RECOMMENDATIONS FOR THE 2018 WASTE ASSIMILATIVE CAPACITY STUDY OF THE HARPETH RIVER FOR ESTABLISHING DEFENSIBLE WASTELOAD ALLOCATIONS (WLAs)

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TDEC, TMDL Committee for Harpeth River TMDL

By E-Mail

RE: Comments and Suggestions for the Harpeth River TMDL Data Collections for Establishing Wasteload Allocations

RECOMMENDATIONS

The following activities need to be done in 2018 in order to have a technically defensible waste assimilative capacity study that can be used to calibrate a wasteload allocation model. The mathematical model is worthless unless it is based on real data and not best guesses of deoxygenation rates, oxygen additions by reaeration and algae, and supporting data as previously outlined. Our suggestions include:

1. Conduct a dye time of travel study and collect a dataset as outlined in 1.c. above;
2. Run 90-day time-series BODs so that the f-factor can be calculated for each discharger. Also measure nitrogen and phosphorous series parameters so that the POTWs can analyze the actual total nitrogen that has impacts to the River (both positive and negative). 30-day BOD analyses are for all practical purposes a waste of time and limited for modeling with the knowledge we have today. One can certainly not establish BOD_5 limits for permits with a 30-day test;
3. Fixed stations for diurnal measurements of DO, temperature, pH and conductivity should be established at selected stations downstream. These data will give the diurnal DO curves that occur at individual stations. These data will be used with the same data collected from the dye cloud as it moves downstream. Both of these are used to be able to determine the net positive or negative addition/subtraction of DO in the Harpeth River;
4. Collect algae samples for identification and numbers plus biomass with dye time of travel. Without algae data, no decisions can be made on the impact of algae on the health of the river. Algae are an important resource for oxygen in the River;
5. Run HPLC analyses with samples collected with dye time of travel. It is especially important to determine the diel cycle of the total chlorophylls and the accessory pigments. The current analyses using spectrophotometric analyses for chlorophyll a only gives us qualitative results that are not reliable for assessing the algae and definitely cannot be used if permit limits that stand-up in court are going to be set by TDEC.
6. The Harpeth River at low flows will have to be modeled using a dynamic model. The Franklin POTW represents the most significant flow in the River (up to 80 to 90%+). This has not been done in the past. Dynamic models typically result in more allocation.
7. Flows should be monitored at each dye of travel station. Stage measurements with barometric pressure recorders should be obtained at several locations down the river. The dynamic hydraulic model should use the same reference on both ends of the model, i.e., flow at Franklin and flow downstream at the last gage location.
8. Bottle rates should not be used. These are only good in the laboratory and are not reality for the more complex stream bacterial populations. Bottle rates can be 500%

greater or more than the actual deoxygenation rate determined from the proper method using rates determined from samples collected with dye time of travel or the ∂t that is being modeled by Tim Wool.

9. A Work Plan should be developed for conducting the 2018 studies. It is impossible to conduct a waste assimilative capacity study and then conduct the subsequent modeling if a definitive plan is not developed and in place before the project begins. This is a fundamental part of any scientific study. The inputs to the model should be developed from the field waste assimilative capacity study and then these data can be used in the calibration of the model without guessing or "curve-fitting the model to meet an arbitrary DO concentration that is constantly changing during the day.
10. At this point, there is no value to calibrating a model where no data exist to calibrate the model. It is possible to build the hydraulic model at this time and have it ready for use with the assimilative capacity study data once it is obtained. The hydraulic model will still have to be calibrated with the flow measurements, flow data from the USGS gages, flow data from the POTWs, and any stage measurements made during the time of travel study.

ANALYSES OF TMDL ACTIVITIES CONDUCTED ON THE HARPETH RIVER IN 2017

AquAeTer offers the following suggestions for the 2018 water quality studies for establishing wasteload allocations on the Harpeth River for Non-point sources (Load Allocations or LA), Point Sources (Wasteload Allocation or WLA), and for establishing a Safety Factor (SF). It is our understanding that the USEPA is calibrating the WASP model currently, but we are unaware of any waste assimilative capacity data ever having been collected on the Harpeth River that can be used for scientifically establishing river deoxygenation rates, except for SOD rates, for the current permitted discharges to the stream.

The Total Maximum Daily Load (TMDL) requires a Load Allocation analysis for nonpoint sources (i.e., nonpoint source contributions over the course of the year) , wasteload allocation(s) (WLA) for point source discharges (i.e., and also non-permitted point or area point sources, e.g., documented organic or inorganic inputs from uncontrolled sites or natural attenuation sites), and a Safety Factor (SF) for future growth or uncertainty due to minimal data to support the basis for waste assimilative capacity. The 2017 data collected has provided some data that can be used to develop background data for the LA part of the analysis, but other data, such as, BOD_{30} and chlorophyll a analyzed using spectrophotometric methods, have no applicability to technically analyzing the inputs required for establishing a defensible TMDL. We will discuss in the following paragraphs suggestions for improving and collecting a defensible waste assimilative capacity dataset for establishing a TMDL that both the public and the permittees can rely on as defensible scientifically and technically for use in setting future permit limits for the permittees.

Historic data collected prior to the current operations at the Franklin POTW are no longer representative of the stream. The current Franklin POTW effluent represents a tributary stream

to the Harpeth that should actually increase the assimilative capacity of the stream, as Jim Greenfield originally stated during discussions on the 2004 datasets collected by USEPA. The Franklin POTW has been meeting very low effluent limits and we expect that the Franklin effluent represents a major resource for improving the assimilative capacity of the Harpeth River due to its contributions to the flow in the River of a well-treated effluent discharge to the Harpeth River. The Franklin POTW is going to require meeting future increased population demands that will increase the flows being treated by the POTW due to the increase in population projected to be 250,000 people. The current treatment system will have to increase its discharge flow, but is currently limited on mass loadings since the facility is already meeting method detection limits or low concentrations for most pollutants. This would require treatment beyond what is currently economically and/or technically achievable. The impact of the NPDES discharges should be documented if an assimilative capacity study is completed that meets the requirements for wasteload allocation modeling as will be outlined below. The Franklin POTW represents the greatest mass loadings to the Harpeth River, but it also represents the major flow in the River during critical low-flow high temperature periods. Flow and temperature are critical for waste assimilative capacity, and with higher flows, increased capacity including higher mass loadings should be achieved along with creating a healthier stream.

Additionally, it is our understanding that one of the issues being discussed and considered is the requirement for additional treatment for nutrients at the Franklin POTW and the other dischargers. The only reason to control nutrients is to control nuisance algae, yet algae are not being monitored throughout the basin nor are the other parameters that have to be monitored, such as, total chlorophylls a, b and c plus accessory pigments using HPLC, biomass and silica. Although TDEC has implemented a nutrient impact analysis that compares total Phosphorous (i.e., organic phosphorous and ortho-phosphate) and nitrate to the Tennessee Macroinvertebrate Index (TMI) macrobenthos scores, their analysis shows that there is no relationship between TMI scores and either total phosphorous and nitrate (i.e., $r^2 = 0$ or no relationship). Organic phosphorous is not toxic and ortho-phosphate is not toxic at >100 mg/L (Kim, et.al. Feb. 7, 2013. "Aquatic Toxicity Assessment of Phosphate Compounds". Nitrate is not toxic at 10 mg/L with a suggested maximum concentration of 3 mg/L for adding a safety factor (Camargo, Alonso and Salamanca. 2005. "Nitrate toxicity to aquatic animals: a review with new data for freshwater invertebrates". Thus the use of these analyses in setting impairments has no technical merit and the literature suggests that these analyses are not defensible, regardless if the science also suggests that there is no viable relationship between TMI and the nutrients in question. Analyzing the impacts of phosphorous to the health of the Harpeth River has to be based on its impacts to the algae populations and coupled with this is the algae productivity a positive impact or negative impact to the DO in the Harpeth River. The predictive model if calibrated properly with defensible data should be able to address the effect of algae on DO resources in the Harpeth River. These data have not been collected to date. Nuisance algae still have to be assessed primarily by river reconnaissance.

Specific Comments

Specific comments that we believe are necessary for actually calibrating a wasteload allocation model follow.

1. ***Collection of Data for Calibrating the Model with Dye Time of Travel.*** The USEPA collected a dataset in 2001 which was used to build a wasteload allocation model and the study results were reported in 2004. They did not collect the data with dye time of travel and thus Harpeth River deoxygenation rates could not be established from this dataset. If the Harpeth was truly a steady-state stream, it may be possible to use independent "random in time" measurements to establish rates as was done by Streeter and Phelps. The Harpeth River is not a steady-state stream at low flows. The Franklin POTW makes up greater than 80 to 90% of the flow in the River at the critical monthly low-flow periods. The Franklin POTW has a variable discharge due to the nature of domestic influents that reach the POTW during various times of the day. That is, maximum flows most likely occur beginning around 7 to 8 am in the morning through the early evening hours and reach very low flows during nighttime hours. There is no way to collect random samples down a stream and determine how they are undergoing decay in the stream since one never knows what slug of water is being sampled. Three things are important here:
    a. A single slug of water has to be followed down the stream to determine how that mass of pollutants is undergoing decay;
    b. Water samples must be collected in the median point of the dye cloud for $CBOD_u$ (at least a 90-day test), nitrogen, phosphorus, Total chlorophylls a, b, and c plus the accessory pigments using HPLC, algae identification, and volatile organic carbon; and
    c. A typical assimilative capacity study that is required to be able to determine river deoxygenation rates including $CBOD_u$ (at least 90-day time-series BOD tests with nitrogen and phosphorous testing as well), Organic-N, and ammonia plus oxygen addition by physical reaeration and algae productivity is described in further detail below. SOD is also required, but does not have to be done concurrently with river deoxygenation rates and oxygen addition rates. A list of assimilative capacity study data collections that are required to be able to calibrate a water quality model include the following:

        A. STATISTICAL MONTHLY FLOW AND TEMPERATURE DATA
        1) Monthly 7Q10's using Adjusted Weibull Method
        2) Monthly 90^{th} Percentile Water Temperatures

        B. FIELD STUDIES
        1) Bathymetric Profiles (during low flow periods and possibly during winter-time low flow period
        2) Sediment Oxygen Demand (During a low-flow period)
        3) Velocity and Stage Continuous Measurements at gage locations and potentially with fixed ADCP units at critical locations (would need to be surveyed)
        4) Dye Time of Travel Study;
            a. Dye Time of Travel by physically tracking dye downstream using a fluorometer for at least 48 to 72 hrs or more. The first 36 hrs should be continuously on a sampling frequency of once every 4 to 6 hrs if possible

i. Dye Time of Travel within Centroid of Mass ~Every 4 to 6 hours

b. Flow measurements at each dye TOT station – preferably using Acoustic Doppler Current Profiler (ADCP)

c. Fixed-Station Diurnal Water Quality Measurements (10 to 12 stations or more depending upon known phenomena in the system)
   i. Dissolved Oxygen, Water Temperature, Conductivity, pH, Rhodamine WT dye

d. In-Situ Water Quality (with TOT)
   i. Dissolved Oxygen, Water Temperature, Conductivity, pH, Rhodamine WT dye
   ii. 90-day Time-Series BOD analyses;
      1. Ultimate CBOD (with TOT and additional samples collected during the study at Fixed stations, such as, effluent collected at or near time 0 of time of travel study and tributaries at or near TOT dye passing by tributary)
      2. Determination of Recalcitrant Organic Nitrogen and Recalcitrant Organic Phosphorus at selected times during the time-series BOD tests – Note: Nitrification can begin as early as time 0 in the BOD bottles if nitrifiers are present in the effluent
   iii. Nitrogen and Phosphorous Series (with TOT and Fixed)
   iv. Algal Parameters (with TOT and Fixed stations)
      1. Algae Identification – Species and Numbers
      2. Algae Biomass Estimate by VOC – Note species biomass are not equivalent between different phylums
      3. HPLC for Total Chlorophylls a, b, and c plus accessory pigments – Note Spectrophotometric analysis is only a qualitative analysis and not sufficient if nitrogen and phosphorous limits are contemplated – Note that Chlorophyll follows a diurnal pattern and one point during the day is meaningless for wasteload allocations for nitrogen and phosphorous
      4. Silicon – required by diatoms which are about 1.3 times more efficient at producing oxygen as other algae species

e. Background and Tributary Measurements
   i. In situ Water Quality
   ii. $CBOD_u$, Nitrogen Series, Phosphorus Series, Algal Parameters
   iii. Determination of Recalcitrant Organic Nitrogen and Phosphorus
   iv. Total chlorophylls a, b, and c plus accessory pigments, algae identification and algal biomass
   v. Silicon
   vi. Flow Measurement(s) and Stage Determinations (existing gages and real-time velocity meters, stage/barometric pressure monitors)

2. ***Understanding the fundamentals of determining river decay rates and oxygen addition rates.***
It is fundamental to determining river deoxygenation rates, reaeration and algae production of oxygen (either negative or positive) for these rates be determined with dye time of travel. Although many people estimate the $CBOD_u$ deoxygenation rates from the laboratory bottle rate(s), this is not based on scientific or technically valid river kinetics, but rather it is a matter of convenience and cost savings or not really understanding the kinetics of river deoxygenation with time (of travel). Streeter and Phelps were able to estimate the River rate by the sheer number of samples they collected and the assumption of steady-state conditions through specific reaches of the River. The River deoxygenation rates cannot be estimated from a fixed water column bacterial population in a laboratory bottle. The real environment in a River is far more complex than this and includes both soluble bacterial populations and attached bacterial populations to sediments, suspended solids in the water column, and/or fixed growth or other objects submerged in the water, e.g., logs as well as algae and other sinks/sources of material. This means that the river bacterial populations and reaction to pollutants travelling down the river cannot be predicted from a bacterial population in a laboratory BOD bottle. These rates are also dictated by any resident seed introduced by a wastewater treatment facility, such as, the Franklin POTW. This works for both $CBOD_u$ and nitrification of ammonia to nitrate (i.e., this also includes organic nitrogen and nitrite nitrogen). For facilities discharging downstream from an upstream-effluent discharge, deoxygenation rates can be higher since a resident, food-starved bacterial population is present and thus acclimated for the new effluent entering the river. This means the Franklin POTW river deoxygenation rate determined from collecting samples in a median point within a dye slug traveling downstream from this effluent may be very low due to the highly recalcitrant $CBOD_u$ being discharged, but the next river deoxygenation decay rate for $CBOD_u$ of the effluent from the next treatment system downstream could be higher since the bacteria are established and the bugs are food-starved. The USEPA did identify in their 2001 studies that the Franklin POTW effluent had a "f-ratio" that indicated that the effluent was highly treated and very recalcitrant, which is expected with the high level of treatment achieved.

In measuring rates in a dye slug, the bottle rates, which can range from 0.05 or less to 1 or more, never match what actually occurs in the river when collecting samples from the same dye slug moving downstream or the true river rate, which typically are in the 0.05 to 0.3 to 0.4/day at today's treatment levels. Understanding the kinetics of BOD decay in the river and in the laboratory is essential to being able to accurately determine wasteload allocation from measured rates in the stream using the same uptake seen in the stream over the time the water is moving downstream (Δt).

Examples of river loss rates measured from water samples collected with dye time of travel sampling are presented in Figures 1 through 5. It is noted that what was measured in the following examples were the total loss rates, which were treated as deoxygenation rates. In fact, settling and other phenomena likely played a part in removing some of the BOD through the systems. The application of these measured rates solely as deoxygenation rates is a conservatism that will be built into the model.

Figure 1. River $CBOD_u$ deoxygenation k_1 rate for Calibration TOT study = 0.85/day @ 20ºC

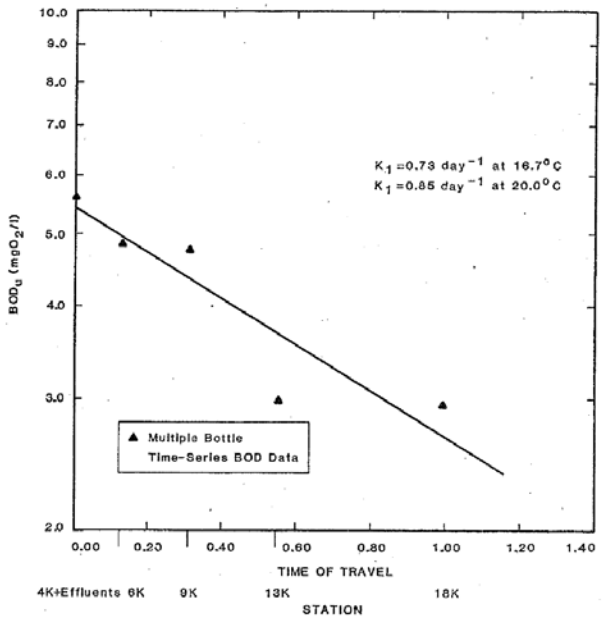


FIGURE 5-6 $K_1 (K_{river})$ FOR PAINT CREEK CALIBRATION SURVEY

Figure 2. River $CBOD_u$ deoxygenation k_1 rate for Verification TOT study = 0.77/day @ 20ºC

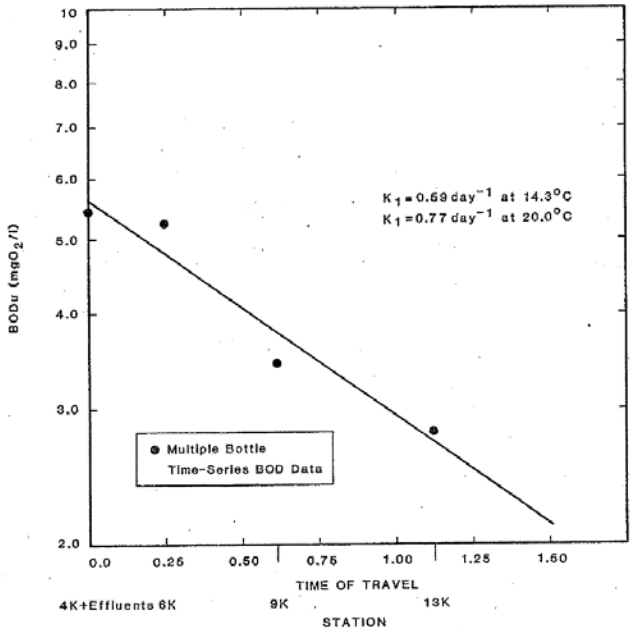
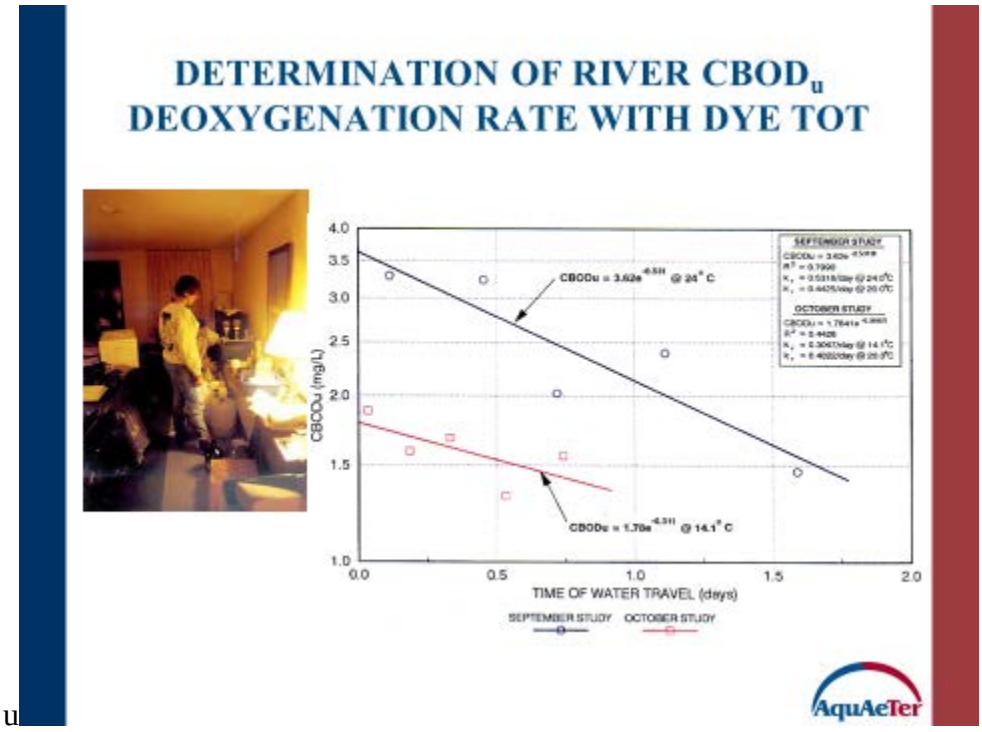


FIGURE 5-7 $K_1 (K_{river})$ FOR PAINT CREEK VERIFICATION SURVEY

The above two $CBOD_u$ rate determinations for calibration and verification studies were collected with dye time of travel on a stream, Paint Creek, receiving effluents from a POTW and a hog slaughtering and meat packing plant. The k_1 rates determined were 0.85 and 0.77 per day. This stream had attached bacterial populations on the bottom gravelly sediments that felt like snot – very similar to attached growth on a trickling filter media and very similar to what was previously established in Liberty Creek. This is an example of a maximum deoxygenation rate that one might see due to the high BOD from the blood sent in the influent to the treatment facility. This high rate is not expected in the Harpeth River.

The next set of k_1's determined from $CBOD_u$ samples collected with dye time of travel is from a municipal POTW. Again a calibration and a verification TOT study were completed and the k_1's determined were 0.44 and 0.4 per day @ 20ºC. Again excellent agreement between the calibration and verification study results were determined.

Figure 3. River $CBOD_u$ Deoxygenation Rates, k_1 determined from TOT Calibration and Verification Studies[M1]



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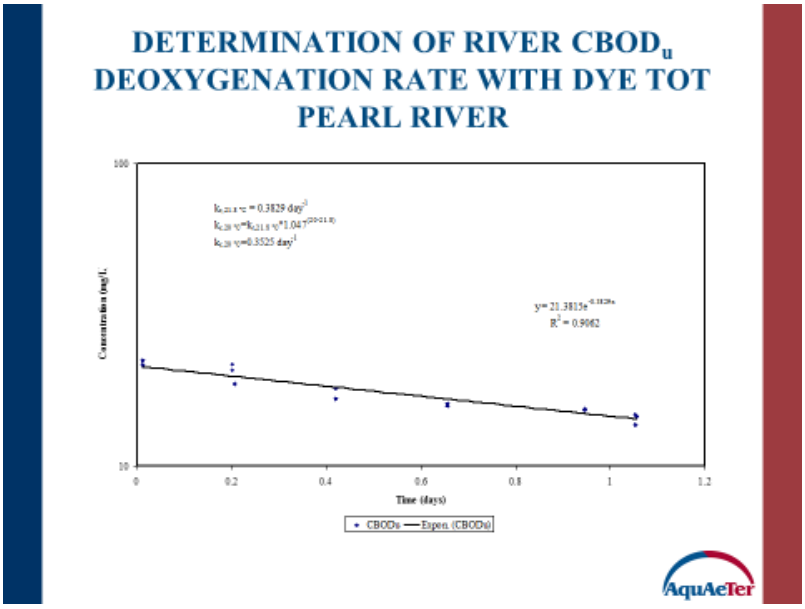
This effluent is now meeting a River $CBOD_u$ deoxygenation rate, k_1 of <0.1/day, based on the true river rate measured from $CBOD_u$ samples collected with dye time of travel. This decrease in the k_1 rate is due to improved treatment by the POTW.

The third study results are for a pulp and paper mill in the southeast where the k_1 rate was determined from the dye time of travel $CBOD_u$ results to be 0.35/day. This degradation rate is higher than most pulp and paper mills but this discharge is downstream from an upstream POTW discharge which has a resident effluent seed reaching this discharge. We have measured this occurrence on two other discharges where the upstream pulp and paper mill had a river k_1 of

around 0.1/day and the downstream pulp and paper mill had a river decay rate of around 0.4/day, for about the same level of treatment from each ASB indicating that if an acclimated seed reaches the downstream mill, the bugs will go after the carbon source rapidly. Bottle rates for pulp and paper mill effluents are routinely reported at <0.1/day to 0.05/day or less but can be as high as 1/day for bottle rates. We have never measured a river k_1 rate of 1/day.

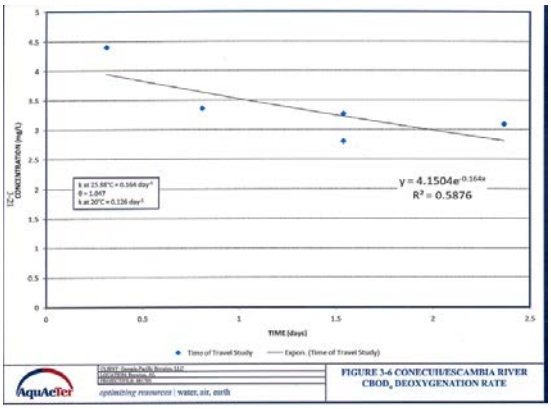
Figure 4. River Deoxygenation Rate determined with dye TOT for a highly recalcitrant Effluent for a discharge downstream from an upstream municipal discharge



The other important thing about this discharge is that ammonia was uptaken within about 1-hour time of travel downstream which was a function of algal uptake of the nitrogen and not nitrification. Algae are expected to outcompete the nitrosomonas bacteria for ammonia, although there are only low levels of ammonia being discharged from the Franklin POTW.

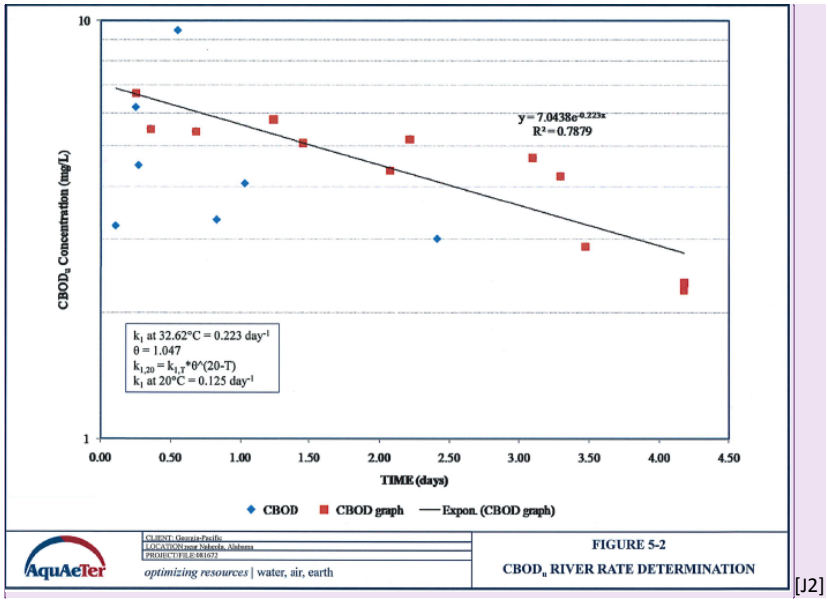
The next curve is also for a pulp and paper mill but there is no significant discharger upstream from this discharge. The k_1 for this discharge based on $CBOD_u$ samples collected with dye time of travel was 0.126/day. The BOD_5 of this discharge was <20 mg/L or well treated.

Figure 5. River $CBOD_u$ deoxygenation k_1 rate for a recalcitrant pulp and paper mill effluent with an upstream POTW discharge



Likewise for another pulp and paper mill meeting around 15 mg/L BOD_5, the k_1 rate was 0.125/day @ 20ºC. The red squares represent samples collected with dye time of travel.

Figure 6. River $CBOD_u$ deoxygenation k_1 rate for a recalcitrant effluent



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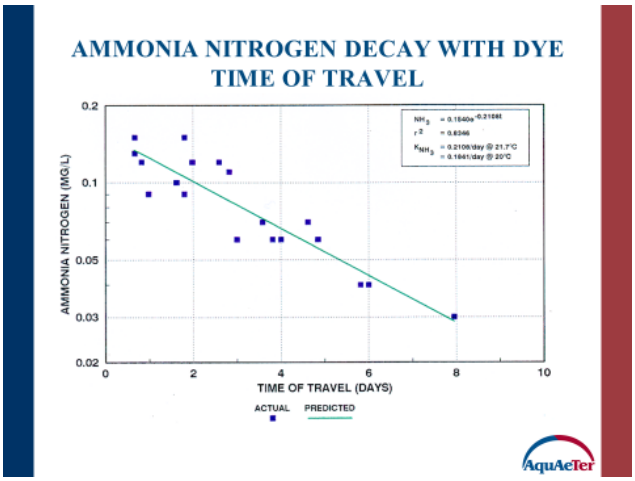
The lowest deoxygenation rate that we have measured from field measurements is around 0.04/day for an effluent achieving BOD_5's of less than 5 mg/L to the detection limit of 2 mg/L. This is in the range of the BOD_5's being discharged at Franklin.

Potential ammonia degradation is also determined from collecting samples in the dye time of travel. However, while nitrification is the dominant form of removal for ammonia in the bottles, ammonia removal by nitrosomonas may not be the dominant method for ammonia removal in the environment.

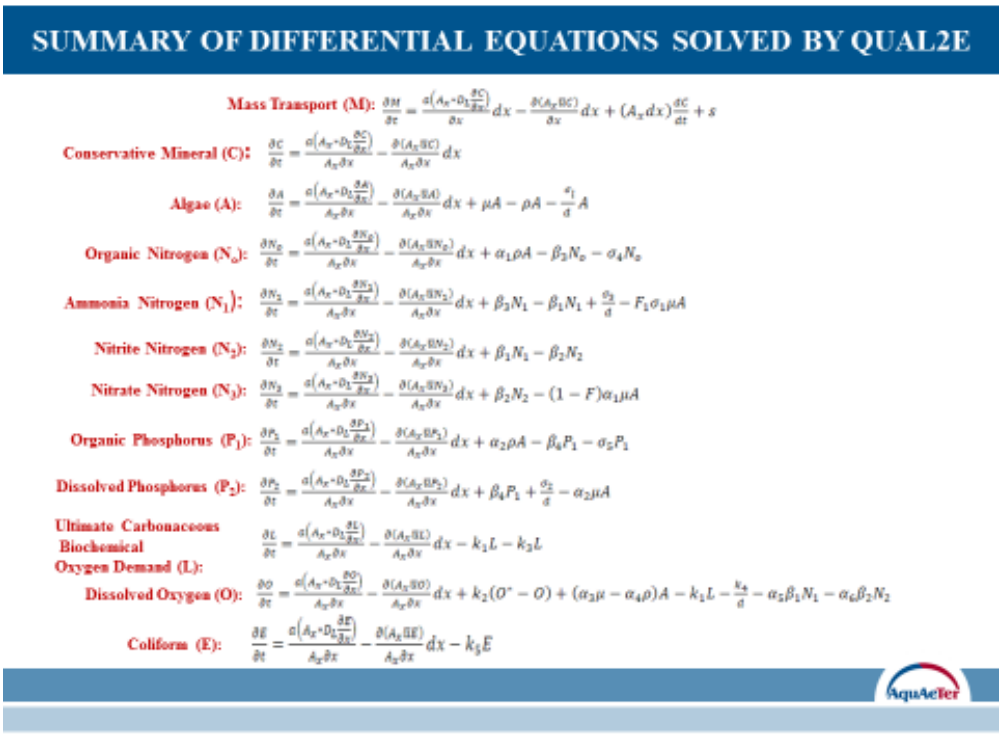
Figure 7. River ammonia deoxygenation $k_{NH3,4}$ rate with dye TOT



Again bottle rates can range from around 0.05 to 1 per day but they only by luck duplicate the true river deoxygenation rate.

3. ***All deoxygenation rates in the model*** follow a first-order decay rate (with the exception of sediment oxygen demand or SOD). These equations were originally proposed by Streeter and Phelps and others for BOD and later for ammonia nitrification. In more recent years, since the late 1970's through the 1990's, additional equations have been added to further divide the oxygen demand into its many components. Part of the fallacy of adding equations is that it takes a tremendous increase in data to be able to properly calibrate a model. Putting in guesses for these rates we have shown using Monte Carlo analyses can lead to significant errors in the analysis which can lead to requirements for expensive treatment whether it is required or not. The assimilative capacity equations that are based on data collected with time of travel of one steady-state slug moving down a river (or upstream/downstream for reservoir and tidal streams) are presented in the following equations given in Figure 8.

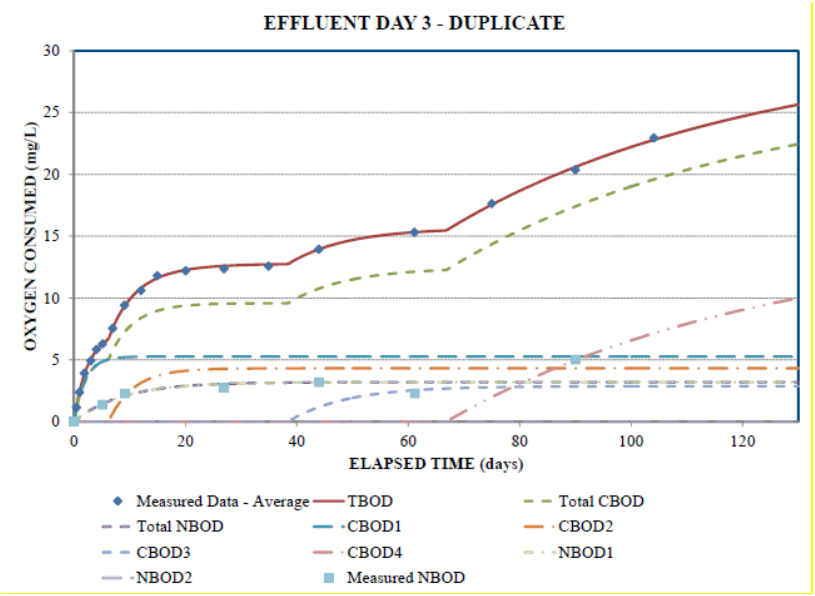
Figure 8. Summary of differential equations used in WASP



These are the basic equations that are in the WASP model. Since these equations have been around for some time, all practitioners or what we like to call the artists in waste assimilative capacity, know that the equations are dependent on time. In the case for $CBOD_u$ and other first-order decay pollutants (e.g., organic and ammonia nitrogen), the decay is with time or $\partial CBOD_u/\partial t$. "t" here is time and time is represented in the model calculations by time-of-travel down the stream. Distance plays no role except in determining what time element you are in as you travel down the stream. This dictates that the data to fill the model must come from the same dye mass or slug of pollutants as it travels down the stream, if you are actually going to model the river system. Collecting random samples on a variable stream at set distances cannot be used to determine a rate unless they are sampled at the appropriate time. There is no way to translate an equivalent time for a bottle deoxygenation rate with an equivalent time with a properly measured river deoxygenation rate. These two are not in the same universe. Bottle rates should never be used as rates representing the "River" unless there are no other data available.

4. ***Determination of Ultimate Carbonaceous Biochemical Oxygen Demand ($CBOD_u$) and Labile and Recalcitrant Organic Nitrogen and Organic Phosphorous.*** Time-series BODs have gradually expanded from the 5-day BOD test to today's 90-day (or more) tests since the time of Streeter and Phelps. Historically, a 20-day test was assumed to be representative of the ultimate carbonaceous BOD. In many instances during the start of the Clean Water Act programs, nitrification did not occur until 21 or more days in a BOD bottle. Today with more and more wastewater treatment facilities achieving nitrification, the effluent from these tertiary treatment facilities not only provide an acclimated seed for the $CBOD_u$ being discharged, but they also provide nitrifiers to the receiving stream. In a time-series BOD test, one can have nitrification in the bottle begin at time 0 as shown in Figure 9.

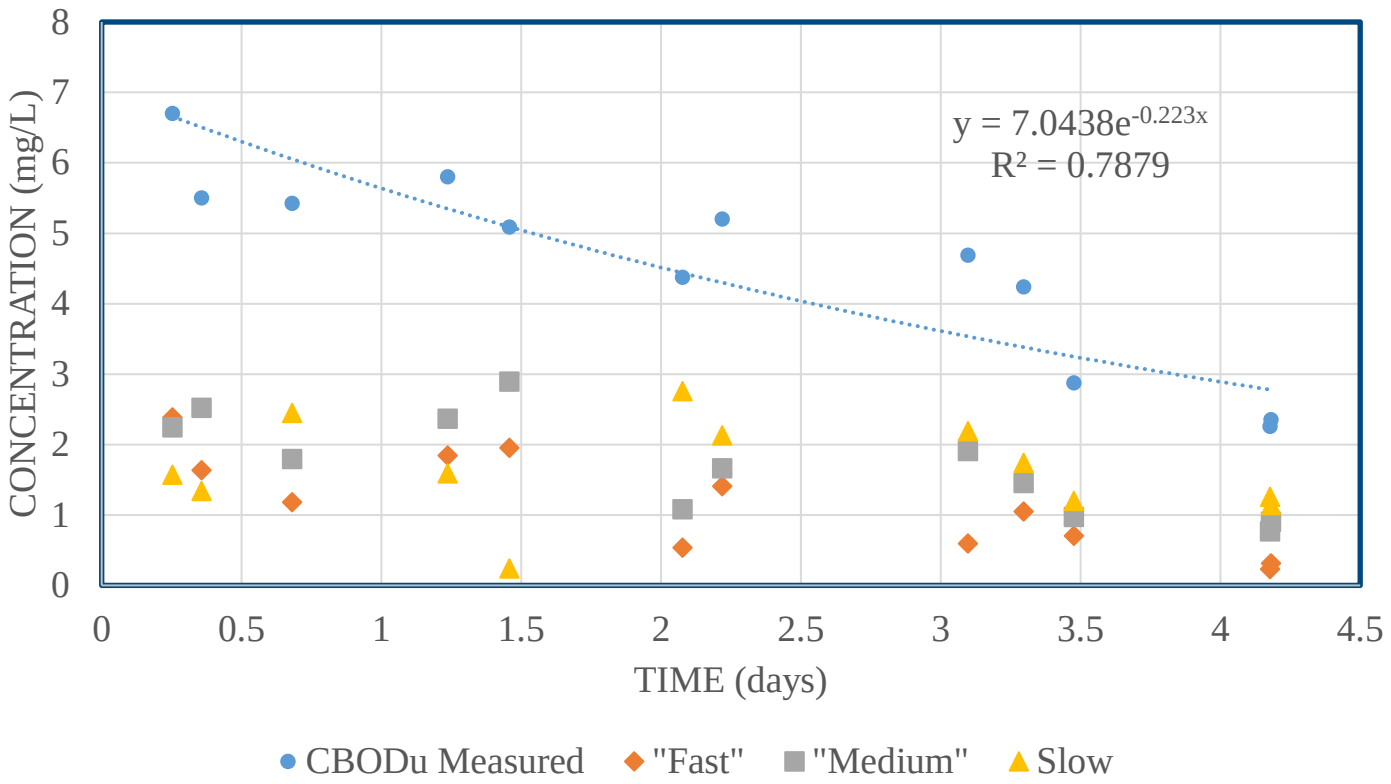
Figure 9. Laboratory time-series BOD analyses for organic carbon and ammonia



As can be seen in Figure 9, the time-series BOD data from the laboratory BOD bottles go through several breaks in the DO uptake. Tim Wool has at least 3 BOD decay rates that can be input into the model. No one has ever actually measured multiple rates in the field (with dye time of travel in the same dye mass moving downstream) so no one can actually scientifically say that this actually occurs. There are many reasons to believe that this does not happen until the bacteria become food-limited since recalcitrant compounds undergo decay to more labile fractions thus allowing the established bacteria to continue their feast.

What is measured in the bottle is a non-specific parameter. That is, without further analytical analyses, the BOD test cannot tell if a sugar is consumed or a tannin or some other organic carbon. We are not aware of any study which attempted to track the same actual carbon through the system. So the "fast" BOD in the bottle may still be present in the system at a time past when it would have been consumed in the bottle. Likewise, a "slow" BOD may be consumed prior to a "fast" BOD when in the River. Likewise, the recalcitrant organics could have been transformed by breaking one of the single chains of the component so that it becomes a labile fraction. All that we can tell is an overall change in the total amount of BOD. The assumption that the breakdown of the bottle BOD fractions can be translated to the River is not born out by the data collected from a dye time-of-travel study. For instance, take the pulp and paper mill influenced stream previously presented. Breaking down the BOD measured into 0 to 5 day, 5 to 30 day, and 30 to 90 day components representing three carbon components, which were seen in all samples, should indicate that the fast BOD should be substantially gone in the 5-day time period of the study, while the "medium" and "slow" should be substantially the same. However, no discernible trend is visible for any component, as shown in Figure 10.

Figure 10. Tracking various fractions of a bottle $CBOD_u$ from samples collected with dye time of travel in a estuarine system (remember it is time not distance or direction)

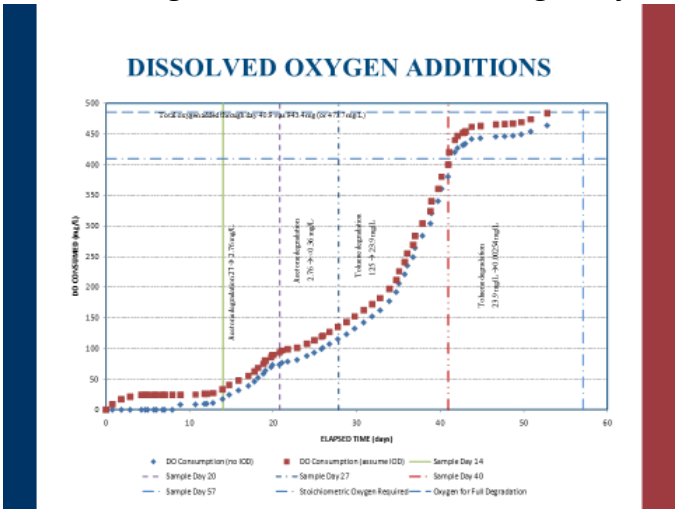


As shown previously in Figures 9 and 10, BOD decay ranged from about 20 to 60% depending on the river decay rate over a 1 to 2 day period (Note: River decay rates ranged from 0.04 to 0.35/day @ 20ºC for variable BOD concentrations).

It is still unproven whether there is one rate or multiple rates in the River. The bacterial population definitely will selectively degrade the more labile constituents first (i.e., the candy) when they are available, but it is uncertain if the decay rate changes until the bacterial population established becomes food limited. We do know that the bacteria in a bottle will selectively decay individual constituents until they are consumed and then continue to degrade the remaining organics, as shown in Figures 11 and 12. These were highly labile pollutants, acetone and toluene, yet individual bottles containing the same water source degraded independently different pollutants at different times. That is, some bottles degraded acetone first and then toluene and another set of bottles degraded toluene first and then acetone even though they were all set-up from a composite sample; however, the degradation rates did not change when one was degraded and the second constituent began to be degraded.

Figure 11. Bacterial populations degrading same source water independently and differently

Figure 11. Bottle bacterial populations require about 2 weeks to establish an acclimated population for the source organics, as well as most biological systems



It is noted that this analysis is applicable to freeze dried bugs used by some to "help" reestablish bacterial populations after an upset condition.

Figure 12. Stoichiometric rates for oxygen required to degrade acetone and toluene

DEGRADATION OF CONSTITUENTS

- **Stoichiometric Conversion**
  - Acetone
    - $C_3H_6O + 4O_2 \rightarrow 3CO_2 + 3H_2O$
    - 25.7 mg/L Acetone requires 52.5 mg/L O_2 to stoichiometrically fully degrade to carbon dioxide and water.
  - Toluene
    - $C_7H_8 + 9O_2 \rightarrow 7CO_2 + 4H_2O$
    - 136.7 mg/L Toluene requires 427.5 mg/L O_2 to stoichiometrically fully degrade to carbon dioxide and water.
  - Total oxygen added = 491.4 mg/L O_2 vs. 480 mg/L required stoichiometrically (note other constituents present in the sample)

Part of the problem with BOD is that the individual organics are not known and thus a stoichiometric rate is almost impossible to estimate without 90-day (or longer) time-series BOD tests.

Some of the individual bottles shown in Figure 11 degraded acetone first and others degraded the toluene first; however, the rates were essentially identical, as shown in Figure 13.

Figure 13. Bottle deoxygenation rates for acetone and toluene

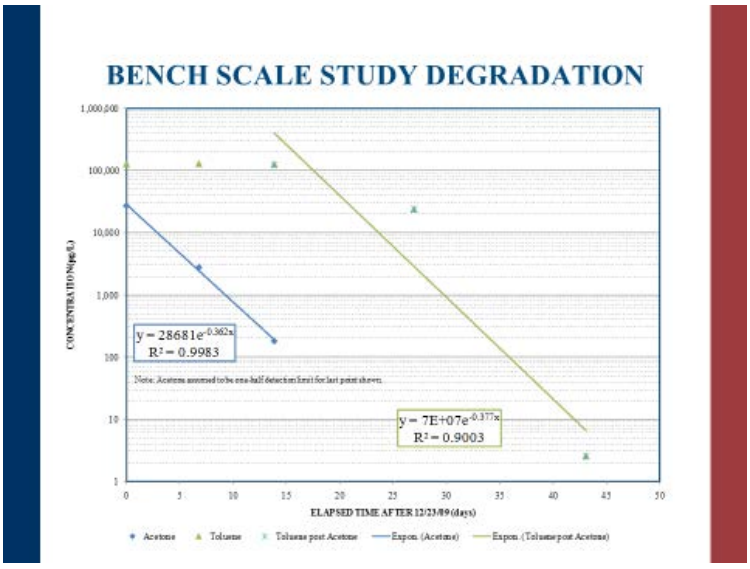


Figure 14. Decay of organics shown in Figure 13.

BENCH SCALE STUDY RESULTS

ELAPSED TIME (days)	TOLUENE (mg/L)	ACETONE (mg/L)
0 (Before 1st bottle)	133	25.7
0 (After 4th bottle)	137	25.6
0 (After 8th bottle)	140	25.8
0 (Average)	136.6	25.7
14.0	127	27
20.8	128	2.76
27.8	125	<0.360
40.9 (yellow-green)	23.9	<0.360
40.9 (black)	5.02	23.6
57.1 (yellow-green)	0.00254	<0.0018
57.1 (black)	0.0339	0.0141

Note: Average is the average of the three time 0 samples.
Yellow-green refers to the visual observation of biological microcosm.
Black refers to the visual observation of biological microcosm.

In other tests for recalcitrant organics, i.e., creosote which is comprised of polycyclic aromatic hydrocarbons or the chicken-wire multi-benzene ringed compounds, the individual PAHs over a 6-month period decayed about the same amount and at about the same decay rate, as shown in Figures 15, 16 and 17

Figure 15. Creosote stained test chamber incubated in the laboratory

Groundwater Sample Containing Free Product

AquAeTer

Figure 16. Decay of individual recalcitrant polycyclic aromatic hydrocarbons (PAHs)

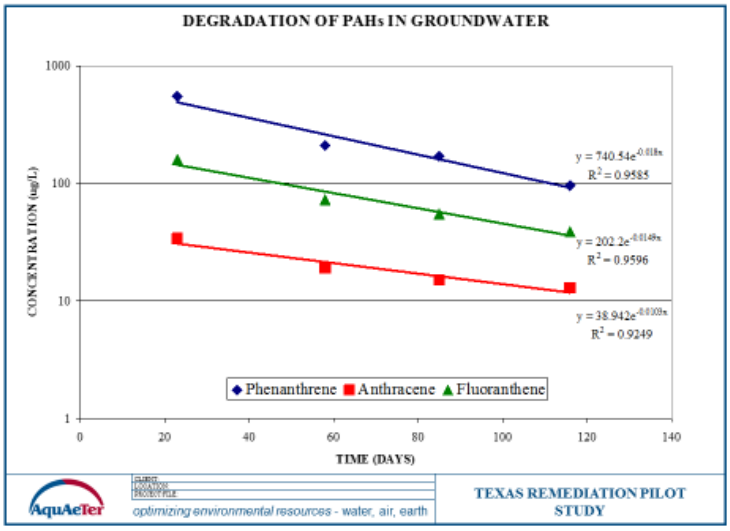
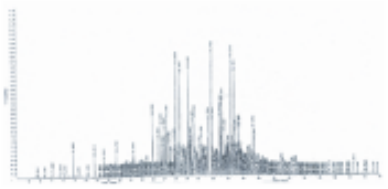


Figure 17. Individual decay rates and chromatograms of beginning and ending decay of PAHs

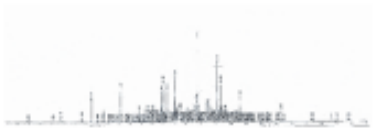
Decay Rates, R^2's and Total Decay for PAHs for Texas

CONSTITUENT	WELL D-4 DECAY RATE (day^{-1})	R^2 (#)	DECAY (%)
Dibenzofuran	-0.0155	0.9026	*81.25%*
2-Methylnaphthalene	1 ug/L or less		*50.00%*
Naphthalene	-0.0216	0.9827	*90.91%*
Acenaphthylene	-0.0310	0.9952	*92.86%*
Acenaphthene	-0.0155	0.9235	*81.00%*
Fluorene	-0.0198	0.9484	*83.33%*
Phenanthrene	-0.0230	0.9342	*90.63%*
Anthracene	-0.0181	0.8099	*87.69%*
Fluoranthene	-0.0232	0.8623	*92.08%*
Pyrene	-0.0223	0.8453	*92.09%*
Benzo(a)anthracene	-0.0259	0.8551	*93.64%*
Chrysene	-0.0250	0.8117	*93.81%*
Benzo(b)fluoranthene	-0.0255	0.8491	*94.23%*
Benzo(k)fluoranthene	-0.0408	0.9336	*94.74%*
Benzo(a)pyrene	-0.0330	0.9330	*93.33%*
Indeno(1,2,3-cd)pyrene	-0.0522	0.9685	*95.45%*
Dibenz(a,h)anthracene	-0.0904	1.0000	*87.50%*
Benzo(g,h,i)perylene	-0.0474	0.8406	*94.44%*
Average	-0.0312	0.9056	88.28%



Initial Chromatograph

Chromatograph Scales are 7:1



Final Chromatograph

These data tend to point out that bacteria utilize the food they have, whether it is recalcitrant or labile based on bottle testing. The fact that the Franklin POTW has a highly recalcitrant effluent does not mean that it will not decay, or that it will be the last organic to decay in the River. Rather, the bacterial population present in the River will consume the organic matter that is available at a steady rate until such time as there is no more food. It is also

noted that another component, such as, benzo(a)pyrene, breaks down into successive more labile fractions that leads to more degradable compounds. Again, there may not be more than one rate in the stream as the same slug of water moves downstream because the recalcitrant organics do not remain recalcitrant forever. The decay of individual PAHs at about the same rate shows that the bacterial populations do eat the available food as shown in Figures 10 and 17. The decay rate is expected to decrease if the bacterial populations ever become food limited and move into endogenous respiration. Again these data strongly suggests that multiple rates for labile and recalcitrant organics probably do not occur. Rather recalcitrant compounds are broken down into labile fractions as shown in Figure 10 with dye time travel samples of the same water slug.

5. ***The Use of a 30-day Time-Series BOD Test.*** Very little information can be obtained from a 30-day laboratory time-series BOD test. First, the time-series BOD test is run to not only determine the "*f-ratio*" or the $CBOD_u/BOD_5$ ratio for setting BOD_5 mass and concentration limits, but also to determine the recalcitrant organic nitrogen in the effluent and in the river, as shown in Figures 18 through 20.

Figure 18. Determination of f-ratio

STATISTIC	STATION	TBOD (mg/L)	$CBOD_u$ (mg/L)	BOD_5 (mg/L)	f-RATIO (_:1)	NBOD (mg/L)
AVERAGE	Upstream	4.1	3.5	1.1	3.2	0.6
	Effluent	5.4	4.2	0.8	5.3	1.2
	Downstream	4.2	3.3	1.0	3.3	0.9
MAXIMUM	Upstream	4.4	3.3	1.3	2.5	0.7
	Effluent	7.1	5.3	0.9	5.9	1.8
	Downstream	5	4.3	1.3	3.3	0.9
MINIMUM	Upstream	4	3.9	0.8	4.9	0.5
	Effluent	4.4	4	0.7	5.7	0.4
	Downstream	3.3	2.4	0.8	3.0	0.7
f-Ratio = $CBOD_u/BOD_5$						

Figure 19. Determination of labile and recalcitrant organic nitrogen

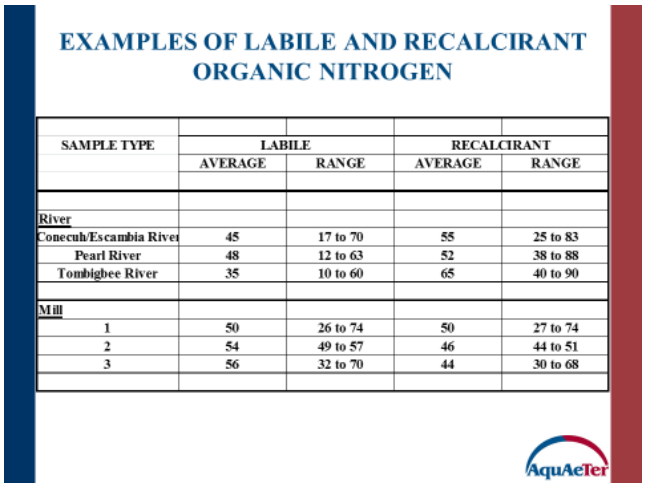


Figure 20. Highly treated effluents have 50 to >90% recalcitrant organic nitrogen

STATISTIC	LOCATION	INITIAL ORGANIC N	FINAL ORGANIC N	LABILE ORGANIC N		RECALCIRANT ORGANIC N	
		(mg/L)	(mg/L)	(mg/L)	(%)	(mg/L)	(%)
AVERAGE	Upstream	0.2	0.1	0.1	79.0%	0.1	45.1%
	Effluent	1.1	0.9	0.3	26.3%	0.9	75.7%
	Downstream	0.3	0.1	0.2	60.0%	0.1	38.2%
MAXIMUM	Upstream	0.2	0.1	0.1	50.0%	0.1	50.0%
	Effluent	1.4	0.6	0.8	42.9%	1.3	92.9%
	Downstream	0.3	0.1	0.2	66.7%	0.2	66.7%
MINIMUM	Upstream	0.2	0.1	0.1	50.0%	0.0	0.0%
	Effluent	1.4	0.6	0.3	42.9%	0.7	50.0%
	Downstream	0.3	0.1	0.1	33.3%	0.1	33.3%

6. ***Labile vs Recalcitrant Organic Nitrogen and Phosphorous.*** Municipal and industrial discharges typically have between 50 and 75% or more recalcitrant organic nitrogen and organic phosphorous in their discharges. So to not determine this in the time-series BOD tests penalizes the permittee if total nitrogen or total phosphorous is an issue. Although it should not be an issue here, it still needs to be confirmed. A 30-day test gives us no useable data for modeling the Harpeth River or for setting permit limits for BOD_5. If these are done in the future, a proper 90-day (or longer) time-series BOD test with measurements for recalcitrant and labile organic nitrogen and organic phosphorous should be conducted. Because of low concentrations of nutrients in most river systems today, organic nitrogen, ammonia and nitrite+nitrate should be monitored.
7. ***Determination of Recalcitrant Organic Nitrogen and Organic Phosphorous.*** Wes Eckenfelder first determined that organic nitrogen in activated sludge plants was about 50% recalcitrant in the 1950's. Since the last few years, several investigators including AquAeTer have measured recalcitrant organic nitrogen and organic phosphorous in time-series BOD tests ranging from 90 to 120 days. Organic phosphorous is difficult to determine since detection levels limit the analysis of the conversion of organic phosphorous to the inorganic ortho-phosphate. An example of recalcitrant vs labile organic nitrogen determinations were previously presented in Figures 19 and 20. Of the several hundred time-series BOD tests that we have conducted on a wide range of effluents including industrial pulp and paper and chemical industries, as well as, municipal discharges, recalcitrant organic nitrogen and organic phosphorous (when detection limits are reliable for determination) vs. labile organic nitrogen (and organic phosphorous) are almost always 50:50.

This is very important to assessing both the NPDES dischargers contributions to nitrogen (and phosphorous) in the stream, as well as, assessing nutrient impacts to the stream. This is a critical determination if total nitrogen and total phosphorous limits are specified in the NPDES permits. Setting effluent limits for nutrients without having this information and information on algae in the stream are not based on sound science and can be challenged if the data are available

to demonstrate otherwise. It is noted that this information cannot be obtained from a 30-day BOD (BOD_{30}) test and thus these tests are not beneficial to the permittees nor to the modeler(s) who need valid data to provide the most accurate model possible. These 30-day tests are ***cheaper*** to run.

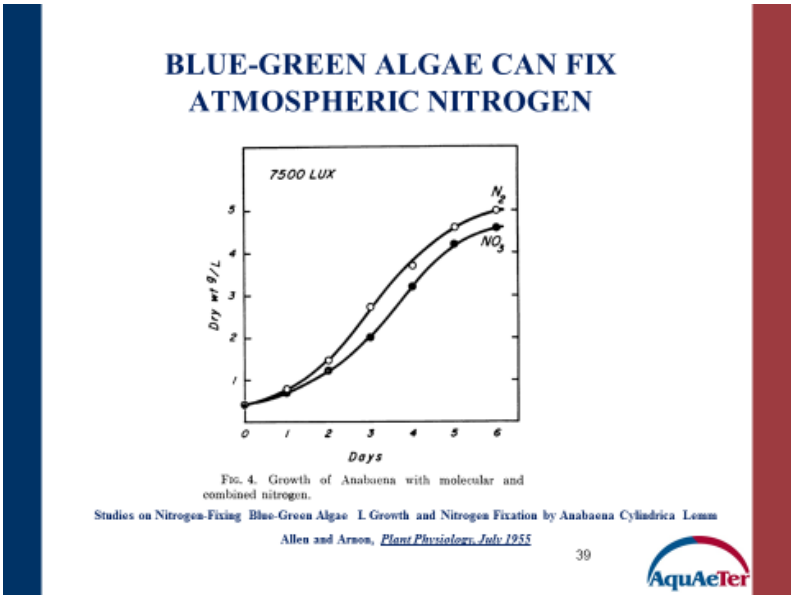
8. ***Determining if the River System has too much of either Nitrogen or Phosphorous.*** The other important technical consideration for determining algae species vs nutrient loadings is the fact that blue-green algae or bacteria do not all have to have nitrogen in a dissolved, inorganic form, rather they can obtain their nitrogen requirements from atmospheric nitrogen (~790,000 ppm N_2). So if we take all of the nitrogen out of a system, we can drive the algae species to an unhealthy population comprised predominantly by blue-green algae. We have seen this in at least two systems and on occasion in Middle Tennessee rivers. Since the Franklin POTW nitrifies in their treatment system, we would expect the Harpeth to be nitrogen limited an a candidate for blue-green dominance. The total nitrogen to total phosphorus ratio (i.e., TN/TP) for a healthy ecosystem is reported in the literature to be 10:1 to 12:1. An example of the total nitrogen to total phosphorous ratios for two streams plus the literature demonstrating that blue-green algae do not need water column nitrogen is presented in Figures 21 and 22.

Figure 21. TN:TP ratios and blue-green algae use of atmospheric nitrogen

SITE LOCATION	TOTAL NITROGEN mg/L)	TOTAL PHOPHOROUS (mg/L)	TN:TP (ratio = 10 to 12))	LIMITATION
Reference 1	0.81	0.03	27.00	P
Reference 2	0.97	0.07	13.86	P
Reference 3	0.94	0.07	13.43	P
Reference 4	0.85	0.06	14.17	P
Reference 5 Lake	0.89	0.07	12.71	good
Average	0.89	0.06	14.87	P
Stream 1 US	0.86	0.12	7.17	N
Stream 2 DS	1.04	0.40	2.60	N
Stream 3 DS	1.27	0.67	1.90	N
Stream 4 DS	0.86	0.31	2.77	N
Stream 5 DS Lake	0.72	0.01	72.00	P
	0.95	0.30	3.15	N

NOTE: Chesapeake Bay TN TMDL was 3 to 5 mg/L

Figure 22. Blue Green algae (bacteria) can utilize atmospheric nitrogen for growth



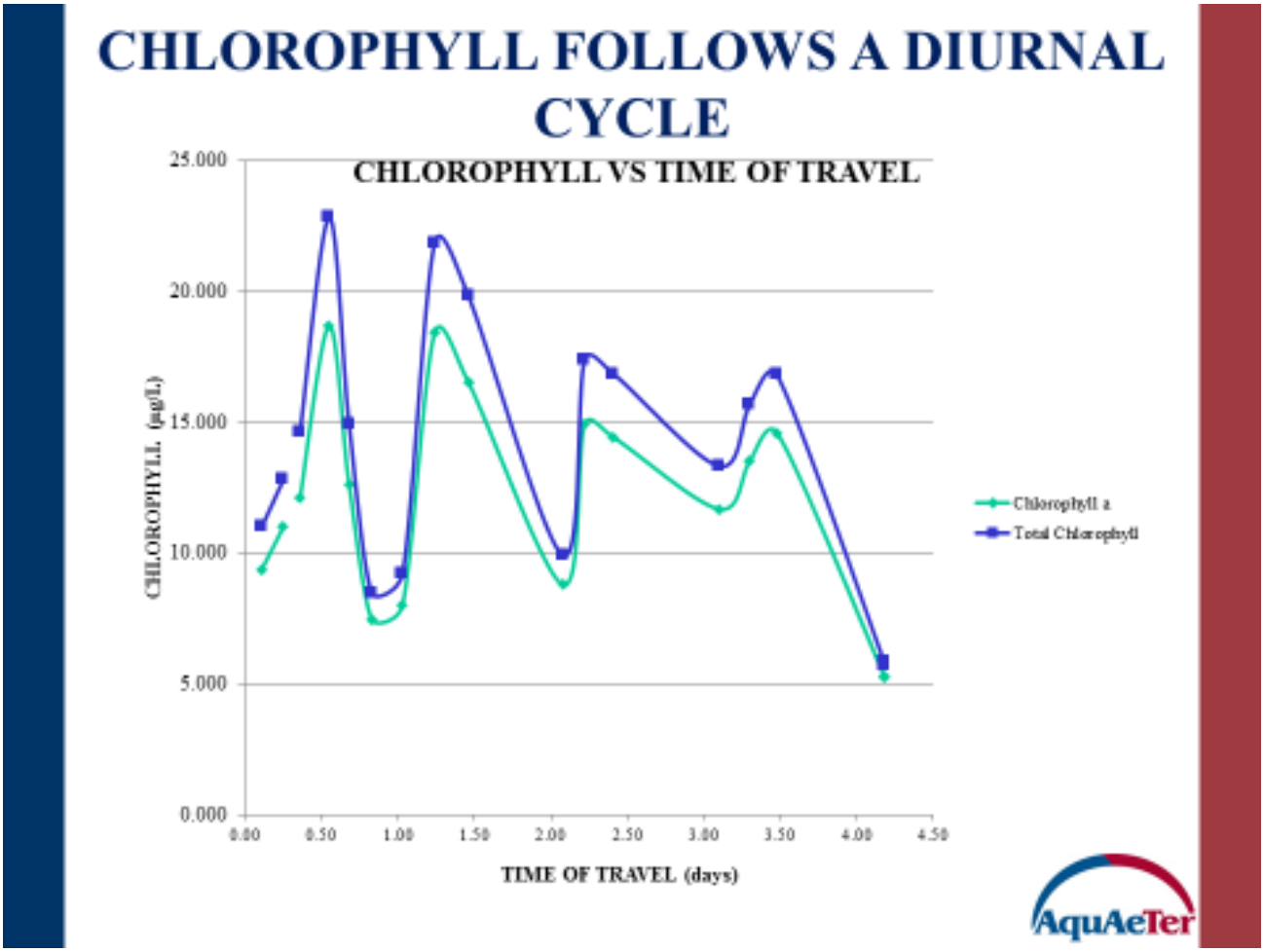
Finally, Franklin nitrifies and there are low nitrogen concentrations being discharged from the POTW. Low nitrogen concentrations are conducive for blue-green algae domination since blue-greens can obtain their nitrogen from the atmosphere, just as the blue-green dominated algae in the gulf hypoxia zone thrive on. The blue-green algae can utilize atmospheric nitrogen for their nitrogen-nutrient source, as has been known since 1955, as shown in Figure 22.

9. ***CHLOROPHYLL a AND BIOMASS ANALYSES. Total Chlorophylls and Accessory Pigments Analytical Methods and Sampling.*** The methods used to measure chlorophyll offer a wide variety of techniques that include both laboratory analyses and *in situ* measurements. The United States Environmental Protection Agency (USEPA) lists three methods: 1) Method 445.0, using fluorescence; 2) Method 446.0, using spectrophotometry; and, 3) Method 447.0, using high performance liquid chromatography (HPLC). Since 1978, multiple studies have evaluated the discrepancies of the three different methods (Jacobson & Rai, 1990). The fluorometric and spectrophotometric methods are unable to quantitatively distinguish between chlorophyll a and other porphyrin pigments that are also common in aquatic ecosystems. Although potentially more expensive (although prices for the laboratory we currently use are equivalent to spectrophotometric analyses), HPLC provides a quantitative means to identify and measure the concentrations of the individual chlorophylls and the accessory pigments, all of which have to be assessed to determine algae species growth and death.

Although we understand that chlorophyll a is being analyzed on random samples using spectrophotometric techniques, this method is at best qualitative and gives no useful information to make million-dollar decisions on wastewater treatment additions. The various algae species

use different chlorophylls including Chlorophylls a and b, Chlorophylls a and c, and various accessory pigments in producing oxygen. Tim Wool has added the ability to model multiple algae types; however, the kinetic equations are basically the same and are based primarily on green algae. Another important point is that Total Chlorophylls and accessory pigments follow a diurnal cycle, as shown in Figure 22 below.

Figure 22. Diurnal cycle for Total Chlorophylls and Chlorophyll a



This impacts the sampling methodology if algae are an important part in determining the WLA. A single random sample in time using a qualitative method offers no technically valid data for modeling and assessing the stream. It is important to develop a trend for the rise and fall of chlorophyll throughout the diel cycle. Many states are beginning to put chlorophyll a limits in permits without any technically defensible sampling regime or understanding of the diel cycle of chlorophyll. It is also noted that the Harpeth River will most likely have to be modeled dynamically for accurate results and thus time of day concentrations are important.

As one can see, a single point in time measurement for chlorophylls is technically questionable as to how you would use the data for modeling impacts to water quality. Likewise using spectrophotometric measurements leads one with a best guess and not one that we would

ever want to defend. Comparative measurements of HPLC measurements and spectrophotometric measurements are presented below in Figures 23 and 24.

Figure 23. Comparison of Spectrometric and HPLC chlorophyll analyses

STUDY	SAMPLE	CHLOROPHYLL a BY HPLC (µg/L)	CHL a AND KNOWN INTERFERENTS BY HPLC[1] (µg/L)	CHLOROPHYLL a BY SPECTROPHOTO-METRIC TRICHROMATIC (µg/L)
I	River 1	1.498	1.828	1.8
	Tributary A	0.465	0.510	2.0
	River 2	2.884	3.146	3.5
	River 3	1.825	2.108	1.4
	River 4	1.490	1.761	2.6
	River 5	6.890	8.040	3.6
	River 6	1.945	2.212	5.5
	Tributary B	0.278	0.419	3.7
	River 7	2.820	3.141	3.7
	Tributary C	2.009	2.432	1.7
	River 8	1.635	1.944	3.4
	River 9	1.994	2.375	5.0
	River 10	2.256	2.553	7.5
	River 11	3.619	4.137	7.3
II	River 2	32.513	33.660	13
	River 3[2]	49.570	52.700	28 43 33 35 38
	River 4	9.285	10.196	7.5

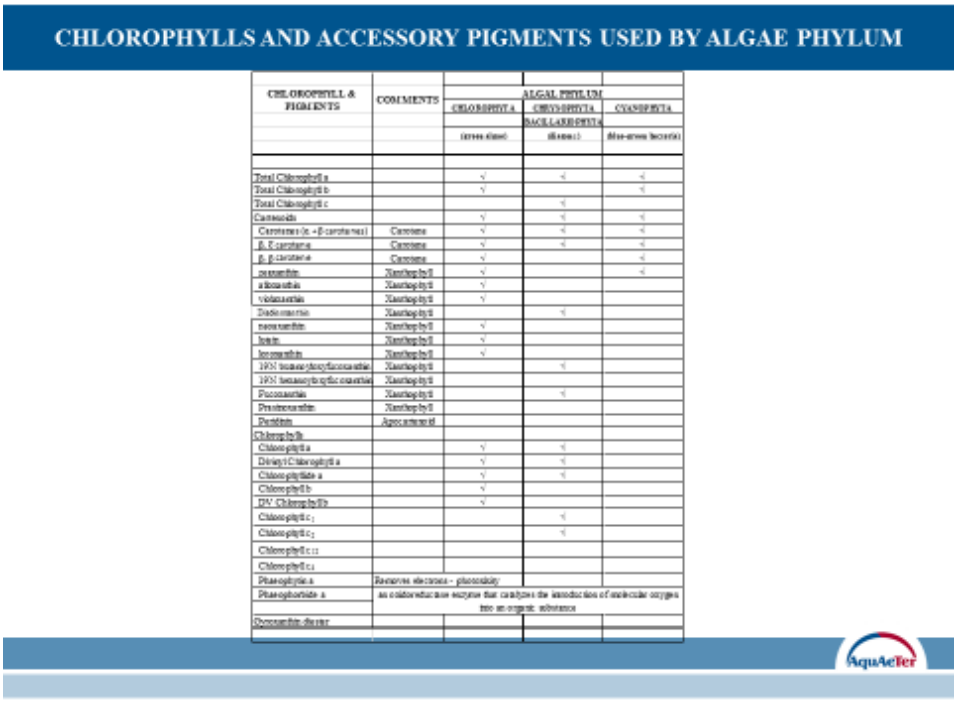
Notes: 1 – Known interferents to the spectrophotometric analysis method include the breakdown products Phaeophytin a, Phaeophytin a prime and Phaeophorbide a pigments.

3 – One sample was split into five for the spectrophotometric analyses.

10. ***The Use of Measuring only Chlorophyll a Using Spectrophotometric Techniques.***

Chlorophylls a, b, c and the accessory pigments are essential in being able to determine the interaction of nitrogen, phosphorous and silica on production of algae in the stream. Algae are an essential part of the stream's oxygen supply which is the basis for the Clean Water Act (i.e., the 5 mg/L DO general standard). HPLC is the only method that gives an accurate measurement of the chlorophylls and accessory pigments. Spectrophotometric measurements for Chlorophyll a are at best qualitative and do not give the information that can be used and defended in determining interactions between nitrogen and phosphorous loads to the River and the need to reduce or to increase these for the health of the River. Research since the 1970's has shown that spectrophotometric and fluorometric tests for chlorophyll are unreliable. Tim Wool has added 5 equations for algae in his current WASP model. There are 8 species of algae but they can easily be estimated by 2 or 3 algae species based on different algae species using different chlorophylls and accessory pigments. We like to divide the algae into the ones that use chlorophylls a and b (Chlorophyta – green algae) and the others that use chlorophylls a and c (Chrysophyta including Bacillariophyta, Chrysophyceae, and Xanthophyceae, and Cryptophyyta and Pyrrhophyta, and the Cyanophyta (blue greens) which use only chlorophyll a (and perhaps chlorophyll f). The blue-green algae being bacteria have a very small cell size and thus their biomass is quite different than all if the other algae. An example of the chlorophylls and accessory pigments are presented below in Figure 24.

Figure 24. Chlorophylls and Accessory Pigments Used by different Algae Phylum



10. Algae Biomass. The model utilizes algal biomass as an input. Unfortunately, no single species of algae has a defined biomass per cell, due to fluctuations in environmental conditions and the cell age. Biomass (volatile organic carbon) is also being measured although biomass per

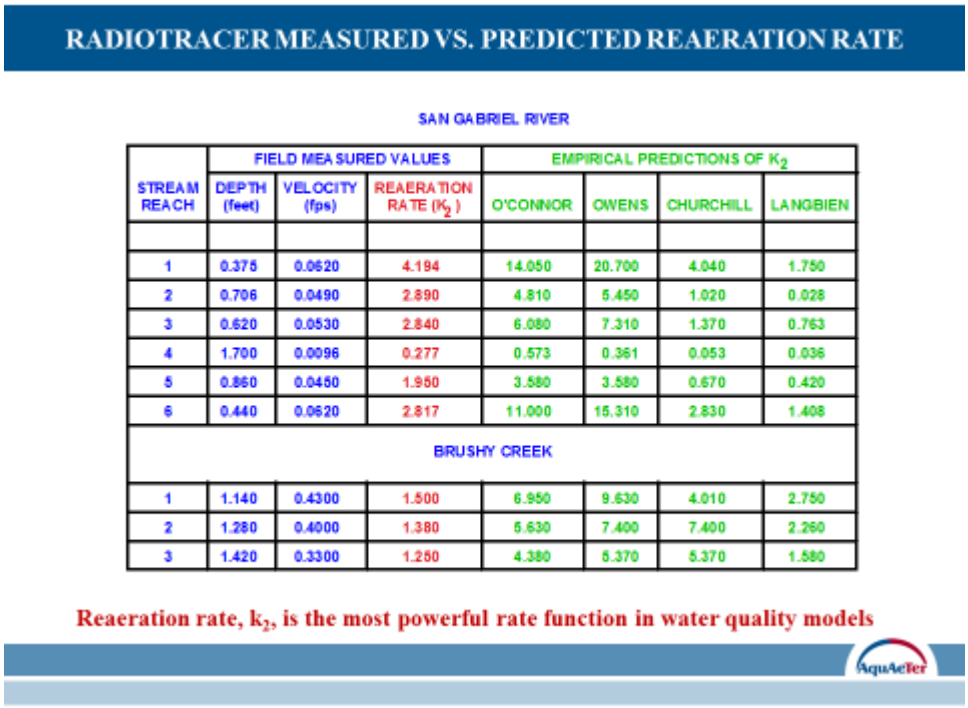
algae species to our knowledge is not a reality for any normal laboratory to measure. It is noted that the volatile organic carbon measurement does not differentiate between organic carbon from algae and organic carbon from other sources. The biomass for green algae, for diatoms, and for blue green bacteria would all be different so the use of biomass in modeling is really a best guess and normally gives an overestimate of algae productivity if you have the appropriate data to model (i.e., a true assimilative capacity dataset). Silicon is also important to monitor for diatoms which are the most productive of the algae (note: about 1.3 times more oxygen produced than green algae). Biomass is the one parameter that we measure, but requires significant adjustment over the normal +/- 25% for sensitivity analyses in order to match the diel DO cycle for algae. This is based on measured values and not best guesses of parameters and rates.

If the data being collected are to be used to set nutrient wasteload allocations, then there needs to be a more concerted effort to collect the data required to be able to make an informed decision versus an unproved theory. There is no substitute for having an assimilative capacity dataset collected with dye time of travel in order to properly calibrate a wasteload allocation model. Not measuring the rates leaves a model that cannot be verified nor is predictive. The data collected to date can be used somewhat for establishing background conditions for modeling the different months of the year, but are not adequate to calibrate a wasteload allocation model. The most sophisticated model is only as good as the dataset used to calibrate the model.

11, ***Lack of Algae Identification and Algae Numbers*** does not allow the modeler to determine if algae are a positive influence or a negative influence on the DO in the Harpeth River. The prime reason for the Clean Water Act was to improve DO resources. The lack of algae identification also does not allow the permit writer, the water quality modeler or the public to have any confidence that there is or is not a problem with nutrient limits or nuisance algae in the River.

12. ***Reaeration Rate Determination***. The reaeration rate was measured in the Harpeth River by the USEPA using a stable Krypton loss rate. Based on side by side comparisons with the radiotracer method using H-3 and Kr-85 radiotracers (the Tsivoglou method), the stable Krypton method is believed to be within +/- 50% of the radiotracer method. This is still much better than the typical predictive equations used by USEPA and many other investigators. This is the most powerful rate in the model and our inability to predict this crucial rate is troubling to the river modeler. Typical differences in measured radiotracer reaeration rates to the predicted reaeration rates from four different predictive equations is presented in Figure 25.

Figure 25. Radiotracer measured reaeration rates compared to typical reaeration rate equations



STREAM REACH	FIELD MEASURED VALUES			EMPIRICAL PREDICTIONS OF K_2			
	DEPTH (feet)	VELOCITY (fps)	REAERATION RATE (K_2)	O'CONNOR	OWENS	CHURCHILL	LANGBIEN
1	0.375	0.0620	4.194	14.050	20.700	4.040	1.750
2	0.706	0.0490	2.890	4.810	5.450	1.020	0.028
3	0.620	0.0530	2.840	6.080	7.310	1.370	0.763
4	1.700	0.0096	0.277	0.573	0.361	0.053	0.036
5	0.860	0.0450	1.950	3.580	3.580	0.670	0.420
6	0.440	0.0620	2.817	11.000	15.310	2.830	1.408
BRUSHY CREEK							
1	1.140	0.4300	1.500	6.950	9.630	4.010	2.750
2	1.280	0.4000	1.380	5.630	7.400	7.400	2.260
3	1.420	0.3300	1.250	4.380	5.370	5.370	1.580

Most of these predictive equations are based on a theory that deeper streams have lower reaeration rates, but this is not a correct assumption. Reaeration rates are based on turbulence and not depth. The Mississippi River has high reaeration rates due to its tremendous turbulence even though it can be over 100 ft deep.

We are conducting a time of travel TMDL study in Middle Tennessee where we have previously measured the reaeration rate using the Tsivoglou method and so we will be able to accurately determine the true impact and importance of the algae to the oxygen balance in the stream. Since we know the "c" coefficient for this stream based on two radiotracer measurements, we will be able to predict reaeration for the monthly 7Q10's for the stream.

13. ***Determining Stage and Flow in the Harpeth River.*** In the previous TMDL modeling for the Harpeth River reported in 2004 by the USEPA, the hydraulic model output had an "unexplained" increase in flow from a few cfs to around 100 cfs in the middle of the model run. We believe that this was caused by the use of stage on one end of the model vs flow used on the other end of the model. This does not work as we found out on a river in Oklahoma that we were using a dynamic model for calibrating the predictive model. In discussions with Bob Olsen who developed the GA EPD RIV1 hydraulic and water quality models, he cleared up this problem stating that either stage or flow has to be used in the model (on both ends in this case) but they cannot be mixed. A couple of ways can be used to help ensure the flows and stages are consistent with conservation of mass include:

a. Measurement of flows at each time of travel station; and

b. Place stage recorders and barometric pressure instruments at strategic locations down the Harpeth River.

Examples of a stage recorder and a barometric monitor are presented in Figure 26.

Figure 26. Stage and barometric pressure recordings can be done economically

13. ***Recommendations for conducting the 2018 river data collections.*** To date, there are no data that have been collected besides the SOD measurements that can be used to calibrate the WLA of a TMDL model nor are data being collected to analyze the effluent dischargers' true impact to the River. Because flow and temperature are the two most important drivers for the wasteload allocation, the Franklin discharge represents a potential important resource to the River and is most likely increasing the wasteload capacity of the River since the Harpeth is a flow-limited stream.

The following activities need to be done in 2018 in order to have a technically defensible waste assimilative capacity study that can be used to calibrate a wasteload allocation model. The mathematical model is worthless unless it is based on real data and not best guesses of deoxygenation rates, oxygen additions by reaeration and algae, and supporting data as previously outlined. Our suggestions include:

11. Conduct a dye time of travel study and collect a dataset as outlined in 1.c. above;
12. Run 90-day time-series BODs so that the f-factor can be calculated for each discharger. Also measure nitrogen and phosphorous series parameters so that the POTWs can analyze the actual total nitrogen that has impacts to the River (both positive and negative). 30-day BOD analyses are for all practical purposes a waste of

time and limited for modeling with the knowledge we have today. One can certainly not establish BOD_5 limits for permits with a 30-day test;

13. Fixed stations for diurnal measurements of DO, temperature, pH and conductivity should be established at selected stations downstream. These data will give the diurnal DO curves that occur at individual stations. These data will be used with the same data collected from the dye cloud as it moves downstream. Both of these are used to be able to determine the net positive or negative addition/subtraction of DO in the Harpeth River;
14. Collect algae samples for identification and numbers plus biomass with dye time of travel. Without algae data, no decisions can be made on the impact of algae on the health of the river. Algae are an important resource for oxygen in the River;
15. Run HPLC analyses with samples collected with dye time of travel. It is especially important to determine the diel cycle of the total chlorophylls and the accessory pigments. The current analyses using spectrophotometric analyses for chlorophyll a only gives us qualitative results that are not reliable for assessing the algae and definitely cannot be used if permit limits that stand-up in court are going to be set by TDEC.
16. The Harpeth River at low flows will have to be modeled using a dynamic model. The Franklin POTW represents the most significant flow in the River (up to 80 to 90%+). This has not been done in the past. Dynamic models typically result in more allocation.
17. Flows should be monitored at each dye of travel station. Stage measurements with barometric pressure recorders should be obtained at several locations down the river. The dynamic hydraulic model should use the same reference on both ends of the model, i.e., flow at Franklin and flow downstream at the last gage location.
18. Bottle rates should not be used. These are only good in the laboratory and are not reality for the more complex stream bacterial populations. Bottle rates can be 500% greater or more than the actual deoxygenation rate determined from the proper method using rates determined from samples collected with dye time of travel or the ∂t that is being modeled by Tim Wool.
19. A Work Plan should be developed for conducting the 2018 studies. It is impossible to conduct a waste assimilative capacity study and then conduct the subsequent modeling if a definitive plan is not developed and in place before the project begins. This is a fundamental part of any scientific study. The inputs to the model should be developed from the field waste assimilative capacity study and then these data can be used in the calibration of the model without guessing or "curve-fitting the model to meet an arbitrary DO concentration that is constantly changing during the day.
20. At this point, there is no value to calibrating a model where no data exist to calibrate the model. It is possible to build the hydraulic model at this time and have it ready for use with the assimilative capacity study data once it is obtained. The hydraulic model will still have to be calibrated with the flow measurements, flow data from the

USGS gages, flow data from the POTWs, and any stage measurements made during the time of travel study.