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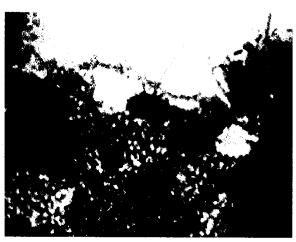
Wastewater Characterization for Evaluation of Biological Phosphorus Removal

by Jae Kwang Park, Jenchie Wang,* and Gerald Novotny**



Abstract

Many treatment plants have been designed or upgraded to remove phosphorus by the addition of chemicals. Problems associated with chemical precipitation include high operating costs, increased sludge production, sludge with poor settling and dewatering characteristics, and depressed pH. Biological phosphorus removal (BPR) systems can offer the benefits of reduced sludge production, improved sludge settleability and dewatering characteristics, reduced oxygen requirements, and reduced process alkalinity requirements. However, pilot-testing and traditional methods for kinetic parameter determination are complex and time consuming, which can make the evaluation of BPR processes too costly for smaller treatment facilities.

A simple COD fractionation method was developed to determine the fraction of readily biodegradable soluble COD, which is vital for biological phosphorus removal design. Simple methods are proposed to determine Y, k_d, μ_{max}, and K_s, which are important for BPR process design. These kinetic parameters and the detailed fractionation results of raw wastewater COD, nitrogen, and phosphorus can be used in biological nutrient removal process design computer programs such as ENBIR, which is based on the model developed by Ekama et al. (1984) and is a public domain computer program, or BIOSIM™, a menu-driven personal computer-based simulation program that solves the equations of the International Association on Water Pollution Research and Control (IAWPRC, now International Association on Water Quality, IAWQ) task group model for activated sludge systems extended for enhanced BPR (EnviroSim Associates 1993). These models can be used to determine the process volume and to evaluate the effect of COD loading, biomass concentration, and sludge age on the nutrient removal efficiency.

The use of a computer package along with the wastewater characterization technique specific for BPR and kinetic parameter determination will allow small wastewater treatment plants or industries to evaluate the feasibility of biological phosphorus removal of their wastewater with minimum cost.

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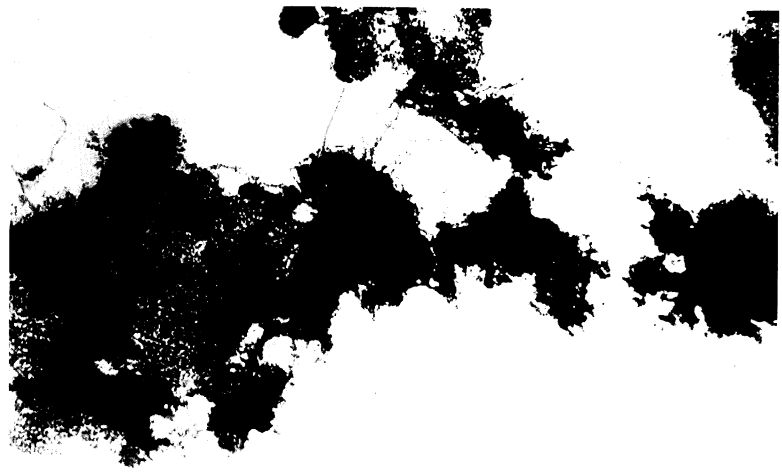


J. Wang

Phosphorus-accumulating microorganisms.

Introduction

Controlling phosphorus discharged from municipal and industrial wastewater treatment plants is a key factor in preventing eutrophication of surface waters. Consistent with International Joint Commission agreements, the Wisconsin Department of Natural Resources (DNR) has, since the mid-1970s, required all municipal treatment facilities that discharge to the Great Lakes basin and have a population equivalent of 2,500 or greater to meet a total limit of 1 mg phosphorus/L (P/L). A new regulation (Ch. NR 217, Wis. Admin. Code), which became effective in 1992, expanded the requirement for phosphorus removal to include the entire state. The new rule requires that all existing wastewater treatment plants discharging in excess of 150 pounds of total phosphorus per month to surface waters meet a 1 mg-P/L effluent limit. This effectively lowers the threshold for the size of plant required to remove phosphorus from about 250,000 gallons/day to 100,000-150,000 gallons/day. The need to retrofit many small- and medium-sized treatment facilities for phosphorus removal has led to increased interest in alternatives to chemical addition. At the same time, biological phosphorus removal (BPR) technology has been steadily developing.

To encourage the use of biological removal techniques, the DNR regulation provides an alternative limit if an enhanced BPR process is used. The alternative limit requires the removal of 90% of the phosphorus that would have been removed to achieve a 1 mg-P/L effluent limit. For example, the Madison Metropolitan Sewage District's (MMSD) Nine Springs Wastewater Treatment Plant has an average influent total phosphorus concentration of 6 mg-P/L. Since the MMSD wastewater treatment plant will fall under the new regulation, it will be required to meet either the 1 mg-P/L effluent standard if chemical phosphorus removal is used or the alternative effluent limit of 1.5 mg-P/L [6 – (6 – 1) x 0.9] if the BPR process is used.

The overall total phosphorus removal obtained in a conventional biological wastewater treatment is generally less than 20% and is even less in wastewater treatment plants where anaerobic digester supernatant is recycled to the head of the plant. Since it is not possible to achieve the 1 mg-P/L effluent limit with conventional biological wastewater treatment processes, additional or alternative treatment methods must be employed.

Many treatment plants have been designed or upgraded to remove phosphorus by the addition of chemicals. Chemical precipitation increases the volume of sludge produced and often results in a sludge with poor settling and dewatering characteristics. Also, precipitation with metal salts can depress the pH. If nitrification is required, additional alkalinity will be consumed and the pH will drop further.

Besides reducing or eliminating the need for chemical addition, BPR systems can offer the following benefits:

- reduced sludge production,
- improved sludge settleability and dewatering characteristics,
- reduced oxygen requirements, and
- reduced process alkalinity requirements.

Pilot-scale tests are generally conducted to evaluate the feasibility of biological phosphorus removal processes. However, pilot tests are expensive and time consuming and generate limited data. Because of this, smaller wastewater treatment plants may not be able to consider BPR as an alternative to chemical phosphorus removal.

The development of activated sludge process

design computer programs provides an alternative design method. Computer models can be used to determine the process volume and to evaluate the effect of chemical oxygen demand (COD) loading, biomass concentration, and sludge age on the nutrient removal efficiency. Multiple process design configurations can be evaluated, and the sensitivity of designs to variations in wastewater characteristics can be economically evaluated.

However, several physical, chemical, and biokinetic parameters of the wastewater must be determined in order to use the activated sludge models. The wastewater characterization methods presented in this report will provide the inputs to the computer design programs. These procedures were developed in conjunction with a study in which the ENBIR program[1] was used to evaluate BPR alternatives for the City of Ashland, Wisconsin. Test data from the Ashland study are used to illustrate the characterization methods.

Principle of Biological Phosphorus Removal

The theory of luxury uptake of phosphorus is now well developed (Wentzel et al. 1990; Wentzel et al. 1991). It has been shown that exposing the mixed liquor to an anaerobic/aerobic sequence in the biological reactor selects microorganisms that accumulate higher levels of intracellular phosphorus than other microorganisms. Phosphorus-removing microorganisms are able to rapidly assimilate and store volatile fatty acids (VFAs) and other fermentation products under anaerobic conditions. Phosphorus is released in the anaerobic zone to produce the energy needed to take up the fermentation products, which are stored as poly-β-hydroxybutyrate. Phosphorus-removing microorganisms produce energy by oxidizing the stored fermentation products in the aerobic zone while simultaneously accumulating intracellular phosphate. The ability of phosphorus-removing microorganisms to rapidly assimilate the fermentation products under anaerobic conditions gives them a competitive advantage over other microorganisms and results in their preferential growth in the wastewater treatment system. Thus, the anaerobic-aerobic sequence allows the selection of a large population of phosphorus-removing microorganisms.

In BPR systems, phosphorus accumulates in the biomass and is removed in the form of waste-activated sludge. A recent study showed that nearly all the enhanced phosphorus removal is due to the storage of polyphosphates. This results in an increase in the inorganic sludge mass but no significant increase in organic sludge production when compared to a conventional activated sludge process without chemical addition (Jardin and Popel 1995). Chemical precipitation of phosphorus has been estimated to increase sludge production by an average of 26% (Sedlak 1991).

Several process configurations (some patented, others not) are currently being applied worldwide for biological phosphorus removal. Some process configurations incorporate nitrogen removal by nitrification and denitrification along with biological phosphorus removal. However, all are based on the sequential exposure of microorganisms to anaerobic and aerobic conditions in the biological reactor.

Problems

Conventional activated sludge treatment was initially developed to remove carbonaceous and nitrogenous biochemical oxygen demand (BOD) from sewage. Activated sludge systems have been modified to enhance biological phosphorus removal by providing aerated and non-aerated reactors in series, along with various internal recycle streams. Not only have the system configurations increased in complexity, but the number of design parameters involved in the processes has also increased. Therefore, additional wastewater characteristics are necessary to evaluate the feasibility of biological phosphorus removal and to design a biological treatment process for phosphorus removal.

Objectives

The main objective of this report is to provide a simple procedure to determine wastewater characteristics necessary for the design of BPR systems, with specific emphases on:

- determination of COD fractions of wastewater,
- determination of kinetic parameters (Y, k_d, μ_{max}, K_s), and
- determination of nitrification and denitrification rates using batch reactors.

These parameters can be used in biological nutrient removal process design computer programs such as ENBIR, which is based on the model developed by Ekama et al. (1984), or BIOSIM™, a menu-driven personal computer-based simulation program that solves the equations of the International Association on Water Pollution Research and Control (IAWPRC) (now the International Association on

[1] A public-domain computer program. To obtain a copy, contact Professor Jae K. Park. See "About the Authors," for address.

Water Quality, IAWQ) task group model for activated sludge systems extended for enhanced BPR (EnviroSim Associates 1993). These models can be used to determine the process volume and to evaluate the effects of COD loading, biomass concentration, and sludge age on the phosphorus and nitrogen removal efficiencies. These methods will allow smaller wastewater treatment plants or industries to evaluate the feasibility of BPR of their wastewater with minimum cost.

Wastewater Sampling, Preservation, and Analysis Methods

Sampling

Sampling is an extremely important consideration in properly characterizing wastewater for biological phosphorus removal. Flow rate and wastewater quality change continuously, and these changes may affect the ability of a wastewater treatment plant to achieve consistent biological phosphorus removal. Obtaining samples that will actually represent the wastewater flow throughout the months and years to come is difficult at best. Diurnal fluctuations occur in concentration and flow volume; seasonal fluctuations occur in concentration, flow volume, and temperature; and industrial contributions to the collection system may cause wastewater characteristics to change on a short- or long-term basis. Given the variable nature of wastewater and the necessity of attaining consistent phosphorus removal, it may be necessary to collect samples that will represent "average" characteristics and approximate characteristics under more extreme conditions.

A desirable sampling method is to collect a 3-4 hour composite sample. This will provide data that may be considered representative of average wastewater characteristics throughout the day while minimizing the sample holding time. A careful review of flow monitoring records and reports generated by a facility over the past couple of years will also be helpful in assessing the seasonal characteristics of the wastewater throughout the year. If records reveal a wastewater that is highly variable in flow volume and concentration, further analysis may be required. It is not unusual to find that a particular facility may remove an adequate amount of phosphorus biologically during certain times of the year, with chemical precipitation being required during times when the wastewater characteristics are not as conducive to biological removal.

Preservation

Once a sample is taken, the constituents of the sample should be maintained in the same condition as when collected. When it is not possible to analyze collected samples immediately, samples should be preserved properly. Biological activity such as microbial respiration, chemical activity such as precipitation or pH change, and physical activity such as aeration or high temperature must be kept to a minimum. Methods of preservation include cooling, pH control, and chemical addition. Freezing is usually not recommended. The length of time that a constituent in wastewater will remain stable is related to the character of the constituent and the preservation method used. The *Handbook for Sampling and Sample Preservation of Water and Wastewater* (Environmental Protection Agency 1982) provides detailed guidelines on this topic. These are summarized in Table 1.

Analysis Methods

The National Pollutant Discharge Elimination System permit for each municipal treatment plant dictates effluent limitations and monitoring requirements for that particular plant. For evaluating plant performance regardless of size, biochemical oxygen demand (BOD), total suspended solids (TSS), pH, and flow should be routinely monitored.

Secondary analyses may include total coliform, fecal coliform, temperature, dissolved oxygen, total volatile solids, total solids, settleable solids, nitrogen, phosphorus, chlorine residual, dissolved solids, alkalinity, metals, COD, oil and grease, and organic priority pollutants as required.

Since COD is a better energy measurement than BOD_5 (the 5-day BOD test) for monitoring carbonaceous energy removal (Ekama et al. 1984), it is recommended that COD be analyzed on a routine basis in plants designed to remove phosphorus. In addition, if a plant is designed to remove phosphorus, phosphorus (total phosphorus and orthophosphate) and nitrogen (ammonium, nitrite, and nitrate nitrogen) need to be monitored more frequently in each basin of the treatment process. The recommended routine analytical methods are summarized in Table 2.

Advantage of Using COD over BOD

The BOD and COD tests are currently employed to measure the carbonaceous energy content of wastewater via its oxygen demand. BOD is a regulatory parameter used by the Environmental Protection Agency to monitor water quality.

Table 1. *Required containers, preservation techniques, and holding times.*[a]

Parameter	Container[b]	Preservative	Maximum Holding Time
Bacterial Test			
Coliform, fecal and total	P,G	Cool, 4°C 0.008% $Na_2S_2O_3$	6 hours
Fecal streptococci	P,G	Cool, 4°C 0.008% $Na_2S_2O_3$	6 hours
Inorganic Tests			
Acidity	P,G	Cool, 4°C	14 days
Alkalinity	P,G	Cool, 4°C	14 days
Ammonia	P,G	Cool, 4°C H_2SO_4 to pH < 2	28 days
Biochemical oxygen demand	P,G	Cool, 4°C	48 hours
Biochemical oxygen demand, carbonaceous	P,G	Cool, 4°C	48 hours
Bromide	P,G	None required	28 days
Chemical oxygen demand	P,G	Cool, 4°C H_2SO_4 to pH < 2	28 days
Chloride	P,G	None required	28 days
Chlorine, total residual	P,G	None required	Analyze immediately
Color	P,G	Cool, 4°C	48 hours
Cyanide, total and amenable to chlorination	P,G	Cool, 4°C NaOH to pH > 12	14 days
Fluoride	P	None required	28 days
Hardness	P,G	HNO_3 to pH < 2	6 months
Hydrogen ion (pH)	P,G	None required	Analyze immediately
Kjeldahl and organic nitrogen	P,G	Cool, 4°C H_2SO_4 to pH < 2	28 days
Metals			
Chromium (VI)	P,G	Cool, 4°C	24 hours
Mercury	P,G	HNO_3 to pH < 2	28 days
Metals, except above	P,G	HNO_3 to pH < 2	6 months
Nitrate	P,G	Cool, 4°C	48 hours
Nitrate-nitrite	P,G	Cool, 4°C H_2SO_4 to pH < 2	28 days
Nitrite	P,G	Cool, 4°C	48 hours
Oil and grease	G	Cool, 4°C H_2SO_4 to pH < 2	28 days
Organic carbon	P,G	Cool, 4°C HCl or H_2SO_4 to pH< 2	28 days
Orthophosphate	P,G	Filter immediately Cool, 4°C	48 hours
Oxygen, dissolved probe	G	None required	Analyze immediately
Phenols	G	Cool, 4°C H_2SO_4 to pH < 2	28 days
Phosphorus (elemental)	G	Cool, 4°C	48 hours
Phosphorus, total	P,G	Cool, 4°C H_2SO_4 to pH < 2	28 days
Residue, total	P,G	Cool, 4°C	7 days
Residue, filterable	P,G	Cool, 4°C	7 days
Residue, non-filterable (TSS)	P,G	Cool, 4°C	7 days
Residue, settleable	P,G	Cool, 4°C	48 hours
Residue, volatile	P,G	Cool, 4°C	7 days

[a] Adopted from Environmental Protection Agency Guidelines for handling and preserving samples.

[b] P = plastic, G = glass.

Table 2. *Analytical methods.*

Parameter	Method
BOD_5	Standard Methods[a] 5210
COD	Standard Methods 5220
Total phosphorus	Standard Methods 4500-P
Orthophosphate	Ascorbic Acid Reduction Method, Standard Methods 4500-P
$NH_3 + NH_4^+$-N	Preliminary Distillation; Titrimetic Method, Standard Methods 4500-NH_3
$NO_2^- + NO_3^-$-N	Devarda's Alloy Reduction Method, Standard Methods 4500
TKN	Semi-Micro Kjeldahl Standard Methods 4500-N (organic)
Total suspended solids (TSS)	Standard Methods 2540-D
Volatile suspended solids (VSS)	Standard Methods 2540-E
Alkalinity	Standard Methods 2320
pH	Standard Methods 4500-H^+

[a] Standard Methods refers to *Standard Methods for the Examination of Water and Wastewater* (American Public Health Association 1995).

The BOD test is empirical and performed under strictly specified conditions and procedures. In the 5-day BOD test, the sample of wastewater is diluted with well-oxygenated and nutrient-containing water, and microorganisms adapted to the wastewater are introduced. The initial dissolved oxygen concentration is determined, and the sample is stored in darkness at 20°C for 5 days. The difference in oxygen concentration between the beginning and end of the test period gives the 5-day BOD value. The BOD_5 test is intended to measure only the biochemical degradation of organic material, or "carbonaceous oxygen demand" of the sample, which results in the underestimation of the energy (in terms of oxygen demand) in the sample. In addition, since it takes 5 days to measure the BOD value , it is almost impossible to remedy any upset due to an unusual inflow into a treatment plant, making it difficult to use as an operational parameter.

Deviations in procedure or sample, such as the presence of nitrifiers in a sample, may give rise to uncertain results. Unless nitrification is suppressed by chemical additives, nitrifying organisms in the treated sample may multiply and utilize oxygen to convert NH_3 or NH_4^+ to NO_3^-, giving an inflated value for the carbonaceous energy. If nitrification is inhibited, *Standard Methods for the Examination of Water and Wastewater*[2] (American Public Health Association 1995) requires that the results be reported as CBOD (carbonaceous BOD). Moreover, if the microorganisms are not acclimated to the wastewater, low BOD values may be obtained due to the existence of heavy metals or inhibitory compounds.

On the other hand, the COD test gives a measure of the total energy in terms of oxygen by oxidizing all biodegradable and unbiodegradable organic materials with an oxidizing agent such as potassium dichromate. Since ammonium is not oxidized, the test value reflects only the energy released due to oxidation of the carbonaceous compounds. COD can also be correlated to carbonaceous BOD_5 ($CBOD_5$),and the COD test takes only 2 hours so that the results can be used in the daily operation of a wastewater treatment plant.

Because the COD test oxidizes both biologically degradable and unbiodegradable organic materials, the energy available for biological action is usually overestimated. However, this does not reduce the usefulness of the test. If it is assumed that the fraction of organic material that is not oxidized in the COD test remains constant, then any change in COD between two points in the process provides an assessment (in terms of oxygen) of corresponding energy change. The change in COD then can be used to establish the kinetics of energy conversion in the process, i.e., the energy removal can be directly linked to the COD change. By contrast, BOD_5 values require a correction factor to correspond the energy changes, because the test values do not reflect the total oxygen demand. Albertson (1995) claimed that the results of using $CBOD_5$ data for raw wastewater and primary effluent could result in a 20-40% underdesign and concluded that $CBOD_5$ is an improper test for influent and settled raw wastewater.

Since the energy changes in biological reactions are reflected in the number of electrons transferred, the electron donor capacity can be measured in terms of the oxygen required to oxidize the carbonaceous matter to CO_2. Such a measurement is available through a COD test because COD can be expressed as a chemical reaction.

Another great advantage of the COD test is that it provides a direct estimate of the oxygen or energy potential of the volatile solids. Based on the average stoichiometric composition of activated sludge ($C_5H_2NO_2$), Eckenfelder and Weston (1956) calculated the theoretical mass of oxygen necessary to oxidize the mass of hydrogen ions per unit of

[2] Hereinafter *Standard Methods for the Examination of Water and Wastewater* is referred to as *Standard Methods*.

organic mass: 1 mg of volatile suspended solids (VSS) is equivalent to 1.42 mg of O_2 or 1.42 mg of COD. Therefore, the COD/VSS ratio, f_{cv}, is 1.42. In the absence of more conclusive data, the COD/VSS ratio of 1.42 is generally accepted. However, Ekama et al. (1984) recommended 1.48 for the COD/VSS ratio based on the actual measurement. This relationship between VSS and COD is of greatest use when investigating the kinetics of the activated sludge process. It allows an estimation of the mass balance between the daily energy entering the plant and that leaving via the activated sludge wasted and the effluent.

Wastewater Fractionation

Fraction of COD in Wastewater

Before biological phosphorus removal process design models can be used, it is necessary to determine the various fractions of the influent COD. These fractions are needed to accurately describe the behavior of the biological phosphorus removal process. Figure 1 shows the subdivisions as presented by Ekama et al. (1984). Although the terminology varies, these are the same fractions used in the IAWPRC Activated Sludge Model 1 (Henze et al. 1987).

The first major subdivision of the total influent COD (S_{ti}) is into biodegradable (S_{bi}) and unbiodegradable (S_{ui}) fractions. Each of these is further subdivided. The unbiodegradable COD (S_{ui}) consists of two fractions: unbiodegradable soluble COD (S_{usi}) and unbiodegradable particulate COD (S_{upi}).[3]

[3] Selected symbols used in this report are defined on page 25.

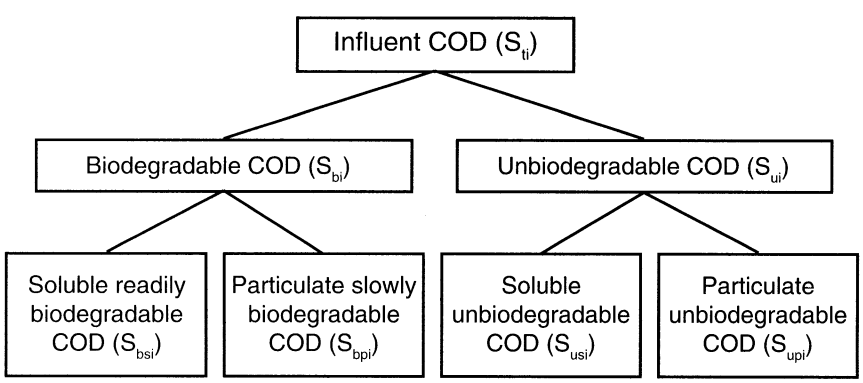


Figure 1. *Division of the total influent COD in municipal wastewater into its various constituent fractions.*

S_{usi} will pass through the treatment process and be discharged with the effluent. S_{upi} is enmeshed in the activated sludge. The mass of S_{upi} entering the system will equal the mass leaving the system via activated sludge wasting. Thus, S_{upi} has the principal effect of increasing the mixed liquor suspended solid (MLSS) concentration.

The biodegradable COD fraction (S_{bi}) is divided into readily biodegradable soluble COD (S_{bsi}) and slowly biodegradable particulate COD (S_{bpi}). S_{bsi} is taken up by activated sludge in a matter of minutes and metabolized, giving rise to a high unit rate of oxygen demand for synthesis. S_{bpi} must first be sorbed onto the microorganisms, and broken down to simple chemical units by extracellular enzymes before finally being metabolized by the microorganisms. The soluble readily biodegradable fraction, S_{bsi}, plays an important role in biological phosphorus removal because phosphorus-removing microorganisms sequester volatile fatty acids (VFAs) in the S_{bsi} fraction, using the energy obtained from cleavage of a phosphate bond of the polyphosphates stored within the biomass.

Readily Biodegradable Soluble COD (S_{bsi})

In the anaerobic zone of a BPR process, only the readily biodegradable soluble COD (S_{bsi}) component is susceptible to fermentation to form VFAs within the short detention time (1-2 hours).

Early evidence of the need for readily biodegradable substrate in phosphorus removal processes was provided by Fuhs and Chen (1975). They proposed that the enrichment of activated sludge with the phosphate accumulating bacteria, *Acinetobacter,* would ensure efficient biological phosphorus removal. The growth of *Acinetobacter* could be ensured by supplying readily biodegradable short carbon chain substrates such as ethanol, acetate, and succinate to an anaerobic zone in the process. Such a carbon source could also be provided by bleeding in fermented primary effluent or anaerobic digester supernatant liquor.

Further evidence of the need for VFAs in biological phosphorus removal was provided by Venter et al. (1978) and Osborn and Nicholls (1978). These experiments indicated that S_{bsi} is mostly utilized in the anaerobic reactor. This concept was also postulated by Nicholls and Osborn (1979)

when they stated that S_{bsi} was taken up into the cell under anaerobic conditions and stored as poly-β-hydroxybutyrate.

In seeking an explanation for the behavior of different phosphorus release patterns, Ekama et al. (1984) found that phosphorus release increased as the readily biodegradable soluble COD (S_{bsi}) increased. Ekama et al. (1984) concluded that a prerequisite for phosphorus release in the anaerobic zone is that the concentration of readily biodegradable soluble COD (S_{bsi}) surrounding the microorganisms in the anaerobic zone must exceed approximately 25 mg/L. Therefore, S_{bsi} is thought to be a very important wastewater characteristic in the process of biological phosphorus removal.

Determination of the COD Fractions

• Biodegradable COD (S_{bi}) Determination

Theory: Biodegradable COD (S_{bi}) may be determined using the total biological demand (T_bOD) concept of Mullis and Schroeder (1971). The T_bOD concept assumes that particulate organic materials are hydrolyzed when the biological oxidation process is completed (normally after 24 hours). This was true in tests performed on wastewaters from several municipalities during this study. Thus, T_bOD is conceptually equal to the biodegradable COD including the soluble readily degradable COD (S_{bsi}) and the particulate slowly degradable COD (S_{bpi}). Using T_bOD as the value for S_{bi} is thought to be adequate for design.

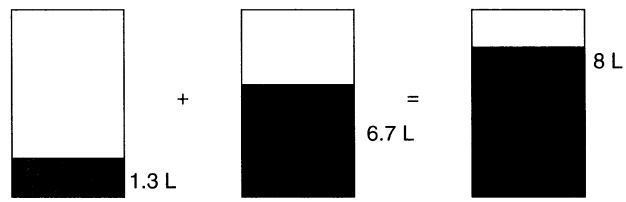
T_bOD can be determined in a batch test simultaneously with the yield coefficient, Y, as described in the section on "Y and k_d Determination by Batch Test" (p. 15). The batch test should be conducted under similar operational conditions of the wastewater treatment plant of interest, including sludge age, food to microorganism ratio (F/M), mixed liquor suspended solid (MLSS) concentration, etc. A worksheet for determination of T_bOD and Y is provided in Table 3. Currently we are trying to develop a simpler method using an electrolytic respirometer.

Apparatus:

10 L bottle (reactor)
Diffuser
0.45 μm glass fiber filter, beakers, pipettes
COD measurement apparatus
VSS measurement apparatus
Filtration apparatus

Procedure: The batch test procedure to determine T_bOD (S_{bi}) consists of the following steps:

1. Obtain 8 L of composite wastewater sample.
2. Measure initial total COD and initial soluble COD (the COD of filtrate passing through a 0.45 μm filter, COD_f) of the wastewater sample. The COD of the wastewater suspended solids is obtained by subtracting soluble COD from total COD.
3. Obtain 8 L of acclimated activated sludge.
4. Place a portion of the wastewater and activated sludge into an 8 L reactor. The dilution ratio used can be the same as the F/M ratio[4] at the treatment plant of interest. For example, the Ashland wastewater treatment plant has the F/M ratio of 0.67; thus, 1.3 L of activated sludge with VSS of 1,840 mg/L can be mixed with 6.7 L of raw sewage with BOD_5 of 240 mg/L to obtain the F/M ratio of 0.67 in an 8 L reactor.



5. Aerate the reactor to reach a dissolved oxygen level of approximately 2 mg/L. If an air pump with a diffuser does not provide sufficient mixing, add a mechanical mixer. The mixture is aerated for 24 hours, and samples are taken periodically.
6. Measure the COD of the mixture (COD_m) and the filtrate passing through a 0.45 μm filter (COD_f). Duplicate or triplicate sample analyses is recommended. The COD of the suspended solids is calculated by subtracting COD_f from COD_m.
7. For Y and k_d determination (described in the section on "Y and k_d Determination by Batch Test," p. 15), measure the VSS of the mixture and COD of the filtrate passing through a 0.45 μm filter (COD_f) at 0, 1, 2, 3, 4, 5, 6, 12, 18, and 24 hours.

Data Analysis: T_bOD is the difference between the initial substrate COD and the final unbiodegradable substrate COD in the reactor:

$$T_bOD = S_{bi} = \text{initial substrate COD} - \text{final substrate COD (final } COD_f), \quad (1)$$

[4] F/M (^{1}/day) = [BOD_5 (mg/L) x Q (L/day)] / [MLVSS (mg/L) x V (L)] where the reaction time in the batch experiments is 1 day.

Table 3. *Worksheet for T_bOD and Y determination.*

Time (hr)	COD_m	Average	COD_s	Average	SSCOD	MLVSS	Average	f_{cv}
0								
0								
0								
1								
1								
1								
2								
2								
2								
3								
3								
3								
4								
4								
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5								
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COD_m = mixed liquor COD (mg/L).
COD_s = filtrate soluble COD (mg/L).
SSCOD = suspended COD = (mixed liquor COD – filtrate soluble COD).
MLVSS = mixed liquor volatile suspended solids.
f_{cv} = SSCOD/MLVSS.

where

initial substrate COD =
initial COD_m – initial biomass COD; and
initial biomass COD =
initial mixture suspended solids COD –
raw wastewater suspended solids COD.

Because the wastewater sample is diluted by adding activated sludge to the reactor, the actual T_bOD is obtained by adjusting the test T_bOD by the dilution factor. An example calculation of T_bOD, using data from Table 4, is provided below.

Calculation of T_bOD from Example Test Data:

1. Use measured total COD and soluble (filtered) COD of wastewater sample (see Table 5) to calculate the wastewater suspended solids COD (SS COD_w).

   SS COD_w = total wastewater COD –
   soluble wastewater COD.
   = 488 – 203 = 285 mg/L.

2. Use measured initial total COD of mixture (COD_m) and soluble (filtered) COD of mixture (COD_s) (see Table 4) to calculate initial suspended solids COD of the mixture (SS COD_m).

   SS COD_m = initial COD_m – initial COD_s.
   = 792 – 153 = 639 mg/L.

3. Calculate the mixture biomass COD as follows:

   Mixture biomass COD = SS COD_m – SS COD_w.
   = 639 – 285 = 354 mg/L.

4. Calculate the initial mixture substrate COD as follows:

   Initial mixture substrate COD = initial COD_m –
   mixture biomass COD.
   = 792 – 354 = 438 mg/L.

5. The final substrate COD of mixture is the measured final soluble (filtered) COD of the mixture. Therefore the test T_bOD is calculated as follows:

   Test T_bOD = initial mixture substrate COD –
   final mixture COD_s.
   = 438 – 71 = 367 mg/L.

6. The test T_bOD must be adjusted by the dilution ratio to obtain wastewater T_bOD as follows:

   Wastewater T_bOD = test T_bOD x
   (volume of mixture/volume of wastewater).
   = 367 x (8/6.7) = 438 mg/L.

Personhours needed: 30 hours + acclimation time (0-30 hours depending on wastewater).

• Soluble Readily Biodegradable COD (S_{bsi}) and Soluble Unbiodegradable COD (S_{usi}) Determination

Theory: Mamais et al. (1993) developed a rapid physical-chemical method for determining the soluble readily biodegradable COD (S_{bsi}) and the soluble unbiodegradable COD (S_{usi}). Flocculation, precipitation, and filtration of wastewater samples allow for the direct measurement of S_{bsi} and S_{usi}.

The method is based on the assumption that the influent unbiodegradable soluble COD (S_{usi}) is equal to the truly soluble effluent COD from an activated sludge plant treating the wastewater with a sludge age > 3 days. Flocculation and precipitation of the samples remove colloidal material that normally passes through a 0.45 μm membrane filter.

Thus,

$$S_{bsi} = (\text{total truly soluble } COD_{inf}) - (\text{soluble unbiodegradable COD, } S_{usi}). \quad (2)$$

The total truly soluble COD of the raw wastewater is determined by flocculating the wastewater influent with $Zn(OH)_2$ at pH = 10.5, filtering with a 0.45 μm filter, and then measuring the COD of the filtrate. The unbiodegradable soluble COD (S_{usi}) is determined by performing the above test with the effluent under the same assumption described above. Subtracting S_{usi} from the total soluble COD of the raw wastewater yields the influent soluble biodegradable COD fraction (S_{bsi}).

Apparatus:

Magnetic stirrer, stirring bar, and pH meter
0.45 μm glass fiber filter, beakers, pipettes
Filtration apparatus
VSS measurement apparatus
COD measurement apparatus

Procedure: A detailed flocculation method is described as follows:

1. Add 1 ml of a 100 g/L zinc sulfate solution to a 100 ml wastewater sample and mix vigorously with a magnetic stirrer for 1 minute.
2. Adjust the pH to approximately 10.5 with 6 Molar sodium hydroxide solution (NaOH).
3. Settle quiescently for a few minutes.
4. Withdraw clear supernatant (20-30 ml) with a pipette and pass through a 0.45 μm membrane filter.
5. Measure COD of the filtrate.

Personhours needed: 5 hours.

Table 4. *Example calculation of T_bOD from batch experiment data. (Ashland sewage of July 16, 1994: 1.3 L activated sludge + 6.7 L sewage).*

Time (hr)	COD_m	Average	COD_s	Average	SSCOD	VSS	Average
0	792	792	153	153	639	484	484 [a]
0	790		150			472	
0	794		156			484	
1	763	763	140	143	620	492	496
1	763		143			512	
1	763		145			500	
2	770	777	120	121	655	484	508
2	784		126			508	
2	777		116			532	
2.5	775	770	108	108	663	520	520
2.5	765		108			524	
2.5	770		108			516	
3	755	756	100	101	654	508	503
3	758		96			500	
3	755		108			500	
3.5	730	725	105	100	625	496	512
3.5	720		95			520	
3.5	725		100			520	
4	766	766	97	96	671	492	501
4	766		98			504	
4	766		94			508	
4.5	710	715	91	92	623	508	509
4.5	720		92			504	
4.5	715		93			516	
5	740	735	88	92	644	500	510
5	735		94			528	
5	730		94			504	
6	760	763	87	87	676	480	499
6	766		87			504	
6	763		87			512	
8	678	677	84	83	594	476	485
8	672		82			496	
8	682		83			484	
12	710	710	78	78	632	464	495
12	720		76			480	
12	701		80			540	
24	715	715	70	71	634	412	497
24	710		68			548	
24	721		74			532	

[a] The value of 472 was eliminated due to its large standard error.

Table 5. *Ashland wastewater analysis of July 16, 1994.*

Parameters	Influent (Raw)	Activated Sludge	Effluent
BOD_5, mg/L	240	-	6
COD, mg/L	488	-	25
Soluble COD, mg/L	203	-	13
TSS, mg/L	228	2,840	9
VSS, mg/L	206	1,840	7
TKN, mg/L	40	-	4
NH_3-N, mg/L	25	-	2
$NO_3^- + NO_2^-$-N	0.6	-	20
Alkalinity, mg/L as $CaCO_3$ (pH = 4.5)	325	-	45
pH	7.5	-	7.1
Orthophosphate, mg/L	4.3	-	0.5
Total P, mg/L	5.6	-	0.6

- **Particulate Slowly Biodegradable COD (S_{bpi}) and Particulate Unbiodegradable COD (S_{upi}) Determination**

From the influent biodegradable COD (S_{bi}) (determined by the T_bOD method) and the influent soluble readily biodegradable COD (S_{bsi}), the particulate slowly biodegradable COD (S_{bpi}) can be obtained:

$$S_{bi} = S_{bsi} + S_{bpi}.$$

Finally the particulate unbiodegradable COD (S_{upi}) is obtained by:

S_{upi} = total influent COD (S_{ti}) – biodegradable COD (S_{bi}) – soluble unbiodegradable COD (S_{usi}).

Once the values of S_{bsi} and S_{usi} are obtained together with T_bOD (or S_{bi}), the COD fractionation of wastewater is completed. Table 6 lists the typical COD fraction in municipal wastewater. Table 7 provides actual test data from the Ashland wastewater treatment plant.

Fraction of Nitrogen in Wastewater

In untreated domestic wastewater, nitrogen will be found primarily in the form of organic and ammonium nitrogen (NH_4^+-N). Little (< 1%) ammonia nitrogen (NH_3-N) exists in a normal domestic wastewater with a pH of 7. A typical total nitrogen in domestic wastewater consists of about 60% ammonium nitrogen and 40% organic nitrogen with less than 1% in the form of nitrate and/or nitrite.

Analytically organic nitrogen and total ammonia nitrogen are measured simultaneously with total Kjeldahl nitrogen (TKN). If nitrate or nitrite is present in the wastewater, the TKN test will not include them.

Table 6. *Typical average South Africa municipal wastewater characteristics (Ekama et al. 1984).*

Wastewater Characteristics	Raw Wastewater	Settled Wastewater
Biodegradable COD fraction, S_{bi}	0.75 - 0.85	0.80 - 0.95
Readily biodegradable COD fraction, S_{bsi}	0.08 - 0.25	0.10 - 0.35
Unbiodegradable soluble COD fraction, S_{usi}	0.04 - 0.10	0.05 - 0.20
Unbiodegradable particulate COD fraction, S_{upi}	0.015 - 0.025	0.02 - 0.03

Table 7. *COD fraction for samples taken from the Ashland wastewater treatment plant on March 30, May 23, July 16, and December 1, 1994.*

Fraction/Concentration (mg/L) (Percentage of Total COD)	3/30	5/23	7/16	12/1
Influent COD (S_{ti})	345	283	488	565
Biodegradable COD (S_{bi})	302 (88%)	220 (78%)	438 (89%)	463 (82%)
Unbiodegradable COD (S_{ui})	43 (12%)	63 (22%)	50 (11%)	102 (18%)
Soluble readily biodegradable COD (S_{bsi})	71 (21%)	85 (30%)	137 (28%)	107 (19%)
Particulate slowly biodegradable COD (S_{bpi})	231 (67%)	136 (48%)	298 (61%)	356 (63%)
Unbiodegradable soluble COD (S_{usi})	14 (4%)	20 (7%)	19 (4%)	29 (5%)
Unbiodegradable particulate COD (S_{upi})	29 (8%)	42 (15%)	34 (7%)	73 (13%)

Table 8. *Nitrogen content of domestic sewage (Sedlak 1991).*

	Type of Sewage		
Nitrogen Form	Strong	Medium	Weak
Organic-N, mg/L	35	15	8
Ammonium-N, mg/L	50	25	12
Total N, mg/L	85	40	20

Table 8 gives a range of typical nitrogen concentrations found in untreated domestic wastewater.

Nitrogen transformations that can occur in biological treatment systems are shown in Figure 2. Organic nitrogen can be converted to ammonium through bacterial decomposition and hydrolysis of urea. Nitrification is the process whereby ammonium is oxidized to nitrate by 2 different genera of microorganisms, *Nitrosomonas* and *Nitrobacter*. Denitrification is the process that transforms nitrates to

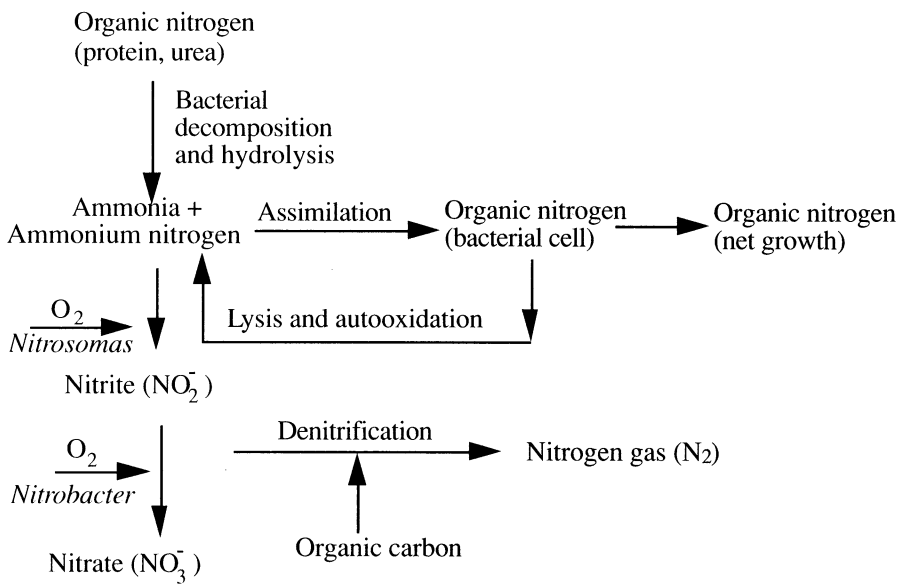


Figure 2. *Nitrogen transformations in biological treatment processes (Sedlak 1991).*

nitrogen gas by denitrifying microorganisms in the absence of oxygen. Denitrification requires an organic carbon source. Biological treatment systems can be designed to nitrify and denitrify by providing the proper conditions for the nitrifying and denitrifying microorganisms. TKN, NH_3 + NH_4^+-N, and NO_2^- + NO_3^--N can be analyzed according to *Standard Methods* (American Public Health Association 1995).

As with COD, nitrogenous material can also be subdivided into fractions as shown in Figure 3. It is difficult to fractionate organically bound nitrogen into biodegradable and unbiodegradable soluble and particulate fractions, N_{oi}, N_{ui}, and N_{pi}. Ekama et al. (1984) suggested that each fraction can be estimated only by comparing the observed response of laboratory scale processes with that predicted by the theoretical model. Although it is necessary to fractionate N_{ui} and N_{pi} for better data fitting, it is not critical to have accurate estimates since these fractions are small. Therefore, the unbiodegradable particulate organic nitrogen, N_{pi}, is simply expressed as 10% of the unbiodegradable particulate volatile solids in the influent, i.e., 0.1 S_{upi}/1.48 (or 1.42) (see "Advantage of Using COD over BOD," p. 3). The unbiodegradable soluble organic nitrogen, N_{ui} is reported to be 0.00-0.04 of TKN for raw wastewater and 0.00-0.05 of TKN for settled wastewater (Ekama et al. 1984). Therefore, biodegradable organic nitrogen (N_{oi}) can be obtained by subtracting N_{ai}, N_{ui}, and N_{pi} from N_{ti}.

Nitrate is the product of nitrification (see Figure 2). Domestic sewage without agricultural runoff normally contains little nitrate. In the aeration basin, nitrifiers oxidize ammonium and form nitrate. Since nitrifiers cannot compete with heterotrophic microorganisms in consuming oxygen, they grow in the latter part of the oxidation basin where little organic substance is present and heterotrophic microorganisms are depressed. Because the maximum growth rate for *Nitrobacter* is much higher than the maximum growth rate of *Nitrosomonas*, very little nitrite is normally present in a biological treatment system. If nitrite accumulates in a treatment plant it may be the result of toxicity to *Nitrobacter*. A nitrifying wastewater treatment plant contains nitrate in its effluent. If nitrifiers oxidize all the ammonia and ammonium nitrogen in the sewage influent, the effluent will contain up to 20-30 mg NO_3^- nitrogen/L (N/L).

The generally accepted theory for biological phosphorus removal is that sequential anaerobic-aerobic contacting processes result in selection of phosphorus-removing microorganisms. Nitrate can be introduced into the anaerobic zone by returned activated sludge from final clarifiers (in the case of a conventional activated sludge treatment plant) or by direct circulation flow (in the case of an oxidation ditch). The introduction of nitrite and nitrate depletes the readily biodegradable substrate (S_{bsi}), which is necessary for the phosphorus-removing microorganisms. Therefore, the presence of nitrates in the recycled stream significantly reduces the biological phosphorus removal potential.

The amount of biodegradable substrate that may be depleted due to the introduction of nitrate may be calculated as follows:

1. Calculate how much of COD will go to cell production.
2. Calculate the amount of oxygen (COD) that will go to oxidation of the substrate (how much COD is left after cell production).
3. Calculate how much of oxygen needed for step 2 will be supplied by NO_3^-.

Assuming Y is 0.45 mg VSS/mg total COD and the oxygen equivalent of the biological VSS is 1.48

mg O_2/mg VSS, the fraction of total COD that goes to cell production can be estimated as follows:

Fraction of total COD to cell mass

= (1.48 mg O_2/mg VSS) (0.45 mg VSS/mg total COD)
= 0.67 mg O_2 to cell/mg total COD.

Thus, the oxygen used for oxidation

= (1.0 mg O_2/mg total COD) – (0.67 mg O_2 as cell/mg total COD)
= 0.33 mg O_2/mg total COD.

Since 1 mg NO_3^--N is equal to 2.86 mg O_2 from half-cell-reactions for denitrification, the nitrate-nitrogen used to supply an equivalent amount of oxygen

= (0.33 mg O_2/mg total COD) / (2.86 mg O_2 equiv./mg NO_3^--N)
= 0.12 mg NO_3^--N/mg total COD, or 8.56 mg total COD/mg NO_3^--N.

This implies that 8.56 mg total COD may be used for each mg of NO_3^--N added to the anaerobic zone. When Y = 0.30 mg VSS/mg total COD, 5.14 mg total COD will be used for each mg of NO_3^--N added to the anaerobic zone. Since denitrifiers use readily biodegradable substrate (S_{bsi}) more efficiently than phosphorus-removing microorganisms, denitrifiers have the potential to consume 5 to 9 mg total COD/mg NO_3^--N and deplete the readily biodegradable substrate (S_{bsi}) necessary for phosphorus-removing microorganisms.

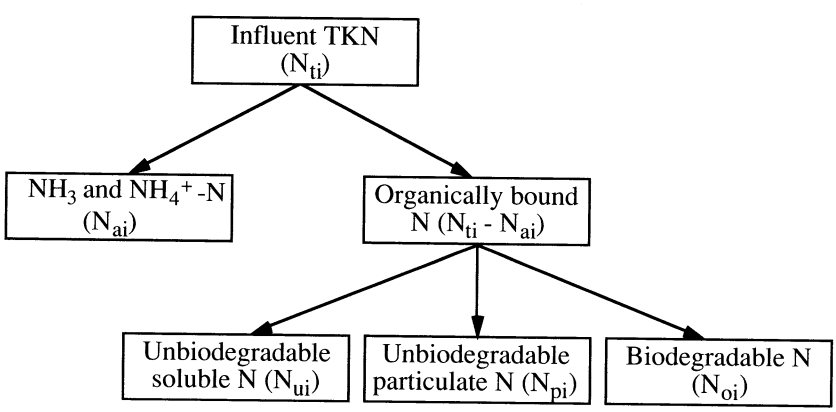


Figure 3. *Fractions of nitrogen in wastewater.*

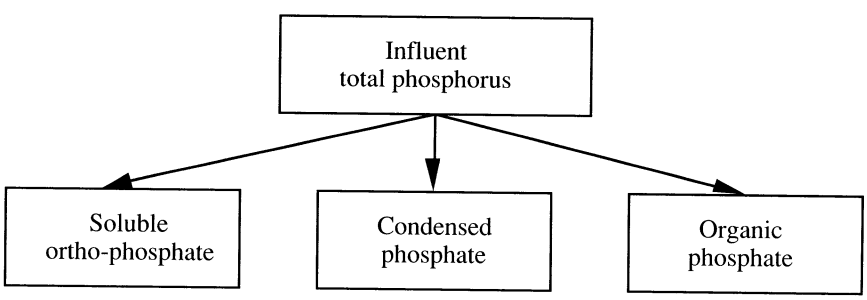


Figure 4. *Fractions of phosphorus in wastewater.*

Table 9. *Chemical form of phosphate in U.S. sewage (Sedlak 1991).*

Phosphate form	Typical concentration (mg-P/L)
Orthophosphate	3 - 4
Condensed phosphates	2 - 3
Organic phosphates	1

Fraction of Phosphorus in Wastewater

Phosphorus is found in wastewater as phosphates. These can be categorized by physical (dissolved and particulate fractions) and chemical (orthophosphate, condensed phosphate, and organic phosphate fractions) characteristics. Orthophosphates applied to agricultural or residential cultivated land as fertilizers are carried into surface waters with storm runoff. Small amounts of certain condensed phosphates (pyro-, meta-, and other polyphosphates) are added to some water supplies during treatment. Organic phosphates are contributed to sewage by body wastes and food residues. Typical concentrations for various forms of phosphorus in raw wastewater in the United States are summarized in Table 9.

In the activated sludge process, condensed and organically bound phosphorus in the influent will be converted to orthophosphate. Phosphorus is removed from the process through activated sludge wasting. Thus, total phosphorus in the effluent will be primarily orthophosphate, although there will be some organic phosphorus contained in any effluent suspended solids. The fraction of phosphorus in domestic wastewater is shown in Figure 4. Phosphate fractions can be analyzed by *Standard Methods* (American Public Health Association 1995).

Biological Kinetic Parameter Estimation

Required Kinetic Parameters

The important kinetic parameters required for biological phosphorus removal process design include the following.

Y - The cell yield coefficient defined as the mass of activated sludge or biomass produced per unit of substrate removed (mg VSS/mg COD).

k_d - The endogenous decay rate or mass of cells lost during endogenous respiration per unit of time (^{1}/day).

μ_{max} - The maximum specific growth rate. The specific growth rate, μ, is the rate of growth per unit of time (^{1}/day).

K_s - The half-saturation constant or shape factor of the Monod equation. K_s equals the substrate concentration (mg/L) at which μ equals 1/2 of μ_{max}.

q_N - The specific nitrification rate, which is measured by rate of $NO_2^- + NO_3^-$ formation (mg $NO_2^- + NO_3^-$-N/mg VSS/hour).

q_D - The specific denitrification rate, which is measured by rate of $NO_2^- + NO_3^-$ removed (mg $NO_2^- + NO_3^-$-N/mg VSS/hour)

The theories and experimental procedures for determining the biological kinetic parameters defined above are discussed in this section. Also discussed are the measurement methods of phosphorus release and uptake rates. Although phosphorus release and uptake rates are not used in the design equations, the rates can provide insight into the design of BPR systems. Therefore, their measurement techniques are presented here.

Theoretical Base of the Kinetic Equations

The cell yield coefficient, Y, is one of the most important parameters used in biological kinetic models. It represents the mass of biomass produced per substrate removed. The endogenous decay rate k_d, represents the rate of biomass loss due to endogenous respiration. The cell yield coefficient, Y, and endogenous decay rate, k_d, are critical for the prediction of waste-activated sludge production. In a BPR process, phosphorus is removed in the form of waste-activated sludge.

The stoichiometry between the organic substrate consumed and microorganisms produced can be expressed as:

$$\frac{dX}{dt} = Y\frac{dS}{dt} - k_d X \qquad (3)$$

where

X = concentration of mixed liquor volatile suspended solids (MLVSS) (mg/L);
t = time (day);
S = substrate concentration (mg/L);
Y = yield coefficient; mass of cells produced per unit mass of substrate utilized (mg VSS/mg COD); and
k_d = fraction of MLSS or cells oxidized by endogenous respiration per unit of time (^{1}/day).

This equation can be rewritten after dividing Equation 3 by X:

$$\frac{dX}{Xdt} = Y\frac{dS}{Xdt} - k_d \qquad (4)$$

It can then be rewritten on a finite time and mass basis:

$$\frac{\Delta X}{X\Delta t} = Y\frac{\Delta S}{X\Delta t} - k_d \qquad (5)$$

where

$\frac{\Delta X}{X\Delta t}$ = amount of specific cell mass produced over unit time, μ (^{1}/day); and

$\frac{\Delta S}{X\Delta t}$ = specific substrate utilization rate, U (^{1}/day).

The growth rate of microbial mass ($\frac{\Delta X}{\Delta t}$) is expressed as the specific growth rate, μ (i.e., the rate of growth per average unit of biomass during the time interval, Δt).

Thus, $\mu = Y \times U - k_d$. (6)

Y and k_d Determination by Batch Test

It is difficult and time consuming to obtain Y and k_d by a conventional method that calls for operating at least four bench-scale, continuous-flow, biological reactors at different sludge ages. These parameters mainly affect activated sludge production and have relatively little effect on predicted effluent quality. However, phosphorus removal in a BPR process

occurs through activated sludge wasting; therefore, Y and k_d are important for BPR design.

It is easy to determine Y and k_d by running a batch test, which is similar to the procedure used for T_bOD determination. Therefore, from the same batch test, T_bOD, Y, and k_d can be determined simultaneously. Since there is little difference in Y and k_d values (VSS basis) for conventional and phosphorus-removing treatment plants (McClintock et al. 1992), it may not be necessary to acclimate biomass for phosphorus removal in Y and k_d determination.

Data Analysis: Some experimental runs may suffer from variability in VSS analyses used to measure biomass growth. If the samples are not carefully taken, the variability in the VSS measurements at each time may be even greater than the net growth of microorganisms, making the kinetic study inaccurate. Thus, the reactor contents must be mixed vigorously to disperse the mixture uniformly before taking samples. Triplicate VSS and duplicate COD samples should be analyzed. It may be desirable to increase the F/M above typical values. In this way, a more noticeable biomass growth may be attained. Idealized cell growth and substrate removal curves are shown in Figure 5. In experimental runs with municipal wastewater, the net growth of microorganisms begins to decrease after several hours and becomes negative after the substrate is consumed. The experimental data are plotted and a smooth "best fit" curve is drawn through the points to average out some of the variability in the test data. These curves can either be drawn by hand or using a computer program to generate a best fit line through the data.

Values of S and X are chosen from the initial portion of the curve where the biomass is in the logarithmic growth phase. These data are transformed into estimates of U, the substrate utilization rate, and μ, the specific growth rate, for each time period (Δt from $i-1$ to i) using the following equations:

$$U_i = \frac{(S_{i-1} - S_i)/\Delta t_i}{(X_{i-1} + X_i)/2} \quad (7)$$

$$\mu_i = \frac{(X_i - X_{i-1})/\Delta t_i}{(X_i + X_{i-1})/2} \quad (8)$$

Based on Equation 6, μ and U can be plotted and a regression line can be drawn as shown in Figure 6. The endogenous decay rate, k_d, is the Y-intercept. Since k_d is extremely sensitive to the variability of the data points, it may be difficult to determine a reasonable value for k_d using this method. However, k_d can be obtained independently from a respirometer experiment that will be described in the section on "k_d Determination by Electrolytic Respirometer" (p. 18). Forcing a regression line to fit through the independently determined k_d makes the resulting slope a more reliable estimate of Y.

An example illustration of Y and k_d determination from an μ vs. U plot is provided in Figure 7. The values of Y and k_d are determined to be 0.65 mg VSS/mg COD and 0.0026 ^{1}/hour (or 0.07 ^{1}/day), respectively.

Personhours needed: 24 hours + acclimation time (0-30 hours depending on wastewater).

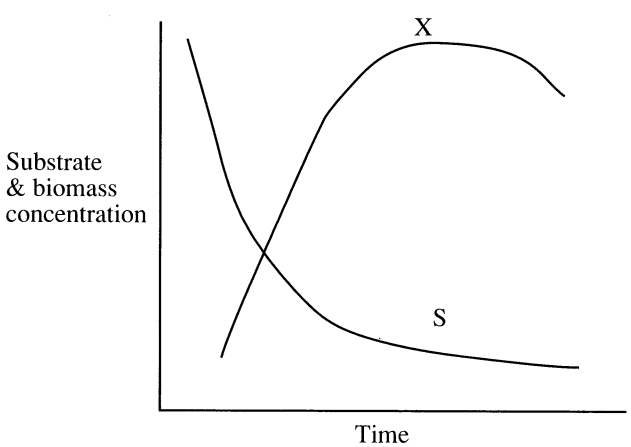


Figure 5. *Generalized substrate consumption and biomass growth with time.*

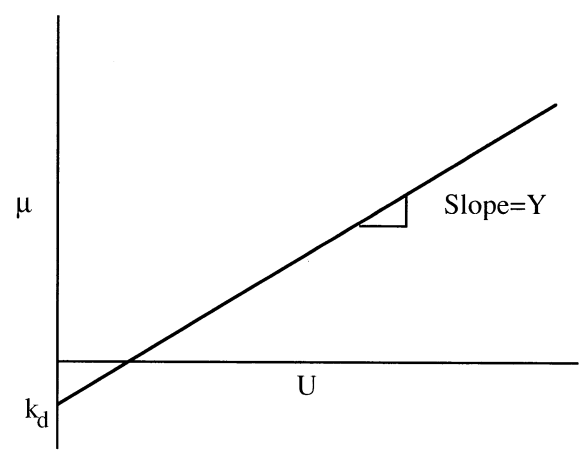


Figure 6. *Plot of specific growth rate (μ) with specific substrate utilization rate (U).*

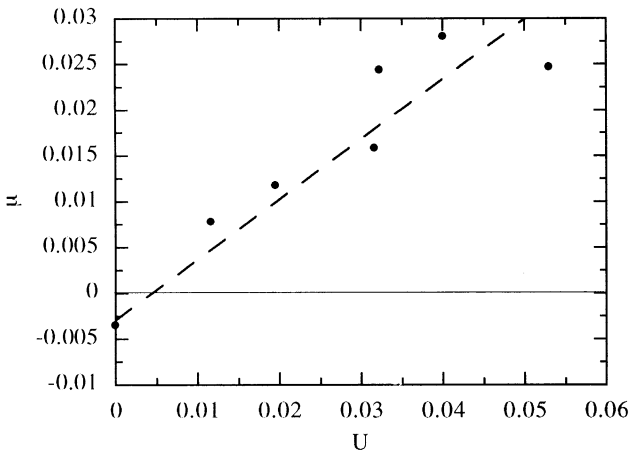


Figure 7. *Y and k_d determination from μ vs. U plot.*

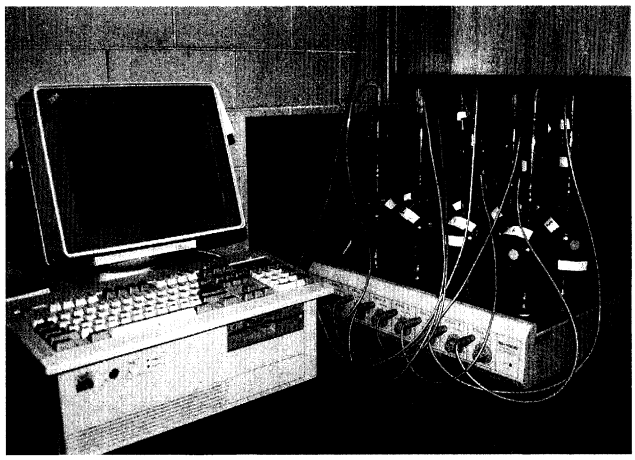


Figure 8. *Electrolytic respirometer.*

μ_{max} and k_s Determination by Electrolytic Respirometer

The electrolytic respirometer is a very useful tool for determining the biokinetic growth constants, μ_{max} and K_s, used in the Monod equation for non-inhibitory wastewater:

$$\mu = \frac{\mu_{max} S}{(K_s + S)} \qquad (9)$$

where

μ_{max} = maximum specific growth rate (^{1}/hour), and
K_s = half-saturation constant or substrate concentration when $\mu = \mu_{max}/2$ (mg/L).

If the wastewater shows inhibition, the Haldane equation should be used. Once the relationship between μ and S is quantified, μ_{max} and K_s in the Monod model can be determined graphically or statistically.

Apparatus:

Electrolytic respirometer
COD measurement apparatus
VSS measurement apparatus
Filtration apparatus

A typical electrolytic respirometer is shown in Figure 8.

Procedure: The procedures to run an electrolytic respirometer may vary slightly, depending on the manufacturer. Basically, the wastewater concentration is diluted by addition of washed activated sludge and added to each reactor cell. Each cell is prepared at a different F/M ratio, and contains a different initial mixed wastewater COD concentration (S_o). The activated sludge should be washed using the following procedure to remove any soluble and adsorbed substrate:

1. Settle the mixed liquor suspended solids.
2. Decant the supernatant.
3. Fill remaining volume with BOD_5 nutrient dilution water containing phosphate buffer, $MgSO_4$, $CaCl_2$, and $FeCl_3$ solution (17 mg of KH_2PO_4, 43.5 mg of K_2HPO_4, 66.8 mg of $NaHPO_4 \cdot 7H_2O$, 3.4 mg of NH_4Cl, 45 mg of $MgSO_4$, 55 mg of $CaCl_2$, and 0.5 mg of $FeCl_3 \cdot 6H_2O$ in 2 L of distilled water).
4. Mix gently and settle activated sludge.
5. Repeat step 2 through step 4 three times.

The oxygen uptake rate is automatically recorded by a computer data acquisition system. The initial mixed wastewater COD concentration (S_o) is used to calibrate the Monod equation. The initial mixed liquor VSS concentration (X_o) and the initial mixed wastewater COD concentration in each reactor cell must be analyzed. If an electrolytic respirometer is not available, a series of batch tests (see "Determination of the COD Fractions," p. 7) for T_bOD determination may be conducted under several different F/M ratios.

Data Analysis: The electrolytic respirometer's data acquisition system records the accumulated oxygen consumption vs. time, which then can be translated into biomass growth data. A typical plot of O_2 accumulation over time is shown in Figure 9.

Oxygen uptake data can be converted into biomass growth curves using the following equation (Rozich and Gaudy 1992):

$$X_t = X_o + \frac{O_2 \text{ uptake}}{1/Y - f_{cv}} \quad (10)$$

where

O_2 uptake = oxygen consumed by biomass (mg/L),
X_t = mixed liquor VSS concentration at time t in each reactor cell (mg/L), and
X_o = mixed liquor VSS concentration at time 0 in each reactor cell (mg/L).

This equation allows the indirect estimation of biomass concentrations over time.

To convert O_2 uptake data to biomass data using Equation 10, values for Y and f_{cv} must be determined. Y can be determined from the kinetic tests described in the section on "Y and k_d Determination by Batch Test" (p. 15). The values of f_{cv} can be assumed to be 1.42-1.48 mg COD/mg VSS. It should be noted that Y and f_{cv} in Equation 10 are assumed to be constant over time under declining substrate concentration conditions. The growth rate is obtained from the following equation:

$$\mu = \frac{\ln(X_2 - X_1)}{(t_2 - t_1)} \quad (11)$$

Thus, when plotting the calculated X with time on a semi-logarithmic paper, the specific growth rate (μ) is the slope of the line. The typical plot of ln X vs. time is shown in Figure 9. The slopes in Figure 9 represent μ values at different substrate concentrations. Table 10 lists the results of specific growth rate (μ) obtained from Figure 9 corresponded with the total substrate concentrations (S), which are predetermined from wastewater in each cell of the electrolytic respirometer. If a lag, stationary, or declining phase is shown in the ln X vs. time plot, the points in these phases should be excluded in the regression analysis. Because of this, only data points up to 10 hours, from Figure 9, were used to determine μ values in Figure 10.

Assuming a wastewater is not inhibitory, the growth rate data (μ vs. S) are fitted to the Monod equation (Equation 9) to determine the values of the biokinetic constants μ_{max} and K_s. An example illustration of a μ vs. S plot used to determine μ_{max} and K_s is provided in Figure 11. Use of statistical computer software is highly recommended for parameter estimation. The curve was obtained from a nonlinear least squares method. The μ_{max} and K_s values were 0.034 1/hour and 209 mg/L, respectively, with the correlation coefficient of 0.99.

Personhours needed: 6 hours.

Table 10. *Results of μ and S determination.*

Cell #	Cell 1	Cell 2	Cell 3	Cell 4	Cell 5
S (mg/L COD)	81	162	244	366	460
μ (1/hour)	0.0083	0.0151	0.0191	0.0216	0.0230

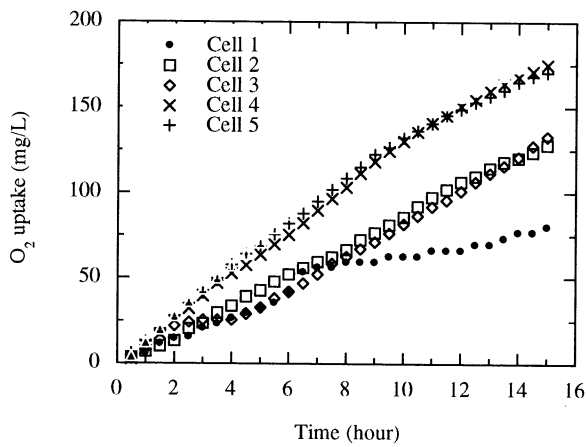


Figure 9. *Typical O_2 accumulated over time.*

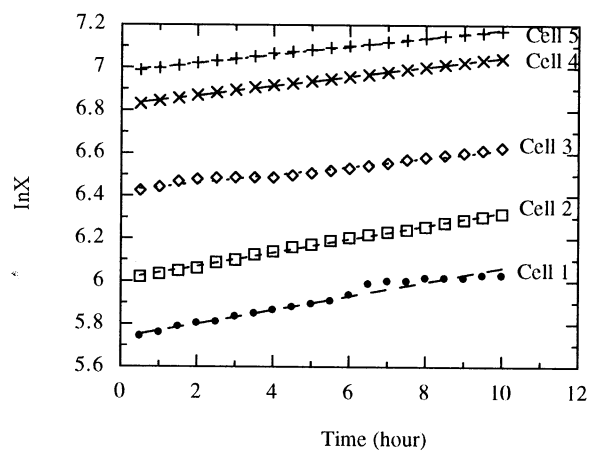


Figure 10. *Typical ln X vs. time plot.*

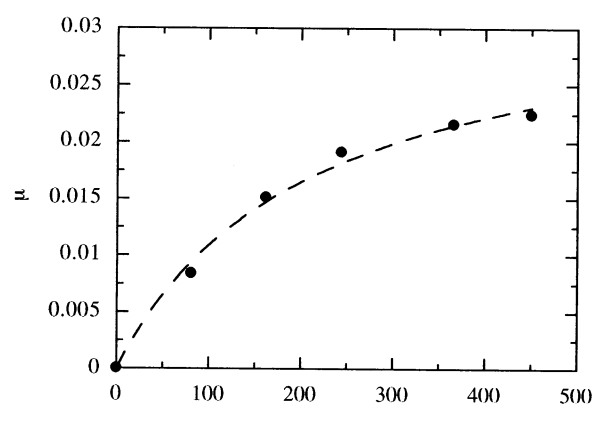


Figure 11. *μ vs. S plot to determine μ_{max} and K_s.*

k_d Determination by Electrolytic Respirometer

Theory: The oxygen consumption rate can be corrected for activated sludge concentration as follows:

$$\frac{dO}{dt} = 1.42 k_d X \qquad (12)$$

The endogenous decay rate, k_d, is defined as the rate of cell mass decrease per unit of mass:

$$k_d = -\frac{dX}{XdX}$$

which can be transformed into

$$X_t = X_o e^{-k_d t} \qquad (13)$$

where

X_t = cell mass at time t (mg VSS/L), and
X_o = initial cell mass (mg VSS/L).

Substituting Equation 13 into Equation 12 yields

$$\frac{dO}{dt} = 1.42 k_d X_o e^{-k_d t} \qquad (14)$$

Taking the natural logarithm, Equation 14 becomes

$$\ln\left(\frac{dO}{dt}\right) = \ln(1.42 k_d X_o) k_d t \qquad (15)$$

In Equation 15, k_d is the slope of the ln (dO/dt) vs. time plot. The dO/dt (rate of oxygen consumption) data can be generated by an electrolytic respirometer.

Apparatus: Electrolytic respirometer.

Procedure: The experimental method to determine k_d by electrolytic respirometer is straight forward. An activated sludge sample is aerated for one day and washed three times with BOD_5 nutrient solution to remove any adsorbed and soluble substrate. Oxygen consumption is measured with washed activated sludge in an electrolytic respirometer, and the rate of oxygen consumption is obtained.

Data Analysis: Figure 12 shows an example of the results of a k_d determination using an electrolytic respirometer. The results indicated there was still residual substrate left in the first 12 hours. The slope of ln (dO/dt) vs. time plot after 12 hours will indicate the endogenous decay rate, k_d. If the activated sludge is washed well after one day aeration without feed, the sharp oxygen uptake rate at the initial phase will be minimized as shown in another run (Figure 13).

Personhours needed: 6 hours.

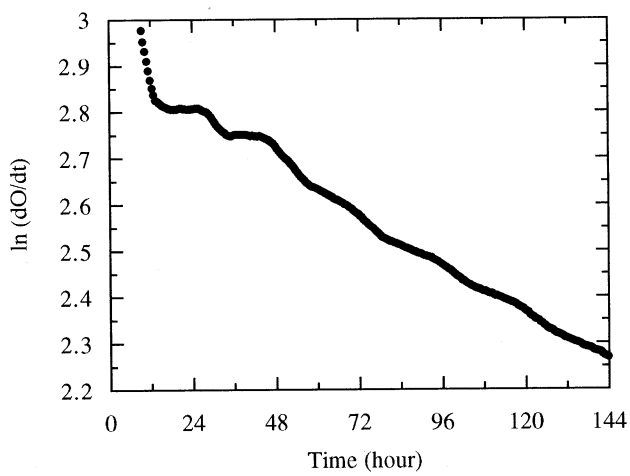


Figure 12. *Endogenous decay rate, k_d, determination without well-washed activated sludge.*

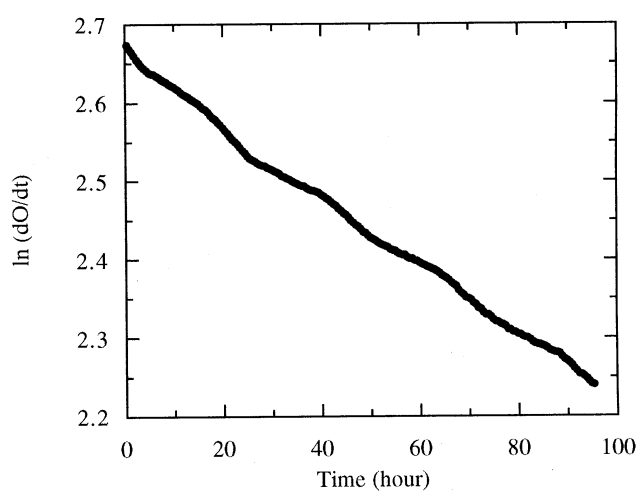


Figure 13. *Endogenous decay rate, k_d, determination with well-washed activated sludge.*

Nitrification and Denitrification Rates Measurement

Nitrification Rate

Theory: Although the kinetics of nitrification have been modeled by zero-order and first-order reactions, a Monod type equation expressing the effect of substrate concentration on the growth of nitrifying bacteria has been found to fit the data in most nitrification studies (Barnes and Bliss 1983). The effect of individual independent limiting substrates on the specific growth rate can also be expressed. Thus, the effects of NH_4^+-N and dissolved oxygen on the growth rate of *Nitrosomonas* are described as follows:

$$\mu_N = \mu_{Nmax}\left[\frac{NH_4^+ - N}{K_N + NH_4^+ - N}\right]\left[\frac{DO}{K_o + DO}\right] \qquad (16)$$

where

μ_N = specific growth rate of *Nitrosomonas* (nitrifiers) (^{1}/hour),
μ_{Nmax} = maximum specific growth rate of *Nitrosomonas* (nitrifiers) (^{1}/hour),
K_N = half-saturation constant for NH_4^+-N (mg/L),
DO = dissolved oxygen (mg/L), and
K_o = half-saturation constant for oxygen (mg/L).

Similar relationships can be written for the oxidation of nitrite to nitrate in terms of *Nitrobacter* and with NO_2^--N as substrate. Because it is generally the rate-limiting reaction, the nitrifier growth rate can be modeled based on the conversion of ammonium to nitrite by *Nitrosomonas*.

The ammonium oxidation rate can be measured to quantify how fast ammonium is oxidized to nitrate. It should be noted that over 99% of the total ammonia nitrogen ($NH_3 + NH_4^+$-N) in normal domestic wastewater pH of 7 is in the form of ammonium (NH_4^+-N). The ammonium oxidation rate (q_N) for activated sludge is often expressed in units of mg NH_4^+-N removed per hour for each g MLVSS in the aeration tank as follows (Barnes and Bliss 1983):

$$\frac{d(NH_4^+ - N)}{dt} = q_N X \qquad (17)$$

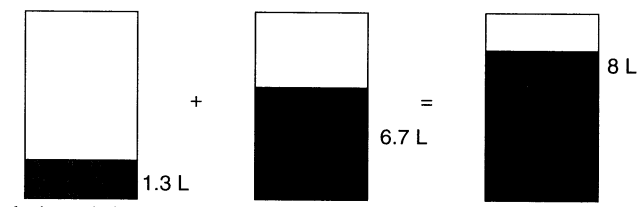
The ammonium oxidation rates (q_N) are commonly 1-3 mg/g/hour (Barnes and Bliss 1983).

Apparatus:

10 L bottle (reactor)
Diffuser
Pipettes
DO meter
$NH_3 + NH_4^+$-N and $NO_2^- + NO_3^-$-N measurement apparatus
VSS measurement apparatus
Filtration apparatus

Procedure: The procedure to determine the ammonium oxidation rate (q_N) is:

1. Obtain 8 L of wastewater sample.
2. Obtain 8 L of acclimated activated sludge.
3. Place a portion of the wastewater and activated sludge into an 8 L reactor. The dilution ratio used can be the same as the F/M ratio at the treatment plant of interest. For example, the Ashland treatment plant has an F/M = 0.67; thus, 1.3 L of activated sludge with VSS of 1,840 mg/L can be mixed with 6.7 L raw sewage with BOD_5 of 240 mg/L to obtain a F/M ratio of 0.67 in an 8 L reactor.



4. Measure VSS of mixture.
5. Aerate the reactor to reach a DO level of approximately 2 mg/L. If an air pump with a diffuser does not provide sufficient mixing, add a mechanical mixer.
6. Determine concentrations of total ammonia ($NH_3 + NH_4^+$-N), nitrite and nitrate ($NO_2^- + NO_3^-$-N) over time (at 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5 hours) in filtrate passed through 0.45 µm membrane filters.

Data Analysis: Since the organic nitrogen will be transformed by bacteria to form total ammonia nitrogen, it is recommended to measure nitrite and nitrate production rates as the indicator of the ammonium oxidation rate. Table 11 and Figure 14 show an example of an ammonium oxidation rate determination. Even though a single sample is analyzed in this example, duplicate sample analysis is recommended.

The ammonium oxidation rate is:

(27.6 – 19.8 mg $NO_2^- + NO_3^-$ /L)/5 hours/ 2,454 mg/L = 6.4 x 10^{-4} mg/mg/hour

where the initial biomass (MLVSS) in the batch reactor = 2,454 mg/L.

Personhours needed: 5 hours + acclimation time (~30 hours depending on wastewater).

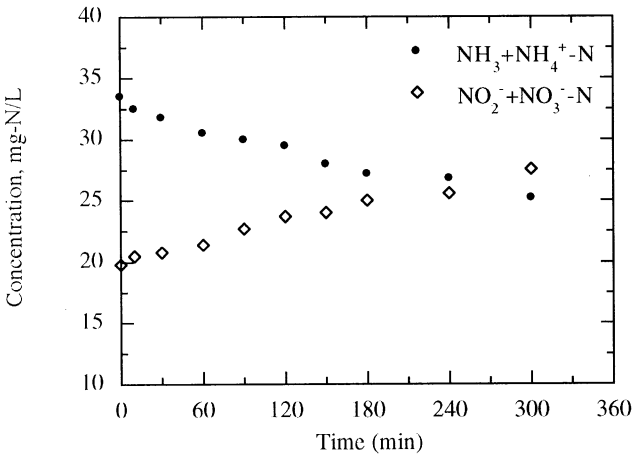


Figure 14. *Ammonium oxidation rate determination.*

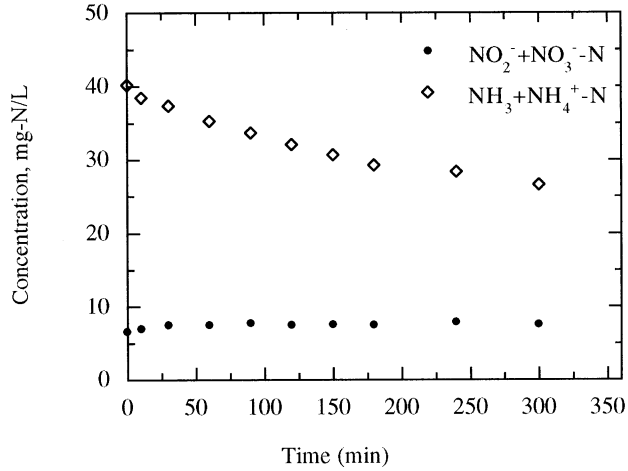


Figure 15. *Denitrification rate determination.*

Table 11. *Example of nitrification determination.*

Time (hr)	NH_3 + NH_4^+-N (mg/L)	Average NH_3 + NH_4^+-N (mg/L)	NO_2^- + NO_3^--N (mg/L)	Average NO_2^- + NO_3^--N (mg/L)
0		33.5		19.8
0				
0.5		31.8		20.8
0.5				
1		30.5		21.4
1				
1.5		30.0		22.7
1.5				
2		29.5		23.7
2				
2.5		28.0		24.0
2.5				
3		27.2		25.0
3				
4		26.8		25.6
4				
5		25.2		27.6
5				

Denitrification Rate

Theory: Carlson (1971) and Christensen and Harremoes (1977) suggested that the kinetic reaction for denitrification by activated sludge can be expressed by:

$$\frac{dN}{dt} = q_D X \qquad (18)$$

where

dN/dt = denitrification rate (mg NO_2^- + NO_3^- -N/L/hour),
N = nitrite plus nitrate concentration (mg-N/L),
t = time (hour), and
q_D = specific denitrification rate (mg NO_2^- + NO_3^--N/mg VSS/hour

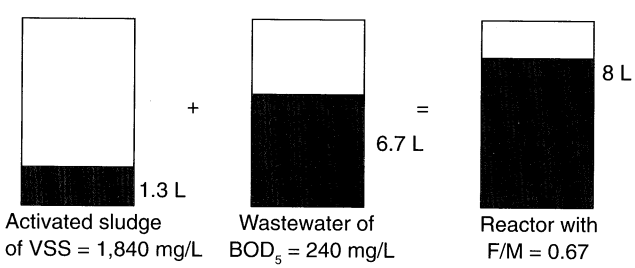
This indicates that the denitrification rate is independent of the nitrate concentration and only a function of the volatile suspended solids concentration.

Apparatus:

Magnetic stirrer, stirring bar, and pipettes
DO meter
Filtration apparatus
NH_3 + NH_4^+-N and NO_2^- + NO_3^--N measurement apparatus

Procedure: The procedure to determine the specific denitrification rate (q_D) is:

1. Obtain 8 L of wastewater sample.
2. Obtain 8 L of acclimated activated sludge.
3. Place a portion of the wastewater and activated sludge in an 8 L reactor. The dilution ratio used can be the same as the F/M ration at the treatment plant of interest. For example, the Ashland treatment plant has the F/M ratio of 0.67; thus, 1.3 L of activated sludge with VSS of 1,840 mg/L can be mixed with 6.7 L raw sewage with BOD_5 of 240 mg/L to obtain the F/M ratio of 0.1 in an 8 L reactor.



4. Measure VSS of mixture.
5. Mix the reactor with a magnetic stirrer and measure DO to ensure a DO level of < 0.1 mg/L.

Table 12. *Example of denitrification determination.*

Time (hr)	NH_3 + NH_4^+-N (mg/L)	Average NH_3 + NH_4^+-N (mg/L)	NO_2^- + NO_3^--N (mg/L)	Average NO_2^- + NO_3^--N (mg/L)
0		6.6		40.2
0				
0.5		7.0		37.4
0.5				
1		7.5		35.3
1				
1.5		7.7		33.7
1.5				
2		7.5		32.1
2				
2.5		7.5		30.7
2.5				
3		7.4		29.3
3				
4		7.8		28.4
4				
5		7.5		26.6
5				

6. Add sodium nitrate ($NaNO_3$), if necessary, to provide an initial nitrate concentration of about 25 mg/L.
7. Determine concentrations of total ammonia (NH_3 + NH_4^+-N), nitrite and nitrate (NO_2^- + NO_3^--N) over time (at 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5 hours) for the filtrate passed through 0.45 µm membrane filters.

Data Analysis: Table 12 and Figure 15 show an example of a denitrification rate determination. Even though a single sample is analyzed in this example, duplicate sample analysis is recommended. From Figure 15, the denitrification rate is estimated to be:

(40.2 – 26.6 mg NO_2^- + NO_3^--N/L) / 5 hours / 2,260 mg/L = 1.2 x 10^{-3} mg/mg/hour

where the initial biomass (MLVSS) in the batch reactor = 2,260 mg/L.

Personhours needed: 5 hours + acclimation time (~30 hours depending on wastewater).

Phosphorus Release and Uptake Rates Measurement

In a biological phosphorus removal process, phosphorus will be released by phosphorus-removing microorganisms under anaerobic conditions and taken up under aerobic conditions. The measurement of phosphorus release/uptake rates is meaningful only when phosphorus-removing microorganisms have been selected. An enhanced culture that removes phosphorus can either be obtained from a full scale BPR plant directly or produced in a laboratory reactor by using enrichment culture techniques.

A sequencing batch reactor (SBR) can be used to develop the enhanced culture in a laboratory. The operational conditions for SBR to develop the enhanced culture depend on wastewater characteristics. The key feature of a SBR is its flexibility to adjust the anaerobic/aerobic retention time depending on the type of wastewater. Figure 16 shows a typical SBR configuration that controls the anaerobic/aerobic stage by a timer.

Operational conditions of the SBR are as follows:

- reactor volume of 6 L; 4 L of fill and withdraw per cycle;
- wastewater feed in 10 minutes at each cycle;
- anaerobic/aerobic retention time = 2 hours/ 5 hours; 1 hour settling and decanting;
- 8 hours/cycle, 3 cycle/day.

When average COD and phosphorus concentrations in the influent are 200 mg/L and 9 mg-P/L, respectively under the above conditions, the effluent phosphorus concentrations were lower than 0.5 mg/L after 14 days of operation at room temperature. Once activated sludge containing phosphorus-removing microorganisms are obtained, phosphorus release/ uptake rates can be measured as follows:

1. For the simulation of the anaerobic conditions, add wastewater and activated sludge to the reactor at a predetermined ratio and mix for a period of time corresponding to the hydraulic retention time of the anaerobic zone of the SBR or full-scale treatment plant. Take samples every 5 to 10 minutes for 0.5-1 hour and analyze for orthophosphate.
2. At the time corresponding to the hydraulic retention time of the anaerobic zone, supply the air using a fine pore diffuser placed at the bottom of the reactor. Take samples every 10 to 20 minutes for 3-4 hours and analyze for orthophosphate.

In order to evaluate the effect of denitrification on phosphorus removal, total ammonia, nitrite, and nitrate concentrations are usually monitored. The rates of phosphorus release and uptake are simply expressed by the increase or decrease in phosphorus concentration per unit biomass per unit time (mg-P/g VSS/min).

The Ashland wastewater was used as an example to determine the phosphorus release/uptake rate. An aliquot of 500 ml of activated sludge from the laboratory SBR, where phosphorus-removing microorganisms were developed, was added to 500 ml of the Ashland composite wastewater to simulate a reaction of influent wastewater with 100% sludge recycle. The activated sludge were taken from the aerobic zone of the laboratory SBRs. The F/M ratio was 0.3. The NO_2^- + NO_3^--N concentration in the initial sludge and in the combined solution were 5 and 2 mg-N/L, respectively. The initial MLVSS was 880 mg/L. Samples were taken every 10 minutes during the anaerobic condition and every 20 minutes

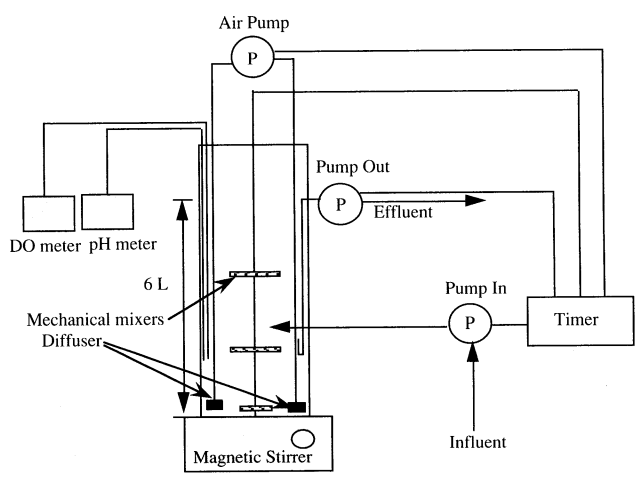


Figure 16. *A typical SBR configuration.*

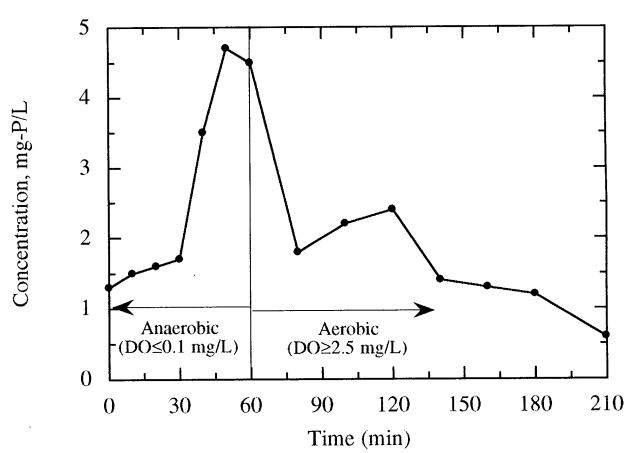


Figure 17. *Phosphorus release/uptake profile of Ashland wastewater.*

during the aerobic condition. This experiment was conducted under room temperature condition. The profile of phosphorus release and uptake is shown in Figure 17.

The phosphorus release was slow in the initial 30 minutes and rapid in the following 20 minutes. For the next 10 minutes, the phosphorus released was taken up slightly (approximately 0.2 mg-P/L). The specific phosphorus release rate was 0.064 mg-P/g VSS/min [(4.7 – 1.3)/60/0.880], and the specific phosphorus uptake rate was 0.034 mg-P/g VSS/min [(4.7 – 1.1)/120/0.880]. The total phosphorus released was obtained from the difference between the initial phosphorous concentration and the phosphorous concentration at the end of anaerobic stage. Even though it is uncertain what causes the lag and bump in the phosphorus release and uptake, the phosphorus release rates are comparable with reported values ranging from 0.042 to 0.056 mg-P/g VSS/min (Kang et al. 1991).

Summary

A simple COD fractionation method was developed to characterize the wastewater specifically aimed at biological phosphorus removal design. Simple methods were also proposed to determine Y, k_d, μ_{max}, and K_s. These kinetic parameters and the detailed fractionation results of raw wastewater COD, nitrogen, and phosphorus can be used in biological nutrient removal process design computer programs to obtain optimum design information for wastewater treatment plants. The models are useful in determining the process volume and evaluating the effect of COD loading, biomass concentration, and sludge age on the phosphorus and nitrogen removal efficiencies. The methods provided for parameter determination will allow smaller wastewater treatment plants or industries to evaluate the feasibility of biological phosphorus removal of their wastewater with minimum cost.

List of Selected Symbols Used in This Report

f_{cv}	COD to VSS ratio of the volatile suspended solids = 1.48 mg COD/mg VSS
F/M	Food to microorganism ratio
k_d	Endogenous decay rate (^{1}/day)
K_o	Half-saturation constant for oxygen (mg/L)
K_s	Half-saturation constant (mg/L)
K_N	Half-saturation constant for NH_4^+-N (mg/L)
N_{ti}	Influent total Kjeldahl nitrogen (TKN) (mg-N/L)
N_{ai}	Ammonia and ammonium nitrogen (mg-N/L)
N_{ui}	Unbiodegradable soluble nitrogen (mg-N/L)
N_{pi}	Unbiodegradable particulate nitrogen (mg-N/L)
N_{oi}	Biodegradable organic nitrogen (mg-N/L)
q_N	Specific nitrification rate (mg NO_2^- + NO_3^--N/mg VSS/hour)
q_D	Specific denitrification rate (mg NO_2^- + NO_3^--N/mg VSS/hour)
S	Substrate concentration (mg/L)
S_{ti}	Influent total COD (mg/L)
S_{bi}	Biodegradable COD (mg/L)
S_{ui}	Unbiodegradable COD (mg/L)
S_{bsi}	Soluble readily biodegradable COD (mg/L)
S_{bpi}	Particulate slowly biodegradable COD (mg/L)
S_{usi}	Soluble unbiodegradable COD (mg/L)
S_{upi}	Particulate unbiodegradable COD (mg/L)
t	Time
μ_{max}	Maximum specific growth rate (^{1}/day)
μ_N	Specific growth rate of *Nitrosomonas* (^{1}/day)
μ_{Nmax}	Maximum specific growth rate of *Nitrosomonas* (^{1}/hour)
U	Specific substrate utilization rate (^{1}/day).
X	Mixed liquor volatile suspended solids (mg/L)
Y	Yield coefficient (mg VSS/mg COD)

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