeuwen *et al.* (1997) and the stoichiometry and kinetics have also been studied by Beun *et al.*, (2000). The metabolic model of PHB storage shown in Figure 4.5(a), suggests that acetate is taken up and converted to acetyl-CoA, which is used to produce biomass and for PHB synthesis. Acetyl-CoA is also used as the energy source. When all the acetate is taken up, stored PHB is hydrolyzed to generate acetyl-CoA under famine conditions. Growth yields under feast conditions have been reported as 0.47 and 0.41 g cellCOD/gCOD; the storage yields as 0.73 and 0.69 gCOD/gCOD and the growth yields on PHB as 0.60 and 0.57g cellCOD/gCOD by Aalst-van Leeuwen *et al.* (1997) and Beun *et al.* (2000), respectively.

Dircks *et al.* (2001), investigated the stoichiometry and kinetics of glycogen metabolism in mixed cultures. The glycogen metabolism is described as shown in Figure 4.5(b). Glucose is taken up and Glucose-6-phosphate (G6P) is produced, which is then converted into glycogen and used for biomass production. G6P is also used for catabolic reactions consuming oxygen. After the depletion of primary substrate, glycogen is used for the synthesis of G6P. Dircks *et al.* (2001) have

reported the storage yield of glycogen as 0.91 gCOD/gCOD and the growth yield in the feast phase as 0.57 g cellCOD/gCOD. The growth yield in the famine phase was given as 0.60 g cellCOD/gCOD.

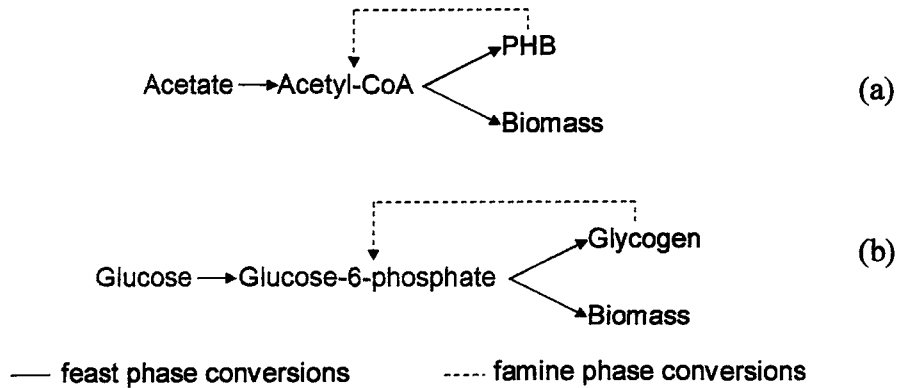


Figure 4.5: Metabolic pathways for (a) PHB, (b) Glycogen storage

Based on the scientific background suggested by metabolic models, the addition of growth process on the primary carbon source to ASM3 was proposed. The modeling studies conducted by **Krishna and van Loosdrecht (1999a)** resulted a better description of the system where the storage yield was adopted as 0.73 gCOD/gCOD; direct growth yield as 0.50 g cellCOD/gCOD; the growth yield on PHB as 0.65 cellCOD/gCOD and the rate of storage was given as 10 1/d, similar to the value reported by **Koch *et al.* (2000)**.

The modified version for the carbon removal processes of ASM3 has been prepared in this section, based on existing scientific information reported in the literature and is given in Table 4.4. The proposed model consists of hydrolysis, endogenous respiration of biomass and respiration of storage products processes, as described in ASM3. Storage of readily biodegradable substrate and primary growth processes were described as simultaneous processes, competing for substrate and electron acceptor. Both processes have reaction rates defined according to Monod kinetics, where growth process has ammonia nitrogen and bi-carbonate limitations. Secondary growth process is inhibited when primary substrate is present in the system. The process uses stored products as substrate and process kinetics is defined as surface reaction kinetics similar to ASM3.

Table 4.4: Matrix representation of the proposed model structure

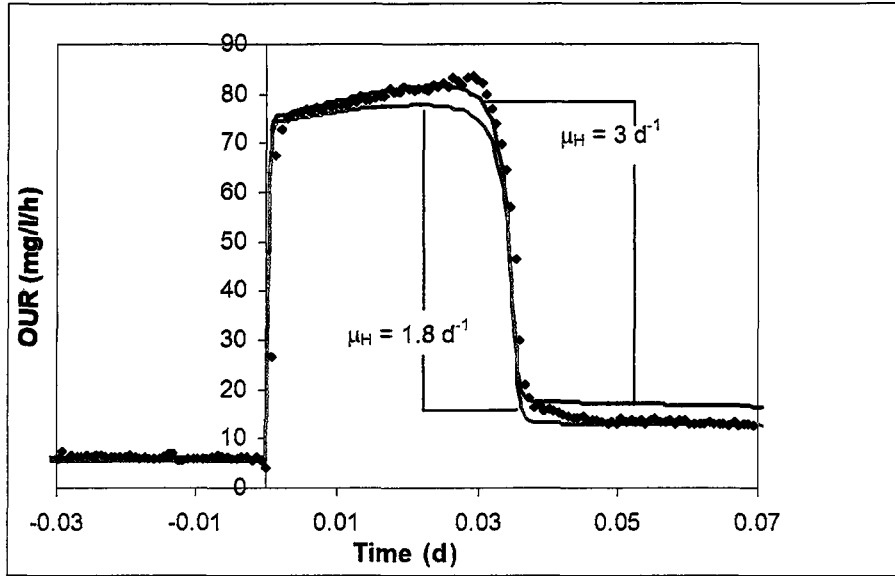
<i>Component</i> <i>Process</i>	S_o O ₂	S_I COD	S_s COD	X_I CO	X_s CO	X_H CO	X_{STO} COD	<i>Rate</i>
Hydrolysis		f_{SI}	$1-f_{SI}$		-1			$k_H \frac{X_S/X_H - X_H}{K_X + X_S/X_H}$
Aerobic Storage of COD	$-\frac{1-Y_{STO}}{Y_{STO}}$		$-1/Y_{STO}$				1	$k_{STO} \frac{S_o}{K_o + S_o} \frac{S_s}{K_s + S_s} X_H$
Growth on S_s	$-\frac{1-Y_{H1}}{Y_{H1}}$		$-1/Y_{H1}$			1		$\mu_{H1} \frac{S_o}{K_o + S_o} \frac{S_{NH}}{K_{NH} + S_{NH}} \frac{S_{HCO}}{K_{HCO} + S_{HCO}} \frac{S_s}{K_s + S_s} X_H$
Growth on X_{STO}	$-\frac{1-Y_{H2}}{Y_{H2}}$					1	$-1/Y_{H2}$	$\mu_{H2} \frac{S_o}{K_o + S_o} \frac{K_s}{K_s + S_s} \frac{S_{NH}}{K_{NH} + S_{NH}} \frac{S_{HCO}}{K_{HCO} + S_{HCO}} \frac{X_{STO}/X_H}{K_{STO} + X_{STO}/X_H} X_H$
Endogenous Respiration	$-(1-f_i)$			f_i		-1		$b_H \frac{S_o}{K_o + S_o} X_H$
Respiration of X_{STO}	-1						-1	$b_{STO} \frac{S_o}{K_o + S_o} X_{STO}$

The model simulation of an OUR curve generated using the proposed model shows the relative impact of direct growth and storage mechanism on oxygen utilization for a set of batch OUR experiments conducted on acetate, evaluated for a selected range of kinetic coefficients (Table 4.5).

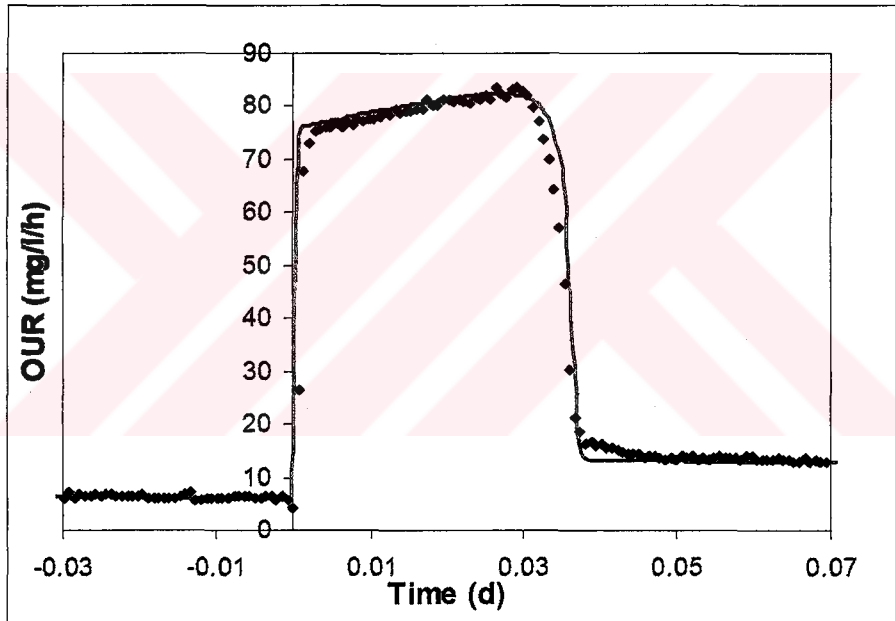
Table 4.5: Kinetic and stoichiometric coefficients obtained for OUR response for a batch test with acetate

Model Coefficient	ASM3	Modified ASM3
$k_{STO}(1/d)$	16	14
K_S (mg/l COD)	4	3
Y_{STO} (gCOD/gCOD)	0.80	0.80
μ_{H1} , (direct growth)(1/d)	-	4
μ_{H2} , (growth on PHB)(1/d)	3 & 1.8	3
K_{STO} (gCOD/gCOD)	1	0.4
Y_{H1} (direct growth)(g cellCOD/gCOD)	-	0.65
Y_{H2} (growth on PHB)(g cellCOD/gCOD)	0.63	0.75
b_{STO} (1/d)	0.24	0.24
b_H (1/d)	0.24	0.24
f_i (gCOD/gCOD)	0.20	0.20

As shown in Figure 4.6, the results have provided strong indication that there was a need for considering direct growth on primary substrate as a significant biological mechanism.



(a)



(b)

Figure 4.6: OUR simulations performed using (a) ASM3 for two different growth rates, (b) modified ASM3

4.2.3 Materials and methods

Experimental studies were conducted with 2 l double jacketed SBR equipped with pH and dissolved oxygen electrodes with a cycle length of 4 hours. The system was operated at pH 7, 20°C and with SRT of 5 days. The culture was enriched on starch and for the batch experiments SBR was fed with glucose during 3 minutes after an idle period of 10 minutes. Aeration and mixing was carried for 130 minutes and sludge was withdrawn in 2 minutes. The settling phase was 90 minutes and the

effluent of 1 l was withdrawn in the last 8 minutes of the cycle. Glucose concentration was adjusted to 180 mg COD/l and the nutrients were supplied in excess amounts. Alythiourea was added to the reactor in the beginning of the cycle to prevent nitrification. The analyses were done as described in the study of **Dircks et al. (2001)**.

4.2.4 Experimental results

Experiments conducted with 182 mg COD/l glucose resulted in the depletion of substrate in 33 minutes (Figure 4.7) and the corresponding glycogen storage was measured as 109 mg COD/l as shown in Figure 4.7. The amount of ammonia nitrogen consumed for growth in the feast phase was 3.5 mg $\text{NH}_4\text{-N/l}$ (Figure 4.7). Assuming a biomass composition of $\text{C}_5\text{H}_7\text{NO}_2$, the corresponding net amount of biomass generated was estimated as 40 mg cellCOD/l. These results for the feast phase were used to calculate the yield for storage of glycogen as 0.90 gCOD/gCOD and the growth yield on glucose was 0.67 g cellCOD/gCOD. Under these experimental conditions 66% of the glucose fed to the system was stored as glycogen and the remaining 34% was used for direct growth. The amount of glycogen consumed in the famine phase was measured as 52 mg COD/l with a corresponding ammonia nitrogen consumption of 1.5 mg $\text{NH}_4\text{-N/l}$ and an estimated net biomass generation of 17 mg cellCOD/l.

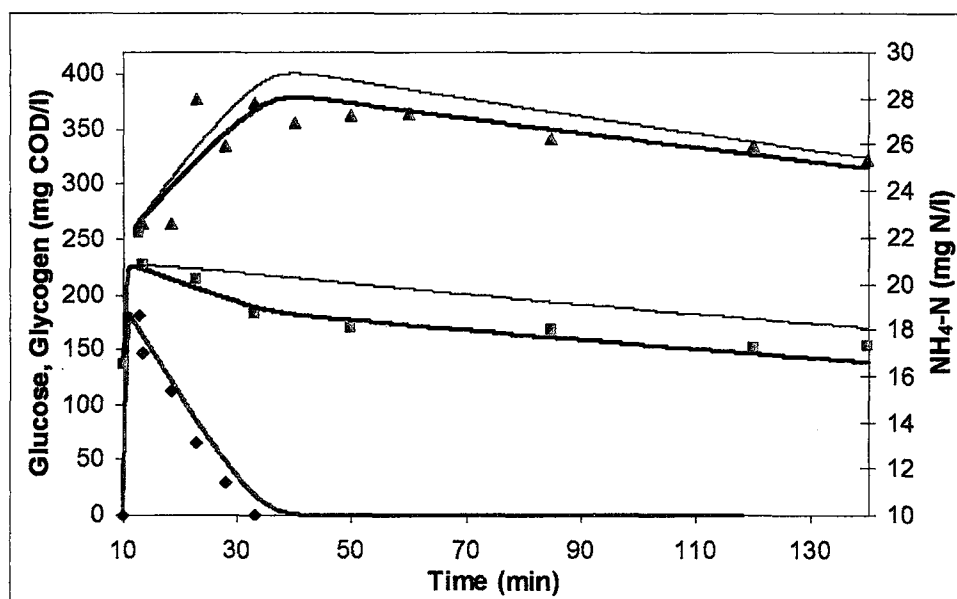


Figure 4.7: Experimental data for ♦ glucose, ▲ glycogen and ■ ammonia and the model simulation results for — ASM3 and - - modified ASM3

The proposed model was simulated for the experimental conditions of the SBR system using AQUASIM[®] (Reichert *et al.*, 1998). The model results are given in Figure 4.8 together with the experimental data.

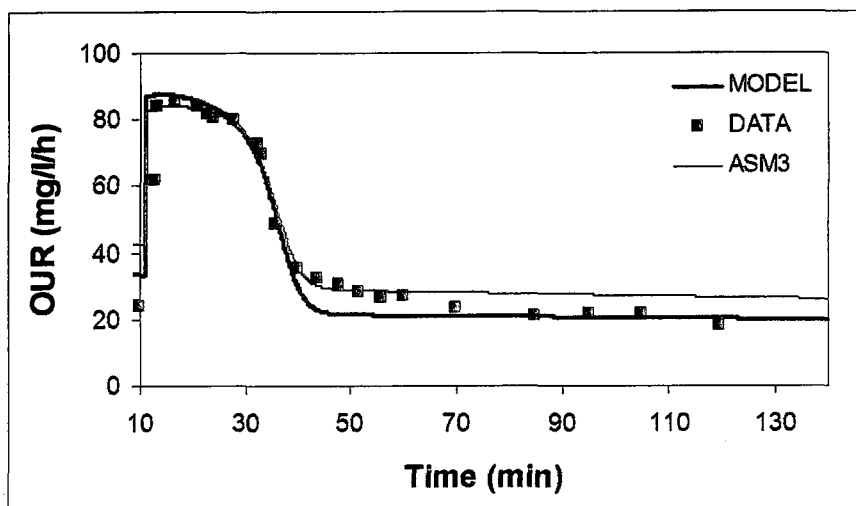


Figure 4.8: OUR data and the model simulation results for proposed model and for ASM3

Respirometry has been extensively used for the experimental assessment of activated sludge models. In this context an OUR profile obtained from the reactor was a useful tool to differentiate ASM3 from its modified version including direct growth on primary substrate competing with storage. The OUR response of the system and the results of model simulation performed for ASM3 and modified ASM3 with the kinetic and stoichiometric coefficients given in Table 4.6 are presented in Figure 4.8.

Table 4.6: Kinetic and stoichiometric coefficients obtained for SBR system fed with glucose

Model Coefficient	ASM3	Modified ASM3
$k_{STO}(1/d)$	12	8
K_S (mg/l COD)	18	18
Y_{STO} (gCOD/gCOD)	0.88	0.90
μ_{H1} , (direct growth)(1/d)	-	2
μ_{H2} , (growth on glycogen)(1/d)	2.5	2.5
K_{STO} (gCOD/gCOD)	1	1
Y_{H1} (direct growth)(g cellCOD/gCOD)	-	0.67
Y_{H2} (growth on glycogen)(g cellCOD/gCOD)	0.60	0.70
b_{STO} (1/d)	0.10	0.10
b_H (1/d)	0.20	0.20
f_I (gCOD/gCOD)	0.20	0.20

The experimental results obtained and the simulations performed with the proposed model have yielded good correlation in terms of the amount of glycogen stored, ammonia nitrogen consumed and the biomass produced. The simulations with ASM3 however, resulted in higher glycogen storage, less ammonia consumption and therefore less biomass production. The substrate utilization and the OUR response of the system under feast conditions were equally well simulated with both models, however ASM3 could not cope up with the decrease of the OUR level in the famine phase. It is clearly seen from the experimental results obtained from ammonia measurements that the feast phase growth rate is higher than that of the famine phase. Thus two different growth rates are necessary to simulate the system response which can also be observed from the OUR data.

The two different growth rates observed in the system are due to two different growth processes occurring, namely growth on primary substrate and growth on storage products. ASM3 with only one growth process can neither predict the amount of sludge produced, nor simulate lower electron acceptor utilization rate in the famine phase. The proposed model with the addition of growth process on primary substrate resulted in two different specific growth rates, where the specific growth rate in the famine phase was 33% of that of the feast phase as shown in Figure 4.9. ASM3 generates a lower growth rate throughout the experiment which gives a lower biomass yield.

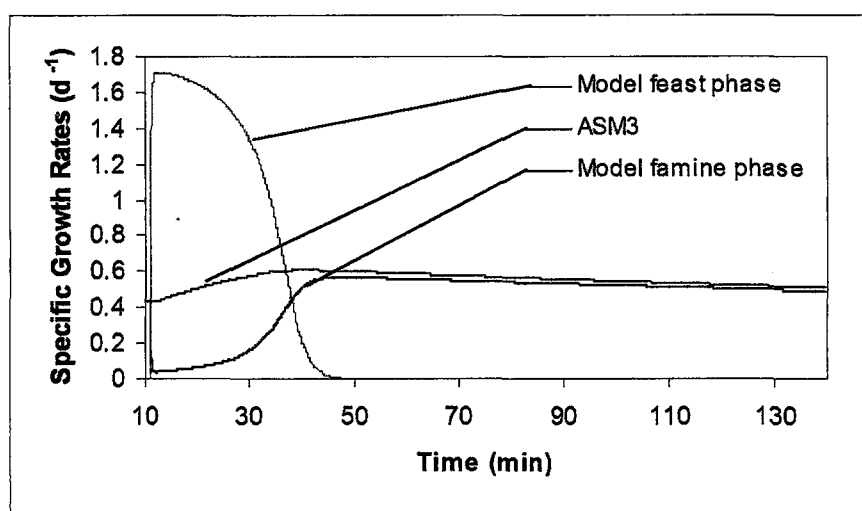


Figure 4.9: Specific growth rates obtained for proposed model and for ASM3

The modeling results for tests conducted with acetate provided a good description of the OUR response. However, the yields of the two growth processes have been found

to be higher than those presented in the literature, since higher endogenous decay rates were assumed for this study according to ASM3, whereas much lower decay rates have been reported for enriched cultures fed with acetate. The modeling results of SBR system fed with glucose yielded a maximum secondary growth rate (μ_{H2}) higher than primary growth rate (μ_{H1}) due to the definition of secondary growth process in terms of surface reaction kinetics.

The main assumption of ASM3 stating that all the readily biodegradable COD is stored and growth only occurs at the expense of storage polymers is not mechanistically valid, since the growth metabolism on the stored products is only activated when external substrate is depleted. Hence, growth on primary substrate occurs simultaneously with substrate storage under dynamic conditions and thus should be taken into account in activated sludge models.

In order to have better predictions for real case applications and batch tests for model parameter determination ASM3 has to be modified in terms of storage and growth process descriptions. The results have provided strong indication that there was a need for considering direct growth on primary substrate as a significant biological mechanism. This study involves a proposed version of ASM3 where a new growth process on external substrate is added. The proposed model gave a better description of the results obtained for batch test in terms of oxygen utilization, glycogen generation and biomass production compared to ASM3 for a selected range of kinetic coefficients.

4.3 Results and Discussion

Respirometric batch tests with acetate have provided consistent data to be used in parameter determination for both aerobic and anoxic conditions. For the determination of aerobic and anoxic model parameters a methodology has been developed. The methodology involved the use of aerobic respirometric data for the estimation of aerobic coefficients by model simulation and substituting the obtained aerobic coefficients in the anoxic data to evaluate the anoxic parameters. The maximum storage rate for acetate was obtained by model simulation applied to the respirometric data. The average storage rate (k_{STO}) of 13 1/d was much higher than the default value of ASM3; however similar results for batch tests have been reported

in the literature. The maximum storage rate found to be representative of the aerobic phase was used for calculating the anoxic correction factor, η_D , using the rate expression for storage. The calculated η_D range was between 0.50 and 0.56 with an average of 0.53. This coefficient would reduce the aerobic storage rate of 13 1/d to approximately 7 1/d under anoxic conditions. The average aerobic heterotrophic growth rate was used in the anoxic simulations to estimate the anoxic heterotrophic growth yield, Y_{HD} . The model simulation outputs yielded an average Y_{HD} value of 0.44 gCOD/gCOD, which is relatively lower than the suggested value of 0.54 in ASM3. The corresponding anoxic correction factor for heterotrophic growth, namely, Y_{HD}/Y_H ratio, was 0.70.

While storage kinetics and stoichiometry could very well be defined, the simulation results of aerobic respirometric data indicated that, it was not quite possible to obtain ASM3 calibration by using a single heterotrophic growth rate, μ_H , value. Apparently two different growth rates were needed in order to simulate OUR data in the storage and growth phases and this finding suggested that the concept of growth on storage products adopted in ASM3 needed to be reevaluated. In this framework, modification of ASM3 considering simultaneous storage and direct growth on readily biodegradable substrate, followed by growth on stored products was considered.

The main assumption of ASM3 stating that all the readily biodegradable COD is stored and growth only occurs at the expense of storage polymers is not mechanistically valid, since the growth metabolism on the stored products is only activated when external substrate is depleted. Hence, growth on primary substrate occurs simultaneously with substrate storage under dynamic conditions and thus should be taken into account in activated sludge models.

Although ASM3 can be used to describe the behavior of activated sludge treatment plants, it fails to simulate the experimental data obtained from batch experiments. The simulation results have shown that, ASM3 predicts higher glycogen storage, lower ammonia consumption and thus lower biomass production. ASM3 cannot cope with the low level of oxygen utilization in the famine phase due to the single growth process, with an average lower specific growth rate. Therefore, the real case applications of ASM3 can have misleading results for sludge production, storage polymer (e.g. glycogen) content of excess sludge and ammonia utilization for heterotrophic growth.

In order to have better predictions for real case applications and batch tests for model parameter determination ASM3 was modified in terms of storage and growth process descriptions. The results have provided strong indication that there was a need for considering direct growth on primary substrate as a significant biological mechanism. This study involved modification of ASM3, where a new growth process on external substrate was added. The proposed model gave a better description of the results obtained for batch test in terms of oxygen utilization, glycogen generation and biomass production compared to ASM3 for a selected range of kinetic coefficients.



5. INVESTIGATION OF SUBSTRATE STORAGE FOR COMPLEX SUBSTRATES

5.1 The Mechanism of Starch Hydrolysis for Substrate Storage[†]

Recognition of substrate fractions with substantially different biodegradation rates may be regarded as one of the most significant milestones in the modeling of activated sludge. This approach differentiates readily biodegradable substrate from its slowly biodegradable counterpart, which represents the major fraction of the complex organics in domestic sewage and industrial wastewaters (Dold *et al.*, 1980; Henze *et al.*, 1987; Orhon and Ubay Çokgör, 1997; Orhon *et al.*, 2002).

Slowly biodegradable substrate, as defined for wastewaters, involves a large spectrum of compounds with different nature and size. In activated sludge models, their biodegradation is conveniently defined by means of a hydrolysis mechanism converting them into simpler readily biodegradable compounds. While this approach provides a useful tool for the interpretation of system behavior and performance, it does not elucidate the true mechanism of the utilization of complex organics. This subject has been an attractive area of experimental research, and starch is selected to represent the slowly biodegradable substrate in the majority of the experiments. Hydrolysis was defined as the rate limiting step of starch utilization (San Pedro *et al.*, 1994). Hydrolytic enzymes were found to be released into the bulk in pure cultures whereas the enzyme activity was mainly associated with the cell surfaces in activated sludge (Goel *et al.*, 1998a).

One of the major concerns in the modeling of activated sludge is the fate of the hydrolysis products. Activated Sludge Model No.1, (ASM1), is structured upon the concept that simpler compounds resulting from hydrolysis are solely utilized for heterotrophic growth (Henze *et al.*, 1987). Activated Sludge Model No.3, (ASM3), involves complete storage of substrate prior to growth (Gujer *et al.*, 2000).

[†] Section 5.1 is a part of the paper entitled: Experimental evaluation of starch utilization mechanism by activated sludge (2005). Submitted to Biotechnology and Bioengineering.

Consequently, there is less emphasis on the extra-cellular hydrolysis of complex organic matter because a significant part of the “slow oxygen uptake rate” is due to growth on stored substrate (Gujer *et al.*, 2000), whereas in ASM1 this is solely due to growth on hydrolyzed substrate. Experiments indicate however that the extent of storage and the level of internal hydrolysis depend much on the parameters of system operation and feast (presence of external substrate) and famine (absence of external substrate) conditions created within the system (Majone *et al.*, 1996). Recent modeling efforts attempt to bring clarification to simultaneous substrate storage and growth (Krishna and van Loosdrecht, 1999a; Beun *et al.*, 2000; Karahan *et al.*, 2003).

The interpretation of the experimental results for design purposes depends on the way in which different COD fractions are defined in the models: ASM3 assumes that the readily biodegradable fraction is the soluble portion of the biodegradable COD after filtration through 0.45 μm membrane filter, while ASM1 postulates that only a part of the soluble portion, which can be quantified by means of respirometry, is readily biodegradable.

This study aims to investigate and evaluate starch utilization in activated sludge by experimental analysis, as an indicative instrument to deepen our understanding of the fate of hydrolysable substrate in suspended growth biological treatment processes. The experiments were designed to generate and interpret data on the rate of starch removal from bulk solution, to evaluate the effect of extra-cellular enzymes on starch removal, the exact site where hydrolysis takes place (*i.e.* floc level or bulk liquid), and the magnitude of substrate storage. The study also includes additional interpretation of experimental results for the kinetic evaluation of starch hydrolysis.

5.1.1 Materials and methods

An aerated sequencing batch reactor (SBR), with a working volume of 2 l was operated at 20°C, with a solids retention time (SRT) of 5 days and 6 cycles/day. The reactor was inoculated with sludge taken from a municipal wastewater treatment plant.

The operation of each cycle started with aeration and mixing and continued for 142 minutes. An idle phase of 10 minutes was provided at the beginning of each cycle, prior to substrate feeding. The total volume of feed was 1 l and was added between

the 10th and 13th minutes of the cycle. Excess sludge was withdrawn during the last 2 minutes the aeration phase. Settling phase was 90 minutes and the effluent was withdrawn during the following 8 minutes.

The feed was added to the reactor in two fractions as carbon source and nutrients. The selected carbon source for the start up and routine operation of SBR was native potato starch (NPS) which was later replaced with soluble starch (SolS), maltose or glucose, for specific periods of experimentation. The experiments on each substrate were carried out as duplicates and/or triplicates and one representative set of results has been presented for each substrate. The COD of the C-sources was adjusted to be around 150 mg/l as the theoretical initial concentration when dump-fed to the reactor, and thus the F/M ratio was maintained as 0.15 gCOD/gCOD in each cycle. The nutrient solution containing 28 mg/l NH₄⁺-N and 16 mg/l PO₄⁻-P was added for nitrogen and phosphorous requirements and to provide additional buffer capacity. The necessary micronutrients were supplied according to the recipe given by **Vishniac and Santer (1975)**. The system was controlled at pH 7.0±0.1 and dissolved oxygen (DO) and pH were monitored with ADI 1060[®] bio-controller and BIODACS[®] software.

Experiments were monitored in the SBR system for each of the four substrates fed to the reactor. The SBR system was operated at steady state, meaning that on-line DO profiles and daily sludge production amounts, in terms of both VSS and TOC, were the same for each cycle. Samples were taken from the reactor at time intervals of 3-5 minutes during the first 50 minutes and 15-20 minutes for the rest of the cycle. These monitoring experimental sets involved measurements of substrate, storage products (*i.e.* glycogen), ammonia, total organic carbon (TOC), dissolved organic carbon (DOC) and oxygen uptake rate (OUR). Allylthiourea was added to the reactor in the beginning of the cycle to prevent nitrification. Glycogen was determined similar to the method described by **Smolders *et al.* (1994)**, namely, by taking 4.5 ml homogenous mixed liquor samples on 0.5 ml 6 M HCl, boiling for 5 h at 100°C, centrifuging and analyzing the supernatant for glucose content with HPLC. Starch measurements were performed with the iodine test (**San Pedro *et al.*, 1994**). DO was always maintained above 2.5 mgO₂/l.

Hydrolytic enzyme activity tests with the effluent of SBR were conducted to spot the portion of hydrolysis taking place in the bulk liquid due to the excreted enzymes.

Data obtained from these tests was also used to quantify the rate of enzymatic hydrolysis and to identify hydrolysis products. For these tests, the effluent of the SBR system was filtered through 0.45 μm membrane filters after centrifugation. Tests were carried out with 500 ml of filtered effluent in an agitated vessel at 20°C. NPS and SolS were added to the system and enzymatic starch degradation was monitored with starch and TOC analysis on the samples taken every 30 minutes during each batch conducted for 3–4 hours.

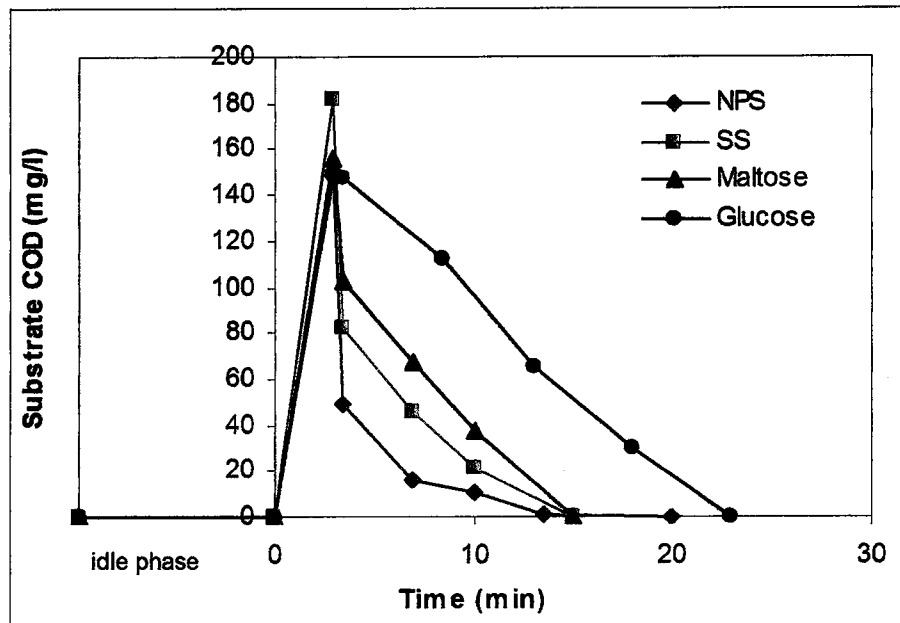
5.1.2 Experimental results

5.1.2.1 Substrate utilization in the SBR system

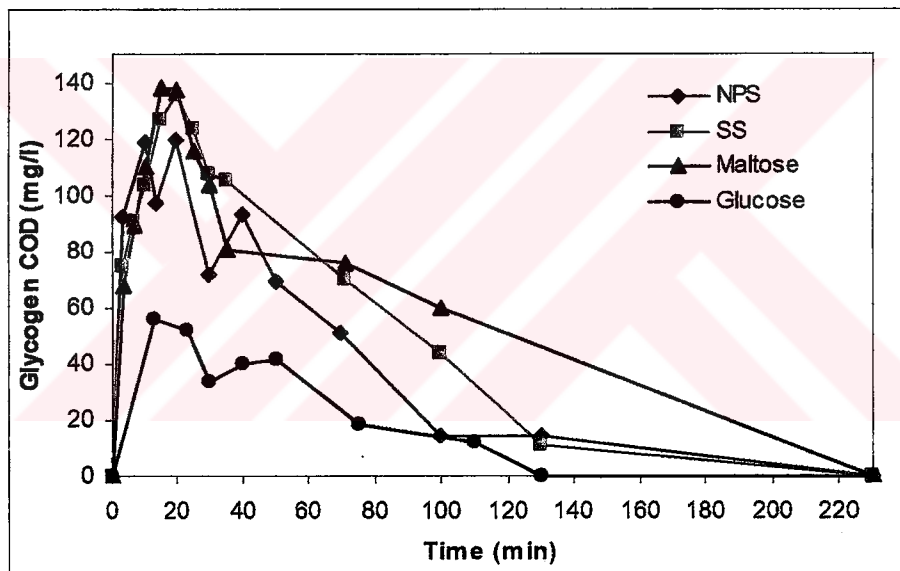
The conversion rate and storage aspects of different substrates were tested in different batch runs with the SBR. The reactor had an average MLVSS concentration of 1250 mg/l and a daily sludge production was 500 mg/d, at a corresponding SRT of 5 days. System responses were monitored when the biomass was fed with native potato starch (NPS), soluble starch (SolS), maltose (as the simplest starch hydrolysis product) and glucose (as the simplest sugar). The experimental results related to the removal of bulk COD are illustrated in Figure 5.1(a) and outlined in Table 5.1.

The results indicate that the removal of all carbohydrates, except glucose, exhibit a similar pattern characterized by a rapid initial uptake, followed by a gradual decrease until complete depletion within 15 minutes after the addition of substrate. However, glucose utilization was slower and completed only after 23 minutes. As indicated in Table 5.1, glucose was taken up at a 50% slower rate than the rates associated with the removal of other substrates

The observations on the removal of different organic carbon sources were coupled with parallel experimental assessment of storage and oxygen utilization. The data on the accumulation and consumption of glycogen in the SBR fed with different substrates is shown in Figure 5.1(b).



(a)



(b)

Figure 5.1: The response of the system to the different substrates in an SBR-cycle
(a) bulk COD concentrations (b) glycogen COD profiles

Maximum amounts of glycogen stored in the biomass were observed to be in the range of 120-138 mgCOD/l for NPS, SolS and maltose but remained at 52 mgCOD/l for glucose, as shown in Table 5.1. Similarly, the highest glycogen accumulation per unit amount of substrate fed was observed for maltose as 0.89 gCOD/gCOD, whereas the lowest storage was associated to glucose as 0.35 gCOD/gCOD. This finding is an indication of the preferential utilization of glucose for direct growth, which is likely to be a direct result of the slower uptake rate of the substrate. It was hypothesized that storage was a balance between substrate uptake and growth rates

(Krishna and van Loosdrecht, 1999a) and much slower glucose uptake in non-acclimatized systems as compared to acclimatized ones was also reported (Dirks *et al.*, 2001). This is most likely due to the need for having a specialized glucose uptake enzyme, which might not be induced in the systems fed with starch. The slow uptake of glucose would result in the prevailing mechanism of growth rather than storage, since there would be no substrate surplus inside the cell which needs to be balanced with the storage process. The rate of glycogen accumulation of glucose was calculated to be only 1/3rd of that of other three multi-unit sugars. The rates of glycogen consumption were observed to be proportional with the amounts of accumulated glycogen, confirming similar previous observations (Dirks *et al.*, 2001).

Table 5.1: Substrate utilization and glycogen results

	Native Potato Starch	Soluble Starch	Maltose	Glucose
Soluble portion of substrate COD (%)	36%	97%	100%	100%
Feast Phase* Length (min)	15	15	15	23
Substrate (mgCOD/l)	150	182	156	149
Substrate uptake rate (mgCOD/l/h)	602	726	624	389
Max. amount of glycogen stored (mgGLY/l)	101	114	116	44
Max amount of glycogen stored (mgCOD/l)	120	136	138	52
Glycogen Storage (mgCOD/mgCOD)	0.80	0.75	0.89	0.35
Glycogen Storage Rate (mgGLY/l/h)	404	458	464	114
Glycogen Consumption Rate (mgGLY/l/h)	28	32	32	13

* The period when external substrate is detected in the bulk liquid.

The oxygen utilization profiles associated with the biodegradation of different substrates tested in the experiments are shown in Figure 5.2. Experimental data gave clear indication that NPS and SolS exhibit a maltose-like behavior, although both compounds first need to be hydrolyzed before utilization. Data displayed in the figure shows that glucose utilization requires significantly higher oxygen consumption as compared to other organic carbon sources.

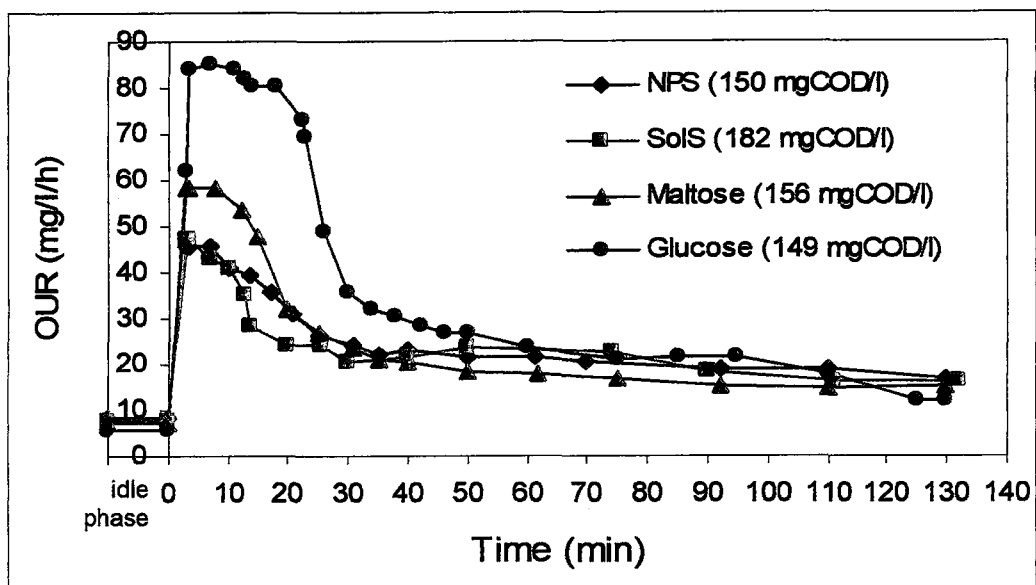


Figure 5.2: Oxygen utilization when different substrates are fed

As outlined in Table 5.2, biodegradation of glucose consumed 58.3 mgO₂/l, about twice the amount of oxygen necessary for NPS, SolS and maltose, for approximately the same level of initial substrate in the reactor. The high amount of oxygen consumption when glucose was fed to the system is a consequence of the lower storage amount and thereby higher amount of substrate being directly consumed for growth in the feast phase.

Table 5.2: Oxygen utilization for different substrates

	Native Potato Starch	Soluble Starch	Maltose	Glucose
Substrate (mgCOD/l)	150	182	156	149
Total Oxygen Consumption (mgO ₂ /l)	52.2	50.8	50.4	72.2
Endogenous Oxygen Consumption (mgO ₂ /l)	19.7	18.1	17.6	13.9
Net Oxygen Consumption (mgO ₂ /l)	32.5	32.7	32.8	58.3

When rapid hydrolysis is concerned, the physical definition of readily biodegradable COD in ASM3 (Gujer *et al.*, 2000), S_s, is not valid, since NPS, SolS and maltose systems consume equivalent amounts of oxygen for the storage of available substrate as glycogen. In this framework, it can be argued that, readily biodegradable COD definition should be based on reaction kinetics rather than physical properties for the correct application of biochemical models. It is however interesting to see that

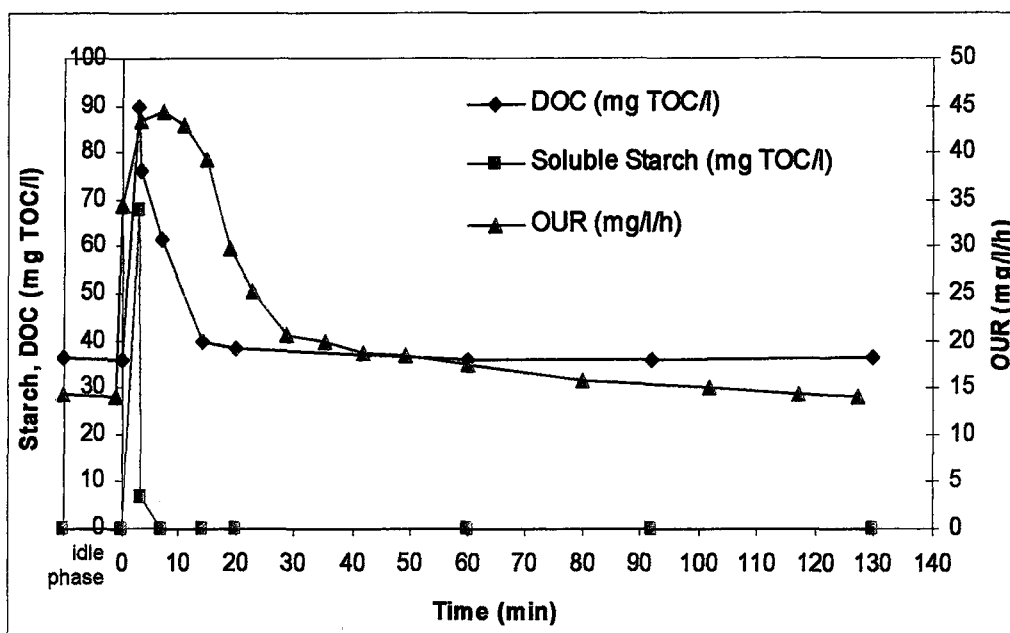
hydrolysable substrate can have the same uptake rates as simple molecules like maltose in this study or acetate in others (Beun *et al.*, 2000).

5.1.2.2 The fate of soluble starch

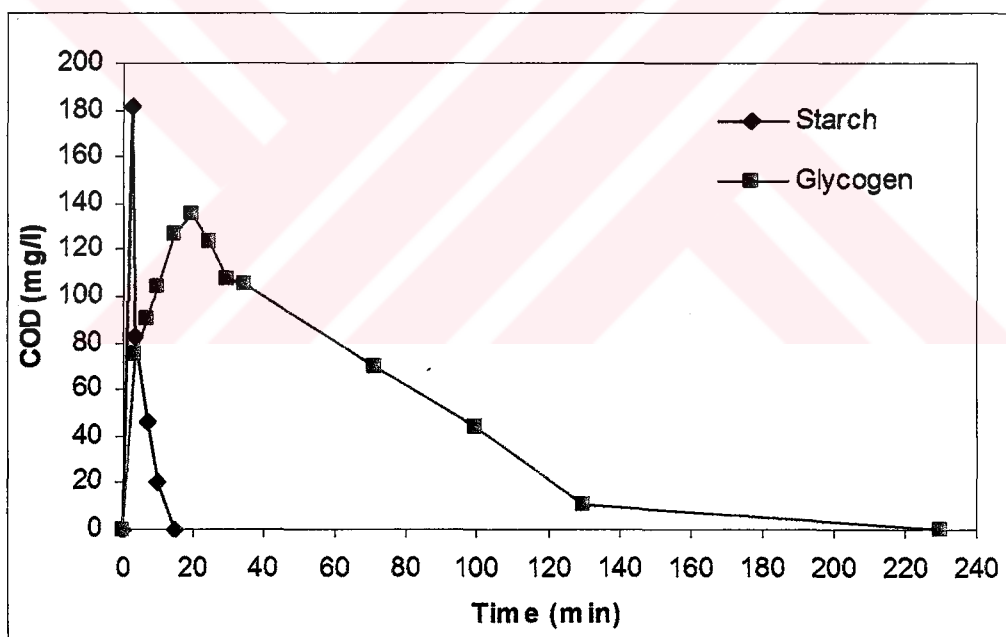
The disappearance of soluble starch, (SolS), in the system was monitored during several cycles of the SBR sequence, in order to achieve a deeper understanding of the related biochemical mechanisms. Filtered mixed liquor samples were taken and analyzed for starch and DOC. Measurements have shown that SolS was immediately removed within the first 10 minutes after addition of the feed due to adsorption in the flocs, while OUR measurement in the reactor showed that substrate was still present but the DOC present was certainly not a sugar. The removed starch was utilized by the biomass leading to a high rate of oxygen consumption during the first 35 minutes after feeding as shown in Figure 5.3(a). When the SBR system was fed with SolS to obtain an initial feed concentration of 182 mg COD/l in the reactor, the immediate disappearance of pulse-fed starch, resulted in 136 mg COD/l glycogen accumulation as shown in Figure 5.3(b).

This is substantiated by the overall mass balance performed on TOC basis as given in Table 5.3. The total TOC in the SolS-fed SBR system was calculated as the sum of DOC (ΔDOC), accumulated glycogen ($\Delta\text{Glycogen}$), produced biomass ($\Delta\text{Biomass}$) and consumed oxygen (ΔOxygen). The biomass produced in the system was calculated from the ammonia consumption in the system using the elemental analysis results which gave an average C:N ratio of the biomass as 5.11 mol/mol.

In order to further substantiate the observation of starch adsorption, 10 ml mixed liquor samples were taken every 5 minutes; 5 ml of these samples containing biomass were directly dyed with iodine and the remaining 5 ml portions were filtered and dyed. The blue color of starch-iodine complex could be observed for 30 minutes on samples with biomass but no more color development was observed in the filtered samples, 8 minutes after feed addition. In this context, the presence of starch could still be detected on the activated sludge flocs for 30 minutes but starch disappeared from the bulk liquid in 8 minutes. This observation, as visualized in Figure 5.4, clearly indicates that starch was rapidly adsorbed on the biomass prior to hydrolysis.



(a)



(b)

Figure 5.3: (a) Oxygen uptake rate, bulk liquid SolS and DOC concentrations (b) COD profiles reflecting starch depletion from the bulk liquid and poly-glucose formation

Table 5.3: The overall mass balance in terms of TOC in the SolS-fed SBR system

Cycle time (min)	Δ DOC (mg TOC/l)	Δ Glycogen (mg TOC/l)	Δ Biomass (mg TOC/l)	Δ Oxygen** (mg TOC/l)	Total TOC (mg TOC/l)
-10.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0
3.0	68.0*	0.0			68.0
3.5	40.1	15.6	12.7	0.9	69.3
7.0	25.7	24.0	14.6	1.9	66.2
14.5	4.0	38.7	18.8	3.9	65.5
20.0	2.5	39.5	21.9	4.8	68.7
60.0	0.1	22.6	33.2	10.0	66.0
92.0	0.0	18.8	35.4	14.3	68.5
130.0	0.3	0.0	49.0	16.5	65.8

* The DOC value used for the 3rd minute is the theoretical amount of SolS fed to the system instead of the measured value of DOC.

**The conversion factor of oxygen to TOC was used as 2.67 mg O₂/mg TOC.

The evaluation of different experimental results and tests on the SBR fed with SolS showed that (i) soluble starch is rapidly adsorbed onto biomass and then undergoes hydrolysis, (ii) some organic metabolic products are released from the flocs as observed by the higher DOC levels in the bulk liquid (Figure 5.3 and Table 5.3), and (iii) hydrolysis is the rate limiting function in the overall utilization of starch, as confirmed by the prolonged pattern of the corresponding OUR curve with respect to depletion of starch. Furthermore, 75% of SolS was stored in the biomass as poly-glucose compounds and was used as the only carbon source after the depletion of external substrate. **Goel *et al.* (1998b)** have reported a carbohydrate accumulation of 282 mg COD/l, when 600 mg/l COD was removed from the bulk liquid after 6 hours, in an aerated batch reactor fed with a starch pulse. The relative amount of starch accumulated as poly-glucose (glycogen, etc.) in this study was observed to be higher than the amount reported by **Goel *et al.* (1998b)**, probably due to different culture history of the biomass and substrate/biomass ratios in the two experiments.

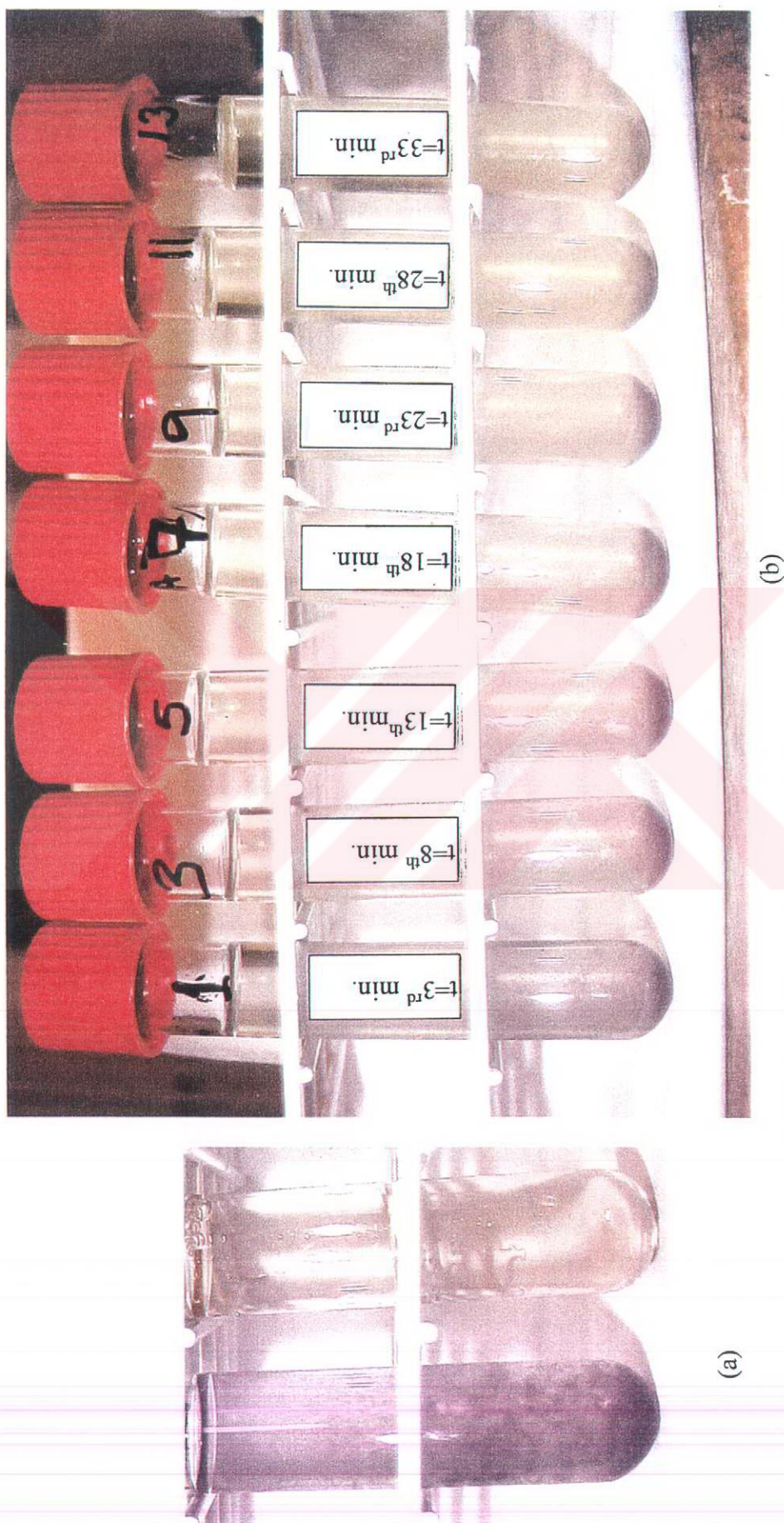


Figure 5.4: Disappearance of soluble starch (a) iodine dyed mixed liquor and filtered samples 8 minutes after feed addition, (b) iodine color fade away in mixed liquor samples

The evaluation of the data presented in Figures 5.3 and 5.4, indicates that after 30 minutes of feed addition OUR decreased accompanied by a similar decrease in the rate of poly-glucose consumption and the sludge was not stained blue anymore: All indicate that after that moment starch is entirely consumed and the measured poly-glucose is most likely internally stored material.

From these experiments it is apparent that in an adapted culture, the hydrolysable substrate, (i.e. soluble starch) is rapidly removed from the bulk liquid, after which a rate limiting hydrolysis process occurs. A significant fraction of the hydrolysis products is stored by the cells, and then used in the famine phase for growth processes.

5.1.2.3 Hydrolytic enzyme activity

Batch tests with the filtered effluent of the SBR fed with NPS were used to determine the hydrolytic enzyme activity. The initial concentrations of NPS and SolS ranged from 280 to 490 mg/l. The amount of starch degraded in each batch is shown in Figure 5.5(a).

It was suggested that the rate of enzymatic hydrolysis of starch can be expressed with a zero-order rate expression (Mino *et al.*, 1995), where the rate constant is a function of temperature (T) and active enzyme concentration (E) as given in equation 5.1.

$$\frac{dX_s}{dt} = k(T, E) \quad (5.1)$$

Starch degradation rates calculated for the enzymatic activity tests in this study were in the range of 1.0-1.7 mg starch/l/min. This level of starch utilization rate is much lower than the reported zero-order process rate (32 mg starch/l/min) in the tests with pure enzyme, α -amylase (Mino *et al.*, 1995) and then the rates of starch hydrolysis in the SBR. This indicates that the amount and therefore the activity of enzymes in the bulk liquid of SBR is low, an observation confirming that starch hydrolysis mostly occurs inside the activated sludge flocs in the SBR system. Another important point to be noted for the hydrolytic enzyme activity of SBR system is that no significant difference has been observed for the decomposition of SolS and NPS, despite the particulate nature of NPS.

A comparison was carried out for hydrolysis with and without biomass. The results have shown that only 5% of the hydrolysis takes place in the bulk liquid and the rest

of starch hydrolysis occurs on the biomass. The rate of starch disappearance with biomass was 18 times faster than that of the hydrolytic activity test without biomass, when the degradation rates were assumed to be zero-order with respect to substrate concentration, as in Figure 5.5(b). Apparently, in activated sludge systems, adsorption mechanism successfully competes with extra-cellular hydrolytic enzyme activity. The experiments verified adsorption as the dominant mechanism and that starch was virtually not hydrolyzed in the bulk liquid.

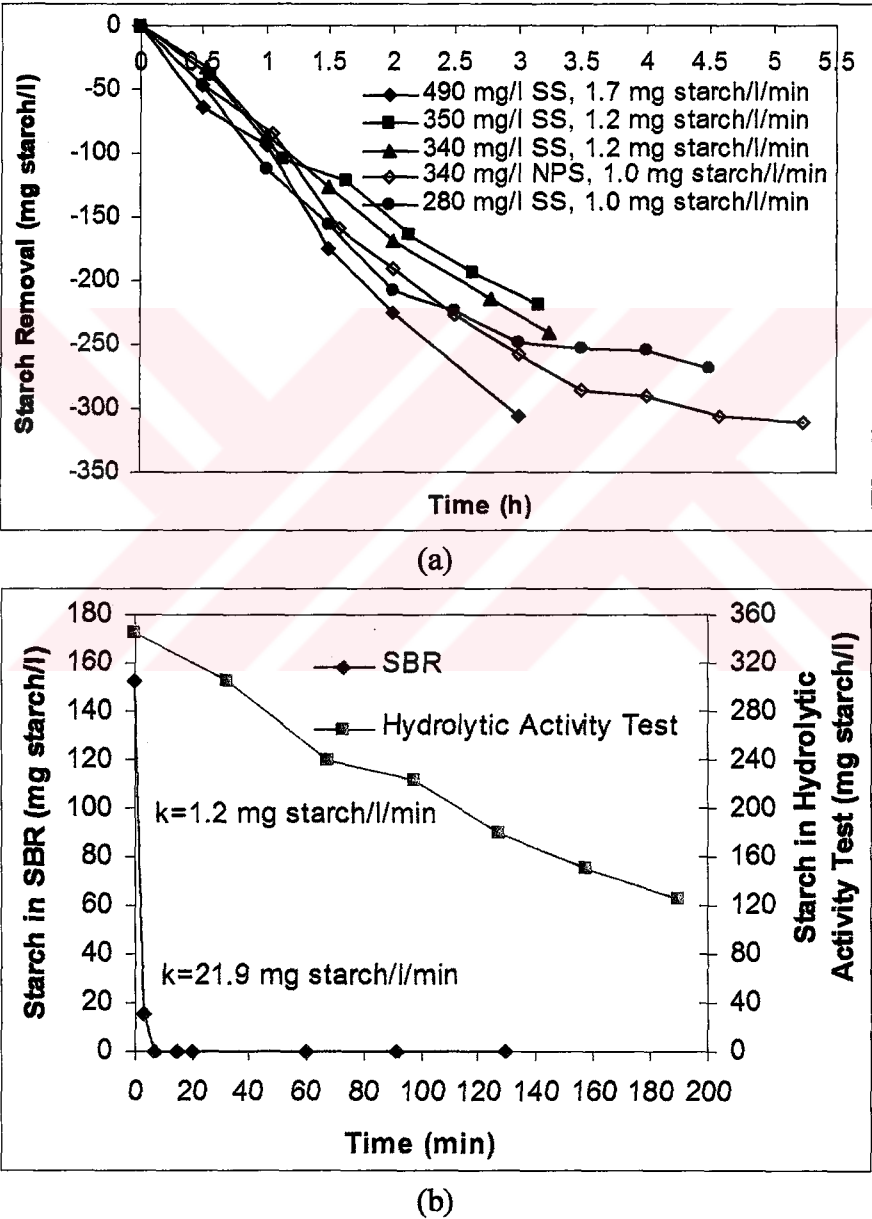


Figure 5.5: (a) The removal of SolS and NPS in the hydrolytic enzyme activity tests run with different initial starch concentrations (b) Comparison of starch removal in the reactor and in the bulk liquid

ASM3, as all current activated sludge models, define hydrolysis as the first step in the sequence biochemical reactions describing the fate slowly biodegradable substrates such as starch. In these models, hydrolysis is associated with a surface limited reaction kinetics involving two rate coefficients, namely the maximum rate of extra-cellular hydrolysis, k_h and the half saturation coefficient of hydrolysis, K_X . ASM1 suggested $k_h = 2$ 1/d and $K_X = 0.1$ gCOD/gCOD as default values for domestic sewage. Orhon *et al.* (1999) reported $k_h = 2.6$ 1/d and $K_X = 0.45$ gCOD/gCOD, characterizing domestic sewage, based on respirometric measurements. Hydrolysis of starch was defined by first order kinetics with respect to starch concentration, with an average rate constant of 3.7 1/d for an aerobic-anaerobic activated sludge culture, independent of starch and MLVSS concentrations (Mino *et al.*, 1995). The high levels for the saturation constant used in other studies (Henze *et al.*, 1987; Orhon *et al.*, 1999) would also imply effectively a first order reaction. Accordingly, it would be more appropriate to describe the hydrolysis of polymers with a first order rate equation.

The kinetic parameters for starch hydrolysis were estimated as k_h as 30 1/d and K_X as 0.3 gCOD/gCOD, according to related surface reaction kinetics incorporated in ASM models. These values, derived from the calibration of the soluble starch concentration profile, are extremely high and they may be interpreted as a supporting indication of the dominant adsorption mechanism responsible for the disappearance of soluble starch from solution. The OUR profile also indicates that the hydrolysis step following rapid adsorption is quite fast, since the microbial culture used in the study was adapted to starch as the only substrate. Furthermore, the affinity constant ($K_X = 0.3$ gCOD/gCOD) is much higher than the starch/sludge ratio immediately after feeding ($F/M = 182/1750 = 0.1$ gCOD/gCOD), again indicating that soluble starch hydrolysis effectively followed a first order reaction kinetics.

The end products of enzymatic hydrolysis of starch analyzed with high-pressure liquid chromatography, (HPLC), revealed maltose as the predominant sugar species instead of glucose as given in Figure 5.6, confirming similar results previously reported (Ubukata 1998; Ubukata 1999).

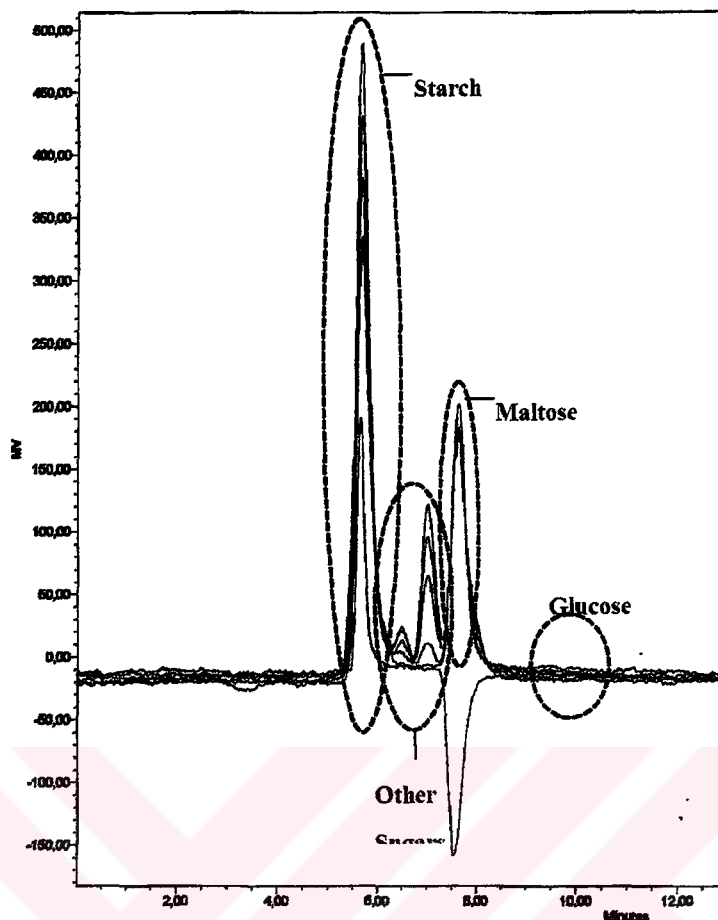


Figure 5.6: HPLC chromatogram for enzymatic hydrolysis products

5.1.3 Discussion

Interpretation of the experimental data collected in this study leads to conclude that adsorption is the dominant mechanism for the removal of both soluble and particulate starch by activated sludge. Starch is adsorbed on the biomass and most of the enzymatic hydrolysis occurs within the flocs. Adsorption proceeds at a much faster rate than hydrolysis and the subsequent storage and growth processes, evidenced by the lag between starch removal, storage polymer formation and oxygen consumption. Starch hydrolysis does not yield glucose but mostly maltose and other multi-unit sugars, which are also stored as poly-glucose.

Starch should be considered a slowly biodegradable (hydrolysable) substrate in ASM models, in contradiction to the physical fractionation of biodegradable COD in ASM3. The evaluation of the experimental results of this study indicates that the hydrolysis rate of starch is relatively fast giving an oxygen uptake response curve similar to that of a rapidly biodegradable substrate. This again underlines that

mechanistic interpretations based on ASM1 and ASM3 can lead to biased conclusions. The hydrolysis process in ASM1 and ASM3 can correctly describe the conversion process, however hydrolysis, by nature, seems to be a first order process.

5.2 Modeling Activated Sludge Systems with Simultaneous Substrate Storage and Growth Concept[†]

Modeling is always regarded as an indispensable tool for the understanding and optimization of substrate utilization by activated sludge. In the early efforts, substrate removal has been solely attributed to microbial growth. This has also been the basis of Activated Sludge Model No.1 (ASM1), which played a pioneering role in multi-component modeling, where microbial growth is defined as the only mechanism for the final utilization of different type of substrate components (Henze *et al.*, 1987). Experimental observations indicated however that for systems subject to a dynamic feeding pattern sustaining sequential *feast and famine* conditions, conversion of external substrate to storage biopolymers becomes the primary metabolic process (van Loosdrecht *et al.*, 1997). A new model (ASM3) was then proposed introducing biochemical storage as the only way for the utilization of the readily biodegradable substrate (Gujer *et al.*, 2000).

Supporting experimental studies using ASM3 for model simulations have mostly been performed with synthetic substrates (Beun *et al.*, 2000; Dircks *et al.*, 2001; Avcioglu *et al.*, 2003). The limited application of the model for domestic sewage (Dircks *et al.*, 1999; Gujer *et al.*, 2000; Karahan *et al.*, 2002) and for industrial wastewaters (Dizdaroglu-Risvanoglu *et al.*, 2004) have has mostly suffered from the definition of readily biodegradable substrate and/or from the correct assessment of storage stoichiometry. In fact, the assumption of total conversion of external substrate to storage, although a useful simplifying approach, may not be sufficiently acceptable in some cases since substrate storage process only serves as a tool to maximize substrate uptake and simultaneous growth on the readily biodegradable substrate should also be incorporated in the model (Khrishna and van Loosdrecht, 1999b; Karahan *et al.*, 2003). Biochemical models on pure substrates also pointed

[†] Section 5.2 is a part of the paper entitled: A new model with simultaneous storage and microbial growth for the utilization of starch by activated sludge systems (2005). Submitted to Biotechnology and Bioengineering.

out that there was substantial evidence for simultaneous utilization of primary substrate for growth and storage for pure cultures (**van Aalst-van Leeuwen *et al.*, 1997**) and for mixed cultures (**Beun *et al.*, 2000; Dircks *et al.*, 2001**).

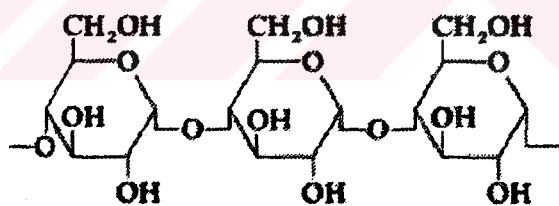
Starch has been used as the model substrate in order to investigate the fate of slowly biodegradable substrate in many studies (**Mino *et al.*, 1995; Goel *et al.*, 1998**). Being slowly biodegradable in terms of microbial utilization, starch first has to go through extra-cellular hydrolysis prior to its consumption by biomass. Recent studies have also shown that starch rapidly disappears from the bulk liquid and is adsorbed on the biomass before being hydrolyzed (**Karahan *et al.*, 2005a**). Consideration of starch as a substrate which undergoes a rate limiting hydrolysis process is also interesting when ASM3 is used, because (i) this model would categorically define soluble types of starch as a readily biodegradable substrate, thus disregarding hydrolysis and distorting process kinetics, and (ii) the concept of dynamic conditions with respect to substrate feeding would not be totally relevant since with the slow hydrolysis process activated sludge culture would be exposed to a low concentration of substrate for a much longer period compared to dynamic feeding, likely to results in primary growth, instead of short duration of high substrate concentrations that would lead to substrate storage. Thus, it is necessary to integrate primary growth in the activated sludge models, simultaneously consuming substrate while the excess substrate taken up by the biomass is stored.

In this context, the objective of the study was to define a new mechanistic model incorporating microbial growth on external substrate with simultaneous formation of storage biopolymers, for the utilization of starch by activated sludge. The model was basically structured as a combination of ASM1 and ASM3, differentiating substrate removal and utilization by including removal of starch through adsorption onto biomass prior to its hydrolysis. Model description and calibration was based upon experiments conducted with particulate Native Potato Starch (NPS) and Soluble Starch (SolS) selected as model substrates, using a sequencing batch reactor operated at a sludge age of five days. The experiments were designed to elucidate the significant biochemical mechanisms involved interpreting the OUR profile together with measurements of starch and glycogen, the relevant storage biopolymer.

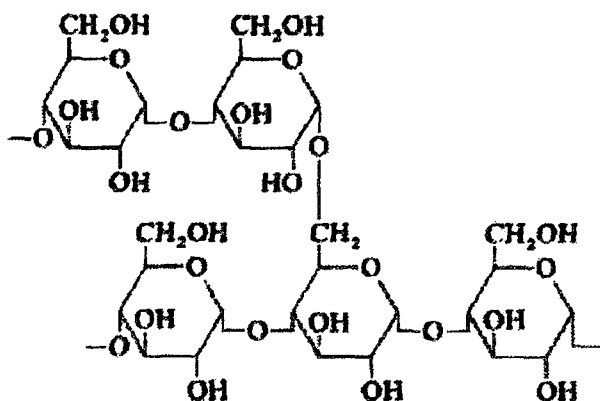
5.2.1 Materials and methods

5.2.1.1 Selection of substrate

In order to investigate the fate of hydrolysable substrates under aerobic conditions starch has been selected as a model substrate, because it is a long chain sugar, which needs to go through extracellular hydrolysis prior to its utilization by the microorganisms (San Pedro *et al.*, 1994). Two types of starch have been selected to predict the biochemical transformations for hydrolysable substrates in this study, namely Native Potato Starch (NPS) and Soluble Starch (SolS). NPS can be characterized by 64% particulate and 36% soluble COD. SolS is on the other hand 97% soluble and has only 3% particulate COD fraction after filtration through 0.45 μm pore sized membrane after sterilization at 110°C. The difference in the solubility characteristics of the two types of starch is due to the difference in their molecular structure. Starch, in general is composed of two different kinds of molecules: a linear molecule with 1,4- α -D glucose linkages, called amylose (Figure 5.7) and a branched molecule with both 1,4 and 1,6- α -D glucose linkages, amylopectin. The ratio of amylose and amylopectin molecules that starch is composed of plays an important role in determining the unique physical properties of each type of starch.



(a) Representative structure of linear amylose



(b) Representative structure of amylopectin, including (1,6)- α -D branch point

Figure 5.7: Two main molecules that compose starch (a) amylose (b) amylopectin

Hydrolysis of starch involves the breakage of the linkages between the glucose units, either enzymatically or by means of a strong acid. Since amylose and amylopectin have a number of sites for hydrolysis, there is nearly an infinite number of combinations of hydrolysis products, such as; glucose, maltose, isomaltose, *etc.* The investigation of starch hydrolysis have also shown that starch is a polysaccharide which is degraded to maltose, malto-tri-ose, and/or other two-three unit sugars by extracellular hydrolysis (Ubukata 1999; San Pedro *et al.*, 1994; Karahan *et al.*, 2005a).

5.2.1.2 Experimental setup

An aerated bioreactor of 2 l was operated at 20°C as an SBR with 6 cycles/day. The reactor was started up with biomass taken from a municipal wastewater treatment plant and was fed with NPS for the enrichment of biomass. The SBR cycle of 4 hours, started with an idle period of 10 minutes, where mixing and aeration was started immediately. The feeding period was adjusted as the first 3 minutes of the reaction phase (130 minutes). After sludge withdrawal in 2 minutes, the settling phase was chosen as 90 minutes and the effluent was discharged in the last 8 minutes of the cycle. The bioreactor was operated with a sludge age of 5 days. The F/M ratio of the reactor was around 0.1 gCOD/gCOD, with an average MLVSS content of 1.5 gCOD/l during the operation period of 6 months. Nutrient addition was secured for nitrogen and phosphorous requirements and to supply additional buffer capacity. The necessary micronutrients were added according to the recipe given by Vishniac and Santer (1975). The SBR system was controlled at pH 7 ± 0.1 and dissolved oxygen (DO), pH and added acid-base amounts were monitored with ADI 1000® bio-controller and BIODACS® software. DO was always above 2.5 mgO₂/l.

5.2.1.3 Analytical program

The SBR system responses to NPS and SolS were monitored with regular sampling during one cycle. Allylthiourea was added to the reactor in the beginning of the cycle to prevent nitrification. Oxygen uptake rate (OUR) was measured in the cycle prior to sampling. The monitoring experiments involved sampling for substrate (starch) and storage products (*i.e.* glycogen). Total and soluble TOC, starch and glycogen analyses were carried out on the samples. The analyses were done as described in the study of Dircks *et al.* (2001). The disappearance of starch was followed with iodine

dyeing as described by **San Pedro *et al.* (1994)** and through glucose measurements with HPLC after acidic hydrolysis of the bulk liquid. Five set of experiments were conducted with the SBR system described above, using NPS for 3 sets and SolS in 2 sets during 6 months of operation.

5.2.2 Conceptual basis of modeling

A new activated sludge model, (Activated Sludge Model for Growth and Storage-ASMGS) is proposed for the utilization of starch, as a combination and adaptation of ASM1 and ASM3, with simultaneous substrate storage and growth concept, together with the addition of adsorption. Based on the experimental observations reported by **Karahan *et al.* (2005a)** and supporting information by **San Pedro *et al.* (1994)**, the mechanism of starch utilization adopted for the structure of the new model has been schematically described in Figure 5.8: Starch, whether NPS or SolS (X_S), is first adsorbed on the biomass (X_{Sads}), then goes through extracellular hydrolysis and is converted mostly to maltose (S_S). Hydrolysis is almost always considered as the rate limiting process for further utilization of generated readily biodegradable substrate. Accordingly, maltose is taken up by active transport into the cell and used primarily for the generation of new biomass (X_H). This assumption basically relies upon the fact that a microbial community under balance growth, *i.e.*, a preset net growth rate at steady state, develops and sustains a protein synthesis system that would optimize substrate utilization, diverting it to growth to the extent that is necessary (**Grady *et al.*, 1996**). Depending on the operating conditions of the system, *i.e.*, steady state growth rate of the activated sludge culture, it is expected that maltose uptake rate would be faster than the rate of growth on maltose, creating a maltose surplus in the cell. Excess amount of maltose present is converted to glycogen (X_{STO}) polymer. After the consumption of the primary readily biodegradable substrate, secondary growth process is carried on the stored glycogen in the famine phase.

The mechanism described above is used to formulate the proposed the new activated sludge model, ASMGS, incorporating simultaneous growth and substrate storage with adsorption, differentiating between substrate removal and substrate utilization, when adsorption and the rate limiting hydrolysis processes are present as in the case of starch. The matrix representation of the proposed model is given in Table 5.4.

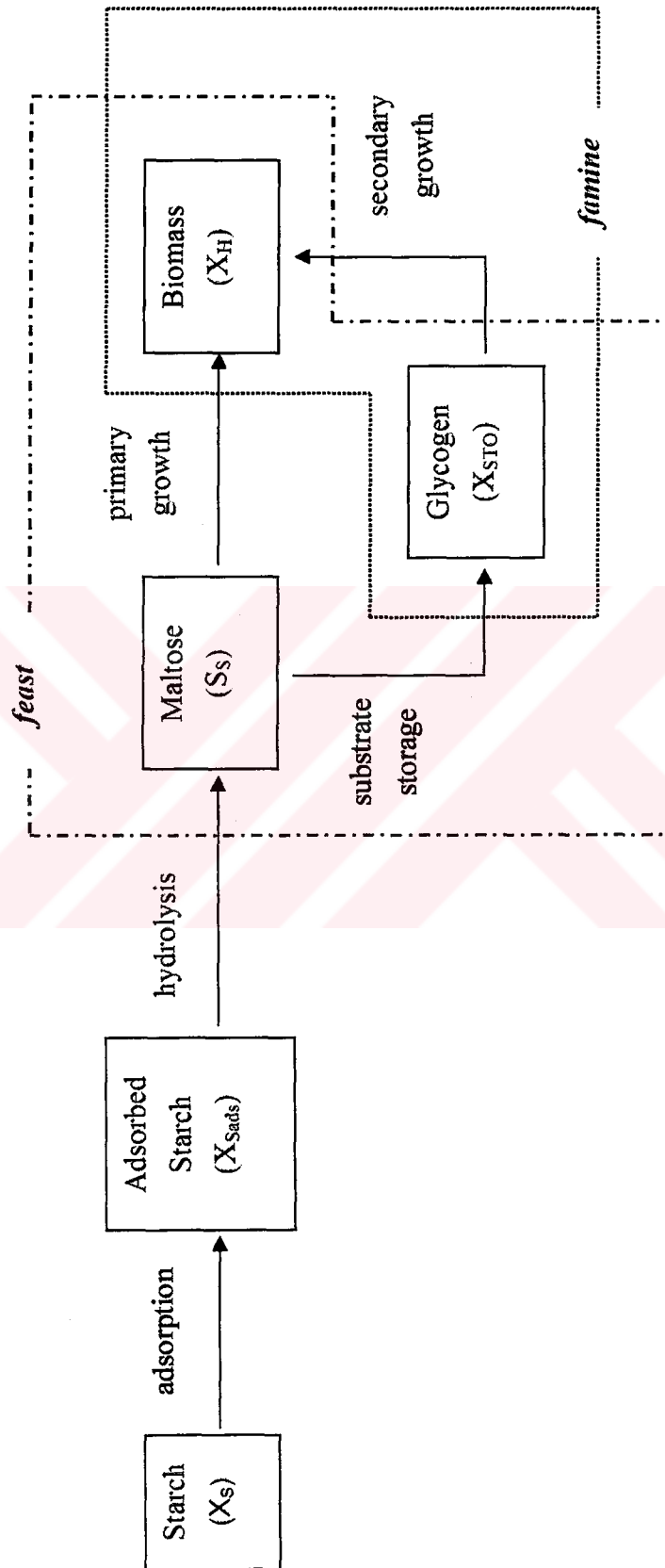


Figure 5.8: The Proposed Mechanism of Starch Utilization for Modeling

Table 5.4: Matrix representation of the proposed model for starch utilization

Component	S_o	S_i	S_s	X_i	X_s	X_{Sads}	X_H	X_{STO}	Rate
	O_2	COD	COD	COD	COD	COD	COD	COD	
Adsorption					-1	1			$k_{ads} X_S$
Hydrolysis		f_{SI}	$1-f_{SI}$			-1			$k_H \frac{X_{Sads}/X_H}{K_X + X_{Sads}/X_H} X_H$
Growth on S_s	$-\frac{1-Y_{H1}}{Y_{H1}}$		$-1/Y_{H1}$				1		$\mu_{H1} \frac{S_o}{K_o + S_o} \frac{S_{NH}}{K_{NH} + S_{NH}} \frac{S_{HCO}}{K_{HCO} + S_{HCO}} \frac{S_s}{K_{S1} + S_s} X_H$
Aerobic Storage of COD	$-(1-Y_{STO})$		-1					Y_{STO}	$k_{STO} \frac{S_o}{K_o + S_o} \frac{S_s}{K_{S2} + S_s} X_H$
Growth on X_{STO}	$-\frac{1-Y_{H2}}{Y_{H2}}$						1	$-1/Y_{H2}$	$\mu_{H2} \frac{S_o}{K_o + S_o} \frac{S_{NH}}{K_{NH} + S_{NH}} \frac{S_{HCO}}{K_{HCO} + S_{HCO}} \frac{K_{S1}}{K_{S1} + S_s} \frac{X_{STO}}{K_{STO} + X_{STO}} \frac{X_H}{X_H}$
Endogenous Respiration	$-(1-f_i)$			f_i			-1		$b_H \frac{S_o}{K_o + S_o} X_H$
Respiration of X_{STO}	-1							-1	$b_{STO} \frac{S_o}{K_o + S_o} X_{STO}$

Adsorption process in the new model has been defined with first order kinetics, since substrate disappearance was reported to be rather fast (**Karahan *et al.*, 2005a**). Slowly biodegradable substrate, X_S , is converted into X_{Sads} , and the adsorbed substrate is hydrolyzed with a rate characterized by surface reaction kinetics as in ASM models. Hydrolysis process is presumably rate limiting for all readily biodegradable substrate utilization processes. Since a slower pace of substrate utilization is dictated by hydrolysis, it is highly possible for the biomass culture to primarily utilize the available substrate via growth. Simultaneous substrate storage and growth concept has been modeled as described previously by **Karahan *et al.* (2003)** and secondary growth process is suppressed with a switch function during the presence of primary substrate. Both primary and secondary growths are dependent on dissolved oxygen, ammonia nitrogen and alkalinity.

5.2.3 Model evaluation of experimental results

Model evaluation utilized experimental data obtained from a laboratory-scale SBR operated at steady state with a sludge age of five days. The operation was started with Native Potato Starch (NPS) feeding which was switched to Soluble Starch (SolS) for related experimental assessment. The experimental data basically involved observation of the concentration profiles of the two significant model components, starch and glycogen, together with the oxygen uptake rate, (OUR), profile throughout selected operation cycles at steady state. Results of five sets of experiments conducted with the SBR system, using NPS in three sets and SolS in the other two sets were calibrated and evaluated with the proposed model. Selected representative results are given in Figures 5.9-5.14. Detailed evaluation of the experimental data in terms of starch utilization mechanism has been presented in the study of **Karahan *et al.* (2005a)**.

5.2.3.1 Support of process stoichiometry for modeling

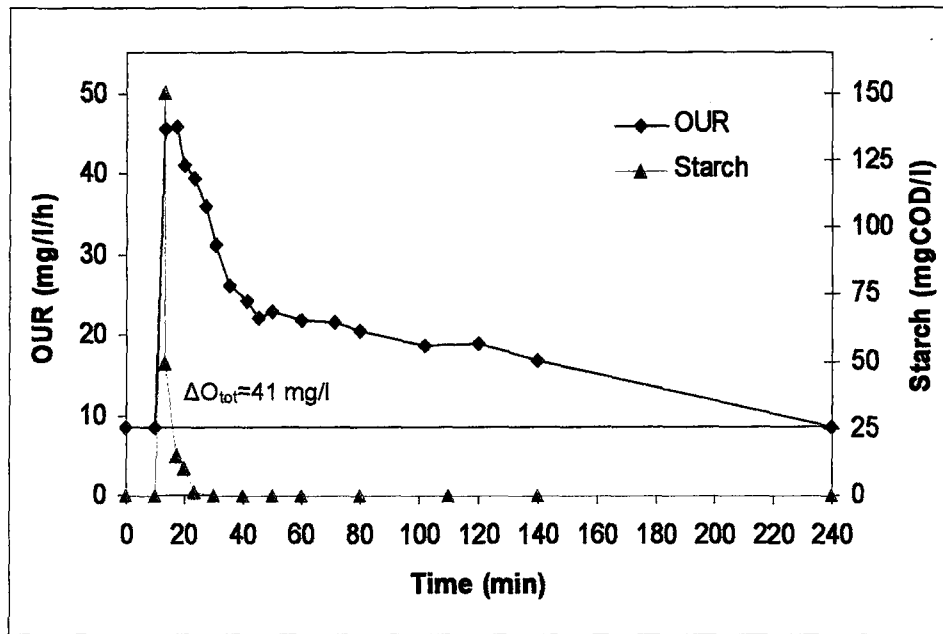
A sound modeling approach can only be possible when the system under concern is mechanistically well defined. Obtained experimental data, therefore should be used to reveal information on the biochemical transformations in the system. In this respect, the observed OUR response, together with the substrate and glycogen data, obtained for the experiments conducted for different cycles of SBR, can be used to evaluate the stoichiometric relationships in the system. Experimental results of

conducted experiments are given in Table 5.5. The evaluation of the transformations in the system however, is dictated by the assumptions on which different models are based. Table 5.5 includes the calculated amounts of oxygen consumed for different phases described and the relevant stoichiometric parameters estimated in the models, ASMGS and ASM3.

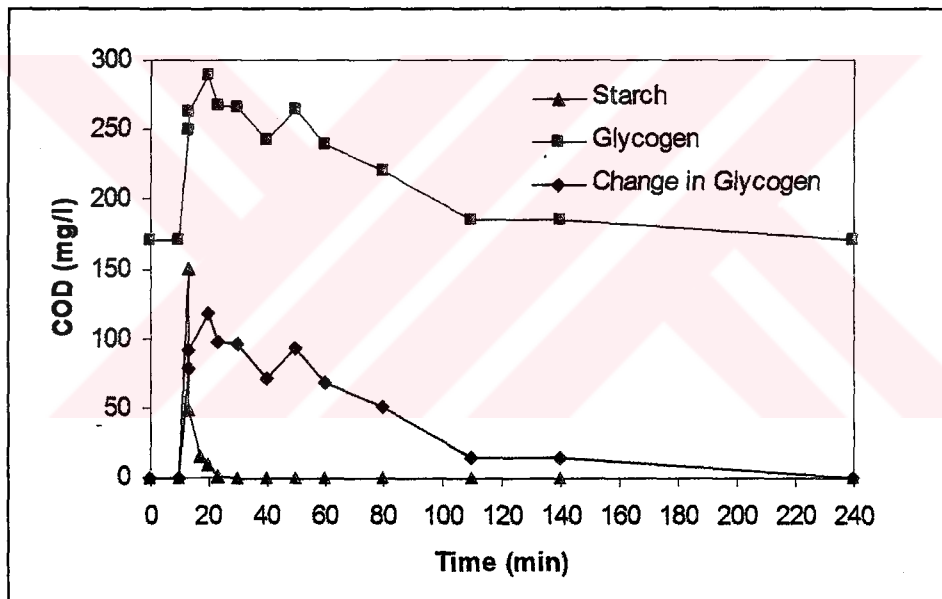
Table 5.5: Stoichiometric evaluation of experimental results

Parameter	Unit	NPS Experiments			SolS Experiments	
		Set 1	Set 2	Set 3	Set 4	Set 5
Starch feed (S_{S1})	mgCOD/l	150	200	183	142	183
Max. amount of glycogen stored (X_{STOmax})	mgCOD/l	119	161	150	108	136
Overall net oxygen consumption (ΔO_{tot})	mgO ₂ /l	41	51	49	30	34
Oxygen consumption for storage ($\Delta O_{stoASM3}$)	mgO ₂ /l	9.4	11.4	9.6	9.5	10.0
Oxygen consumption for growth ($\Delta O_{groASM3}$)	mgO ₂ /l	31.6	39.6	39.4	20.5	24.0
Feast phase length according to OUR	minutes	25	27	24	23	23
Oxygen consumption in the feast phase (ΔO_{feast})	mgO ₂ /l	13	16	17	13	13
Oxygen consumption in the famine phase (ΔO_{famine})	mgO ₂ /l	28	35	33	17	21
Y_{NET}	gCOD/gCOD	0.73	0.74	0.73	0.79	0.82
$Y_{STO,ASM3}$	gCOD/gCOD	0.94	0.94	0.95	0.93	0.95
$Y_{H,ASM3}$	gCOD/gCOD	0.78	0.79	0.77	0.85	0.86
$Y_{STO, GLY}$	gCOD/gCOD	0.79	0.81	0.82	0.76	0.74

A typical data set (Set 1 fed with NPS) is given in Figure 5.9, to provide illustrative explanations on the stoichiometric considerations for modeling presented in Table 5.5. As Figure 5.9 shows, the amount of substrate fed to the system was already determined by the experimental setup and OUR and glycogen concentration in the system was monitored during the cycle. Since the system is an SBR operated under steady state conditions, there is already a glycogen pool present in the mixed liquor activated sludge culture and therefore the amount of glycogen produced and consumed in the system during one cycle is demonstrated with the change in glycogen due to starch fed in the beginning of the cycle, as shown in Figure 5.9(b).



(a)



(b)

Figure 5.9: Typical cycle measurement results, (a) OUR response and fate of starch, (b) glycogen accumulation and degradation observed (Experimental data from Set 1 with NPS)

When the system is disturbed by a pulse feed of substrate, the observed net yield, Y_{NET} , is defined as the overall yield of the system until the system reaches its initial endogenous response. This whole period involves substrate utilization, growth either on primary or stored substrate, and the endogenous decay of produced biomass due to the substrate fed. This period also provides information on the observed net yield of the system when fed with a specific substrate. The observed net yield, Y_{NET} , therefore, shows how efficient a substrate can be utilized by the system. This

parameter may well be used to evaluate the differences in the system response for NPS and SolS and as an indication of the value of heterotrophic yield for modeling purposes. Y_{NET} can be calculated using the OUR curve. The area under the whole OUR curve and above the endogenous OUR level, determined in the beginning of the cycle before substrate addition (Figure 5.9), represents the amount of oxygen utilized for the consumption of the specific amount of substrate fed to the system (ΔO_{tot}). The net heterotrophic yield observed, Y_{NET} , is estimated using the following relation, with the example for Set 1.

$$Y_{NET} = 1 - \frac{\Delta O_{tot}}{S_{S1}} = 1 - \frac{41}{150} = 0.73 \text{ gCOD/gCOD} \quad (5.2)$$

The storage yield, Y_{STO} , in terms of ASM3 can also be calculated using the OUR curve, with the assumption that both NPS and SolS as readily biodegradable. The amount of oxygen utilized for substrate storage is determined using the area under the OUR curve and above the straight line drawn from the endogenous decay level to the inflection point on the OUR curve, which is the end of the feast phase according to ASM3, as given in **Karahan *et al.* (2002)**. Figure 5.10 shows the specific area used for the assessment of Y_{STO} , as dictated by ASM3 if the substrate is readily biodegradable ($\Delta O_{stoASM3}$). Both types of starch with rapid observed disappearance can be assumed as readily biodegradable for ease in numerical evaluation. The storage yield can be estimated as in equation 5.3.

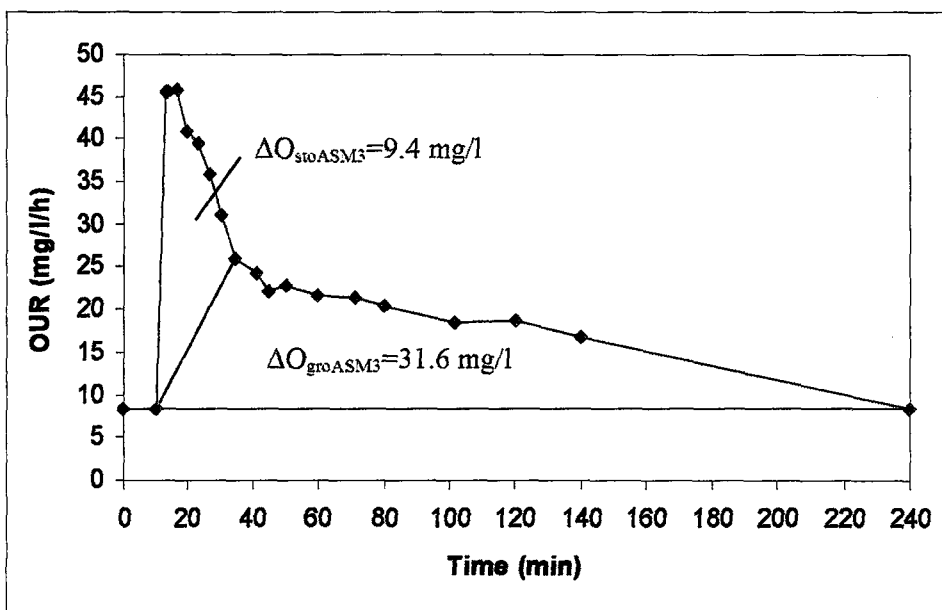


Figure 5.10: Interpretation of OUR data according to ASM3

$$Y_{STO,ASM3} = 1 - \frac{\Delta O_{stoASM3}}{S_{S1}} = 1 - \frac{9.4}{150} = 0.94 \text{ gCOD/gCOD} \quad (5.3)$$

The remaining amount of oxygen consumed in the system ($\Delta O_{groASM3}$) is devoted to heterotrophic growth on storage polymers according to ASM3 and can be calculated as given in expression 5.4.

$$Y_{H,ASM3} = 1 - \frac{\Delta O_{groASM3}}{X_{STOmaxASM3}} = 1 - \frac{31.6}{150 \cdot 0.94} = 0.78 \text{ gCOD/gCOD} \quad (5.4)$$

Although glycogen measurement results suffer from the presence of starch at the beginning of the cycle, the maximum amount of glycogen stored is a very important parameter for understanding the conversions in the system. Thus, the storage yield, $Y_{STO,GLY}$, can also be calculated using glycogen data, according to ASM3. The maximum amount of glycogen stored is used to calculate the observed storage yield for each set of experiment. Equation 5.5 presents an example for the estimation of observed glycogen storage for Set 1. The calculation, given in equation 5.5, is subject to errors, since the amount of glycogen used for growth in the feast phase is omitted. As seen from the results obtained in equations 5.3 and 5.5, when ASM3 is used assuming starch being readily biodegradable, higher amounts for stored glycogen ($X_{STOmaxASM3}$) are predicted than the observed results. Although this estimation embodies some error, the differences obtained with equation 5.3, using OUR data, and equation 5.5, using glycogen data, are far too large to be accounted for the amount of glycogen used for growth.

$$Y_{STO,GLY} = \frac{X_{STOmax}}{S_{S1}} = \frac{119}{150} = 0.79 \text{ gCOD/gCOD} \quad (5.5)$$

If both substrates are assumed to be readily biodegradable, the estimated $Y_{STO,GLY}$ values are found as 0.80 gCOD/gCOD for NPS and 0.75 gCOD/gCOD for SolS, which are much lower than the values obtained using the OUR data, namely $Y_{STO,ASM3}$ (Table 5.6). This observation shows that it is not possible to predict the amount of glycogen storage using ASM3 and thus these results indicate that the substrate is not totally utilized for storage but simultaneous growth on the substrate is also a significant process in the system.

The growth yield for ASM3 ($Y_{H,ASM3}$) of NPS is estimated as 0.78 and that of SolS is found to be 0.85 gCOD/gCOD. The higher secondary growth yields obtained for SolS can be due to possible derivatives of glycogen, *i.e.* different poly-glucose molecules, stored. This result shows that the poly-glucose compounds generated from the hydrolysis products of SolS and consumed for heterotrophic growth, are energetically more efficient than that of NPS, due to the molecular structure of the stored polymer. This observation is also confirmed with lower Y_{NET} value of 0.73 gCOD/gCOD for NPS and higher values of 0.79 gCOD/gCOD for SolS, showing that SolS is utilized more efficiently than NPS.

According to the simultaneous storage and growth approach (ASMGS), the amount of oxygen utilized for external substrate in the feast phase is the sum of electron acceptor consumption of substrate storage and primary growth processes. Iodine dyeing has shown that both NPS and SolS were rapidly adsorbed on the biomass in the system (Karahan *et al.*, 2005a) and thus, it is only possible to determine the duration of the feast phase, using the respirometric response of the system. The disappearance of substrate in the bulk liquid was very rapid, in the order of minutes, however the OUR profile indicated a period of around 20-30 minutes for the utilization of external substrate as shown in Figure 5.11. Thus the end of the feast phase is accepted as the break point on the OUR curve, where a sharp change in the slope of the curve is observed.

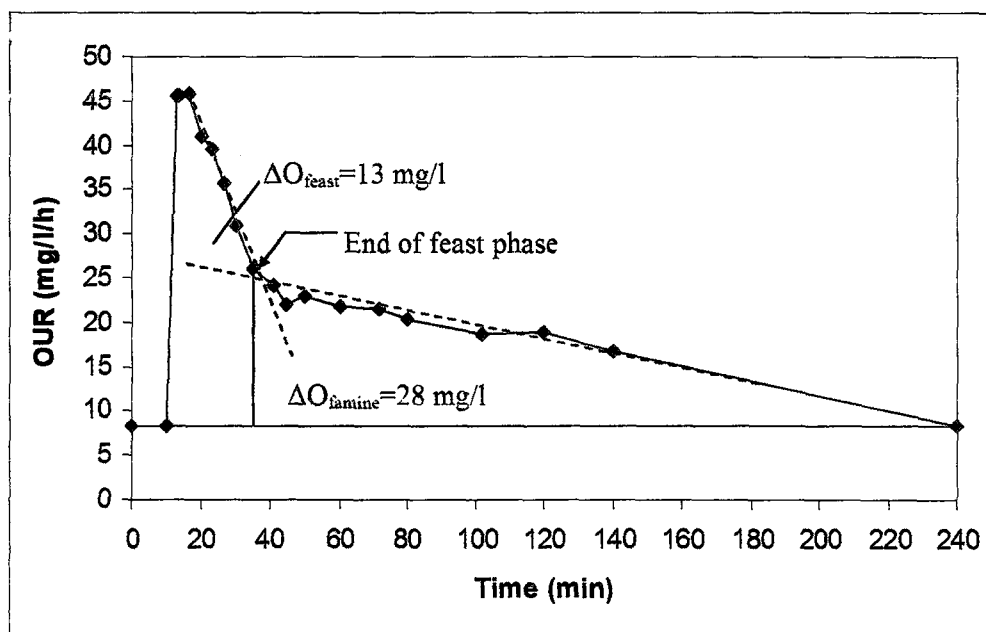


Figure 5.11: Oxygen consumption in the feast and the famine phases

5.2.3.2 Calibration and evaluation of experimental data by modeling

Modeling studies were conducted using Aquasim[™] (Reichert *et al.*, 1998) software for the dynamic simulations of batch experiments. The model was run to simulate the response of SBR system fed with NPS and SolS. The process kinetics associated with the five sets of experimental data were estimated by model simulations, using substrate (starch: NPS or SolS) consumption, oxygen utilization and glycogen production and consumption. Model calibration and evaluation results are illustrated in Figures 5.12, 5.13 and 5.14. Kinetic and stoichiometric coefficients of the proposed model providing optimum calibration of the experimental data are given in Table 5.6.

Table 5.6: Kinetic and stoichiometric coefficients providing optimum calibration of the experimental data

Model Coefficient	Maltose	NPS	SolS
	(Karahane <i>et al.</i> , 2005a)	Experiments Sets 1,2&3	Experiments Sets 4&5
Y_{STO} (gCOD/gCOD)	0.91	0.91	0.91
Y_{H1} (g cellCOD/gCOD)	0.7	0.73	0.79
Y_{H2} (g cellCOD/gCOD)	0.7	0.78	0.84
f_{SI} (gCOD/gCOD)	0.05	0.05	0.05
f_I (gCOD/gCOD)	0.10	0.10	0.10
b_H (1/d)	0.05	0.10	0.10
b_{STO} (1/d)	0.05	0.05	0.05
k_{ads} (1/h)	-	15	15
k_H (1/d)	-	30	30
K_X (gCOD/gCOD)	-	0.15	0.15
μ_{H1} (1/d)	-	3	3
K_{S1} (mgCOD/l)	-	20	20
k_{STO} (1/d)	26	25	25
K_{S2} (mgCOD/l)	15	2	2
μ_{H2} (1/d)	1.8	4	4
K_{STO} (gCOD/gCOD)	0.2	0.4	0.4

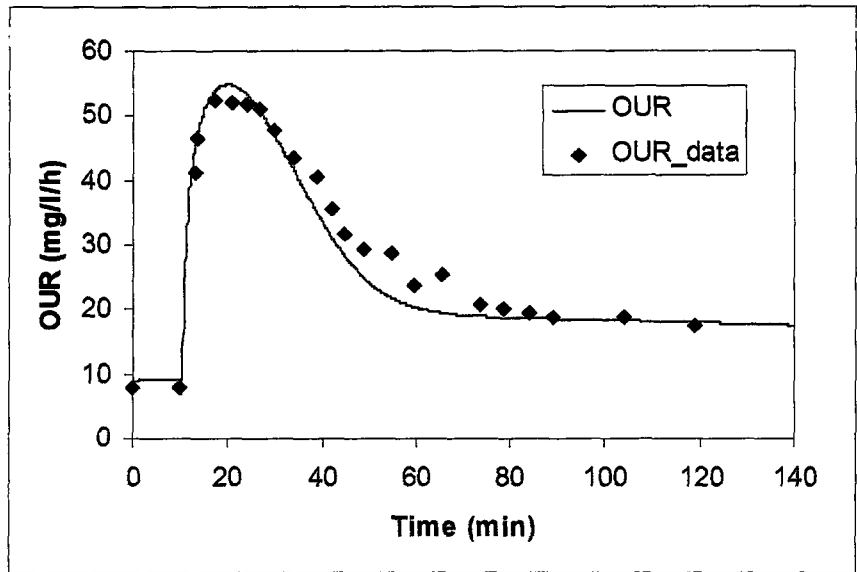
The proposed model involves adsorption and hydrolysis of both types of starch and simultaneous storage and primary growth on the hydrolysis products. Hydrolysis plays an important role for this model since the substrate can only be utilized after extracellular hydrolysis. It should be first mentioned that the structure of the model does not allow assessment of the yields for the substrate storage and primary growth processes involved with substrate consumption, separately from the OUR curve, as previously done for ASM3. The interpretation of the OUR curve for computing the amount of oxygen utilized by each process is even more difficult, when the substrate has to go through hydrolysis. However, it is possible to estimate the electron acceptor utilization for each process with the help of the stoichiometry obtained for the end product(s) of hydrolysis. In other words, maltose, previously identified as the end product of starch hydrolysis (Karahana *et al.*, 2005a), can be used as the model substrate for the stoichiometry of substrate storage and growth for both types of starch. Maltose formed would then be utilized for storage and growth. Therefore it is possible to assume that starch is totally hydrolyzed down to maltose prior to its utilization as the primary substrate in the system for modeling purposes. Accordingly, the storage yield in the modeling studies were accepted to be the same as maltose, as 0.91 gCOD/gCOD and the primary growth yields were selected in agreement with the overall observed yields (Y_{NET}). Simulations have shown that the primary growth yield of NPS was 0.73 gCOD/gCOD and that of SolS was 0.79 gCOD/gCOD (Table 5.6). This result shows that the growth yield of SolS is higher than NPS and is supported by the argument that growth on SolS is more efficient, since the hydrolysis products of SolS are presumably sugars with linear structure and thus taken up and consumed more easily by the heterotrophs.

Interpretation of the experimental data with the proposed model indicates that relatively high rates previously defined for starch hydrolysis (Karahana *et al.*, 2005a) are also applicable in this study. As can be depicted in Figure 5.12, the high OUR response at the beginning of the experiments can only be simulated using high amounts of readily biodegradable substrate and therefore high hydrolysis rates are required. The rapid hydrolysis of starch results in a fast storage process with a maximum rate, k_{STO} , of 25 1/d and a low half saturation constant, K_{S2} value of 2 mgCOD/l. Primary growth rates (μ_{HI}) were estimated to have a moderate value of 3 1/d with a relatively high K_{S1} value of 20 mgCOD/l. These results are in favor of a

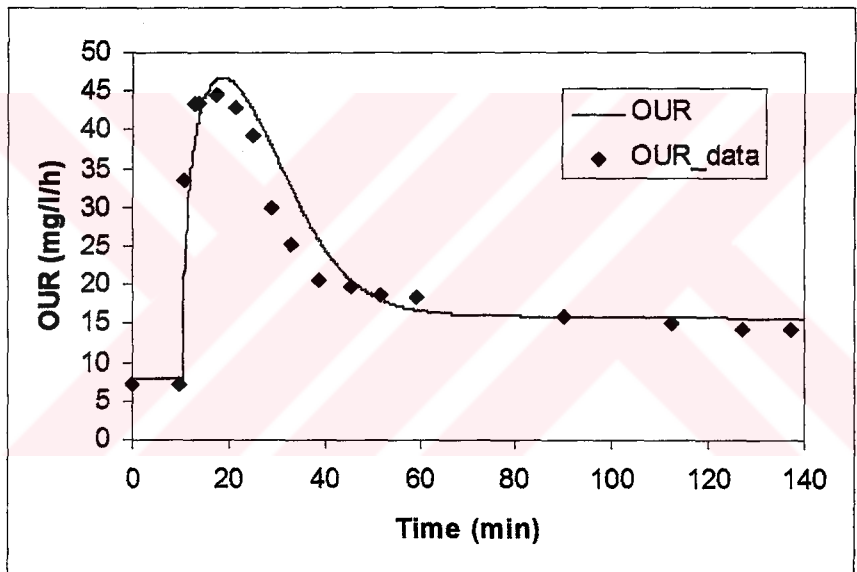
rapid substrate storage accompanied by an optimized primary growth on the available substrate, when excess substrate is present in the system. These findings also support the idea of heterotrophs using substrate storage as a tool for maximizing substrate uptake, while retaining optimum growth rates (**van Loosdrecht *et al.*, 1997**). The secondary growth rates, namely the maximum growth rates on stored polymers- in this case glycogen or poly-glucose are higher than the maximum rates of primary growth as given in literature (**Karahan *et al.*, 2003**), but of course secondary growth process is slower than primary growth since the process is basically defined as a surface reaction kinetics.

The proposed model provides, as illustrated in Figure 5.12, a good simulation of the OUR profiles for both NPS and SolS for the adopted process stoichiometry and kinetics in Table 3. It should be noted that the validity of the proposed model is verified, not only with OUR, but simultaneously with the starch and glycogen data related to NPS and SolS for all the five experimental sets (Figures 5.13 and 5.14).

The simulation results of the proposed model provides an accurate prediction of starch removal from bulk solution as shown in Figure 5.13, since the model is structured on the observation that starch is first adsorbed on biomass and rapidly disappears from the bulk liquid. Hence, the disappearance of starch can not be simulated by activated sludge models unless adsorption is also incorporated in the model. Figure 5.13 displays together with the simulations for the disappearance of starch (X_s), model simulations of the fate of adsorbed starch (X_{sads}) and the fate of readily biodegradable substrate produced through hydrolysis (S_s). These modeling results explicitly show that hydrolysis is the rate limiting process in the system since the produced S_s is immediately utilized for storage and growth. The modeling results for two different types of starch have shown that the removal and the utilization of starch by activated sludge culture are two distinct phenomena which require careful and separate evaluation.

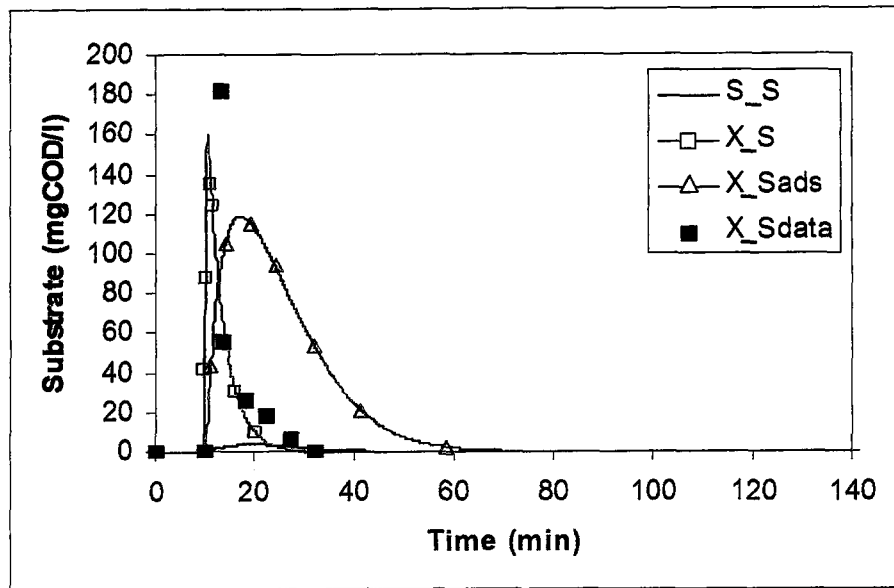


(a)

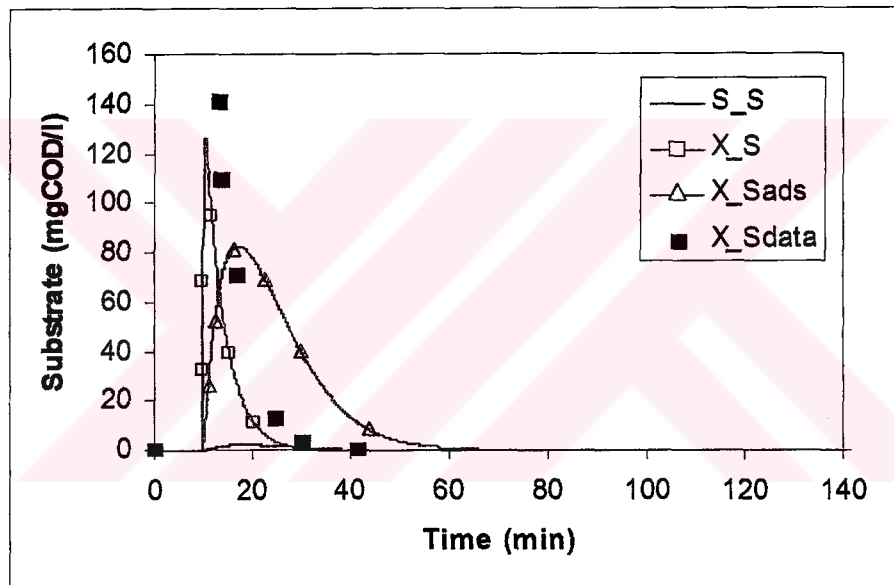


(b)

Figure 5.12: Model calibration of the OUR profiles for (a) NPS, Set 3 and (b) SolS, Set 4



(a)

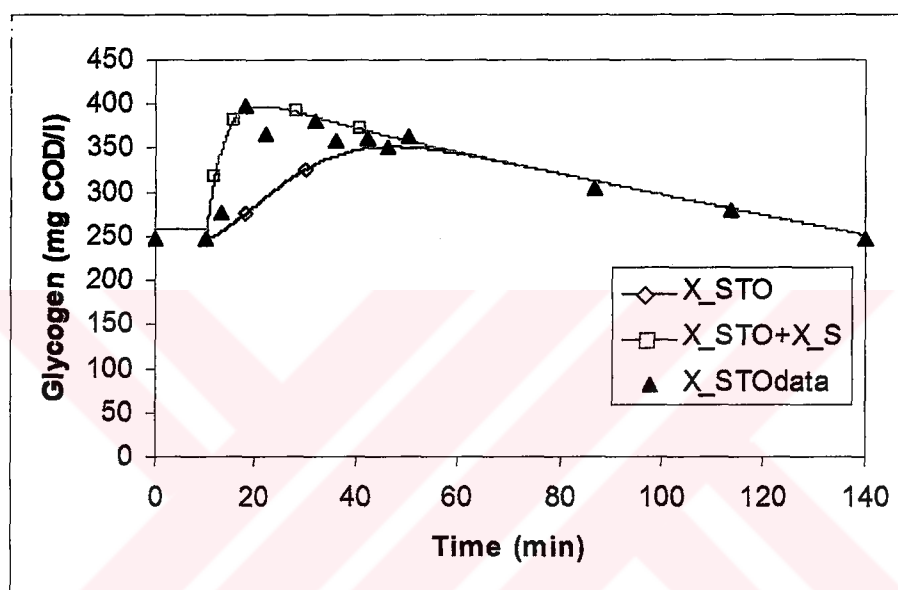


(b)

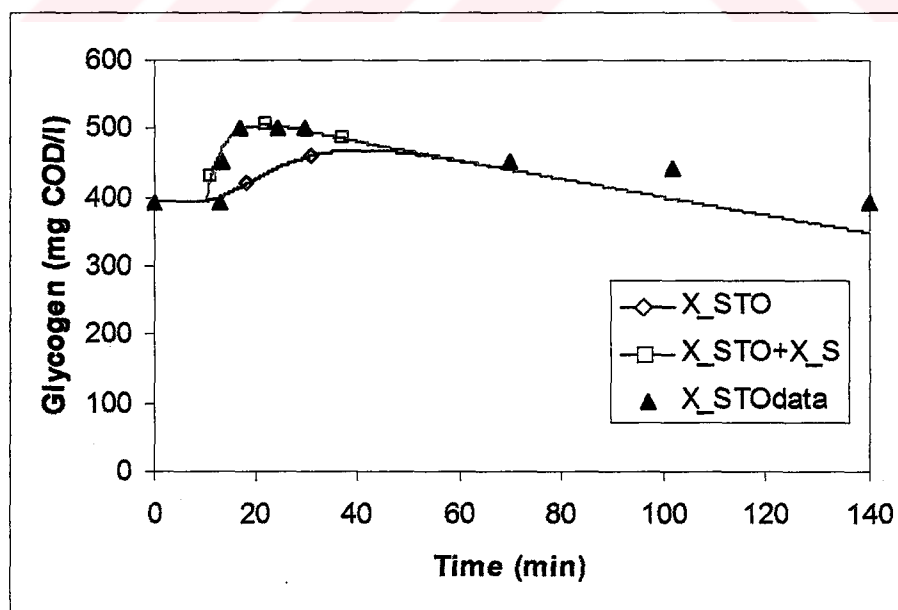
Figure 5.13: Model calibration of starch removal and simulation of the hydrolysis of adsorbed starch for (a) NPS, Set 3 and (b) SolS, Set 4

Model calibration of the glycogen concentration profiles highlights a major issue that merit careful evaluation: Figure 5.14 displays a discrepancy between experimental data and model calibration, indicating a glycogen surplus for the first hour of the experiments. This discrepancy can be explained in view of the fact that glycogen measurements are not capable of differentiating between starch, (X_S) and stored glycogen, (X_{STO}) and the results of the analysis is the sum of the two sugars ($X_{STO}+X_S$). As shown in Figure 5.14, the experimental data presented as glycogen is bound to include some starch adsorbed onto biomass and still present in the system, at the beginning of the experiments. Accordingly, model predictions for glycogen

generation and consumption (X_{STO}) have also accounted for the amount of starch still present in the system at the beginning of the experiment (X_S), in order to provide a better simulation of the real observation. Therefore, glycogen simulation results have been reported with two curves as given in Figure 5.14. This way, the model presents good simulation results for glycogen storage and consumption, basically showing that the selected stoichiometric coefficients for the model were appropriate also providing substantial support for the mechanisms of rapid starch adsorption with further hydrolysis of the adsorbed starch within biomass.



(a)



(b)

Figure 5.14: Fate of glycogen and adsorbed starch for (a) NPS, Set 3 and (b) SolS, Set 4

5.2.3.3 Evaluation of experimental data with ASM3

In this part of the study, the experimental data was also simulated with the original ASM3 model, for comparative evaluation of the results obtained with the proposed model. The critical issue when dealing with ASM3 is the basic assumption that classifies and processes all biodegradable soluble substrate as readily biodegradable, which then undergoes through biochemical storage without the intermediate hydrolysis step. Since SolS is almost entirely soluble and NPS, only slightly (36%) soluble, this assumption is likely to have a significant impact on the interpretation of the experimental results using ASM3. In this framework, when modeling with ASM3, both NPS and SolS, were first considered as readily biodegradable and then as slowly biodegradable substrate.

Accordingly, the first ASM3 modeling approach (ASM3a) is based on the assumption that both NPS and SolS behave like readily biodegradable. The stoichiometry used for these simulations was adopted as given in Table 5.6, where the storage yields of NPS and SolS were found as 0.95 gCOD/gCOD. The second ASM3 modeling approach (ASM3b) used for the dynamic evaluation embraces the assumption that starch, both NPS and SolS, should be totally hydrolyzed and consumed as maltose by the heterotrophic biomass. The stoichiometry of storage in these simulations was accepted to be the same as maltose. In other words, the storage yield was selected as 0.91 gCOD/gCOD as estimated by **Karahan *et al.* (2005a)**. The level of agreement between optimum model simulation and experimental results is illustrated in Figures 5.15 and 5.16 for SolS as an example. Figure 5.15 gives glycogen production and consumption simulated by the two different interpretations of ASM3 for best fits of all experiments, with a clear indication that both models are incapable of describing the experimental observations, due to the complete storage of substrate concept inherently associated with ASM3.

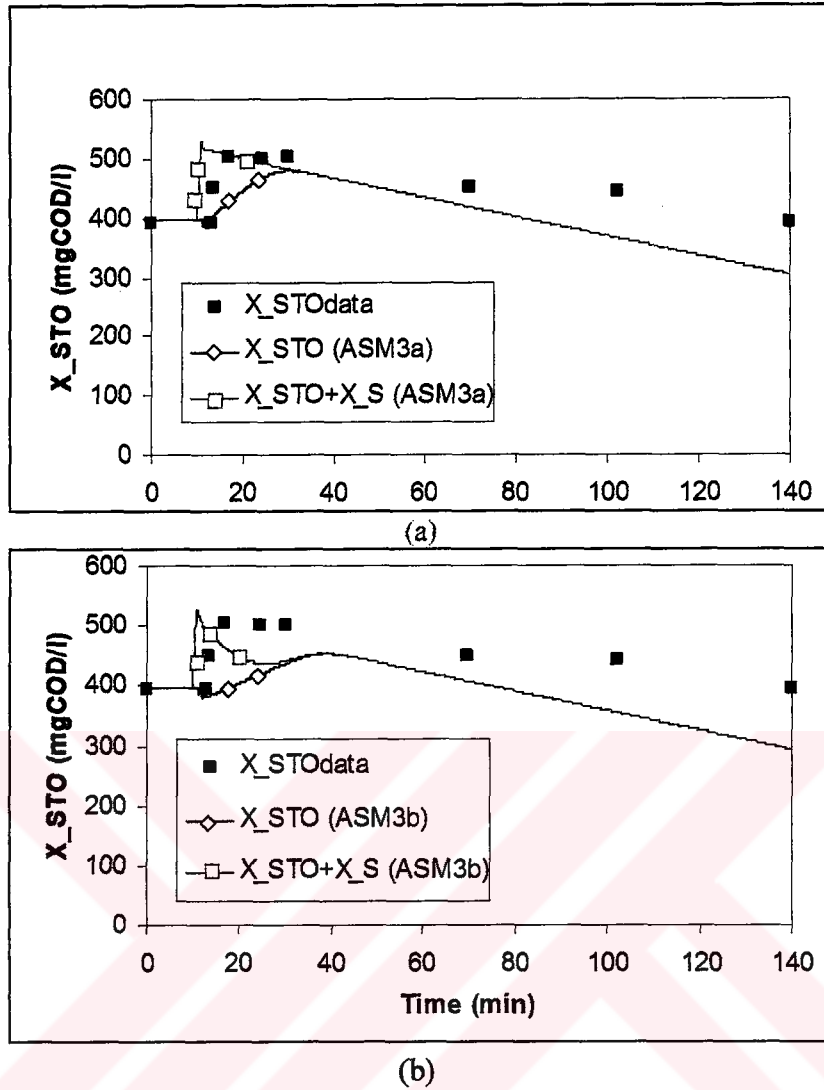


Figure 5.15: Simulation of the glycogen concentration profile with (a) AMS3a and (b) ASM3b

As shown in Figure 5.16, ASM3a is capable of simulating the high OUR response in the beginning, but has a sharp transition towards the second OUR level, which does not fit well with the experimental data. The OUR response and transition from the feast phase to the famine phase (when external substrate is depleted) is smoother in ASM3b, depending on the hydrolysis rate parameters. However, this smooth transition also brings about a prolonged feast phase, which is more obvious in the case of SolS.

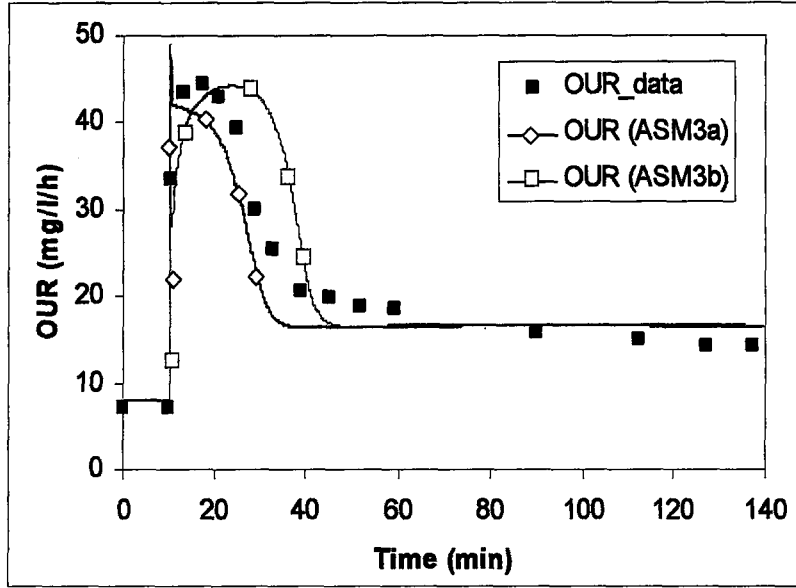


Figure 5.16: Model simulation of the OUR profile with ASM3

In contrast to the results obtained with ASM3, the results given in Figure 5.14 show that the proposed model can predict both the initial OUR response and the smooth transition to the famine phase. This is provided by the fast storage process, which determines the initial OUR response dominantly together with the growth process, competing for substrate, especially at lower substrate concentrations, around the substrate affinity coefficient of growth: K_{S2} values. It should be mentioned that the addition of primary growth process to ASM3 brings about more complication to the interpretation of the OUR data. The main problem in modeling is the distribution of substrate and its counterpart-oxygen, among storage and growth processes. The stoichiometry of defined in the model is not solely capable of determining the amount of substrate and oxygen allocated by the simultaneous processes. Oxygen utilization due to substrate consumption is defined by process kinetics with the following relationships:

$$\left[-\frac{dS_o}{dt} \right]_{\text{Substrate Consumption}} = \left[-\frac{dS_o}{dt} \right]_{\text{Substrate Storage}} + \left[-\frac{dS_o}{dt} \right]_{\text{Primary Growth}} \quad (5.6)$$

$$\begin{aligned} \text{OUR}_{\text{Substrate Consumption}} &= (1 - Y_{\text{STO}}) \cdot k_{\text{STO}} \frac{S_o}{K_o + S_o} \frac{S_s}{K_{S2} + S_s} X_H + \\ &\quad \left(\frac{1 - Y_{H1}}{Y_{H1}} \right) \cdot \mu_{H1} \frac{S_o}{K_o + S_o} \frac{S_{NH}}{K_{NH} + S_{NH}} \frac{S_{HCO}}{K_{HCO} + S_{HCO}} \frac{S_s}{K_{S1} + S_s} X_H \end{aligned} \quad (5.7)$$

The process rates and the substrate affinity constants are highly effective for the simulation of OUR response of substrate storage and primary growth. The rate couples, namely; μ_{H1} - K_{S1} and k_{STO} - K_{S2} couples have to be chosen meticulously in order to simulate the OUR response.

The model interpretation of the OUR profile as displayed in Figure 5.17, indicates that a relatively small amount of substrate is used for primary growth. However, it is still necessary to incorporate primary growth simultaneously utilizing substrate with the storage process in the activated sludge models. The primary growth on substrate may proceed at low levels in some cases, depending on the type and structure of the substrate and the operating conditions, but certain conditions, like presence of excessive substrate concentrations in the system, would favor the process in terms of substrate and electron acceptor utilization. Thus, activated sludge models should involve simultaneous storage and primary growth processes, as evidenced in this study for starch, especially for a better understanding and interpretation of the utilization of slowly biodegradable substrates.

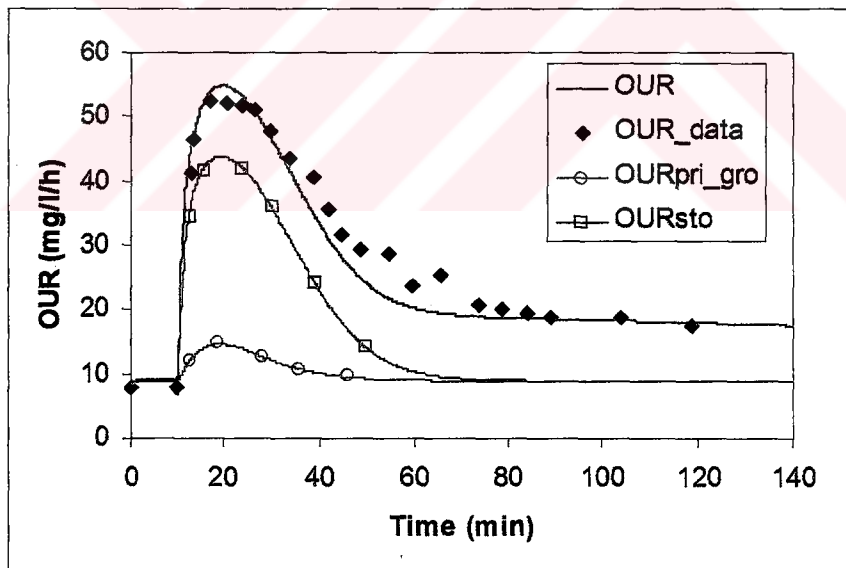


Figure 5.17: OUR fractions for storage and primary growth obtained with the proposed model

5.2.4 Discussion

This study provided the mechanistic description of a new activated sludge model incorporating primary microbial growth on external substrate, with simultaneous formation of glycogen as the storage biopolymer, for the utilization of starch by

activated sludge. The proposed model calibrated and verified with an acceptable accuracy, concentration profiles of two types of starch, Native Potato Starch (NPS) and Soluble Starch (SolS) monitored throughout the entire cycle of a sequencing batch reactor operated at steady state. Model evaluation also included the other model components, namely glycogen and oxygen uptake rate profiles obtained at five different cycles. The following observations may be further emphasized as the significant remarks of the study:

Model evaluation of the experimental data clearly indicated the need to differentiate between removal of starch from the bulk solution by rapid adsorption and utilization of the adsorbed starch onto biomass through hydrolysis. Hydrolysis of starch to maltose proceeds at the same rate for both NPS and SolS with totally different solubility characteristics and appears to be the rate limiting step for substrate storage and primary growth.

The adsorption mechanism for the slowly biodegradable substrate is generally overlooked in activated sludge models, leading to presume instantaneous uptake and start of the hydrolysis step. The evaluation showed however that adsorption mechanism is likely to have a significant impact on modeling the fate of glycogen, as the current measurement techniques fail to differentiate stored glycogen and remaining starch adsorbed onto biomass.

One of the major drawbacks of current activated sludge models (ASM3) is the categorical classification of substrate as either readily biodegradable or slowly biodegradable, solely on the basis of their solubility. In fact, two different versions of ASM3, which assumed starch either as a readily or slowly biodegradable substrate, did not provide an equally acceptable fit for the glycogen and OUR curves, implying the need to consider hydrolysis as the central mechanism for substrate utilization regardless of the type of starch.

The evaluation clearly underlined the merit of incorporating the primary growth process in the model structure for an accurate interpretation of the experimental data since. This process is likely to have a significant impact on substrate and electron acceptor utilization in the system, especially when high concentrations of slowly biodegradable substrate is present.

The study also underlined the extreme importance of using the appropriate relationships for the proposed model. Therefore, any selected model should be investigated in detail, concerning the assumptions involved and the correct stoichiometry being carefully determined. The selected values of the stoichiometric parameters should be determined according to the experimental data obtained for the three major system components, namely substrate, storage polymers, and oxygen.

Comparison of the degradation of two different types of hydrolysable substrates (particulate NPS and soluble SolS), leads to conclude that although the substrates are hydrolyzed with similar rates, primary and secondary growth processes on SolS are more efficient, with higher yields, due to the more easily utilizable products of SolS, both in terms of extracellular hydrolysis and the hydrolysis of stored poly-glucose. The results have also indicated that the yield of secondary growth depends on the type of the storage polymers, in this case type of glycogen (poly-glucose), which is established by the incorporated monomer, mostly maltose, for this study.

6. CONCLUSIONS AND RECOMMENDATIONS

Substrate storage under dynamic conditions is recently regarded as a significant process for activated sludge systems. Substrate concentration gradients are always present in wastewater treatment plants, which are caused by dynamic conditions due to changes in wastewater composition, treatment plant scheme, reactor hydraulics and variations in the modes of operation of treatment plants. The dynamic conditions and substrate gradients convey activated sludge cultures to develop a storage response when external substrate is present in the system. Activated sludge culture adapts to the dynamic conditions by storing the substrate when available and surviving on the stored substrate when external substrate is not present.

Substrate storage is incorporated into activated sludge modeling with Activated Sludge Model No.3 (ASM3) and biochemical models for pure substrates. ASM3 has been proposed for activated sludge systems both for aerobic and anoxic conditions. Introducing storage phenomena has also introduced a number of stoichiometric and kinetic coefficients making the model rather complicated with many degrees of freedom. Although some default values have been given in the model, calibration in terms of kinetic and stoichiometric parameters is still needed for various applications. A part of this study aimed to reduce the complexity brought by the numerous parameters in ASM3 by providing a systematic approach for the estimation of the essential kinetic and stoichiometric parameters.

In this context, ASM3 was investigated in detail for organic carbon and nitrogen removal. The conventional activated sludge system for carbon removal and the conventional pre-denitrifying single sludge system were considered in terms of process stoichiometries, according to ASM3. It is necessary to obtain the steady state solutions of the mass balances based on the model, in order to describe the fate of each parameter in the system. The mass balance equations constructed using ASM3 involved the input, the output and the generation of the parameter under concern, without any accumulation in the system.

An experimental procedure was developed for the respirometric determination of bacterial storage yield as defined ASM3. The proposed approach is based on the oxygen utilization rate (OUR) profile obtained from a batch test and correlates the area under the OUR curve to the amount of oxygen associated with substrate storage. Model simulation was used to evaluate the procedure for different initial experimental conditions. The procedure was used to determine the storage yield, Y_{STO} , associated with acetate, glucose and domestic sewage, together with mixtures of acetate/glucose and acetate/domestic sewage at different initial F/M ratios. Y_{STO} was calculated as 0.78 gCOD/gCOD for acetate, 0.87 gCOD/gCOD for glucose and 0.96 gCOD/gCOD for domestic sewage. The Y_{STO} of substrate mixtures was found to reflect the characteristics of the dominant fraction in the mixture.

This study also presents the effect of substrate composition and specifically culture composition on the observed respirometric responses under anoxic conditions. In this context different mixtures of readily biodegradable substrates have been investigated. The results of the study provide examples and data on the experimental assessment of storage yield for different substrates and heterotrophic growth yield based on nitrate utilization rate (NUR) tests. In the tests conducted with biomass acclimatized to a 4-compound substrate mixture of acetate, propionate, ethanol and glucose, the observed anoxic storage yields were assessed as 0.70 gCOD/gCOD when fed with the same mixture and 0.71 gCOD/gCOD when fed only with acetate and propionate. However for the second culture enriched with acetate-propionate mixture, the observed storage yields were estimated as 0.61 gCOD/gCOD when fed with the 4-compound mixture and 0.71 gCOD/gCOD when fed with acetate and propionate. This result has been evaluated as a possible consequence of culture adaptation. The anoxic growth yields in the tests were calculated to be equivalent to an average of 0.64 gCOD/gCOD. The study could serve as a new perspective for the experimental determination of model parameters for the design of activated sludge systems for different substrate compositions.

A methodology has been developed for the determination of aerobic and anoxic model parameters of ASM3 in this study. The average maximum storage rate (k_{STO}) for acetate was obtained as 13 1/d was much higher than the default value of ASM3; however similar results for batch tests have been reported in the literature. The calculated anoxic correction factor, η_D , range was between 0.50 and 0.56 with an

average of 0.53. This coefficient would reduce the aerobic storage rate of 13 1/d to approximately 7 1/d under anoxic conditions. The anoxic heterotrophic growth yield, Y_{HD} was estimated as 0.44 gCOD/gCOD on the average and the corresponding anoxic correction factor for heterotrophic growth, namely, Y_{HD}/Y_H ratio, was 0.70.

While storage kinetics and stoichiometry could very well be defined, the simulation results of aerobic respirometric data indicated that, it was not quite possible to obtain ASM3 calibration by using a single heterotrophic growth rate, μ_H , value. Apparently two different growth rates were needed in order to simulate OUR data in the storage and growth phases and this finding suggested that the concept of growth on storage products adopted in ASM3 needed to be re-evaluated. This study provides the structural framework for the proposed modified version of ASM3, where direct heterotrophic growth on readily biodegradable substrate is included as a new process and provision is made so that growth on internal storage compounds is started sequentially, after the depletion of the external primary substrate pool. The results have provided strong indication that there was a need for considering direct growth on primary substrate as a significant biological mechanism.

The study also aimed to explore the conversion processes of hydrolysable substrates by activated sludge. Parallel batch tests were run with Native Potato Starch (NPS, particulate), soluble starch (SolS), maltose and glucose for comparative evaluation. Results indicated that adsorption was the dominant mechanism for starch removal with subsequent enzymatic hydrolysis inside the flocs and the role of bulk liquid enzyme activity was minimal. Starch was observed to hydrolyze to maltose rather than glucose. The behavior of NPS and soluble starch was quite similar to maltose in terms of poly-glucose formation and oxygen uptake. Since the simplest hydrolysis product was maltose, the biomass was not acclimated to glucose and thus, glucose exhibited a significantly different removal and storage pattern. The study showed that differentiation of readily biodegradable and slowly biodegradable COD should better be based on the kinetics of their utilization rather than simple physical characterization.

Finally, the study presents a new mechanistic model incorporating microbial growth on external substrate with simultaneous formation of storage biopolymers, (ASMGS), for the utilization of starch by activated sludge. Model description and calibration utilized experimental data of an SBR fed with particulate NPS and SolS

selected as model substrates. The proposed model, basically modified ASM3, to include adsorption of starch, its hydrolysis and simultaneous growth and glycogen formation using the hydrolysis products, which was mainly maltose. Model simulations indicated hydrolysis of the adsorbed starch as the rate limiting process. The proposed model calibrated well the fate of all major model components, namely, starch, glycogen and OUR. Particulate NPS and SolS were hydrolyzed with similar rates; however, primary and secondary growth processes on SolS were more efficient, with higher yields, due to the more easily utilizable products of SolS, both in terms of extracellular hydrolysis and of stored poly-glucose. Two different versions of ASM3, assuming starch either as a readily or slowly biodegradable, did not provide an equally acceptable fit for the glycogen and OUR curves, this way supporting the need to consider primary growth together with storage as defined in the proposed model.



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