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Introduction

The South Florida Water Management District (District) and Lee County are partnering to reduce nutrient concentrations and loads in the C-43 Canal (Caloosahatchee River) upstream of the S-79 water control structure east of Ft. Myers and in the Caloosahatchee Estuary (Estuary) downstream of S-79. Elevated concentrations of nitrogen and phosphorus in these water bodies are contributing to impairment of beneficial uses in the Caloosahatchee River and Estuary, primarily by generating excessive algae blooms and resulting in decreased water clarity and dissolved oxygen content. The primary focus of the District’s and Lee County’s efforts is reduction of bioavailable forms of nitrogen and ultimate compliance with the nitrogen Total Maximum Daily Load (TMDL) requirement for the Estuary. While the focus is on nitrogen (N) load reduction, loads of phosphorus (P) and suspended solids are also of concern and are being considered.

The effect of excessive nutrient loads from the C-43 Basin (including Lake Okeechobee) is exacerbated by unnaturally high and variable flows that bring excessive nutrients and humic/tannic colored water into the river and estuary. One component of this restoration effort mandated by the Northern Everglades and Estuary Protection Program of 2007 is implementation of a Water Quality Treatment Area (WQTA) just upstream of S-78 on the south side of C-43. Other activities in this overall restoration plan that are outside the consideration of this review but may be critical for the success of the WQTA project include a proposed water storage reservoir (C-43 Storage Reservoir) to restore some normality to flows and source controls in the basin to reduce nutrient and solids loads in the C-43 Canal.

A 1,750-ac site has been purchased by the District and Lee County for the proposed WQTA. Preliminary studies and engineering for the proposed WQTA facility are being conducted by
CH2M HILL (Consultant) under contract with the District. A number of project deliverables have been completed by the Consultant to identify the best option(s) for achieving the project goals of nutrient reduction in C-43 and the Estuary. Key activities completed by the Consultant under this contract included:

- Initial Data Collection and Total Nitrogen Reduction Technologies Assessment
- Water Quality Evaluation and Characterization of Dissolved Organic Nitrogen (DON)
- C-43 Water Quality Treatment Project Test Facility Conceptual Plan Development

The Consultant’s work efforts have resulted in a proposed treatment train of natural nutrient removal technologies (i.e., “green” wetland and aquatic processes that rely more on solar and other natural energy inputs/plants and processes and less on the consumption of fossil fuels or chemicals) to reduce concentrations of bioavailable nitrogen and phosphorus compounds in C-43 prior to discharge to the Estuary. The Consultant has prepared a conceptual plan for development of a research/demonstration facility on the C-43 WQTA site as the next step prior to full-scale implementation of nutrient removal in the Caloosahatchee River.

The District is conducting a peer review of the Consultant’s findings and recommendations prior to proceeding with final design and implementation of the research/demonstration project. A panel of three technical experts (Panel) with extensive credentials in water quality treatment and wetland and natural systems, were selected for this peer review:

- Dr. Robert Knight, Panel Chair (Wetland Solutions, Inc. [WSI] and University of Florida)
- Dr. Alex Horne (University of California Berkley)
- Dr. John White (Louisiana State University)

The District has directed the Panel to complete the following four tasks as part of this review effort:

- Task 1 - Review and Evaluate Consultant Deliverables including the following:
  - Total Nitrogen Reduction Technologies Review (April 2008);
  - Organic Nitrogen Methodology Screening Analysis (Deliverable 3.2.1 Draft, November 2009);
  - Findings Memorandum (Deliverable 3.1.2 Final, December 2009),
  - Water Quality Treatment Area Test Facility Parameter Plan (January 2010); and
  - C-43 Water Quality Treatment Area Draft Conceptual Plan Technical Memorandum (Deliverable 4.2.8 Draft, March 2010).
- Task 2 - Provide guidance on the C-43 WQTA Test Facility design, including:
  - Identify promising approaches to TN removal; and
  - Recommend Test Facility changes as needed to evaluate these promising approaches.
• Task 3 - Recommend parallel work efforts (experimental and/or data review) to improve the information derived from the Test Facility; and

• Task 4 - Participate in a two-day workshop to discuss and review Panel findings and to reach consensus on the conceptual plan for the proposed C-43 WQTA Test Facility.

Each of the three Panel members have submitted detailed draft review comments to the District (see Appendices A, B, and C attached). Each of the Panel members attended and made a Power Point presentation at a two-day workshop held with District and Lee county staff in West Palm Beach, Florida on July 12 and 13, 2010 (see Appendix D). This technical memorandum (TM) provides a final summary of the panel’s technical conclusions concerning the Consultant’s plan for the C-43 WQTA project implementation. The District’s specific questions/requests are used to organize the consolidated memo that follows. A consolidated list of Panel conclusions and recommendations is provided at the end of this TM.

Task 2 – Review Consultant Deliverables

Question 2.2.1: Are the major conclusions of the Total Nitrogen Reduction Technologies Review valid and supported by the information presented?

The eight principal conclusions in the Consultant’s report are addressed as follows.

2.2.1(a) Dissolved organic nitrogen (DON) is essential to the nutrient budget of harmful algae blooms in South Florida waters.

The Panel’s consensus is that all available nitrogen forms are important and must be considered when evaluating the potential for algal blooms in the C-43 Canal and Caloosahatchee Estuary. While DON is often the predominant form of nitrogen, it is by no means the only form of nitrogen and is essentially the least available for algal blooms. Concentrations of total inorganic nitrogen (TIN) must be considered first and foremost as the most readily available nitrogen forms that stimulate algal blooms. Since selection for organisms that specialize in the enzymatic degradation of recalcitrant or refractory DON (RDON) is dependent upon low TIN concentrations (<150 µg/L) the first step in any treatment process should focus on TIN removal prior to trying to stimulate removal of RDON. Since a variable fraction of DON is typically bioavailable in most surface waters (including C-43), mineralization of this nitrogen fraction (BDON) to TIN is the next priority for a conceptual design. There is also some seasonality to expression of eutrophic conditions, and therefore it may be more important to reduce N concentrations in the spring and summer than at other times during the year when algal blooms are rarely present. More detailed discussions of the Panel’s finding concerning the most important nitrogen transformation processes are included in the detailed review memos attached to the end of this report.

2.2.1(b) DON bioavailability can be divided into recalcitrant, semi-labile, and labile fractions in terms of algal uptake. Semi-labile forms need to be converted by bacteria to become labile. Categorizing these fractions simply by molecular weight shows no general relationship. Assessing treatment efficacy of DON will require insight into their bioavailability.

The Panel agrees with the first part of this conclusion. Dr. Horne points out that until TIN is sufficiently depleted, removal kinetics for DON are not maximized. There is also evidence of
heterotrophic nitrogen use by some phytoplankton such as marine dinoflagellates. Dr. Knight concludes that the complex diversity of DON compounds exceeds the ability to deal with compound specific removal rates in treatment system design. All of the Panelists agree that finding a trustworthy and cost effective analytical method or bioassay for assessing bioavailability would be desirable for rapid assessment of potential eutrophication effects.

2.2.1(c) Total nitrogen (TN) treatment performance analysis is necessary but not sufficient to achieve treatment goals. Background concentrations of TIN (e.g., C*NH3, C*NOx) approach analytical detection limits. There will always be a residual concentration of DON in surface waters (C*DON) that cannot be further degraded by a natural treatment technology. The portion of this RDON that is labile and ultimately available must be determined.

The Panel members are in essential agreement with these conclusions. However, Dr. White emphasizes that the actual C*DON is not really known because existing natural treatment systems have not been optimized to explore this limit. Dr. Horne points out that the actual C* values for TIN are also not that well known but are less relevant since DON is the predominant form of nitrogen. Dr. Knight makes the point that the focus of the full-scale design should be on removing the greatest amount of “biologically available nitrogen” (BAN) that is possible by a NTS, including all particulate and dissolved forms. He suggests an operationally-based definition of BAN as any form of inorganic or organic nitrogen that has an environmental half-life in an NTS of about 30 days or less.

2.2.1(d) Advanced analytical and bioassay methods are an obligatory portion of the proposed C-43 Water Quality Treatment Area (WQTA) research and demonstration project.

Dr. White and Dr. Knight generally agree that the EIS-MS methodology for screening individual DON compounds is not quantitative, too experimental at this time, and not likely to be of much help for project implementation. The Panel is in agreement about the need for and benefits of a repeatable analytical test or bioassay method as an indication of project success. An ideal analytical or bioassay method should follow standard protocols to the extent possible, be repeatable, and should be applicable to indicating potential eutrophication effects in both the freshwater and saltwater portions of the Caloosahatchee. All of the Panelists agreed that the ultimate measure of success for the Project is a quantified reduction in TN concentrations and loads to the Caloosahatchee River and Estuary.

2.2.1(e) Floating aquatic plant (FAV) dominated natural treatment systems (NTS) appear to be the best candidate for the C-43 project. Emergent wetlands are concluded to be the next best option. Open water systems dominated by algae and periphyton are concluded to be the least applicable NTS for this project.

The Panel does not whole-heartedly agree with this conclusion. While all Panel members understand the benefits of FAV systems for control of higher TN and TP pollutant concentrations, these systems are notorious for their management difficulties. Dr. Knight points out that floating aquatic plant systems are susceptible to catastrophic die off due to frosts and pathogens, require fertilization for high growth, are typically harvested to maximize growth and nutrient removal, and are easily affected by extreme winds such as those that result from hurricanes in south Florida. Dr. White points out that the concentration of total suspended solids in these systems is often high. Dr. Horne and Dr. Knight both point out the limited basis for the Consultant’s conclusion that FAV systems are more effective for DON removal than the
other NTS plant communities. Unlike emergent wetland systems, only two relatively limited data sets were offered by the Consultant to reach this conclusion.

2.2.1(f) Soil Aquifer Treatment (SAT) and Riverbank Infiltration (RBF) are recommended by the Consultant for testing based on existing limited evidence.

The Panel is in agreement that these proposed technologies and others that require percolation of water through soils (such as slow rate land application) may have considerable hydraulic constraints for implementation at this scale and will need considerable research and development to evaluate their applicable design criteria and expected performance for DON removal. Dr. White agrees with preliminary testing of soil-based nitrogen treatment as a potential implementation tactic for source control in the watershed; Dr. Knight recommends additional literature review from similar Florida systems (e.g., the C-43 and C-44 Reservoir Test Cell projects) to better assess feasibility of soil aquifer treatment prior to any field tests; and Dr. Horne does not support further expenditures on these technologies.

Dr. Knight points out that the evaluation of other possible conventional technologies for TN reduction provided by the Consultant was not comprehensive and may not fully document or justify the Consultant’s ranking of preferred alternatives. However, the Panelists were in agreement that based on their combined experience in the field of nitrogen removal technologies, a wetland-based NTS was likely to be most cost effective for the District. This shared experience includes the Panel’s consideration of all of the alternatives discussed by the Consultant, as well as others like slow rate land application, rapid infiltration basins, biological nutrient removal, and others not specifically addressed in the Consultant’s report.

2.2.1(g) The recommended treatment train for the C-43 WQTA project and estimated fractional land area requirements is: FAV to Emergent Aquatic Vegetation (EAV) (combined at 75%) to Submerged Aquatic Vegetation (SAV) (10%) to SAT (10%). The recommended FAV system is proposed to be dominated by floating tussock growth.

The Panel provided a number of qualifications and concerns about these conclusions. The Panel does not agree that the Consultant’s proposed treatment train is best or likely to be the best overall alternative for a full-scale project.

2.2.1(h) Conventional nitrogen treatment technologies do not offer any advantages over NTS systems. Of the conventional technologies considered, drinking water technologies (e.g., advanced oxidation, coagulation, reverse osmosis, ultra-filtration, etc.) offer the most reasonable model but are not competitive based on cost and proven reliability.

The Panel agrees that the costs for implementing energy-intensive conventional potable water treatment technologies are prohibitive for full-scale implementation. Dr. White and Dr. Knight both suggest that the Consultant could have provided a more defensible comparison between NTS and conventional processes than provided in this report.

Question 2.2.2: Did the assays (salinity release, photolysis, bioavailability) presented in the Organic Nitrogen Methodology Screening Analysis adequately quantify the fraction of the DON pool that could become available to bacteria and algae?

The Panel had difficulty understanding or accepting the results of the bioassays. Some issues were methodological (goals, short duration, poor replicability, sampling issues, ineffective
photolysis, and focus on algal pigments rather than bacterial biomass). Some concerns were related to data interpretation (variable inoculum ecology, death and lysis of inoculum cells and possible release of nutrients, unconventional data presentation), and use of non-standard methods when there are standard tests available (e.g., the Algal Growth Potential test).) The Panel did not recommend any specific bioassay test as being preferable for assessing the effectiveness of this Project. However, the Panel did agree that a modified analytical procedure similar to the test for total Kjeldahl nitrogen test might be helpful for assessing the BAN component of these waters.

**Question 2.2.3: Are the seasonal shifts in DON availability/recalcitrance supported by the chemical and biological evidence presented?**

The Panel agreed that there are differences in the DON bioavailability during the wet and dry seasons. The significance of those differences was discussed by Dr. White who felt that they may be based as much on the source of DON (increased runoff from agricultural areas) as on the age of the water and associated DON. Dr. Knight noted that upstream-downstream data from stations in C-43 indicate that a sizable fraction of the DON is assimilated during its seasonal flow from Lake Okeechobee and tributary inputs to the Caloosahatchee Estuary. He also noted that the Consultant’s FAV-dominated stations had some of the highest measured TN and DON concentrations and that TIN makes up as much as 20% of the TN in these waters (the target mass reduction of TN in the Total Maximum Daily Load [TMDL] is 23%). The Panel also noted that there is substantial variability from year-to-year in both the seasonality of water flows and of nitrogen fractions in the C-43/Callosahatchee River system.

**Question 2.2.4: Is the preliminary surrogate method for determination of biologically available DON reasonable and supported by the evidence presented?**

The Panel did not support this conclusion. While a bioassay of some sort is desirable, the one proposed by the Consultant was not sufficiently developed to fill this need.

**Question 2.2.5: Are the conclusions of the Findings Memorandum supported by the data and its analysis?**

The Panel reviewed the two primary conclusions of this report as described below.

2.2.5(a) Data show that nearly all nitrogen in these surface waters is organic and that inorganic forms are typically an order-of-magnitude lower than DON. The correlation between TOC and TON and DOC and DON are more apparent in the dry than in the wet season.

Dr. Horne indicated general agreement with these conclusions. Dr. White felt that the analysis and comparison of the data was lacking. He indicated that it might be confusing to compare data from the Stormwater Treatment Areas (STAs) to data from C-43 and its watershed, that more careful analysis should have been made of the wet and dry season data sets, and that collection of water quality samples from FAV-dominated areas may have been misleading. Dr. Knight also recommended a more detailed analysis of these data with more attention paid to the subtle differences rather than the overall wet/dry season means. A review of the historic data set for C-43 shows very high variability between flows, nitrogen concentrations, and loads between years and between the theoretical wet and dry seasons of south Florida. Quantification of this full range of variability is important for any full scale design project.
2.2.5(b) These datasets provide descriptive information useful in assessing the findings of the bioassay, photolysis and salinity, and compound-specific tests.

The Panel agrees with this conclusion except that the Panel finds that these data alone have limited applicability for design of the Project since they were collected within a relatively small range of spatial and temporal variation.

Question 2.2.6: Are the conclusions of the Parameter Plan supported by the data and its analysis?

2.2.6(a) The basis of the Parameter Plan is that there is an apparently reproducible pattern of change both at the compound-level analyses and total pigment changes in bioavailability assays as DON is transformed from bioavailable to recalcitrant forms. The pigment changes in the bioavailability assays are proposed as a method to track DON transformations using the compound-level analyses for verification.

Dr. White is concerned that the compound-specific analyses are not quantitative and that some compounds are not detected by the methodology. Therefore he recommends more work on a less specific, more integrative test such as a modified analytical test as described above or a more easily interpreted bioassay. Dr. Horne agrees that there is apparent reproducibility in the compound-specific test but that the bioavailability tests, as presented, are not clear and would be better with a more conventional presentation. Dr. Knight concluded that all of the tests cited in this report are not adequately developed at this time to form the basis for implementation of a critical water quality treatment project. Existing methods for analysis of nitrogen and its various interchangeable forms (e.g., nitrate+ nitrite, total ammonia N, and TKN) should be relied upon as much as possible to serve as the basis for project implementation. As mentioned above the Panel supported the idea of evaluating a modified TKN analysis to better assess the fraction of biologically available organic nitrogen in the total DON fraction.

Task 2.3: Evaluate the scientific validity of and identify advantages and disadvantages of the proposed approach to N removal and transformation in light of the low level of TN in the water being treated.

The Panel members offered the following conclusions related to the scientific validity of the Consultant’s proposed approach in light of the low level of TN in the water to be treated:

- Dr. Alex Horne
  - The TN in the water is not really low for surface waters (TKN > 2,000 µg/L; DIN>200 µg/L) and may not be limiting algal growth.
  - DIN is high enough that algae are not pressured into using DON as a preferred source of nitrogen.
  - Light may be the environmental factor limiting algal proliferation.
  - The dominance of ammonia in the DIN indicates low oxygen conditions and nitrogen recycling.
  - Removal of ammonia in NTSs is slower than nitrate and cannot be assumed to occur in a FAV or emergent wetland cell without adequate retention time.
• Recommends acceptance of NTS wetlands for TN-DON reduction but rejects the Consultant’s concept to focus on labile DOC in the wet season.

• Recommends additional inquiry into the effectiveness of reducing TIN to limiting levels and for evaluation of photolytic degradation of DON.

• Dr. John White

  o TN treatment in C-43 is liable to be more challenging than TP treatment was for the Everglades ecosystem because of the difference in particulate forms.

  o Reliance on ion resonance mass spectrometry for compound characterization is not recommended.

  o Reliance on FAV has some merit as a treatment process.

  o The Consultant’s idea of using an SAV-dominated plant community as a final cell appears to conflict with the project goal due to likely nitrogen fixation at low N concentrations.

• Dr. Robert Knight

  o The use of a wetland treatment system as a center piece for this project is supported by ample evidence from other Florida systems. However, the focus of the project should be on removal of the bioavailable forms of nitrogen, including especially TIN and the easily mineralized forms of DON (urea, uric acid, amino acids and sugars, amides, etc.) rather than on conversion of BDON to RDON. There should also be more emphasis on the design basis for phosphorus reduction.

  o This reviewer concludes that the evaluation of NTS technologies was not well balanced and put too much emphasis on a few relatively small data sets.

  o The evaluation of conventional technologies may be insufficient to provide a defensible argument for their rejection from further consideration.

  o Emphasis on review of emergent wetland data is justified considering the large data base, but focus should be on more Florida systems built on a range of soils from inorganic to organic and with differing plant community dominance.

**Task 2.4: Recommend that the District accept, reject, or revise the Consultant’s proposed approach.**

The Panel members generally recommended that the District revise the Consultant’s approach based on the comments summarized above and detailed in the individual Panel memos (attached in Appendices A, B, and C).
Task 3 – Recommend Feasible Approaches

Task 3.1: Each Panel Member shall identify and recommend feasible approaches to TN removal using wetland-based technologies in light of the low TN levels at the study site and elsewhere in the District.

The Panel members offered the following independent evaluations of feasible wetland-based NTS for the proposed C-43 Project:

- Based on a detailed evaluation of alternative wetland plant communities and configurations Dr. Horne’s order of ranking indicated that either tall emergent aquatic vegetation (EAV) or SAV-dominated wetlands are preferred over FAV. Based on combination systems with two plant communities, Dr. Horne ranked EAV-SAV and FAV-EAV considerably higher than FAV-SAV. Based on further evaluation of enhanced alternatives, Dr. Horne ranked Pulsed EAV, Wet-Dry EVA, and Algal Turf Scrubber, followed by a modified periphyton-based system highest overall and FAV and aerated FAV the lowest.

- Dr. White concluded that EAV, SAV, and FAV should be considered individually as separate treatments on mineral-based (sandy) soils to fully evaluate their performance. He also offered the concept of horizontal flow through a limerock berm as a final polishing system prior to discharge to surface waters, if feasible based on hydraulic considerations. The contribution of light to nitrogen reduction, either artificial or natural should also be investigated.

- Dr. Knight concluded that based on existing data from Florida systems the preferred ranking of individual alternatives to meet the goals of the C-43 Project (from most cost effective to least) is: shallow and deep emergent wetlands (=EAV), non-harvested ponds dominated by FAV/SAV, ponds dominated by algae, harvested FAV ponds, algal turf scrubber technologies, and subsurface flow wetlands consisting of vertical and horizontal flow. Dr. Knight further concluded that only the first two technologies (EAV and non-harvested FAV/SAV) are promising enough to warrant study/demonstration at the C-43 WQTA Test Facility.

Task 3.2: Review the existing proposed conceptual design of the Test Facility in light of the approach suggested by the Consultant and any approaches identified in Subtask 3.1. Can the recommended approaches be evaluated experimentally using the current design of the Test Facility?

The Panel members had the following comments about the suitability of the proposed Test Facility design to test the Consultant’s recommended treatment scheme:

- Dr. Horne commented that overall facility design appears to be adequate. He specifically suggests a revised treatment train for wetland options preceded by a pond for nitrification prior to an EAV cell followed by an FAV cell for DON reduction. He also recommended inclusion of a periphyton-enhanced oxidative phytodegradation wetland to better evaluate benefits of DON photolysis.
• Dr. White suggested that the Consultant’s proposed mesocosm facility be re-designed to include side-by-side comparisons of individual plant communities. He also stresses a need to look at the effects of soil conditions on DON release. Dr. White generally agreed with the Test Cell design but recommended the ability to test individual units in parallel.

• Dr. Knight concluded that the proposed Test Facility and Parameter Plan is overly ambitious from a cost and time perspective. Based on the number of mesocosms and test cells in series, and the possible flaws in the selected treatment train, the proposed Test Facility will be very expensive to construct and operate and may not provide data relevant to full-scale project implementation.

Task 3.3: Identify and suggest changes to the proposed design of the Test Facility with the goal of providing a sound and robust evaluation of any proposed approach.

• Dr. Horne suggests a revised treatment train consisting of a pond for nitrification prior to an EAV cell followed by an FAV cell for additional conversion of biologically available DON to DIN. He also recommended inclusion of a periphyton-enhanced oxidative phytodegradation wetland to better evaluate benefits of DON photolysis.

• Dr. White suggested that the proposed mesocosm facility be re-designed to include side-by-side comparisons of individual plant communities. He also stresses a need to look at the effects of soil conditions on DON release and the need to test more plant combination options at the larger demonstration scale.

• Dr. Knight concluded that the focus of the Test Facility should be on developing optimal design criteria for full-scale project implementation. The only NTS technologies that are currently developed to the point of serious consideration are constructed wetlands dominated by emergent, submerged, and/or floating plants. SAT and RBF should not be included in the Test Facility design unless further evaluation of their technical feasibility and applicability at C-43 is conducted. Testing and demonstration at the C-43 WQTA test facility should emphasize the benefits and problems related to realistic ranges of plant community dominance, water depth, hydraulic loading rates, and antecedent soil conditions. The use of mesocosms is not recommended at this facility, and if included, should only be used to look at process-level design issues such as substrate and plant community effects on N removal. Test Cells should be larger (10 to 40 acres each) to eliminate edge effects and to provide realistic plant establishment/maintenance experience; should not be used for replicated experiments; and should only be used to test distinctly different NTS alternatives that are supported by an updated feasibility assessment. A larger Demonstration Cell or Treatment Train should only be put into operation once preliminary data are available from the proposed Test Cells. The Demonstration Cell(s) will be comparable to the Everglades Nutrient Removal (ENR) cell and will provide lessons in full-scale project implementation.

Task 3.4: Suggest experimental and/or data collection needs that could be run in parallel to improve the information output of the facility.

• Dr. Horne suggested that the laboratory photodegradation tests be repeated for a longer duration and with different water, location, and season. He also emphasized the need to
look more at initial nitrification of ammonia to nitrate at the front end of the treatment train.

- Dr. White suggested that testing of SAT in the orange grove drainage rows would be worth pursuing as a potential source control in the watershed.

- Dr. Knight suggested the following parallel work efforts while the Test Facility Plan is implemented: review of additional relevant data sets on N dynamics in a variety of Florida wetland and reservoir systems with a focus on systems with low TON outflows; calibrate the P-k-C* model for each system and for each substrate and vegetation type and develop a dynamic nitrogen removal model for these systems to compliment the DMSTA v.2 model for total phosphorus; continue to develop reliable and cost effective surrogates for DON fractionation into available and recalcitrant fractions; prepare a preliminary conceptual plan for a WQTA capable of achieving the overall project goal of TN TMDL compliance and reduction in nuisance algal blooms; and coordinate the overall functionality of the proposed C-43 West Storage Reservoir and the C-43 WQTA projects and consider possible trade offs in footprint of the two systems to optimize effectiveness for project goals.

**Final Conclusions and Recommendations**

The Panel has completed the review of the Consultant’s deliverables related to the C-43 WQTA Project. Detailed technical review comments are provided in the attachments and briefly summarized above. A workshop was held on July 12 and 13, 2010 in West Palm Beach with District staff and with representatives of Lee County to present and discuss these review comments. During the workshop, District staff presented informative background information concerning the District’s experience with NTS water quality projects and the existing conditions in the Caloosahatchee River (C-43) and Estuary. Each Panelist presented their individual technical review in the form of a PowerPoint presentation (attached as appendices to this report). On the second day of the workshop the Panel met to develop a consensus concerning technical findings and recommendations. The Panel’s consensus conclusions and recommendations are summarized as follows:

1. The six reports and appendices produced or commissioned by the Consultant provided a good basis to begin to understand the changes in both bioavailable (labile) and unavailable (refractory) dissolved organic nitrogen (BDON & RDON respectively) in both natural and simulated natural conditions of the Caloosahatchee River and its estuary. As many as 5,000 individual DON compounds may be involved compared with only 3 for DIN (Dissolved Inorganic Nitrogen). Typically DIN as nitrate is physically removed in wetlands to atmospheric N₂ though the denitrification transformation process.

2. The panel recommended a change in direction from promotion of the reaction BDON → RDON, to a direct TN reduction (BDON &/or RDON → N₂). The main change recommended by the Panel would be to focus on mineralizing as much DON as possible to DIN which could then be nitrified (if needed) and denitrified with the certainty that maximal TN reduction would occur. Only conversion of the DIN present initially in C-
43 water was part of the Consultant approach. Thus the innovative Consultant suggestion to focus project design on the reaction BDON \(\rightarrow\) RDON was not supported.

3. Use of floating aquatic vegetation (FAV) for DON removal was a primary recommendation by the Consultant to meet the TMDL for TN with a Natural Treatment System (NTS = wetlands). The concept was that the FAV would convert BDON to RDON. The resulting TMDL would thus require a change in definition where TN would be replaced with BDON + DIN (dissolved inorganic-N, primarily nitrate + ammonia). In essence any RDON in the water would not be counted by the regulators as TN following the Consultant’s concept. Support for the FAV mechanism by the Consultant relied heavily on a single small plot experiment using FAV, EAV and SAV cells in series in two parallel sets. The Panel did not support the Consultant’s redefinition of the TN limit in the TMDL and noted a lack of sufficient good science to support the overall FAV-BDON to RDON concept based on these cells.

4. The various alternative NTS plant communities that would be most effective for the conversion of DON to \(\text{N}_2\) were discussed by the Panel. Five unit processes that hold most promise included: a very shallow (<15 cm) emergent wetland marsh for nitrification, a classic emergent wetland (about 30 to 45 cm deep) for denitrification, a deeper water mixed wetland or slough dominated by a mix of FAV, SAV, and tolerant rooted plants for long hydraulic residence time and conversion of BDON to DIN, an innovative POP (Periphyton-enhanced Oxidative Photodegradation) mixed open water-wetland system with pulsed operation for physical DON degradation, and a final polishing emergent marsh for removal of DIN and algal solids..

5. It was the Panel’s conclusion that there has been little previous effort to develop an emergent wetland NTS to reliably break down RDON or BDON to DIN at the low levels of TN present in the Caloosahatchee River system. The Panel recognizes that this is similar to the uncertainty concerning the FAV reliance recommended by the Consultant. However, the Panel concluded based on best available information that an emergent wetland NTS possibly supported by other ecologically engineered add-ons is most likely to be successful for this application, and certainly the most direct way for the District to meet its TMDL requirement for TN. The Panel noted that existing wetland treatment systems constructed on sandy soils typically have lower DON levels in their effluents than wetlands built on organic soils. This finding indicates that lower DON and TN concentrations are likely to be achieved in the C-43 basin than in other basins (e.g., the Everglades Agricultural Area) in the District that have a prevalence of organic soils. This difference was highlighted by the Panel as an important avenue for further evaluation and testing.

6. The need to reduce DIN to minimal concentrations prior to maximum DON breakdown was recognized by all Panel members. Lowering DIN to N-limiting levels would force microbes in the core DON reduction wetlands to seek their N in DON compounds as the only option for growth. In practice the initial cell(s) would be similar to the recommendations of the District’s Consultant but with more attention to nitrifying the existing ammonia and nitrite present in the Caloosahatchee River. The induction of undesirable \(\text{N}_2\)-fixation (\(\text{N}_2\rightarrow\text{NH}_4\)), probably by blue-green algae, at low DIN levels was considered less of a problem by the Panel than by the Consultant.
7. A revised plan for the C-43 WQTA Test Facility was recommended by the Panel. This plan recommended construction of five 20 to 40-acre Test Cells that would be operated and carefully monitored for water and pollutant mass balances over a period of two or more years. The relatively large size of the Test Cells is based on the need to avoid scale-up issues apparent in smaller test units (such as the ENR Test Cells) and to be able to accurately evaluate issues related to full-scale construction, plant community establishment/maintenance, and performance estimation. Each of the five Test Cells would have a separate unit process as follows:

a. Cell 1 – Nitrification Cell: an emergent wetland cell operated at minimum water depth (<15 cm) to accelerate flow velocity, maximize diffusion of atmospheric oxygen, and optimize nitrification of ammonium to nitrate;

b. Cell 2 – Denitrification Cell: a classic emergent wetland dominated cell with average water depth between 30 and 45 cm, probably dominated by cattails and a variety of subdominant rooted wetland plants;

c. Cell 3 – Slough Cell: a deepwater (>60 cm) wetland dominated by a mix of floating, submerged, and rooted wetland vegetation that would include algae/periphyton, open water, and relatively long hydraulic residence times to allow a greater variety of biological processes to convert DON to DIN;

d. Cell 4 - POP (Periphyton-enhanced Oxidative Photodegradation) Cell: open water over an engineered substrate (limerock or similar), followed by interior deep and shallow areas and receiving pulsed flows to stimulate photo- and fungal degradation of DON;

e. Cell 5 – Polishing Marsh Cell: a final wetland cell dominated by emergent macrophytes (possibly a mix of sawgrass and more desirable native wetland plants) for final removal of suspended solids and algal-fixed DIN prior to final discharge back to C-43.

All of these cells would be tested over a realistic range of hydraulic loading rates and hydraulic residence times to provide adequate data for calibration of an improved model for nitrogen removal in wetland natural treatment systems.

8. Several macrocosm experimental cells should be set up at the same time as the larger Test Cells to evaluate individual biological and physical processes at a scale that can be easily manipulated and replicated. Key processes that may be evaluated at this scale are: the effects of different soil/substrates on C*, plant growth requirements for individual plant species and combinations, effects over a wider range of HLR and HRT, and detailed water chemistry and DON bioavailability occurring in different plant communities. This recommendation is a reduced version of the several kinds of experiments and mesocosms suggested by the Consultant, but essentially serves the same purpose.

9. The Panel recommends continuing development of an affordable bioassay or analytical approach to rapidly assess bioavailable DON. This test might consist of some more standard laboratory algal test and/or a chemical analysis of DON based on sequential washes of chemical reagents of increasing strength but less reactive than the Kjeldahl
metal-catalyzed boiling acid test (e.g., (1) water at pH 7, (2) water at pH 2, (3) 0.1 or 0.3 N sulfuric acid-cold (4) 0.1 or 0.3 N sulfuric acid – hot and (5) sulfuric acid at Kjeldahl strength but cold). The Consultant’s recommended bioassay approach was found by the Panel to be too complex to carry out and hard to interpret.

10. Some kind of NTS rather than conventional drinking water treatment technology was agreed by the Panel as best for the District, primarily based on the large volume or water to be treated (600 – 6,000 MGD), the low concentrations of various N-compounds (~ 1-2 mg/L TN vs. 3-25 mg/L in conventional systems), and the high color in the water (often ~ 90 PCU). This is essentially the same recommendation as that made by the District’s Consultant.
Appendix A – Dr. Alex Horne

Review of Wetlands-Based Nitrogen Removal Techniques for the South Florida Water Management District (June 23, 2010)
REVIEW OF WETLANDS-BASED TOTAL NITROGEN REMOVAL TECHNIQUES FOR THE SOUTH FLORIDA WATER MANAGEMENT DISTRICT

Alex J. Horne
23 June 2010

SUMMARY

The complex chemistry and bioavailability of DON in aquatic ecosystems is not reflected in the TMDL regulations faced by the District because all N-species are lumped as TN. Nonetheless, a lesser level of knowledge may suffice to solve the District’s TMDL concerns. Reports from CH2M and others have made a commendable job of summarizing the old information and creating new knowledge about DON that is an essential step to removing it in Natural Treatment Systems. The focus evolved in the CH2M work was converting a fraction of labile DON (when present in the wet season) in the District’s canals and wetlands to a smaller amount of refractory DON plus some total inorganic nitrogen (where TIN $\rightarrow$ N$_2$). So far this reaction would reduce some TN which is not unexpected since most DON is not bioavailable over the short time periods important for algal blooms. In contrast, the current TMDL assumes all TN is bioavailable. That may be true, at least in part, for the different chemical conditions of the Caloosahatchee Estuary. Experiments made by CH2M also show that the proposed FAV solution will at best reduce TN by 30% and require the use of a new type of floating treatment wetland. Floating wetlands have been used for many years but have a shaky record for pollution control and have not been used for DON removal. Overall reduction in TN in full scale wetlands may be lower than that measured in the small test cells where conditions are easier to impose. The CH2M position is that if wetlands treatment releases only refractory DON the eutrophication problem is resolved. Although true, the concept will be a difficult but not impossible sell to regulators who worry that what is refractory to one alga may be less so to another.

$\Sigma$DON breakdown. The ideal solution is reducing most TN by breaking down refractory DON. This will be an easier sell for regulators than a change in chemical state from labile to refractory DON. There is still some room to pursue the Holy Grail for direct TN reduction in wetlands by testing to see how to speed up the reaction RDON $\rightarrow$ LDON $\rightarrow$ DIN $\rightarrow$ N$_2$. The reaction may involve light and initial TIN scrubbing before FAV which is not part of the current plan for C-43. I recommend that this be followed up before committing all efforts to the floating wetlands concept, good idea though that may eventually prove to be.

UV experiment. A most surprising finding was the lack of effect of UV light on DON breakdown. Although there is no reason to doubt the specific results of the university investigator used by the District, there is reason to double that the short, single experiment covered all the conditions typical of DON in the District’s waters or the Caloosahatchee Estuary. The degradation time of DON-DOC was reported at days to weeks in the Estuary so one would expect some breakdown with intense UV found on sunny days in Florida. Different kinds of UV experiments should be repeated, with a different set of circumstances to determine their applicability. The normal path of breakdown of RDON is likely to involve photo-degradation of
some form so this avenue is worth pursuing in the C-43 experiments as a route to attacking the 70% of DON that apparently resists degradation by FAV or the tested design of an EAV.

**N-limitation in the Caloosahatchee Estuary but not the District’s Waters.** An important point is that the inorganic-N (TIN or DIN) in the CH2M samples never fell to what I consider N-limiting levels (< 150 ug/L). Thus there was never a pressure for the wetland bacteria to break down DON for its N-content. In contrast, phytoplankton in the target area the Caloosahatchee Estuary is (potentially) growth-limited by N as shown in the recent experimental work by Loh. The TIN concentrations also reported by Loh during the estuary growth season are less (mean ~ 70 ug/L) and sometimes very much less (< 5 ug/L) than those I used mentioned above as likely N-limiting in the natural environment (< 150 ug/L) or that were present in the District’s waters. Nonetheless, in the rather turbid waters of the Caloosahatchee Estuary, light may be a more important growth-limiting factor than nutrients and this might be checked before assuming what is the driving force for eutrophication in the estuary. Loh assumes but does not fully prove cycling rates of overall DON-DOC in the Estuary are days to months and more precisely 1-2 weeks. If so then the same processes should be replicable in the C-43 tests. The lesson for the District is that DIN may need reduction to lower levels before RDON degradation with a FAV cell can be contemplated. Use of a good denitrifying EAV cell with ample labile organic-C in the vegetation (e. g. cattails-Typha) as the first cell in the treatment train should be tested in the C-43 pilot work. A TIN goal of < 70 ug/L, mostly as nitrate is suggested. How to convert the large ammonia fraction of the District’s water TIN to nitrate with the time and space available is not clear (but see below). In addition, if the bacteria were breaking down DON for its carbon energy, rather than N, then C-limitation would provide the driver to getting more RDON removed. Bacterial metabolism in wetlands is usually C-limited in the warm season and temperature limited otherwise. Given the rainy season in the summer as in Florida it is not clear to me which season should be C-limited.

**Reduced N concentrations.** There is a surprising (to me) amount of reduced or semi-reduced compounds (ammonia, nitrite & perhaps some DON) in the District’s waters. In contrast water in the Caloosahatchee Estuary is dominated by nitrate, the most oxidized form of N. The TIN in these Florida samples was made up of an unusually high amount of ammonia and nitrite relative to ammonia - a situation I have not tested for N-limitation. In most open waters with reasonably high algal biomass, the oxygen produced by photosynthesis keeps most DIN as nitrate. The large amount of reduced soils in the STA wetlands, canal bottoms and perhaps BOD in the Lake Okeechobee outflow may account for this problem. The every-present humic acids must reduce photosynthetic oxygen production and may be the reason for the persistence of these reduced TIN species. Since I think DIN reduction is needed to spur degradation of RDON in the FAV or other wetland. It is not clear how to increase oxygenated forms of N for such a large scale or at the C-43 site scale. Options are discussed in the response to question Task 3

**The main concern** is thus that the CH2M route leads to FAV by a logical progression that RDON degradation requires darkness (= absence of N₂-fixing algae). The net reduction of ΣDON (= most TN) is so far lowish (max. 30%). My examination of the data now available suggests that the alternative route via photodegradation &/or RDON use at low TIN levels (~70 ug/L) requires light. Both FAV, EAV and SAV may be used to give both light and dark reactions but both conditions needs clarification before the tests at C-43 are carried out.
DETAILED RESPONSES TO SPECIFIC QUESTIONS ASKED BY THE DISTRICT: TASKS 2, Six questions

Task 2.2. While Experts are encouraged to make constructive comments as they see fit, they should address the following questions in accordance with their expertise. This task lists six specific questions that are answered below.

Question # 1 Task 2.2. Are the major conclusions of the Total Nitrogen Reduction Technologies valid and supported by the information presented (see pages 33-35 of the report for summary of conclusions)?

Answer # 1. In part, details are shown for each of the conclusions in Table 1 below. At any one time a few compounds of DON probably dominate but may change as the organisms releasing them follow their annual cycles. Comprehensive testing of removal of DON by wetlands or any other process is thus hampered by detailed knowledge of the ecology of DON.

Table 1. Conclusions from the TN-reduction Technologies & reviewer Horne’s comments.

<table>
<thead>
<tr>
<th>Individual conclusions quoted from the CH2M Findings Memo</th>
<th>Supported by data &amp; analysis?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dissolved organic nitrogen is a dynamic element of estuarine and coastal nitrogen budgets. The preponderance of evidence implicates DON as essential to the nutrient budget of harmful algae blooms in a variety of marine locations, including South Florida waters.</td>
<td>Not shown in this report but may be irrelevant for this TMDL whose assumptions are that it is.</td>
</tr>
<tr>
<td>The bioavailability of DON varies widely, depending on DON sources and the ecology of receiving waters. Conceptually, DON can be divided into recalcitrant, semi-labile, and labile fractions in terms of algal uptake.</td>
<td>Yes, but biodegradation for the N in DON may require TIN to be at limiting levels.</td>
</tr>
<tr>
<td>Semi-labile fractions need conversion by bacteria to become labile to algae. In specific terms, attempts to categorize these fractions simply by molecular weight have shown no general relationships across a variety of surface waters and wastewater sources.</td>
<td>Yes, but some semi-algae like dinoflagellates may use DON directly since they can be heterotrophic.</td>
</tr>
<tr>
<td>Assessing treatment efficacy of DON will require insight into bioavailability of DON within treatment system effluents.</td>
<td>Yes</td>
</tr>
<tr>
<td>Traditional methods of DON analysis entail subtraction of ammonia, nitrate, and nitrite from total nitrogen. TN treatment performance analysis is a necessary element of DON analysis for treatment systems, but it is not sufficient to achieve treatment goals.</td>
<td>Yes</td>
</tr>
<tr>
<td>There will always be a ( k_{NC} ) value below which a given natural treatment system cannot remove nitrogen.</td>
<td>Yes</td>
</tr>
<tr>
<td>Because the ( k_{NC} ) value for ammonia, nitrate, and nitrite is very close to zero, ( k_{NC} ) …</td>
<td>No, these values not well known but not relevant here</td>
</tr>
<tr>
<td>… ( k_{NC} ) is comprised almost entirely of DON.</td>
<td>Yes, ( \Sigma TN \sim DON ) so ( k_{NC} ) for DIN less relevant for Florida</td>
</tr>
<tr>
<td>Statement</td>
<td>Recommendation</td>
</tr>
<tr>
<td>--------------------------------------------------------------------------</td>
<td>----------------</td>
</tr>
<tr>
<td>To the extent that constituents of kNC* are bioavailable to algae in receiving waters, treatment efficacy is compromised.</td>
<td>Yes</td>
</tr>
<tr>
<td>Recalcitrant kNC* is not an issue.</td>
<td>Perhaps, but regulators will disagree</td>
</tr>
<tr>
<td>The kNC* “black box” will need to be opened to understand how treatment affects the split between labile and non-labile fractions.</td>
<td>Yes</td>
</tr>
<tr>
<td>Additional analytical methods that appear at this time to be obligatory for monitoring the C-43 project are a combination of EIS-MS and bioavailability assays of treated effluent that emulate receiving water conditions.</td>
<td>Maybe. I need to see the bioassay data expressed in the conventional way rather than the (to me) inappropriate probability graphs</td>
</tr>
<tr>
<td>DON is comprised of hundreds of compounds that EISMS divides into compound-specific spectra. Spectra change with biological treatment or utilization of DON compounds. Association of these spectra with bioavailability or lack thereof may allow tuning of treatment diagram to produce the lowest bioavailable kNC* effluent TN.</td>
<td>Yes, this would be a good way to proceed in any upcoming tests at C-43</td>
</tr>
<tr>
<td>FAV systems appear to be the best candidate NTS technology for DON removal, followed by EAV. This conclusion has emerged from the study of five different wetland databases that contain sufficient TN data to infer DON treatment.</td>
<td>No or maybe. Experiment used was in series so only first cell data is valid. Both FAV (better) and EAV (about half as good in one test) may work</td>
</tr>
<tr>
<td>The key to DON removal is to limit light and maximize bacteria biomass.</td>
<td>No or partially. Only about 1/3 of DON is removed by bacterial degradation. Removal of the other 2/3 may need light &amp; no bacteria. Reduction of DON to &lt; 70 ug/L as NO3 may be the key.</td>
</tr>
<tr>
<td>Systems with a significant algal component, such as PSTA basins or algae dominated lagoons, clearly are not effective for DON removal and can increase DON (and TN) in some instances.</td>
<td>No-maybe. Experiment used was in series so only first cell data is valid. Both FAV (better) and EAV (about half as good) may work</td>
</tr>
<tr>
<td>There is additional information in the scientific and engineering literature that SAT may be effective at DON removal. Most evidence is indirect. The SAT literature typically reports removal of DOM or DOC. Because DON is an element of DOM and is associated with DOC, significant DON removal can reasonably be inferred by substantial DOM or DOC removal.</td>
<td>Yes, but it must be slow due to percolation rates &amp; clogging in the soil. Bank filtration has substantial drawbacks in general although specific sites may work well in Germany.</td>
</tr>
<tr>
<td>In the C-43 project, SAT is recommended as a demonstration element of the large treatment system. It also motivates investigation of the Citrus Grove Filter System as a BMP to be applied to existing agricultural irrigation infrastructure.</td>
<td>No, SAT is not an obvious candidate except for small volumes</td>
</tr>
<tr>
<td>The recommended conceptual NTS process diagram starts with an FAV cell. Most DON removal will occur in the FAV cell.</td>
<td>No-maybe. Experiment used was in series so only first cell data is valid. Both FAV (better) and</td>
</tr>
<tr>
<td>Statement</td>
<td>Conclusion</td>
</tr>
<tr>
<td>--------------------------------------------------------------------------</td>
<td>---------------------------------------------------------------------------</td>
</tr>
<tr>
<td>EAV (about half as good) may work. To make the FAV cell remove RDON an initial nitrification-denitrification EAV is more suitable as # 1 cell.</td>
<td></td>
</tr>
<tr>
<td>There will also be removal of BOD, TSS, and phosphorus in the FAV cell.</td>
<td>Not much, since there is nowhere to store TSS &amp; TP in the FAV unless harvested</td>
</tr>
<tr>
<td>The design intent with FAV cells is to create floating tussocks of native species.</td>
<td>Yes, good idea but needs testing for scale &amp; longevity</td>
</tr>
<tr>
<td>An EAV cell follows the FAV cell system primarily to polish BOD and TSS from the FAV cell, and secondarily for limited removal of phosphorus and DON.</td>
<td>No, The EAV cells main purpose should be denitrification of DIN produced by DON breakdown</td>
</tr>
<tr>
<td>The EAV cell will be followed by SAV cells to oxygenate effluent.</td>
<td>OK for day but will reverse at night</td>
</tr>
<tr>
<td>A demonstration SAT cell will complete the process diagram.</td>
<td>I would not do this. Soil percolation is just too slow for the volume of water</td>
</tr>
<tr>
<td>Of the area available for treatment, approximately 75 percent will be EAV and FAV cells, 10 percent will be SAT demonstration cells, 10 percent SAV cells for aeration of effluent prior to discharge, and 5 percent will be devoted to the pump station and pilot systems.</td>
<td>Yes or maybe, The role of light needs consideration before FAV is taken too far. Yes for aeration but maybe before EAV as well? No for SAT.</td>
</tr>
<tr>
<td>Conventional treatment of Caloosahatchee River water cannot be recommended at this time given questions of efficacy and high costs.</td>
<td>Agreed, the volumes are just too high for drinking water methods &amp; the need may be questionable.</td>
</tr>
<tr>
<td>The NTS is a superior choice based on cost and an equivalent choice based on efficacy.</td>
<td>Probably but 30% removal at best sometimes is not really high enough for TN reduction. But these are early days and modification of the NTS for DON removal should improve performance</td>
</tr>
</tbody>
</table>

In particular, the choice for main testing at the C-43 site rests on an in-series wetland test cell experiment (as distinct from an in-parallel set of cells) as far as I can tell from the flow diagram presented. Thus only the first cells in the two trains (FAV and EAV) were real tests of bioavailable DON. My conclusion rests on the fact that any bioavailable DON would be removed in the first cells regardless of type, leaving only refractory DON to be processed by the succeeding four wetland types.

While the in-series test does represent the proposed full NTS treatment train it lacks the ability to pick out the DON removal capacity of each kind of wetland, especially SUV and PSTA. Nonetheless, the experiment does clearly show the superiority of the FAV over the EAV under some conditions. I cannot imagine PSTA working at the scale required unless it
was a POP cell (see later). The fact that no wetland worked at some times of year (dry season) also means that picking a clear favorite wetland type may be premature.

*Question # 2 Task 2.2. Did the assays (salinity release, photolysis, bioavailability) presented in the Organic Nitrogen Methodology Screening Analysis adequately quantify the fraction of the DON pool that could become available to bacteria and algae?*

*Answer # 2. I think so be I am not sure. I would have expected a conventional bioassay result presentation. It appears from Exhibit # 14 that there were 200% increases in chlorophyll in the wet season with most site and smaller increases in the dry season. However, no control data was shown and linking the individual data points from all the sites as if they were a continuum on the x-axis seems non standard practice. Although this probability graphs used for Exhibit 14 is appropriate for some kinds of water treatment in small, uniform concrete tanks, is seems unwieldy and inappropriate for this wide area of wetlands. I would like to see a conventional bar graphs of each station expressed as a percent of control. Nonetheless, within the limits of the experiments and tests, the fraction of the DON that was potentially bioavailable for algae and bacteria was quantified accurately. However, the actual amount of measured stimulation from any labile DON was not clear to me. My concerns are shown below.*

*Answer # 2a. Unnecessarily short duration of the tests: TN/DON reduction. All of the tests were fairly short in duration; the main bioassay tests were 120 hours (5 days) and the UV experiment was only 8 hours (although under ideal conditions). While it is true that a water residence time of ~ 1-2 weeks is typical for many treatment wetlands, the experimental times seem a bit short to measure potential changes. In the early stages of method development it might take 20-30 days for indirect DON removal to show up. Indirect removal would meet the TMDL goals. If even slow indirect removal could be shown, then modifications to speed up the process could be examined in the C-43 pilot test. Indirect reduction of the TN via labile DON (LDON) to refractory DON (RDON) + DIN (removable rapidly in the emergent vegetation wetlands) was discussed in the CH2M report but not well emphasized and has a maximum of 30%. That may be why the District has concerns. The process of LDON to RDON + DIN was stated in the CH2M report as due to hydrolysis. There may well be methods of speeding up hydrolysis in many kinds of wetlands which would give a wider range than just the floating wetlands proposed (with an emergent second cell to remove TIN).*

*Answer # 2b. Understanding the algal-bacterial bioassays. As far as I could tell there were no bacterial growth assays. I would expect to see some parameter such as ATP or similar living biomass measure monitored under dark conditions for bacteria.*

*Answer # 2c. Use of wetlands-derived DON for substantial growth in the target estuarine and marine algae. The stimulation of algal growth by DON alone was first mooted by Professor Pearsall in the English Lake District in the 1920s, was similarly examined by myself in the same lakes in the 1960s with regard to blue-green algae and N fixation. DON has been a subject of great concern since. There are two views; one is that, at least in fresh waters, there is no need to invoke DON to explain the seasonal wax and wane of algal populations. Using Occam’s Razor the science ends there. The second is that the dissolved organic carbon fraction (CON that necessarily includes DON) stimulates growth*
indirectly possibly via chelating of toxic or bioavailable metals. Here I exclude urea which is not a common excretory product in aquatic ecosystems since there is no need for its water-conserving function in terrestrial organisms. Urea is rapidly converted to ammonia in soil.

The uptake of some $^{15}$N labeled DON into biomass in algae described in the literature presented in the CH2M reports does not give me a firm understanding that this uptake is either ecologically important or real since true axenic (bacteria-free) cultures are very hard to grow at field rates and field samples contain bacteria that can convert the very bioavailable amino acids and urea used in tests to DIN.

In the open blue-water ocean DIN can be so low that other methods such as N$_2$-fixation become important and I can believe that urea at least can be used by phytoplankton. This still leaves the source of the urea to be found so far from the shore. Indirect DIN production via bacteria decomposition still is the more likely method and has not been fully studied.

Finally, estuarine and near-shore algae, may well be stimulated by DON or an associated compound. Dinoflagellates are known to be heterotrophic and thus some species can take up both dissolved and particulate DON along with other nutrients. Why they would need the additional-N is water with reasonably high TIN is difficult to see from a simple element consideration but may be a more efficient way to acquire some amino acids that synthesizing them themselves. Or it may be the carbon, metals or chelation potential that is the key effect in stimulation.

The net result of these uncertainties is that the actual concentration of DON that should be part of the TMDL goal for TN is not well established scientifically. Thus the target of TN and DON for the District is hard to gauge using wetlands or any other technology. The concern is critical for wetlands technology since most effective large-scale wetlands produce a DON in the 0.5 mg/L range. In my experience getting lower concentrations is not easy but also has not been tested with the unit process wetlands that are used for other pollutants.

Specific comments on the Organic Nitrogen Methodology Screening Analysis

The photolysis experiment was too short for wetlands typical water residence time but did show clearly that little DON was broken down in 8 hours. However, other research on some DOC-DON compounds, particularly humic substances, indicates that UV light is a major factor in their breakdown. Turnover time of DON-DOC in the Caloosahatchee Estuary was given as less than a fortnight. The level of dissolved oxygen (DO) and temperature may be important in DOC-DON breakdown. Since these may be high in the POP and open water cells of unit process NTS wetlands, this question is still not resolved.

The salinity release did seem to show applicable results. Nevertheless, there seems little reason to think the high cation water would cause rapid breakdown of DON. However, such water does normally cause precipitation of colloidal or micro-particles which could reduce DON if a brackish water wetland were considered in the NTS spectrum.

The bioavailability test was obscured by its graphical presentation and text. I would prefer the conventional presentation (see above).

Question # 3, Task 2.2. Are seasonal shifts in DON bioavailability/recalcitrance supported by the chemical and biological evidence presented?
Answer #3. Yes, the van Krevelen diagrams of summer and winter DON compound classes and the same diagrams of the bioassay convince me that the seasonal shifts are true. Further support comes from the bulk analysis of the DON in summer and winter at the many sites within and outside the STAs.

*Question #4. Task 2.2. Is the preliminary surrogate method for determination of biologically available DON reasonable and supported by the evidence presented?*

Answer #4. No. This is a very important question and much may depend on it. I am not certain that the bioassay method is robust enough to depend on. I had difficulty in understanding the bioassays for algal growth based primarily on chlorophyll (+ phaeophytin). As far as I could tell from the highly derived results presentation there was more growth in the summer water with its higher LDON. It may be the presentation that caused my problems. As stated earlier, in my experience, algal bioassays are usually presented in either a simple bar chart with before, control, and + nutrient(s) and/or a chart of the same three tests over about 10-14 days with time as the x-axis. Without this base data it is impossible for a reviewer to determine what actually happened. Since this test is proposed as a surrogate for LDON (or the DIN from it) it is important that the test is on firm ground. It may merely be that there is a need for more explanation and basic data in the report but the fact that the report also stated in the text that there was not much difference in growth over time.

*Question #5. Are the conclusions of the Findings Memorandum supported by the data and its analysis?*

Answer #5. Yes, these conclusions are shown in detail in Table 2 below.

<table>
<thead>
<tr>
<th>Conclusion from Findings Memo (quotation)</th>
<th>Supported by data &amp; analysis?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preliminary Characterization data shows that nearly all nitrogen in these surface waters is organic nitrogen</td>
<td>Yes</td>
</tr>
<tr>
<td>Preliminary Characterization data shows that nearly all the organic nitrogen DON.</td>
<td>Yes</td>
</tr>
<tr>
<td>DIN and NH3 are an order of magnitude lower than the DON, and may therefore be negligible in many circumstances when calculating TON and DON.</td>
<td>Yes</td>
</tr>
<tr>
<td>These data are consistent with data extracted from DBHYDRO and formerly presented in the Total Nitrogen Technologies Review Report.7</td>
<td>Yes</td>
</tr>
</tbody>
</table>

*Question #6. Are the conclusions (see page 15) of the Parameter Plan supported by the data and its analysis?*

Answer #6. See Table 3 below for details.
Table 3. Conclusions from the Parameter Plan and comments by the reviewer.

<table>
<thead>
<tr>
<th>Conclusion from Findings Memo</th>
<th>Supported by data &amp; analysis?</th>
</tr>
</thead>
<tbody>
<tr>
<td>This parameter plan is derived directly from the Organic Methodology Screening Analysis.</td>
<td>Yes</td>
</tr>
<tr>
<td>It is based on the observation that there is an apparently reproducible pattern of change in compound-level analyses as dissolved organic nitrogen is transformed from bioavailable to recalcitrant forms. This observation forms the basis of the parameter plan.</td>
<td>Yes</td>
</tr>
<tr>
<td>It is based on the observation that there is an apparently reproducible pattern of change both total pigment changes in bioavailability assays as dissolved organic nitrogen is transformed from bioavailable to recalcitrant forms. This observation forms the basis of the parameter plan.</td>
<td>Not clear, may be a presentation that I find not the best for algal bioassays. Sites indicated as a continuum but that cannot be so.</td>
</tr>
<tr>
<td>The pigment changes in bioavailability assays are proposed as a method to track DON transformation with the compound-level analyses used as a quality control method to ensure that pigment change behavior reflects transformation mechanisms.</td>
<td>Maybe – need a more conventional bioassay presentation for the many wetland site</td>
</tr>
</tbody>
</table>

Task 2.3 Evaluate the scientific validity of and identify advantages and disadvantages of the proposed approach to N removal and transformation in light of the low level of TN in the water being treated.

The first response to this question in Task 2.3 is to examine the data collected from the dry and wet seasons, April-May and June-July 2009. Many sites were collected and many form of N, C and P analyzed. A summary Table 4 is shown below.

Table 4. Summary of selected N and P compounds collected in the District’s area in 2009. All values are means of two separate collections made roughly a month apart. All values are in ug/L for the element, e.g. NO3-N. TKN was measured in the unfiltered sample but was similar to the filtered sample which is an indication that most TKN, other than ammonia, is soluble. Data from Final Findings Memo, Appendix D: Water quality measurements.

<table>
<thead>
<tr>
<th>Season</th>
<th>TKN</th>
<th>DON</th>
<th>DIN</th>
<th>NH4</th>
<th>NO3</th>
<th>NO2</th>
<th>TP</th>
<th>O-PO4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>April</td>
<td>2,500</td>
<td>2,200</td>
<td>190</td>
<td>98</td>
<td>48</td>
<td>40</td>
<td>48</td>
<td>16</td>
</tr>
<tr>
<td>May</td>
<td>2,300</td>
<td>2,000</td>
<td>294</td>
<td>157</td>
<td>97</td>
<td>40</td>
<td>50</td>
<td>23</td>
</tr>
<tr>
<td>MEAN</td>
<td>2,400</td>
<td>2,100</td>
<td>242</td>
<td>128</td>
<td>73</td>
<td>40</td>
<td>49</td>
<td>20</td>
</tr>
<tr>
<td>Wet</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>June</td>
<td>2,200</td>
<td>1,900</td>
<td>200</td>
<td>120</td>
<td>60</td>
<td>25</td>
<td>80</td>
<td>40</td>
</tr>
<tr>
<td>July</td>
<td>1,800</td>
<td>2,100</td>
<td>210</td>
<td>120</td>
<td>64</td>
<td>22</td>
<td>80</td>
<td>40</td>
</tr>
<tr>
<td>MEAN</td>
<td>2,100</td>
<td>1,850</td>
<td>205</td>
<td>120</td>
<td>62</td>
<td>23</td>
<td>80</td>
<td>40</td>
</tr>
</tbody>
</table>
**Question Task 2.3 asks “...in light of the low level of TN in the water being treated.”**

**Answer.** TN in the water is not really low high for surface waters because the TKN was over 2,000 ug/L. Assuming that only low TIN was meant by the question, the answer is that TIN (DIN) is probably high enough for natural waters that N-limitation probably did not occur. Depending on the amount of algae present, I generally assume that N-limitation begins about 150 ug/L (TIN-DIN). The Florida wetlands and canals showed a lowest DIN value of 190 ug/L and generally averaged about 200-290 ug/L (Table 4). So there would be no real pressure for the phytoplankton to use DON in these systems. There is probably a light limitation for algal growth in these Florida waters due to color (humic substances) and some turbidity, especially in the canals. Thus there is more TIN than needed to grow lots of algae, once the algae can get light.

Included in TIN are ammonia, nitrate and nitrite but the District’s waters had an unusual balance between the three. As mentioned earlier, normally in surface waters nitrate dominates since it is by far the most stable form and there is energy to be gained when microbes oxidize ammonia and nitrite to nitrate. In both wet and dry seasons ammonia dominated and nitrite was unusually high. The conclusion is that most of these waters were reducing or have low dissolved oxygen somewhere in the water column or sediments. Under reducing conditions, nitrate is used for respiration producing ammonia and nitrite as an intermediate. The other explanation is that there is a nitrogen shortage and ammonia is being cycled by fish and other organisms. This latter explanation is unlikely since with N-limitation, ammonia concentrations would be more like 5-40 ug/L not the > 200 ug/L observed.

**Uncertainty of removal of TIN in the EAV cell proposed to follow the FAV cell**

The removal of the TIN component was taken for granted in the CH2M reports and assumed to occur via denitrification in the EAV cells where labile DON $\rightarrow$ DIN $\rightarrow$ N$_2$ is the goal. However, TIN dominated by ammonia will not be easy to denitrify since nitrate is the normal substrate for denitrification. The bacteria that carry out denitrification use the oxygen in the nitrate as a terminal electron receptor so respire the oxygen and cannot do so with ammonia which contains no oxygen. There is one other denitrification reaction, the AMMONOX process but this still requires one or other oxidized form of nitrogen. The importance AMMONOX in nature is still uncertain and seems to be restricted to special conditions albeit in both high and low concentrations of ammonia.

**Improvement of removal of TIN**

The reports supplied by the District and other data from the Everglades indicate that overall and eventually wetlands reduce TIN to very low levels (~ 10 ug/L). The process may take several months. For the District there will only be a week or two for denitrification. Denitrification will require most ammonia to be oxidized rapidly to nitrate. Using a wetland as a nitrification site is not efficient since, as a rough guide it takes 10 times the area to convert inflowing ammonia $\rightarrow$ nitrate $\rightarrow$ N$_2$ gas than for nitrate $\rightarrow$ N$_2$. Depending on the rate of the nitrification the volume needed would require a prohibitively large area for the District.
Task 2.4 Recommend that the District accept, reject, or revise the Consultant’s proposed approach.

- I recommend that the District accept the main thrust of the Consultant’s proposed approach which I define as the use of NTS wetlands for TN-DON reduction.
- I recommend that the District reject the Consultant’s concept of only reducing any (labile) LDOC in the wet season. This approach will only remove 15-30% of the TN and more reduction is needed.
- A specific recommendation is that degradation of refractory RDON be further pursued. One promising method would be to reduce the TIN to limiting levels before the RDON removal by FAV (no light) or some other system (with light) is used. N-limitation may stress the bacteria so that they decompose RDON more rapidly as the apparently do in the definitely N-limited Caloosahatchee Estuary.
- A second specific recommendation is to re-examine the Consultant’s rejection of light as a key factor in degradation of RDON (and thus main role of FAV) despite the negative results of the 8 hour UV test.

TASK 3 RECOMMEND FEASIBLE APPROACHES TO TN REMOVAL AND EVALUATE PROPOSED CONCEPTUAL DESIGN

3.1 Each Panel Member shall identify and recommend feasible approaches to TN removal using wetland-based technologies in light of the low TN levels of at the study site and elsewhere in the District. At a minimum, each Panel Member should comment on the feasibility of four or five approaches from the literature just to be certain that every possibility is on the table. Each Panel Member shall work with the Panel Chairperson to provide final comments, recommendations, and consensus design recommendations.

The levels of TN present in the Florida wetlands are not really low in terms of most of the World’s natural waters. An average of just over 2,000 ug/L TN with presumably mostly bioavailable TIN > 200 ug/L (Table 4) is much lower than is found in wastewaters but perhaps 4 times what one might have expected in the original Everglades (see my responses to Task 2 questions). The only importance of this distinction here is that the bacteria are not being forced into metabolism of RDON by N-limitation. I do not know the exact value of the enzyme half saturation constant (Monod or Ks) for the three DIN compounds for the bacteria present in the Florida wetlands. Given their small size I expect that is will be lower than the similar Ks values for algae. In that case at least, ammonia at concentrations averaging about 120 ug/L in both wet and dry seasons (range ~100 to 160 ug/L, Table 4) should provide an adequate N supply. The lower concentrations of nitrate (mean ~ 60-70 ug/L) and nitrite (mean 20-40 ug/L) should also be a reasonable N-source. Many algae, and likely bacteria, can use TIN levels down to 10 ug/L for each compound, although the rate of uptake may be slower than at saturation levels. I note that in the Caloosahatchee Estuary DIN is either low or very low except for occasional winter spikes.
Bearing in mind that N-limitation may not be a major driving force, a major recommendation from my review is that more attention be paid in the upcoming tests at C-43 to overall reduction of TN in the new wetlands outflow rather than removing RDON from the TN requirement. Considered in a somewhat different way from the CH2M report, DON removal can come from two processes which are indirect in the wetland. Basically, there is a further need for a process that will rapidly (days to 2 weeks) break down RDON to TIN. The alternatives are:

- RDON\(_1\)→LDON→RDON\(_2\) + DIN where DIN→N\(_2\) (where RDON\(_1\) and 2 are different DONs and RDON\(_2\) is small relative to RDON\(_1\))
- RDON\(_1\)→RDON\(_2\) + DIN where DIN→N\(_2\)

In both cases it is assumed that bacterial denitrification will convert DIN to N\(_2\) gas which will be vented to the atmosphere

\textbf{RDON\(_1\)→LDON→DIN→N\(_2\) + RDON\(_2\).} It is known from the studies carried out by CH2M that dry season wetlands in Florida release only RDON but that in the wet season LDON was carried in presumably from surface runoff &/or in-wetland plant degradation and released by the wetlands. The wetlands are primarily a mixture of emergent macrophytes, open water, and some submerged macrophytes. In general the wetlands were not designed from scratch but are flooded land with old ditches and other depressions that give less than desirable hydrologic flows. Thus a variety of desirable and undesirable aquatic habitats are present. Unfortunately, at present we do not know which habitat is best to break down RDON quickly.

What controls the rate of the reaction LDON→RDON + DIN? It is presumably the numbers and activity of a small fraction of the total amount of bacteria present in the wetlands. The guiding principle in Ecological Engineering that is the basis of the design of treatment wetlands suggests that making life comfortable for these particular bacteria that break down RDON should be the basis of the design for TN removal via LDON to TIN to N\(_2\). Denitrification of TIN is the other key element but a lot of work is available on how to make that process work faster.

Floating wetlands do not seem to have any unique ability to promote either the bacteria needed for LDON→RDON + TIN or denitrification. The CH2M report recognizes part of this concern and provides a denitrification wetland to follow the floating wetlands.

What is needed to increase TN (RDON) breakdown? The 8 factors controlling the rate of reaction LDON→RDON + TIN are:

- **Substrate concentration: LDON or RDON feed in.** Concentration of the LBOD or RDON fractions are not controllable except in gross amounts of water added to the wetland. This volume will probably be set by the TMDL needs rather than ideal bio-reaction kinetics.
- **Product Release: RDON & TIN flow out.** Removal of the reaction products to prevent mass feedback (hard to control but pulse flow may be a solution combined with several unit process wetlands)
- **Bacterial food-energy supply.** Availability of other labile fractions, primarily organic matter from decaying leaves and stems of the wetlands plants. This energy powers the reactions and thus the more other labile material there is the better. Note that this labile
carbon is not soluble but is present as cellulose in the dead detritus so does not necessarily add to DOC and DON.

- **Co-factors or co-metabolism.** Many bacteria carry out several reactions and can be stimulated by one factor which effectively increases the rate of reaction of several reactions, sometimes including the desired one. In this case a general hydrolysis catalyst or co-factor may improve performance for RDON → LDON.

- **Temperature.** An optimum temperature for the reaction (LDON→RDON + DIN). Normally higher temperatures are better and the simple fact that wet season temperatures at the root-litter zone will be lower in the wet season may explain the measured results for percentages of LDON and RDON.

- **Micro-hydraulics.** Flux rate of LDON to the site of the bacteria reduction. In the final stage the flux is via diffusion but higher overall rates can occur if advective flow of the contaminant to the site is improved by better mixing (natural or forced). The kinetics of LDON→RDON + DIN are not known and difficult to determine from the data presented (at least on this review level).

- **Predation-grazing.** Control of predation or other biological losses in real open wetlands is rarely considered. However, grazing on the target bacteria that carry out the reaction LDON→RDON + DIN would lower the rate of reaction. Since the site of the wanted bacteria is not known, reduction of their predators is not clear. However, assuming they live in an at least partially oxygenated site, the slime on the litter and plant stems is a likely place. Predation on the slimes is primary by grazers such as snails and shrimps. One snail can clear a large area in one day. Grazing be controlled by manipulating the kinds and numbers of fish or birds. In a treatment wetland such ecosystem such manipulations are possible.

- **Oxygen-redox potential.** The reaction LDON → RDON + TIN is stated with good support in the CH2M report to be a hydrolysis with is also an oxidation. In contrast denitrification TIN → N₂ is a reduction. A priori, this suggests that different wetlands are needed for the two sub-reactions as suggested by CH2M. Will the proposed floating wetland be the best site for LDON → RDON + TIN? Ignoring the N₂-fixation argument made by CH2M for the present, bacterial slimes on floating roots will require oxygen at the root hairs. My extensive research on the redox state of roots and root hairs indicates that the immediate exterior of any wetland plant root is also likely to be oxidizing at least some of the time. However, other studies show that the amount of oxygen leaking from wetland plant roots is small ~ 2% of total oxygen production. The strategy of floating plants is to lower oxygen in the water so as to release nutrients from anoxic sediments so the bulk water under the floating wetlands is likely to be anoxic which would not favor hydrolysis-oxidation. CH2M recognize this concern and suggest that aeration may be needed at least sometimes.

### POSSIBLE ALTERNATIVE WETLANDS DESIGNS TO REDUCE TN IN THE OUTFLOW

Available wetlands of possible utility for dissolved TN removal, broadly defined, are listed in Table 5.
Table 5. Types of wetland and key features that might be used to reduce soluble TN.
Almost all system can be aerated or given pulsed water flow or both. Internal channeling is a problem with almost all these wetlands except the turf scrubber. Use for TN-DON see Tables 2-3.

<table>
<thead>
<tr>
<th>Name</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emergent aquatic veg. EAV-tall</td>
<td>Most common treatment type, plants to 2 m+, typically cattail (<em>Typha</em>), bulrush (<em>Scirpus</em>), Reed bur (<em>Phragmites</em>). Easy to maintain in almost mono-specific stands. <em>Typha</em> &amp; to some extent <em>Scirpus</em> support denitrification. Algae generally shaded out.</td>
</tr>
<tr>
<td>Emergent aquatic veg. EAV-short</td>
<td>Rarely used for treatment due to overgrowth but can be maintained by mowing. Tends to have good neritic algal growth so would release PON unless filtered by a EAV-tall. Depending on the plants should support denitrification (soft plants better than reeds or rushes).</td>
</tr>
<tr>
<td>Floating Aquatic veg. FAV-large</td>
<td>Large plants (water hyacinth, <em>Eichhornia</em>, water cabbage, <em>Pistia</em>). Can be harvested due to size, Not clearly proven to remove nutrients very well at lower concentrations compared with other methods. Needs aeration or rapid water flow to avoid anoxia. Likely to be difficult to maintain in clean water.</td>
</tr>
<tr>
<td>Floating Aquatic veg. FAV-small</td>
<td>Small plants (duckweed, <em>Lemna</em>; the large floating fern <em>Salvinia</em> &amp; the small water fern, <em>Azolla</em>). Not clearly proven to remove nutrients very well at lower concentrations compared with other methods. Hard to maintain above 1 acre due to wind-blown pileups. Needs aeration or rapid water flow to avoid anoxia. Likely to be difficult to maintain in clean water. <em>Azolla</em> hosts a N2-fixing blue-green algae so is to be avoided for the FWMD TN reduction wetlands.</td>
</tr>
<tr>
<td>Submerged Aquatic veg. SAV</td>
<td>Little used deliberately for treatment but is major component in shallow lake biomanipulation. Various leafy pondweeds (<em>Potamogeton</em>). Hydraulic channeling probably largest drawback. Very variable light and DO conditions day/night. Easy to maintain normally if water &gt; 4 ft but die off may occur in winter. (Florida too warm?).</td>
</tr>
<tr>
<td>Pond + riparian fringe</td>
<td>Fringe riparian wetland around a pond is a common method but is inefficient for treatment due to hydraulic short-circuiting through the open water. Does combine oxic &amp; anoxic zones. Algae (PON) grow in pond so EAV filter needed as end cell.</td>
</tr>
<tr>
<td>PSTA</td>
<td>Periphyton growing on short EAV tested in Florida. Difficult to maintain but removes low levels of TP. For TN, N2-fixation likely (<em>Nostoc</em>). Algae likely to flake off so filtering EAV needed as end cell.</td>
</tr>
<tr>
<td>Turf scrubber</td>
<td>Plastic grass in a channel with a similar method to PASTA but much higher flow rate. Periphyton on mat must be squeegeed off periodically. Not likely to work for DON breakdown due to lack of bacteria and conditions to favor them.</td>
</tr>
</tbody>
</table>

**Ranking wetlands for TN-DON removal potential**

Using the data from the CH2M report on TN-reduction technologies a form of ranking can be made if it is assumed that a main mechanism for TN breakdown is DOC $\rightarrow$ TIN $\rightarrow$ N$_2$ gas. The rankings are shown in Table 6. Surprisingly, the CH2M recommended FAV does not rank highly on its own due to a lack of processing TIN. However, as noted and recommended in the CH2M report, the combination of FAV + EAV ranks highest Table 6. Also ranking highest was SAV + EAV, although the CH2M work indicated that this SAV the DON conversion percentage
was not as high as the peak FAV removal. However, there were less clear tests made for SAV so the preliminary rankings are the same.

Table 6. Ranking of likelihood of removal of TIN via TON in various proposed wetlands. From Exhibit 20 in the CH2M report on TN-reduction technologies. Since the tests were run in two series with floating wetlands at the head of one train and emergent vegetation at the head of the other, there may have been no LDON left to remove in the SAV, PSTA and other emergent cells. The main removal was in cell #1 for each train. Value shown are for high and low flows and their mean.

<table>
<thead>
<tr>
<th>Wetland</th>
<th>Conversion of RDON to LDON</th>
<th>TIN removal</th>
<th>Average 2 = worst 1 = best</th>
<th>Overall Rank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single unit cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Floating normal</td>
<td>47,12 x = 30 (1)</td>
<td>2</td>
<td>1.5</td>
<td>3</td>
</tr>
<tr>
<td>Dense tall emergent</td>
<td>24, 1.5 x = 13 (1.5)</td>
<td>1</td>
<td>1.25</td>
<td>2</td>
</tr>
<tr>
<td>SAV</td>
<td>29 (partial) (1)</td>
<td>1.5</td>
<td>1.25</td>
<td>2</td>
</tr>
<tr>
<td>Σ% removable</td>
<td>47, 22 x = 37 (1)</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Combinations</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FAV + EAV</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>FAV + SAV</td>
<td>1</td>
<td>2</td>
<td>1.5</td>
<td>3</td>
</tr>
<tr>
<td>EAV + SAV</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Is it possible to improve the design using alternatives?

Normally in Ecological Engineering a process is found to occur in nature and then tests are made to improve the performance. The CH2M work demonstrated some labile DON breakdown and nicely showed why this occurred (hydrolysis). So far no effort has been spent on taking this data and upgrading the method. Based on the 8 controlling factors listed above, what alternative designs are possible? The alternatives are summarized in the table below (Table 7)

Table 7. Summary and ranking of alternatives for wetlands to reduce overall TN compared with the floating wetlands proposed by CH2M based on the 7 factors controlling the desired reaction LDON → RDON + TIN and then LDON → RDON + TIN. Note that this table does not include direct DON removal which was covered in Table 2. **Lowest score is best.** Key: 1 = advantage, 2 = disadvantage, 0 – neutral or not easily controllable in a treatment wetland. POP = periphyton-enhanced oxidative phyto-degradation wetland (differs from the SFWMD periphyton cells in the algae grow on the bottom concrete or membranes in very shallow water; no other vegetation is permitted).

<table>
<thead>
<tr>
<th>Wetland</th>
<th>LDON in</th>
<th>TIN RDON out</th>
<th>Factor</th>
<th>Temp</th>
<th>hydraulics</th>
<th>grazing</th>
<th>oxygen</th>
<th>Total 12=worst 1=best</th>
<th>Overall Rank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Floating normal</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Floating aerated</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>Dense tall emergent</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>Aerated emergent</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>6</td>
<td>2</td>
</tr>
</tbody>
</table>
### Table 1: Wetland Performance Rankings

<table>
<thead>
<tr>
<th>Wetland Type</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulsed emergent</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Wet-dry emergent</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Turf scrubber</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>POP cell</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Aerated lagoon + riparian fringe</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>Lignin (bulrush)</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>Labile C cattail-grass</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>SAV</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>7</td>
<td>3</td>
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</tbody>
</table>

1. It would be hard to pulse (dry out) floating or SAV wetlands which would suffer damage to the roots. 2. Under normal operation a turf scrubber has such a low WRT that all products are removed rapidly.

The assumptions in Table 7 are:

The wetlands will have a complete cover of the desired vegetation. If aeration ponds are needed for any wetland it is assumed that they will be > 6 feet deep and well stirred mechanically to discourage N₂-fixing blue-green algae. The aerated lagoon is similar to the small ponds provided for various reasons in many unit process treatment wetlands but is also similar to some aeration lagoons used in conventional sewage treatment. The lignin (bulrush) and cellulose (cattail) are EAV wetlands dominated by that genus. Other cellulose rich wetland plants such as aquatic grasses or soft plants could be used but would be hard to manage in a large area without overgrowth of cattails or bulrush. It is also assumed, as in the CH2M plan, that a conventional denitrification emergent plant cell with follow the DON wetland to remove nitrate.

Not surprisingly pulsed flow and wet-dry EAV and the turf scrubber ranked #1 and FAV last at #5. This ranking corresponds to their relative popularity for treatment systems. FAV based on water hyacinth at San Diego grew well but data showed nutrient inflow to have the same concentration as those in the outflow showing zero removal for a treated wastewater system. A similar system located in a greenhouse at Hercules, California was abandoned for lack of performance.

However, improvement in ranking for FAV occurred if it was aerated (Table 7), suggesting this option be used. Aeration was also mentioned in the CH2M reports although no specific recommendation or design options were made.

### Sequences of the final wetland design

The proposed CH2M design is surface water inlet → FAV → EAV → outlet back to surface waters as its main option. The purpose of the EAV is primarily to denitrify any DIN produced by degradation of RDON in FAV cell(s). Based on the partially ambiguous test treatment trains used in the CH2M report, SAV and EAV might also be used as cell # 1. However, since DIN might not be really limiting for bacteria, some further decrease in DIN, especially ammonia might be valuable. To further decrease ammonia it must be nitrified and then denitrified before
the water arrives at the FAV wetland (if that was chosen). Nitrification of ammonia (a highly reduced substance) in wetlands is inefficient since they have anoxic soils by definition. So an alternative sequence might be:

Canal water $\rightarrow$ nitrification cell (wetland?) $\rightarrow$ EAV $\rightarrow$ FAV $\rightarrow$ EAV

The nitrification cell requires air, sand or similar solid substrate for the nitrification bacteria, probably a labile carbon source, and warm temperatures. These conditions do not seem easily translated into large-scale wetlands of the type envisaged for DON-TN reductions. Possibly an oxidation-type water body perhaps with scattered EAV would suffice as the needed passive nitrification.

**DETAILED RESPONSES TO SPECIFIC QUESTIONS ASKED BY THE DISTRICT:**

**TASK 3 Recommend feasible approaches to TN removal and evaluate proposed conceptual design**

3.1 Each Panel Member shall identify and recommend feasible approaches to TN removal using wetland-based technologies in light of the low TN levels of at the study site and elsewhere in the District. At a minimum, each Panel Member should comment on the feasibility of four or five approaches from the literature just to be certain that every possibility is on the table. Each Panel Member shall work with the Panel Chairperson to provide final comments, recommendations, and consensus design recommendations.

**Question Task 3.2 Review the existing proposed conceptual design of the Test Facility in light of the approach suggested by the Consultant and any approaches indentified in Subtask 3.1. Can the recommended approaches be evaluated experimentally using the current design of the Test Facility?**

**Answer.** Much of the Test Facility design concerns details of size, location, use of orange groves while the experiment proceeds, access, road, and power. Much of this is beyond my specialized knowledge but seems to be adequate. Yes, the layout of the wetlands and plans seem to be adequate to select and reject the proposed treatment options at a more realistic size scale.

In terms of the layout of the wetland cells and other experiments these are discussed in detail in the other parts of my report. In summary these are:

- Sequence of wetlands (treatment train). May require a nitrification pond and EAV to give N-limitation prior to the FAV or similar DON reduction wetland.
- Photodegradation of RDON & LDON. Needs to be further considered, either by longer lab experiments with UV or better some kind of shallow POP cell in the C-43 test site.

**Question Task 3.3 Identify and suggest changes to the proposed design of the Test Facility with the goal of providing a sound and robust evaluation of any proposed approach.**

**Answer.** See above. Summary:

- Modified test treatment train(s). Test SAV & FAV first in train
• Some field photodegradation tests. Longer-term in open water cells to get at why photodegradation does not occur. Photodegradation seems so likely a method to degrade RDON.
• Evaluate nitrification de-nitrification of ammonia and nitrite prior to FAV cell to reduce TIN to N-limiting levels to push bacteria to degrade RDON for its N.

**Question Task 3.4** Suggest experimental and/or data collection needs that could be run in parallel to improve the information output of the facility.

**Answer**. See above (summary)

• Repeat laboratory photodegradation tests. Test over more than 8 hours &/or with different water (sites, season?) Again as in 3.3 to get at why photodegradation does not occur.
• Work on how to get inflowing TIN into a nitrate form then reduced down to < 70 ug/L to give a really N-limited system that might improve the 30% maximum performance of the FAV cell in degrading RDON (and maybe improve LDON breakdown).

• **Deliverable 3.0**: Draft report that includes recommendations for feasible approaches to nitrogen removal, an evaluation of the currently proposed design of the Test Facility, recommendations of possible alternate design(s) to optimize project goals and suggestions for any additional experimental or data gathering efforts. Descriptions of alternative approaches as well as recommendations must be supported by reference to literature of known quality, preferably from the peer-reviewed scientific and/or engineering literature.

**REFERENCES** (apart from those supplied by the District)


Horne, A. J. 2003. San Diego Creek Watershed Natural Treatment System Master Plan. Prepared by Geosyntec Consultants, Portland Oregon. This is the formalized unit process wetland first design concepts that were put in to use in various sites since then. March 2003 ~ 200 pp.

Appendix B – Dr. John White

C-43 Water Quality Treatment and Demonstration Project Technical Review (July 29, 2010)
TECHNICAL REVIEW

C-43 WATER QUALITY TREATMENT AND
DEMONSTRATION PROJECT (FINAL)

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July 29, 2010
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Overview

As per "Exhibit A" of the Expert Assistance Scope of Work and the Project Kick-off meeting, the task included reviewing the following four technical documents:

1) Review of Total Nitrogen Reduction Technologies Review

2) Review of Organic Nitrogen Methodology Screening Analysis (Draft)

3) Review of Findings Memorandum (Final)

4) Review of Water Quality Treatment Area Test Facility Parameter Plan

Based on the review of those documents, the following questions were to be considered in accordance with expertise.
I. Are the major conclusions of the Total Nitrogen Reduction Technologies valid and supported by the information presented

Major Conclusions

A. There is a baseline value of DON treatment capability

The North American treatment wetland database (NADB), as mentioned in the report, can only be considered a guide. The systems are highly variable, deal with wide-ranging concentrations and quality of water and don’t require treatment down to the level which is being sought in this system. The same could be said for TP. The NADB has a much lower mean removal rate for TP than is achieved in many of the South Florida Water Management Treatment systems and this is due to the fact that treatment focuses on this particular contaminant while the performance of many different systems are likely looking at a balance in treatment for many water quality parameters, the least which may be DON in most systems. Therefore, this does not mean that the DON treatment can’t be substantial better than this mean, given a system whose primary design goal is to reduce DON. Having said that, what the reasonably achievable level is unknown and there will be some low level concentration that a range of treatment technologies will not be able to breach.

B. No easy method to identify all – bioavailability assay is most useful

The review has done a good job of underscoring the difficulty of identifying the myriad of compounds that comprise DON in water. Further, the bioavailability of several thousand compounds can only be estimated at best. It is this very complex nature of DON which requires a more general assay or measurement tool to determine overall bioavailability to the aquatic ecosystem.

The % removal of bulk DON ranging from 0 – 70 % demonstrates the need to have some compound-specific or compound-class quantification of the DON in order to monitor the treatment efficacy and potential bioavailability of the effluent. However, It is likely that compound-specific/compound class may not be as valuable unless there are a low number of specific compounds which comprise the DON pool, which does not appear to be the case. Since current efforts are in their infancy, an easily replicated bioassay would appear to be needed.
C. FAV systems appear to be best candidate NTS for DON removal followed by EAV

If bacterial degradation of the DON pool is the primary goal with considerable concern over N-fixers, then the selection of FAV does provide the conditions for which this can be maximized. Given the problems with wind as well as water flow, selection of the dominant FAV species is critical, as mentioned in the report. I agree with the statements that water hyacinth has an advantage over some other species due to a vigorous growth rate and root system which could support biofilms dominated by bacteria whilst providing shade to prevent algal N-fixers.

I am not convinced that TSS will be low in this type of system. From my experience with STA-1W – Cell 5 in the early years of treatment when floating and submerged vegetation dominated, sampling within the FAV mats as well as the sediment under these mats contained easily suspended organic material. During wind events, the TSS of the water column was high in these areas which would suggest material, shedding off of the plants and roots, were contributing to this TSS. However, the design proposed does not call for this to be the outflow cell but rather followed by an EAV cell, and those types of systems are well proven for reduction of TSS, provided the hydraulic loading is not overwhelming.

I am unfamiliar with engineered tussock systems and cannot provide any guidance as to whether they would be superior to less engineered SAV system in terms of ease of implementation or treatment effectiveness.

D. SAT may be effective at DON removal, albeit indirect evidence. Test area should be set aside to evaluate this.

The discussion of SAT, especially using the current land modification should be evaluated given the potential for basin-wide adoption of this practice which may reduce the watershed DON load. It is appropriate that this test facility evaluate this potential treatment technology given the presence of the relict and active groves on-site. I understand the concern over costs on building a truly Vertical Flow Treatment System to remove the DON/DOM but I am not sure if this should be ruled out at the mesocosm scale, as this technology may provide a final polishing of the water. If the literature supports some DON/DOM removal, then regardless of cost of a full-scale system, this should be evaluated even on a small scale. Essentially, the SAT is the less engineered version of a true SSF treatment wetland whether the water goes down vertically or through bank infiltration on a more lateral trajectory.
E. FAV cell – EAV cell – SAV cell possible SAT

This proposed design, in terms of the FAV followed by the EAV has merit as mentioned earlier. Having the final cell as an SAV could be problematic in relation to TSS, something that many of these systems create in quantity. The final treatment cell/area will be dependent, likely, on what the physical and chemical characteristics of the water discharged from the treatment system will be required to meet in the future. Will the water discharged from the treatment facility solely need to meet DON and perhaps, P standards? Then an SAV cell would provide the well oxygenated water. The cell will have to be maintained free of FAV, likely though herbicides, which will create TSS and likely DON/DOM as the material decomposes. There was also concern in the report that an open water environment low in nutrients would be colonized by N-fixing algae. Therefore, I am unsure why this would not also be a concern for a final SAV polishing cell.

There is the mention that the water may then pass through a SAT treatment, though the design of the facility may provide an opportunity to test that, but this does not appear to be a definitive, preliminary aspect of the proposed design.

F. Area available for each component

The general breakdown of treatment areas appears to be reasonable. Designing the large-scale system without some data from mesocosm work is somewhat problematic. However, the district does have a successful history of building, monitoring and subsequently modifying demonstration project all while conducting detailed mesocosm manipulative experiments. The only concern here is whether this single sequential design is the best design based on the limited available data. The two day workshop will provided the opportunity to consider several other mesocosm configurations and perhaps even increase the number of mesocosm units to provide for more extensive evaluations. The District is moving into fundamentally uncharted waters on the treatment of TN which is primarily composed of DON and providing the maximum opportunity to evaluate alternatives would be desirable and prudent.

G. Conventional treatment is not good averaging 3 mg N/l, twice the level in the Caloosahatchee River.

The discussion here underscores the problem of using data for conventional treatment which do not focus on the DON removal requirement. Conventional treatment technologies average
3 mg/L because that is satisfactory for their purposes, where overall treatment is for a range of water quality parameters and likely DON is not the major concern. This has a corollary to the work the District has done in the past with TP removal rates. If you consider that the TP concentrations exiting from the Everglades Agricultural Areas ranged from 150 – 280 ppb and compared those values with the literature on P treatment in wetlands, at first glance you would throw up your hands in defeat. This being due to the fact that an average value for TP treatment effluent on a wide number of systems is an order of magnitude higher than these original EAA values. If the level of DON in the Caloosahatchee River water is \( \frac{1}{2} \) what the conventional systems produce, then conventional treatment should not be ruled out as they might perform even greater with a significantly lower input of DON concentration.

The character of the water, as was noted in the report, contains high DOM concentrations. For P, the use of chemical amendments was forsaken for a number of reasons, not the least of which was due to the large amount of chemicals required (as in the example of Alum) to remove a small amount of P as the DOM was removed first. Again, on this I can appreciate the cost considerations, but there may be a technology or a component part of conventional technology which may be adapted here and therefore, should not be ruled out summarily based on historical treatment levels which did not primarily seek to remove DON or cost of implementation at this stage.

**H. Conventional treatment is subjected to the same analytical constraints — Look at compounds and bioavailability — Costs are very high for drinking water technologies**

On this point, I am in agreement, that treating the Caloosahatchee River to drinking water standards and then placing the water back into the Caloosahatchee River for discharge into the river could be expensive. I am not familiar with the details of the several drinking water treatment technologies, but it seems like they should at least be evaluated on their performance in addition to the cost of implementation if for no other reason, than the District can report in the final recommendations that every option was evaluated.

**II. Did the assays (salinity release, photolysis, bioavailability) ONMSA quantify the fraction of the DON pool that could become available to bacteria and algae?**

The salinity assay that investigated the release of ammonium from the DON pool was conducted using a concentrated sea salt and addition by dilution to the required salinity range.
I am unclear as to what the salinity study was meant to produce. Under what mechanisms was the inorganic salt responsible for breaking down the DON pool? Is the first question that came to mind. While it may be true that as DON enters an estuarine environment, there can be breakdown, this would be biologically mediated. It is also unclear as to whether these samples were sterilized. If not, then the microbial consortia may have taken up any N liberated from the DON, though salt shock may have slowed microbial activity in the higher salinity treatment given the short-time span. Regardless, this study did demonstrate in no uncertain terms that a treatment facility cannot rely on salty water to breakdown DON as an inorganic treatment.

The photolysis assay was conducted in quartz glass bottles floated in a lake to receive a full day’s sunlight (8 hours in this case). This study sought to investigate whether sunlight could significantly breakdown the DON to inorganic N as net N release. This result of very little release as a % of the total DON pool is not surprising as the native DON is exposed to the full sun spectrum close to the surface of the Caloosahatchee River water and there is little removal of the DON in the field. It is unclear if this assay was done on sterile samples. The small amount of bioavailable N released is not sufficient to drive an algal bloom. While this was an important assay to run, it might be prudent to follow this study up with the effects of UV light on the DON pool. Banks of UV lights are used in several treatment wetlands in Louisiana in an attempt to sterile harmful pathogens that may be in the wastewater prior to discharge into the environment by damaging the DNA (Hijnen et. al., 2005). There is some evidence that a small fraction of DON can be degraded by natural UV light (Wiegner and Seitzinger, 2001) thus it would be an interesting concept to test this technology with banks of UV lights integrated into a greater treatment system on some scale. There are cost issues as well as implementation issues associated with this treatment technology so it would be worthwhile to conduct a lab study on how resistant this native DON pool would be to UV light. The light bank would have the distinct advantage of providing more direct exposure to the water column. Minimal exposure time for DON degradation would have to be determined and not all water might require passage through a light system.

There should be general concern that the bioassays produced very different results for duplicate samples. If the inoculum did not thrive in one but did in another replicate, as has been suggested, that suggests the bioassays are unreliable. It would have been important to document if the inoculum had indeed survived the placement into the vessels to attempt to narrow down conflicting results on the bioassay. Only after determining that as well as having analytical evidence that the DON was the same in the duplicates, can we suggest that there may have been some procedural reason for the disparate results. Therefore, it is difficult to deduce how much of the DON pool would be available to the bacteria or algae. It is a relatively simple procedure to verify if the inoculum is live by stain and high powered microscopy. Total numbers would not be needed, just an overall assessment of the consortia.
There is some useful information to be gleaned from this series of studies. However, I do not agree that the draft results, as presented, provide a definitive assessment of what fraction of the DON pool is available to bacteria or algae. The primary limitation on the data is the high variability on the replicates which suggests either the DON was not a true replicate, the inoculum was inconsistent among reps or there was some experimental procedural problem in carrying out the assay. Without an accounting of the viability of the inoculum at the termination of the assay, the results are difficult to put into context.

III. Are seasonal shifts in the DON bioavailability/recalcitrance supported by the chemical and biological evidence presented?

One might expect a difference between wet and dry season samples based solely on the source of the material. The wet season rains are likely to translocate terrestrial and perhaps fresh DON material from the watershed into the Caloosahatchee River. The dry period DON pool is likely that pool within the Caloosahatchee River or produced along the edge that is available for breakdown by either bacteria or algae and therefore more recalcitrant. This recalcitrance may also be related to residence time.

While the UHR-Mass Spec method is not quantitative, comparisons can still be made on the general makeup of the DON pool. There does appear to be a difference in the overall makeup of the DON pool from the wet to the dry season. However, since this method is not quantitative, it is difficult to deduce exactly how much of the wet vs dry season greater bioavailability is characteristic. This may be deduced from the spectral difference based on the DON concentration of each season's samples as an approximate measure.

This attempt at characterizing the DON pool is in its infancy and certainly more work needs to be supported in this arena to see if using both electrospray and APPI may yield more conclusive results. This work is currently being done for DOM in ocean water (D'andrill et al, 2010).

While the spectra indicate that the bioassay in the wet season underwent some changes, the percentage change is difficult to assess as the bioassay results proved somewhat inconclusive as the variability of the data outweighed any significance. If a bioassay if to be used to determine the treatment effectiveness of the proposed wetland treatment system, the reason for this high variability needs to be determined.

The Mass Spec data did provide some evidence that the DON had a more stable distribution of the compounds detected in the dry period and a more dynamic pool in the wet season. However, the assays need to be more closely linked to this result. In the end, a bioassay if the most reasonable method for determining recalcitrance of the DON pool for numbers of
samples, cost and time. Perhaps instead of dealing with bacteria from the coast, which will change significantly from collection time to collection time, a standard inoculum might be considered.

IV. Is the preliminary surrogate method for determination of biologically available DON reasonable and supported by the evidence presented?

The use of pigments to track changes in abundance and activity of photosynthetic organisms is well documented. Chl a is a non-specific pigment while the pheophytin pigment is a general degradation product of Chl a.

As mentioned in the consultant report, there is a real concern about using this method for determining the DON availability as any numbers of nutrient limitations and changing conditions can stem/prevent algal growth. Changes in phytosynthetic pigment in the STAs during the dry season could as well be related to the increased decomposition of the organic soil and release of DOM associated DON. Therefore, suggesting that the DON production can only be related to increased N fixation is not fully supported (Devesa-Rey et al, 2009; Parsons et al, 2006).

This method could show promise to determine the bioavailability of DON if the water samples were spiked with P and micro nutrients such that N was the limiting nutrient to growth and that the majority of the N in the same was the DON, which appears to be the case for the Caloosahatchee River water. This approach is mentioned in the report but it was not clear if this approach has been evaluated in this early stage of development.

V. Are the Conclusions of the Finding Memorandum supported by the data and its analysis?

The Finding Memorandum details the sampling and analyses of a range of sites including Lake O, the Caloosahatchee River and several STAs. All analytical protocols and COC sheets are in order and appear to have been conducted using standard accepted procedures.

I am uncertain if plotting all the data from these stations into dry season and wet season graphs provides us the information we need to assess the Caloosahatchee River DON but does provide an integrative view of the surface waters within the watershed. The approach on page 16 of the Findings Memorandum investigating the C:N ratio of the dissolved pool is a useful one but the STA samples probably should be eliminated from the plot to concentration on the dissolved C:N ratio in the Caloosahatchee River water. It is clear from the data that there is a strong
correlation to DOC and DON across these water bodies and hence, many of the assumptions which related DOM to DOC and DOM to DON seem validated by this data.

It is also interesting to note the DON concentration range is more constrained (1.25 – 2.5 mg/L) with a significantly lower average concentration than the dry season which ranges from just below 1 mg/L up to 4.0 mg/L for the averages. I am unsure if the concentration differences are in response to dilution, wet season having greater water and hence lower concentrations and any water level information might provide some insight to that. The Scope of Work and Report mention there is no difference in wet vs dry season DON and that may be true for the median but the much wider distribution of values in the wet season is important to consider.

The Findings Memorandum conclusion that the vast majority of the TDN is DON appears supported by the characterization and available water quality data. There are some details as to what micro-environments the samples were collected within. There is mention made to sampling within a floating mat and the equipment and procedures followed. One important question is “Were all samples from within floating mats in all systems and if not, does the DON concentration of the Caloosahatchee River water away from the mats vary from those concentrations? The data includes three FAV stations which were presumably in the Caloosahatchee River.

Relating this characterization data to the other studies, if the DON values are lower in the wet season and more bioavailable, as there appears to be some evidence, then an important question to ask is where did all this nonbioavailable DON come from in the dry season since the concentration range is much greater? Were the highest samples from the STAs or Lake O or the Caloosahatchee River? There are graphs of TSS, carbon and phosphorus (which forms?) but none for DON in the Appendix. If all the high values of DON were not from the Caloosahatchee River, then the DON would show no seasonal effects as stated on Page 4 of Exhibit A Purchase Order. In that case, treatment would only be possible in the wet season based on the assumption of increased bioavailability.

VI. Are the Conclusions (page 15) of the parameter plan supported by the data and its analysis?

The conclusions state “there is a reproducible pattern of change in compound-level analyses and total pigment change in the bioavailability assays as DON is transformed from bioavailable to recalcitrant forms.”

It is around these analyses that the design plan for the treatment system has been constructed. Reviewing my comments earlier in this report, the compound-specific analysis is not
quantitative but gives a qualitative assessment of trends of compound change for the compounds for which this method can "see". Dr. Cooper's very recent published work on DOM suggests that spectra determined using an alternative to electrospray, atmospheric pressure photoionization would provide a different signature (D'Andrill et al, 2010). I am only providing this insight to underscore that some compounds are not characterized by the suggested method and therefore, the entire DON pool along with associated shifts is still unknown at this time. This is expected as the reports cited the difficulties in determining the compound-specific distribution and goes with the task/challenges of developing a new analytical methodology.

Therefore, a more rational approach to determining the bioavailable pool likely falls to a bioassay as a more routine measure. The variability of the bioassay is of concern and more work should go into standardizing the bioassay with a standard inoculum and checks on viability to focus the differences on the bioavailability of the DON pool as opposed to experimental artifacts.

I am unclear as to why the total DON is not also being used in conjunction as a standard measure. From the data presented in the review documents, it appears that the wet season has lower concentrations AND some evidence that the DON is more bioavailable. Therefore, the problem for treatment is going to fall to the Dry Season when the DON concentrations are the highest and may be more recalcitrant. From my experience with water quality standards and the TMDL process, the TMDL process focuses on load but water quality standards focus on concentrations. There is an inherent flaw in this configuration but due to implementation issues, exists. An ecosystem response to nutrient loads will be different when compared to a concentration which provides no information on the total amount of that nutrient exported from the system. However, outfitting every conveyance with a flow meter and the difficulties in dealing with non-point sources has led to the concentration approach. Therefore, the concentration requirements for TN, of which the majority is DON, need to be a parallel focus.
Overall Comments on the Scientific Approach

Treatment for low level DON is going to be more challenging than the District’s treatment for low level TP for protection of the Everglades ecosystem because the TP load had a significant fraction in the particulate form, which can be settled out while the component SRP form was large and could be immobilized using any number of natural or chemical treatments. As with DOP, DON is not well studied, difficult to characterize and currently, there are no large scale natural treatment technologies specifically designed for removal.

Setting the difficulties aside, the contractor has suggested an approach which uses natural wetland treatment, keeping in step with desirability of design and cost. The approach of transforming bioavailable DON to recalcitrant DON is certainly one approach that can be taken to reduce the overall impact of the DON load to the coastal ocean. This approach may not actually lower the total DON load, but instead transform it into a more stable form which would be more slowly available to the ecosystem and hence, be less likely to trigger coastal algal blooms.

There is sufficient evidence shown that, for this region, there is a good agreement between DON and DOC. Therefore, it follows that both are also well correlated to DOM. This is a critical link because much of the characterization work on the dissolved fraction has been and is being done on DOM. The work done on Fourier transform ion cyclotron resonance mass spectrometry has primarily been directed at DOM. This method may provide some insights into the DON pool but I am unsure, especially at its infancy, if it should be relied upon to direct a wetland treatment design. The qualitative nature of the method is similar to NMR used to investigate P fractions in that respect and while it provides insight into treatment, it was not used to design the treatment system, though the work on NMR and Mass Spectrometry for P has many more years of study to this point and has been refined (El-Rifai et al, 2008).

Assuming for the time being, that a TN water quality standard would be flexible enough to consider the bioavailability of DON, then the proposed FAV has some merit as the bacteria could breakdown the bioavailable pool making the DON pool more recalcitrant. Data from the report show that the highest range of DON concentrations are in the dry period when the characterization data show less labile forms as opposed to the lower range of concentrations of DON in the wet season, where the DON may be more bioavailable. (I am assuming for the sake of this report that the coastal algal blooms of concern coincide with the wet season DON flows.
which would lend additional support that the DON in the wet season is more bioavailable.) This
difference in concentrations of reported DON may be and artifact due to dilution and therefore
the greater mass load of DON occurs in the wet season (concentration x flow).

Given that the proposed treatment system would not degrade the recalcitrant fraction, which
may be so slowly available as to be considered unavailable, this system would be ineffective
during the dry season assuming all assumptions on bioavailability are correct. Therefore,
considering a range of treatments at the mesocosm and perhaps, larger (hectare-size) test cells
would be advisable. There are still questions to be answered for the FAV treatment system
(floating vs tussocks) and the possibility of increased TSS. If the concern is that N-fixation
increases bioavailable DON, the concept of adding an SAV cell proximal to the outflow appears
in conflict. A shallow, open water cell with low N would favor N fixation, especially in a soil that
is not proving a flux of ammonium through mineralization of the macrophytes, algae, and soil
organic matter which occurs in the STAs. Please find specific comments related to the design
below.

My expertise lies in biogeochemical cycling and while I evaluate wetland treatment in my
research, design engineering is not an expertise of mine. Therefore, I will leave the component
of the engineering design criteria evaluation to the engineers on the review panel and will
constrain my comments to the scientific merit of the approach.

A. Specific Comments on Mesocosms

I have worked extensively with mesocosms for P removal in the STAs as well as sampling in the
larger experimental test cells. On the design of the mesocosms, I would suggest a modification
of the proposed design. The research at this site, which is going to be required to deal with this
complex issue of DON treatment to low levels, would be best served by a number of different
approaches tested and perhaps not in series, initially. The design for triplicate experimental
units is a good one and for the future, if the mesocosms can be plumbed to be run in series, all
the better. For right now, it might be in the best interest of the District to evaluate more
approaches to treatment instead of configuring the mesocosms for evaluation of the single
large-scale design. Most of the District’s STA experience is on very organic soils and this does
not appear to be the case with the Water Quality Treatment Area Test Facility site. Therefore,
there may be limited usefulness of relying on DON treatment in STAs due to the high potential
of production of DON from the Soil. I anticipate a list of alternative approaches to be finalized
at the two day technical meeting as recommendations from the review committee to be
evaluated in the mesocosm test facility should this modification be agreed upon. The negative
aspect of the mesocosm approach as described is that it is limited in the ability to investigate alternatives. The positives are that they can be linked in series to investigate the transformation of the water as it moves through the system. I believe the current design in not scaled to the different sized cells of the large-scale facility but this does no appear to be a significant limitation.

B. Specific Comments on Test Cells

The test cells will provide a more reliable assessment of promising treatment technologies due to the scale. Ideally, they will also provide some information on anticipated challenges in scaling up from the mesocosm scale to the full scale facility. This facility should provide for the evaluation of several of the most promising natural treatment technologies, which it appears the contractor has provided with the inclusion of these into the facility design.

Given that the District is working at the low end of the nutrient removal curve, these intermediate test cells should be of adequate size to allow a longer retention time while maintaining realistic operating condition (e.g. water still flowing). Since nutrient removal rates are low at the low end of the curve, providing enough treatment time or treatment area is critical to determine the removal capacity of the entire treatment area under the vegetation treatment under evaluation.

C. Specific Comments on Large-Scale facility

The benefits of the proposed facility will give the District a scaled-up version of the technology which can be adapted over time as data from the smaller systems is collected. The large scale system which uses both tussocks and areas that utilize water hyacinths will provide the opportunity to test the hypothesis that the DON pool can be degraded by the bacteria dominated films. This would need to be tested at a larger scale than the mesocosm scale.

The negatives associated with this approach would be the potential for production of high TSS, and decomposition of the plants and sloughing/grazing of the roots producing DON.

Again, the soils in this facility are very different in character than the organic soils upon which the STAs were constructed. It would be interesting to know if the organic soil production of DON, in addition to N fixation are the major reasons for relatively poor DON treatment in STAs.
Over time, no matter which natural wetland treatment option is adopted, there will be an accretion of organic matter which can cycle DON back to the water column. However, that will likely not be an issue in the early operation as this soil is not a histosol. I do agree that the general approach the contractor presents does have some merit but I am unsure if the large scale should not be subdivided such that more than one type of vegetation and option can be explored in case this experimental approach is not the best. For example, if there are to be FAV cells, then ones with the described tussocks could be evaluated coincident with Water Hyacinths. Of course, I would favor a mesocosm approach first for suitable natural treatment options but as I stated earlier in this report, the District does have a successful record of investigating treatment at different scales simultaneously with the preference for adaptive management.

Specific Comments on SAT (bank infiltration)

The opportunity to evaluate the removal of DON using SAT in the drainage rows of the citrus agricultural lands is a good and opportunistic one at this site. I do not know the relative number of opportunities in the upper watershed or along the Caloosahatchee River to use this potential treatment technology but as long as it does not affect the orange production, then it appears to have merit for two important reasons; 1) the soils for citrus production are generally fine sands which would have adequate hydraulic conductivity to permit movement of some water and provide treatment prior to discharge into the Caloosahatchee River; 2) If implemented basin-wide and successful, this may help significantly reduce the load of DON that needs to be treated (source reduction). This approach is similar to the retention of drainage water on the sugarcane farms for a few days post precipitation event that significantly reduced the TP exported from the farms and therefore was less P that the STAs needed to remove. Even limited application in the watershed or at the treatment facility would be desirable.

There is very little published information on TN removal by bank infiltration and nearly all is focused on nitrate removal (Mayer et al, 2010; Schipper et al, 1993). However, treatment by the soil is generally through utilization by bacterial films and if the water does not contain substantial quantities of fertilizer N, then there is the possibility that a bacterial population would enzymatically attack the DON to satisfy their N requirement.
Conclusions on the Design Approach of the Water Quality Treatment Facility

Considering the relative unknown characterization of DON and the fact that no large-scale treatment systems exist to the treatment level being sought here, the design facility has some promising features. The test cells along with an expansion and decoupling, at least initially, of the mesocosms will provide the District with the opportunity to evaluate a range of natural treatment options. The current design seems focused on the degradation or conversion of the bioavailable DON to a more recalcitrant form of DON in the large scale system. Since the characterization of DON is qualitative and still very much in the development stages and given that the bioassays provided some inconsistent replicate results, the District may not be able to adequately determine the performance of the large-scale facility until these characterization methods have been developed further. Therefore, the immediate evaluation will fall to what is likely to be a water quality standard based around TN, of which DON comprises the vast majority. It would appear more prudent to section the large-scale system such that several different systems can be evaluated. It is likely that the FAV, whether water hyacinths or tussocks will require implementation and evaluation at this larger scale because of the inherent challenges of this FAV approach. Any natural technology that might provide an overall reduction of DON should be evaluated. Much along the previous research path for low-level TP reduction, a low concentration stream might provide better treatment in these systems, in particular on soils of lower organic matter concentration. The inclusion of the SAT is opportunistic and may hold some promise for implementation in the watershed. Even if the SAT provided a modest reduction of 5 – 10%, that would still be some DON that the treatment facility does not have to treat.

The timeline for the District implementation will also likely influence the final development of the water quality facility and this information will need to be discussed with District personnel during the 2-day meeting.

Alternative Natural Treatment Options

I have the most experience with STA-like systems and consequently, one option that could easily be tested in a mesocosm would be the emergent and SAV systems on this more mineral soil. The District has a great deal of data on N and P removal in studies focused on P removal however just about all these systems save for a few PSTA systems on limerock have been on organic soils. One of these studies of which I was involved was the mesocosm study which collected inflow water to STA-1W (prior to the addition of cell 5, so essentially the old
Everglades Nutrient Removal Project). There were two published papers from this study that were solely focused on the various fractions of P (White et al, 2004; 2006). A copy of the 2006 paper is provided in appendix 1 for details of the experiment. There is data that the District possess that investigates all the N fractions. I am currently working with a District scientist on the publication of this N data and I have provided a copy of the relevant figure in Appendix 1. As you might expect, the DON inflow varied from 1 mg L\(^{-1}\) with occasional spikes to 4 or 5 mg L\(^{-1}\) over the two year study. As with so many of these waters, the DON was the dominant fraction and there was no relative treatment regardless of a drawdown of the water or emergent vs. submersed. Coincidentally there was no FAV treatment over the concern of increased TSS and associated P. One important finding from this study was that 2 drydown periods (1 month in length each) created a spike in DOP and in several cases, DON. This might not be as big a concern with a more mineral soil as the organic wetland soils have a large microbial pool capable of mineralizing the soil organic matter (White and Reddy, 2001). Therefore, in addition to the FAV, I would suggest the EAV – which the consultant report suggests for the preliminary cell and SAV, suggested for a polishing cell.

It has been suggested that using limerock as a true vertical flow system would be too expensive and difficult to maintain, and as the primary treatment, I agree. However, there are systems which provide aeration and potential removal of the dissolved nutrient fraction using slightly elevated crushed rock berms. The water is delivered by pump delivery to sprinklers which allow the water to cascade or trickle through the rock filter. Since the site is underlain with a porous limerock (according to surface borings), a substantial source of the material may exist onsite. My suggestion for this technology is as a polishing treatment. The biofilm which forms over the surface of the rocks in low nutrient conditions would favor bacterial decomposition of the DON without providing a potential source, as with the decomposition of particulates and the SAV tissue. I am unsure if this could be implemented on the scale called for here, but as a concept provides an intriguing alternative to the proposed polishing cell, albeit a bit more engineered.

Therefore I suggest that EAV, SAV and FAV be considered as separate treatments to determine the efficacy of DON removal with these mineral soils and also evaluated for the potential of creating DON in the treatment process. If feasible from an engineering perspective, using bacteria biofilms on the surface of a crushed limerock berm may provide a possible alternative scrubbing system for the low DON water prior to discharge.
Additional Notes on Timing of Blooms and Nutrient Delivery

Algal blooms generally appear in the Spring and Summer months driven not only by available nutrients but also other conditions including water temperature, water clarity, light. Therefore, one question that needs to be address is “Does N loading from the Caloosahatchee during the winter months, for example, have any effect on algal blooms”. It is likely that treatment of N in just those time periods when blooms are likely, would be sufficient to prevent the expression of eutrophic conditions.
References


Appendix I

Previous STA DOP and DON removal study
Hydrologic and Vegetation Effects on Water Column Phosphorus in Wetland Mesocosms


ABSTRACT

Historic phosphorus (P) loading from agricultural areas has been identified as one of the major causes for ecological changes occurring in the Florida Everglades. The restoration plan for the Everglades includes construction of large stormwater treatment areas (STAs) to intercept and treat this relatively high nutrient water down to very low total P (TP) concentrations. One such STA has been in operation for approximately 10 yr and contains both emergent aquatic vegetation (EAV) and submerged aquatic vegetation (SAV) communities. The surface water TP concentrations in areas near the outflow range from 0.02 to 0.05 mg TP L⁻¹. To simulate these areas, we investigated the interaction of vegetation type; EAV or SAV; and hydrology continuously flooded or periodic drawdown; on the P removal capacity in mesocosms packed with peat soil obtained from STA-1W. The surface water had low TP concentrations with an annual mean = 0.023 mg L⁻¹. For SRP and TP, hydrologic fluctuations alone had no discernable impact on P treatment while vegetation type showed a significant impact. Infiltration soluble reactive P (SRP) decreased by 49% for the SAV treatments compared with 41% for the EAV treatments, irrespective of hydrology treatment. The reduction of dissolved organic P (DOP) was also higher for the SAV treatment averaging 33% while showing a reduction of 11% for the EAV treatments. There was no significant difference in the treatment efficiency of particulate P (PP) across the treatments. For TP, SAV treatments removed 45% of TP while EAV removed significantly less at 34%. By mass calculations, the EAV required 85% more P for plant growth than was removed from the water column in 1 yr compared with only 47% for the SAV. Therefore, the EAV "mined" substantially more P from the relatively stable peat soil, translocating it into the detrital pool.

Phosphorus retention by constructed wetlands may include the following processes: surface adsorption on soil minerals, precipitation reactions, microbial immobilization, and plant uptake (Reddy et al., 1995). These processes may be combined into two distinct P retention pathways for wetlands: sorption and burial (Reddy et al., 1999). Phosphorus sorption includes both adsorption and precipitation reactions as mechanisms for the removal of phosphate from the soil solution to the solid phase. As plants senesce, some of the P contained in detrital tissue can be recycled within the wetland, and released into the water column. Remaining refractory detrital tissue may eventually become incorporated as organic matter in the wetland soil profile as organic matter accrues. Accretion of organic matter has been reported as a major sink for P in wetlands (Craft and Richardson, 1993; Reddy et al., 1993). Wetlands tend to accumulate organic matter due to the production of detrital material from biota and experience relatively low rates of decomposition under flooded conditions (DeBusk and Reddy, 1998). P accretion rates for constructed wetlands are generally on the order of millimeters per year, although accumulation rates in productive natural systems such as the Everglades have been reported as high as 1 cm or more per year (Craft and Richardson, 1993; Reddy et al., 1993). Over time, productive constructed wetland systems will accumulate organic matter that has different physical and biological characteristics than the original preconstruction soil. Eventually, this new material settles and compacts to form new soil, which may exhibit a different P removal capacity than the original soil.

Phosphorus accretion increases with P loading to the wetland (Reddy et al., 1993). However, an increase in accretion does not assure low surface water outflow P concentrations, especially for intermittently flooded wetland systems where decomposition of organic detritus releases available P back into the water column. Decomposition of detrital material was found to increase under high P conditions (DeBusk and Reddy, 2003, Wright and Reddy, 2001) lower water levels (White and Reddy, 2000), and higher redox conditions (White and Reddy, 2001).

Scientific investigations of P reductions in constructed wetlands generally focus on wetlands receiving much higher inflow concentrations (>0.100 mg L⁻¹) than this study. The goal of this study was to investigate the P treatment capacity of EAV and SAV communities under both continuously flooded and under periodic drawdown with a mean inflow TP concentration of 0.023 mg L⁻¹.

STUDY AREA

The Everglades is an internationally recognized oligotrophic ecosystem and more than half of the original 1.17 million ha has been lost to drainage and development (Davis and Ogden, 1997). Today, the Everglades wetland ecosystem is comprised of three Water Conservation Areas (WCAs) and the Everglades National Park (Fig. 1). These areas are being negatively impacted by hydrologic changes and nutrient-rich runoff generated from urban and agricultural sources (Davis and Ogden, 1997). Predrainage estimates of nutrient loading, reconstructed from written records and paleoecological assessments made in nearly pristine areas of the Everglades, have indicated that the Everglades flora and fauna had adapted to a very low nutrient regime (McCormick et al., 2001). Contemporary monitoring has shown that TP concentrations in south Florida rainfall are generally...
<0.010 mg L\(^{-1}\), and water column samples from the relatively unimpacted areas have TP concentrations between 0.004 and 0.010 mg L\(^{-1}\) (McCormick et al., 2001). Over time, increased nutrient loading to a system adapted to extremely low nutrient conditions, resulted in a decrease in the native periphyton assemblage and a shift from a sawgrass dominated emergent marsh to a cattail dominated system (Davis, 1991).

In 1994, the State of Florida enacted the Everglades Forever Act (EFA) (Section 373.4592, Florida Statutes) that mandates both hydrologic modifications and nutrient reduction to protect the remaining Everglades. As

Table 1. Soil physiochemical properties including total C, N, P, bulk density, and moisture content from the soil samples collected from the mesocosms at Year 1. Values are means ± one standard deviation (n = 3).

<table>
<thead>
<tr>
<th>Treatment combinations</th>
<th>Vegetation†</th>
<th>Soil Interval</th>
<th>Total C (g kg(^{-1}))</th>
<th>Total N (mg kg(^{-1}))</th>
<th>Total P (g cm(^{-3}))</th>
<th>Bulk density</th>
<th>Moisture content (wt %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>continuously flooded SAV</td>
<td>0-5</td>
<td>259 ± 23.0</td>
<td>11.7 ± 1.8</td>
<td>227 ± 21.3</td>
<td>0.32 ± 0.07</td>
<td>74.4 ± 4.16</td>
<td></td>
</tr>
<tr>
<td>periodically flooded SAV</td>
<td>0-5</td>
<td>279 ± 17.4</td>
<td>14.9 ± 3.1</td>
<td>243 ± 3.8</td>
<td>0.31 ± 0.02</td>
<td>73.8 ± 8.65</td>
<td></td>
</tr>
<tr>
<td>continuously flooded EAV</td>
<td>0-5</td>
<td>264 ± 27.2</td>
<td>13.8 ± 3.5</td>
<td>227 ± 25.6</td>
<td>0.29 ± 0.05</td>
<td>74.5 ± 5.17</td>
<td></td>
</tr>
<tr>
<td>periodically flooded EAV</td>
<td>0-5</td>
<td>261 ± 34.5</td>
<td>16.0 ± 12.2</td>
<td>232 ± 42.7</td>
<td>0.31 ± 0.05</td>
<td>73.2 ± 2.48</td>
<td></td>
</tr>
<tr>
<td>continuously flooded SAV</td>
<td>5-30</td>
<td>215 ± 48.8</td>
<td>7.9 ± 3.9</td>
<td>214 ± 64.7</td>
<td>0.4 ± 0.03</td>
<td>65.4 ± 2.48</td>
<td></td>
</tr>
<tr>
<td>periodically flooded SAV</td>
<td>5-30</td>
<td>253 ± 65.8</td>
<td>11.1 ± 4.4</td>
<td>213 ± 55.5</td>
<td>0.39 ± 0.08</td>
<td>67.2 ± 7.2</td>
<td></td>
</tr>
<tr>
<td>continuously flooded EAV</td>
<td>5-30</td>
<td>260 ± 25.4</td>
<td>11.7 ± 1.6</td>
<td>219 ± 5.0</td>
<td>0.34 ± 0.03</td>
<td>68.8 ± 2.71</td>
<td></td>
</tr>
<tr>
<td>periodically flooded EAV</td>
<td>5-30</td>
<td>282 ± 18.6</td>
<td>13.3 ± 1.2</td>
<td>257 ± 26.4</td>
<td>0.34 ± 0.03</td>
<td>70.6 ± 2.52</td>
<td></td>
</tr>
</tbody>
</table>

† EAV - emergent aquatic vegetation, SAV - submerged aquatic vegetation.
part of the nutrient reduction program, the EFA requires the South Florida Water Management District (SFWMD) to construct several large treatment wetlands (about 17,000 ha) called STAs to reduce nutrient levels in runoff to a design target of 0.05 mg TP L⁻¹ before discharging into the Everglades. The EFA also requires the SFWMD to conduct research to optimize nutrient removal performance by the STAs in an effort to produce outflow concentrations less than the design target, and provide operational guidance to maintain and improve the long-term P retention of the STAs.

These large STAs have been designed as passive, wetland removal treatment systems, primarily dominated by emergent and submerged vegetation. The maximum and minimum standard operating water depths range between a low of 0.15 m to a high of 1.22 m, depending on dominant vegetation and watershed runoff volumes. However, with increased drainage and development of south Florida, the pressure for freshwater supplies during the seasonal dry periods and prolonged droughts may reduce the volume of runoff water available to the STAs, resulting in a temporary dry out of the system and potential release of P from the system. A significant increase in the mean TP outflow concentration could result from climatic and hydrologic conditions depending on the degree and extent of P flux (Olila et al., 1997; White et al., 2004).

These effects may be even more critical in areas near the outflows, resulting in export of P. Depending on the degree and extent of the P flux, a significant increase in the mean TP outflow concentration from the STA could result. We conducted a controlled mesocosm study to determine the effects of water level drawdown and rehydation and vegetation type (EAV vs. SAV) to better understand the potential effects of dry-out and vegetation type on STA P removal performance. There is a distinct paucity of information on the detailed P dynamics at very low (0.010-0.10 mg L⁻¹) TP concentrations. Therefore, this study provides critical information on the detailed P dynamics at low P concentrations typical of the surface water P concentration proximal to the outflow of the STA.

MATERIALS AND METHODS

Experimental Design

The mesocosms were situated at the surface water outflow at the south end of STA-1W, a 2700-ha constructed wetland built on previously farmed agricultural land located 25 km west of the city of West Palm Beach in Palm Beach County, The site borders the northwest corner of WCA-1 (260’ N and 800’ W) situated proximal to the outflow of STA-1W (Fig. 1). Mesocosms consisted of 12 fiberglass-lined plywood tanks measuring 5.9 m long by 1.0 m wide by 1.0 m deep. Each mesocosm contained 30 cm of previously farmed peat taken from STA-1W, Cell 5, before its construction. The soil was overlaid with 40 cm of STA-1W outflow water and the mesocosms were open at the top, exposed to direct sunlight and precipitation. STA-1W outflow water was pumped from the outflow canal into a head tank located at the site and then gravity fed into a PVC distribution system. The flow-through mesocosm system operated with an average design hydraulic loading rate (HLR) of 2.61 cm d⁻¹ that resulted in a nominal hydraulic retention time of 15.4 d. The HLR was controlled through calibrated pipette tips that were replaced weekly.

There were 12 mesocosms, six of them planted with 32 Typha sp. plants and six were not planted. The mesocosms were flooded at the HLR previously described. The following treatments were evaluated in triplicate:

- Continuously Flooded with EAV (planted with 32 mature cattail plants)

Table 3. Mean annual inflow and outflow concentrations of soluble reactive P (SRP), dissolved organic P (DOP), particulate P (PP), and total P (TP) and percent reductions over the inflow concentration. Data are means ± one standard deviation for replicate mesocosms (n = 3).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>P parameter</th>
<th>Mean outflow concentration</th>
<th>Mean % reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flooded-STA</td>
<td>SRP ⁹</td>
<td>0.065 ± 0.001</td>
<td>45.4 ± 2.1</td>
</tr>
<tr>
<td>Drawdown-STA</td>
<td>SRP ⁹</td>
<td>0.065 ± 0.001</td>
<td>45.7 ± 3.3</td>
</tr>
<tr>
<td>Flooded-EAV</td>
<td>SRP ⁹</td>
<td>0.066 ± 0.001</td>
<td>39.7 ± 2.6</td>
</tr>
<tr>
<td>Drawdown-EAV</td>
<td>SRP ⁹</td>
<td>0.065 ± 0.001</td>
<td>45.1 ± 0.6</td>
</tr>
<tr>
<td>Flooded-STA</td>
<td>DOP ⁹</td>
<td>0.003 ± 0.002</td>
<td>41.2 ± 8.2</td>
</tr>
<tr>
<td>Drawdown-STA</td>
<td>DOP ⁹</td>
<td>0.004 ± 0.002</td>
<td>42.5 ± 4.3</td>
</tr>
<tr>
<td>Flooded-EAV</td>
<td>DOP ⁹</td>
<td>0.004 ± 0.003</td>
<td>40.7 ± 7.2</td>
</tr>
<tr>
<td>Drawdown-EAV</td>
<td>DOP ⁹</td>
<td>0.005 ± 0.003</td>
<td>56 ± 5.0</td>
</tr>
<tr>
<td>Flooded-STA</td>
<td>PP ⁵</td>
<td>0.005 ± 0.002</td>
<td>48.2 ± 8.7</td>
</tr>
<tr>
<td>Drawdown-STA</td>
<td>PP ⁵</td>
<td>0.005 ± 0.003</td>
<td>46.5 ± 4.2</td>
</tr>
<tr>
<td>Flooded-EAV</td>
<td>PP ⁵</td>
<td>0.005 ± 0.001</td>
<td>38.2 ± 5.5</td>
</tr>
<tr>
<td>Drawdown-EAV</td>
<td>PP ⁵</td>
<td>0.006 ± 0.003</td>
<td>40.5 ± 4.1</td>
</tr>
<tr>
<td>Flooded-STA</td>
<td>TP ⁵</td>
<td>0.013 ± 0.004</td>
<td>47.3 ± 5.4</td>
</tr>
<tr>
<td>Drawdown-STA</td>
<td>TP ⁵</td>
<td>0.014 ± 0.005</td>
<td>42.4 ± 4.1</td>
</tr>
<tr>
<td>Flooded-EAV</td>
<td>TP ⁵</td>
<td>0.016 ± 0.004</td>
<td>33.4 ± 4.1</td>
</tr>
<tr>
<td>Drawdown-EAV</td>
<td>TP ⁵</td>
<td>0.015 ± 0.006</td>
<td>35.9 ± 1.7</td>
</tr>
</tbody>
</table>

Inflow water

SRP ⁹ 0.010 ± 0.0024
DOP ⁹ 0.005 ± 0.0031
PP ⁵ 0.009 ± 0.0059
TP ⁵ 0.024 ± 0.0079

¹ Different letters indicate significantly (p < 0.05) different concentrations between each treatment within each P fraction.
Vegetation Sampling and Analyses

A single randomly selected cattail was reserved for nutrient analysis from each of the EAV treatment mesocosms at project inception. However, no SAV were sampled at this time because these communities had not become established. After 1 yr of operation, a 0.25-m² quadrat was used to collect the above-ground vegetation at two randomly located sites within each tank for both the EAV and SAV treatments. The plant material was dried, weighed, and ground before analyses. The roots were collected from the soil coring procedure listed below. Samples were analyzed for total C, and total N using a Carlo-Erba NA-1500 CNS Analyzer (Haak-Buchler Instruments, Saddlebrook, NJ), and TP was determined from a total Kjeldahl digestion and analyzed colorimetrically for P (Method 365.1; USEPA, 1993).

Soil Sampling and Analyses

Duplicate soil cores were collected from each mesocosm by driving a 10-cm diam. aluminum tube to the bottom of each tank for both the initial soil sampling and after 1 yr. The depth of the soil was noted; the core was sealed, and removed. Each core was sectioned into two intervals; 0 to 5 cm and 5 cm until the bottom of the mesocosm (5–30 cm). The core sections were extruded into a labeled plastic bag, sealed, and stored on ice until return to the laboratory the following day. All roots and rhizomes were removed, dried, weighed and digested for TP determination. The soil samples were weighed, homogenized, and transferred to 2-L polyethylene storage containers and refrigerated at 4°C until analysis. Soil redox (Eh) was measured each week at the 5- and 10-cm depths using permanently installed platinum electrodes.

Gravimetric moisture content was determined on subsamples by weight percent change of the initial soil samples and

![Image](https://via.placeholder.com/150)

**Fig. 2.** Soluble reactive P concentrations for surface waters entering (inflow) and discharging (outflow) the mesocosms for the continuously flooded treatments containing submerged aquatic vegetation (SAV) and emergent aquatic vegetation (EAV).
samples dried at 70°C until constant weight. Bulk density was calculated for each soil core on a dry weight basis. Total C and N content of detritus and soils was determined on dried, ground samples using a Carlo-Erba NA-1500 CNS Analyzer (Haake-Buchler Instruments, Saddlebrook, NJ). Total P concentrations were determined on dried, ground subsamples by ashing at 550°C and subsequent hydrochloric acid digestion (Anderson, 1976) followed by analysis of P by an automated ascorbic acid method (Method 365.4, USEPA, 1993).

The soils were also analyzed for various inorganic P fractions following the sequential inorganic P fractionation scheme developed for Histosols (Reddy et al., 1998). The following P fractions were determined from field moist soils (0.5 g dry weight equivalent):

- 1.0 M KCl-Pi representing labile P;
- 0.1 M NaOH-Pi representing Fe and Al bound P;
- 0.1 M NaOH-Po representing fulvic and humic bound P;
- 0.5 M HCl-Pi representing Ca and Mg bound P;
- residual P representing refractory organic P.

Mass Balance of Phosphorus

The soil component was determined from the TP concentration of the soil multiplied by the mass of soil in the mesocosms. The P mass within the area of the core was normalized to a square meter. The aboveground and belowground biomass was collected in two locations within a 25 cm by 25 cm quadrant. The collective dry mass of plants collected within the total 50 cm by 50 cm area, multiplied by the TP concentration, normalized to a m² basis and reported as g m⁻². The fourth component was the mass of TP in both the inflow and outflow water determined by sum and was calculated by multiplying the concentration of P by the total hydraulic loading at each time step (week) and dividing by the area of the mesocosms to obtain g P m⁻² yr⁻¹.

Data Analysis

Soil characteristics were statistically related using Pearson's product moment correlation and regression analysis. Analysis of variance (ANOVA) and Fisher's least significance difference (LSD) tests were used to compare the treatments. A statistical comparison was made for outflow concentrations for SRP, DOP, PP, and TP for each of the treatments. Concentration and percentage data was calculated at each time step and means and standard deviations were determined over the year long experiment.

RESULTS AND DISCUSSION

Soil Characteristics

Soil characteristics were determined for both the 0- to 5-cm soil intervals and the subsurface intervals (5-30 cm). Dry weight bulk density averaged 0.306 g cm⁻³ in the surface soil interval and was slightly higher at 0.369 g cm⁻³ in the subsurface soil interval (Table 1). The dry weight bulk densities are somewhat higher than expected for

[Graphs showing periodic drawdown - SAV and EAV]

Fig. 3. Soluble reactive P concentrations for surface waters entering (inflow) and discharging (outflow) the mesocosms for the periodic drawdown treatments containing submerged aquatic vegetation (SAV) and emergent aquatic vegetation (EAV).
this organic soil and were due to significant bits of limestone incorporated in the soil. The limestone bedrock is close to the surface of the soil in the area where the soil was excavated. The higher bulk densities in the subsurface were also reflected in average moisture contents of 68 vs. 74% in the surface interval. The total C and N content of the soil averaged 260 and 13.7 g kg⁻¹ with no significant differences in the surface and subsurface soil intervals. The TP of the surface soil interval was 232 and 226 mg kg⁻¹ in the subsurface soil interval with no significant difference with depth (Table 1). There was no significant difference in any soil parameters from the initial sampling and the sampling at the end of 1 yr.

Soil Phosphorus Forms

On average, the soil contained 62% organic (Table 2) vs. 38% inorganic P (Table 2). The inorganic P fractions were comprised of KCl-extractable P, which is the total of the porewater P and exchangeable P. This most available fraction comprised only 0.01% of total soil P. The Fe-Al bound P comprised 1.6% of TP and the final inorganic component was the Ca-Mg bound P 36.5% of TP. The organic P fractions include the moderately labile humic and fulvic P, which collectively made up 6.9% of TP while the residue P represented all fractions not extracted with salt, acid, or base and is generally composed of recalcitrant organic compounds comprised 55%.

In total, the largest pools of P in this soil were the residual organic P and the Ca-Mg bound P (Table 2) in concert making up 91.5% of the TP. The Ca-Mg bound P is relatively stable in these soils due to the pH (>7.0). However, anaerobic decomposition processes and accumulation of organic acids can potentially solubilize this pool of P.

Vegetation

The mesocosms with EAV were dominated by *Typha sp.* while the SAV treatments were colonized by, predominately, *Chara carum* and *Hydrilla verticillata*. Plant tissue from the SAV treatments averaged 221 and 202 g C kg⁻¹ and 6.68 and 9.24 g N kg⁻¹ for the continuously flooded and periodic drawdown treatments, respectively. Plant tissue from the EAV treatments averaged 383 and 384 g C kg⁻¹ and 5.46 and 7.08 g N kg⁻¹ for the continuously flooded and periodic drawdown treatments, respectively. The TP of the plant tissue for the SAV treatment averaged 249 mg P kg⁻¹ with a higher average for the EAV treatments at 365 mg P kg⁻¹. The C/P ratio of the EAV was 1063:1 with a lower ratio of 851:1 for the SAV. There was no statistical difference in plant variables related to hydrologic treatments.

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**Fig. 4.** Total P (TP) concentrations for surface waters entering (inflow) and discharging (outflow) the mesocosms for the periodic continuously flooded treatments containing submerged aquatic vegetation (SAV) and emergent aquatic vegetation (EAV). Shown are the Interim TP standard and the Draft (in the current rulesmaking process) TP standard for discharge waters.
Water Quality

The mesocosm inflow characteristics are similar to the outflow surface waters of STA 1W. The inflow water contained, on average, SRP concentrations of 0.010 mg L\(^{-1}\), and TP concentrations of 0.024 mg L\(^{-1}\) over the year (Table 3). The inflow water TP concentrations ranged from a low of 0.013 mg L\(^{-1}\) to a high of 0.044 mg L\(^{-1}\) during the experimental period. Concentrations of SRP also varied over the year ranging from 0.005 to 0.017 mg L\(^{-1}\) (Fig. 2).

The outflow concentration for the continuously flooded treatments had a tendency to fluctuate along with the concentration of the inflow waters (Fig. 2). This same pattern was also seen in the treatments which underwent periodic drawdown (Fig. 3). However, these treatments also experienced increased outflow concentrations for several weeks after the reflood of the first drawdown event.

Total P concentration did not vary with changes in inflow TP concentration unlike the pattern seen with SRP (Fig. 4 and 5). The continuously flooded-SAV treatment had outflow concentration of 0.010 mg L\(^{-1}\) or lower 30% of the time while the continuously flooded-EAV treatment did not reach below 0.010 mg L\(^{-1}\) at any time during the year (Fig. 4). For either vegetative treatment (EAV vs. SAV), the outflow TP concentrations were higher than the inflow water for up to 3 wk after the reflood for the first drawdown event, suggesting the soil and plants were acting as a source of TP to the water column (Fig. 5). There was a small effect of higher outflow concentrations from the second drawdown event on the EAV treatment, which was not apparent in the SAV treatment (Fig. 5).

To investigate the mechanisms that may be responsible for P removal or release to the water column, we elucidated experimental effects on the individual P components in the water. For example, TP contains SRP, DOP, and PP fractions. The reduction of SRP in the water column is mediated by either uptake by microbes and plants and incorporated into organic forms while precipitation with calcium carbonate compounds provides for an inorganic removal mechanism.

There was no significant difference in the reduction of SRP in the SAV treatments based on concentration, whether or not periodic drawdown was imposed (Table 3). Both the drawdown-EAV treatment at 42% reduction and the continuously flooded-EAV treatment at 39% performed statistically worse than the SAV treatments (49%) for the outflow SRP concentrations (Table 3). The data suggests that vegetation type, more than the hydrologic fluctuations, controlled removal efficiency of SRP from the water column for these inflow P concentrations.

Decomposition processes of detrital or soil organic matter can influence the release of DOP and may have liberated the DOP from the organic detrital material or microbial pool. In the case of DOP, the continuously

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Fig. 5. Total P (TP) concentrations for surface waters entering (inflow) and discharging (outflow) the mesocosms for the periodic continuously flooded treatments containing submerged aquatic vegetation (SAV) and emergent aquatic vegetation (EAV). Shown are the Interim TP standard and the Draft (in the current rulemaking process) TP standard for discharge waters.
flooded SAV treatment had the statically best removal of DOP at 41%. Both drawdown treatments and the continuously flooded EAV treatment had lower removal efficiency for DOP ranging from 24 to 10%. While the mean percentage reduction was lower by over one-half for the EAV treatments compared with the SAV treatments, the EAV had a higher variability that likely affected statistical comparisons (Table 3). Conversion of DOP to SRP is mediated by enzymatic hydrolysis (Pant and Reddy, 2001). The DOP fraction, based on percentage removal, appears to be the most difficult fraction for treatment in terms of percentage reductions in these systems.

Treatment for PP, which is a settling process, was not significantly different for each of the four treatments, due in large part to high variability. Mean reductions ranged from a 48% down to 38% (Table 3).

Total P reductions are perhaps of most critical importance in terms of meeting the water quality standards for the Everglades. The highest average reduction was in the two SAV treatments; for the flooded at 0.013 mg L$^{-1}$ with a 47% reduction and for the drawdown at 0.014 mg L$^{-1}$ with an overall reduction of 42%. Significantly lower was the drawdown-EAV treatment averaged 0.015 mg L$^{-1}$ while the continuously flooded-EAV averaged 0.016 mg L$^{-1}$ with reductions of 36 and 33%, respectively (Table 3).

The drawdown events, which appeared to have a much smaller effect on the reduction of TP of the surface water than vegetation type, did not have identical effects on the soil redox. The first drawdown period had a high $E_{s}$ (Fig. 6), usually associated with drier soil conditions, which led to a release of SRP and TP for several weeks after the reflooding event, while this effect was not apparent after the second drawdown (Fig. 3 and 5). The reason for the difference in average $E_{s}$ values during the drawdown period could be related to rainfall patterns. The first drawdown occurred in the late winter, which is the dry period in south Florida while the second dry period occurred in the summer when precipitation is at the highest levels. Since the mesocosms were not covered, to expose these systems to the changing climatic conditions seen over the year, the effect of the differential in precipitation likely maintained the soil moisture at a higher level in the summer than during the winter (Fig. 6). When other experimental mesocosms were maintained for 2 yr, the same pattern of higher redox in the winter drawdown was observed (White et al., 2004).

**Mass Balance of Phosphorus**

There were five major components of the mass balance of P calculated for this study including soil, aboveground biomass, belowground biomass, inflow TP, and outflow TP. There were only very fine root mass for the SAV treatment tanks that comprised < 0.5% of the total vegetation wet weight and were considered insignificant and not analyzed.

The largest component was the soil TP values averaging 21.7 g P m$^{-2}$ for the 0- to 30-cm depth (Table 4). The SAV treatment produced significantly less biomass P for both the periodic drawdown and continuously flooded treatments combined when considering only the above ground biomass at 0.121 g P m$^{-2}$ while the EAV treatments aboveground biomass averaged 0.214 g P m$^{-2}$. When you include the substantial belowground biomass for the EAV treatment, the total biomass P value rises to 0.348 g P m$^{-2}$. On average, all treatments removed 0.057 g P m$^{-2}$ from the water column (Table 4).

**Table 4.** Mass balance of P in various components in the mesocosms after 1 yr. Values are means ± one standard deviation (n = 3).

<table>
<thead>
<tr>
<th>Treatment combinations</th>
<th>Plants</th>
<th>Roots</th>
<th>Soil</th>
<th>Inflow total P</th>
<th>Outflow total P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrology</td>
<td>Vegetation</td>
<td>g P m$^{-2}$</td>
<td>g P m$^{-2}$ yr$^{-1}$</td>
<td>g P m$^{-2}$</td>
<td>g P m$^{-2}$ yr$^{-1}$</td>
</tr>
<tr>
<td>continuously flooded</td>
<td>SAV</td>
<td>0.158 ± 0.033</td>
<td>n.d.</td>
<td>22.3 ± 0.07</td>
<td>0.17 ± 0.004</td>
</tr>
<tr>
<td>drawdown</td>
<td>SAV</td>
<td>0.084 ± 0.029</td>
<td>n.d.</td>
<td>18.9 ± 0.43</td>
<td>0.13 ± 0.002</td>
</tr>
<tr>
<td>continuously flooded</td>
<td>EAV</td>
<td>0.152 ± 0.073</td>
<td>0.133 ± 0.084</td>
<td>21.1 ± 1.25</td>
<td>0.17 ± 0.002</td>
</tr>
<tr>
<td>drawdown</td>
<td>EAV</td>
<td>0.176 ± 0.052</td>
<td>0.137 ± 0.117</td>
<td>24.6 ± 5.3</td>
<td>0.13 ± 0.002</td>
</tr>
</tbody>
</table>

† EAV: emergent aquatic vegetation, SAV: submerged aquatic vegetation.
‡ n.d.: not determined.
The amount of above and belowground biomass P in the emergent treatments could lead to a decline in treatment capacity over time since P is released from detrital material into the water column. The EAV produced twice as much aboveground biomass P when compared with the SAV treatments and significantly more root-associated P (Table 4). The TP of this vegetative matter was far more than was removed from the water column, therefore exploiting the more stable P in the peat for incorporation into EAV biomass. If we assume the plants were able to take up all the P that was removed from the water column, the EAV took up 85% of their P requirement from the peat soil while the SAV would have required less at 47%. The presence of significant EAV rhizosphere influences led to increased nutrient uptake from the interstitial water and likely less uptake of nutrients from the surface water (Moore et al., 1994). The SAV generally has a slower growth rate than EAV due in part, to poor light transmission and the slow diffusion rate of CO₂ in water; however, SAV have shown to reduce P concentration at similar rates as emergent vegetation (Reddy et al., 1987). The SAV have also been shown in other studies to produce small (<10%) total biomass as roots and have been shown to take nutrients directly from the water column and therefore exploiting less P from the soil (Bole and Allan, 1978). Another SAV P removal mechanism is related to photosynthetically driven elevation of water column pH and the concomitant precipitation of P out of solution as Ca-P compounds (Dierberg et al., 2002).

Surface water P removal was greatest in both the continuously flooded and drawdown SAV treatments compared with both hydrologic treatments containing EAV at these low water surface-water P concentrations suggesting that the SAV configuration might perform better than EAV for the outflow half of these large constructed wetlands (STAs) where the surface water TP concentrations are low (14–30 ppb). Generally, the wetland mesocosms provided good SRP and PP removal in these systems. There was relatively poor removal of the DOP fraction. This P fraction may be of concern due to the transport of DOP carried by surface waters into the Everglades where enzymatic hydrolysis can transform the DOP into SRP, the most bioavailable form (Reddy et al., 2005).

CONCLUSIONS

Removal or immobilization of P at these water column concentrations was shown to be effective under both vegetative treatments with the best performance seen for the SAV communities, irrespective of hydrologic treatment. However, there was diminished effectiveness in the short-term P removal after reflooding and there was drawdown events that lasted up to 3 wk before returning to pre-event removal rates. Significant reductions were seen for SAV treatments for SRP and TP when compared with the EAV treatments with no difference seen for PP. The greatest difference in treatment was seen for DOP, with an average of 11% reduction seen for the EAV treatment while the SAV treatment reduced DOP an average of 33%, irrespective of hydrology. Taken individually, the flooded SAV treatment was significantly better than all the other treatments at 41% reduction of DOP.

There was also a significant difference in total biomass P for the type of plant community with greater biomass P in the EAV compared with the SAV (Table 4). The EAV took up at least 85% of their TP requirement from the peat soil assuming they also utilized all the P that was removed from the water column. In comparison, the SAV could have met 53% of their P requirement from utilizing all the P removed from the water column while requiring less P (47%) for biomass requirements from the peat soil. Overall, the EAV community has significantly less effect on SRP and TP removal at these low water column P concentrations and also took up more P from the peat soil than the SAV. This mobilization of P from the more stable peat soil into plant biomass could potentially lead to oxidation of this organic detrital P and the concomitant release of P into the water.

REFERENCES


Appendix C – Dr. Robert Knight

MEMORANDUM

C-43 Water Quality Treatment Area – Expert Panel Review

TO: Jennifer Leeds/SFWMD
    Peter Doering/SFWMD
    Zhiqiang Chen/SFWMD

FROM: Robert L. Knight/WSI

DATE: June 24, 2010

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Introduction

The South Florida Water Management District (District) and Lee County are partnering to reduce nutrient concentrations and loads in the C-43 Canal (Caloosahatchee River) upstream of the S-79 water control structure east of Ft. Myers and in the Caloosahatchee Estuary (Estuary) downstream of S-79. Elevated concentrations of nitrogen and phosphorus in these water bodies are contributing to impairment of beneficial uses in the Caloosahatchee River and Estuary, primarily through the creation of excessive algae blooms and resulting decreased water clarity and dissolved oxygen content. The primary focus of the District’s and Lee County’s efforts is reduction of bioavailable forms of nitrogen and ultimate compliance with a Total Maximum Daily Load (TMDL) requirement for the
Estuary. While the focus is on nitrogen (N) load reduction, loads of phosphorus (P) and suspended solids are also of concern and are being considered.

The effect of excessive nutrient loads from the C-43 Basin (including Lake Okeechobee) is exacerbated by unnaturally high and variable flows that bring excessive nutrients and tannic colored water into the river and estuary (Knight and Steele 2005). One component of this restoration effort mandated by the Northern Everglades and Estuary Protection Plan of 2007 is implementation of a Water Quality Treatment Area (WQTA) just upstream of S-78 on the south side of C-43. Other activities in this overall restoration plan that are outside the consideration of this review but may be critical for the success of the WQTA project include a proposed water storage reservoir (C-43 Storage Reservoir) to restore some normality to flows and source controls in the basin to reduce nutrient and solids loads in the C-43 Canal.

A 1,750-ac site has been purchased by the District and Lee County for the proposed WQTA. Preliminary studies and engineering for the proposed WQTA facility are being conducted by CH2M HILL (Consultant) under contract with the District. A number of project deliverables have been completed by the Consultant to identify the best option(s) for achieving the project goals of nutrient reduction in C-43 and the Estuary. Key activities completed under this contract to-date include:

- Initial Data Collection and Total Nitrogen Reduction Technologies Assessment
- Water Quality Evaluation and Characterization of Dissolved Organic Nitrogen (DON)
- C-43 Water Quality Treatment Project Test Facility Conceptual Plan Development

The Consultant’s work efforts have resulted in a proposed treatment train of natural nutrient removal technologies (i.e., “green” wetland and aquatic processes that rely more on solar and other natural energy inputs/plants and processes and less on the consumption of fossil fuels or chemicals) to reduce concentrations of bioavailable nitrogen and phosphorus compounds in C-43 prior to discharge to the Estuary. The Consultant has prepared a conceptual plan for development of a research/demonstration facility on the C-43 WQTA site as the next step prior to full-scale implementation of nutrient removal in the Caloosahatchee River.

The District is conducting a peer review of the Consultant’s findings and recommendations prior to proceeding with final design and implementation of the research/demonstration project. A panel of three scientists (Panel) with extensive credentials in water quality treatment and wetland and natural systems, were selected for this peer review:

- Dr. Robert Knight, Panel Chair (Wetland Solutions, Inc. [WSI] and University of Florida)
- Dr. Alex Horne (University of California Berkeley)
- Dr. John White (Louisiana State University)

The District has directed the Panel to complete the following four tasks as part of this review effort:

- Review and Evaluate Consultant Deliverables including the following:
Task 1 – Review and Evaluate Consultant Deliverables

Total Nitrogen Reduction Technologies Review (April 2008)

General Comments

The information included in this document is well organized and presented. However the scope is too narrow. This document should treat both nitrogen and phosphorus with equal weight since both elements are thought to be involved in eutrophication of the Caloosahatchee Estuary (Knight and Steele 2005).

The title of this report indicates that it is intended to be a review of existing information about all available and feasible treatment technologies for reduction of TN, with a focus on wetland/natural systems and the biogeochemistry of the most recalcitrant forms of N, including dissolved organic nitrogen (DON). The discussion of the feasibility and cost of using conventional (energy and chemical intensive) nitrogen removal technologies should be expanded and more conclusive.
The discussion of organic nitrogen forms and nitrogen transformations needs more explanation. While the focus is on DON, there should be more discussion on transformations to and from ammonium or nitrate forms and on particulate organic nitrogen (PON). PON consists of living and dead algal cells and to a lesser extent suspended organic sediments. When these particulates are trapped in treatment wetlands a fairly large fraction of the liberated organic nitrogen is bioavailable following ammonification.

This Reviewer suggests a different focus from the one proposed by the Consultant. This suggested alternative approach would highlight the relationship between “biologically available nitrogen” (BAN) and “recalcitrant nitrogen” (RN). The District’s basic goal is to reduce the concentration and load of BAN in the Caloosahatchee River and Estuary, rather than TN or DON. The Consultant has put a considerable effort into defining biologically-available DON (BDON) but the resulting definition should be more comprehensive with better explanation of the assumptions. Suggested definitions are:

- BAN includes all inorganic nitrogen forms (NH$_3$ + NO$_X$) and biologically available organic N (urea, uric acid, amino acids and sugars, amides, etc.); and
- RN includes all other nitrogen forms that will not degrade in the freshwater or salt water environment within a reasonable period (e.g., 30 day half life to be estimated based on eventual dilution and breakdown in the marine environment). RN could be assumed to be the TN remaining in the outflow from a well-designed/constructed treatment wetland that has a nominal hydraulic residence time of at least 30 days.

The DON conceptual model proposed by the Consultant is not comprehensive and ignores significant components of the nitrogen budget in treatment wetlands. Specifically, the conceptual model does not acknowledge the direct release of DON from organic soils or include the contribution of rooted macrophytes as they release nitrogen from the soil pool to the BDON pool or to recalcitrant DON (RDON).

The whole issue of soil interactions with DON should be addressed in this TM. Natural treatment systems built on organic soils may have a higher irreducible background TN concentration ($C^*_{TN}$) than systems built on mineral soils with low organic matter content. Examples include the Iron Bridge Treatment Wetland east of Orlando built on sandy soils with low organic matter content and a $C^*_{TN}$ less than 0.8 mg/L, and the EAA STAs with $C^*_{TN}$ values as high as 2.2 mg/L in the northern EAA with highly organic soils and a $C^*_{TN}$ value of about 1.45 mg/L in STA 6 in the western EAA where soils are sandy and less organic. Exhibit 1 illustrates the monthly outflow TON concentration data from eleven Florida wetland and aquatic systems based on their predominant soil characteristics. These data indicate that treatment wetlands built on sandy or clayey soils have consistently lower outflow TON concentrations than wetlands built on organic soils. On treatment wetland soils saturated with sorbed ammonium nitrogen (from antecedent conditions including fertilization of orange groves or application of wastewater residuals), the release of this inorganic nitrogen form can be very significant.
The focus of this TM and the Consultant’s approach to the reduction of nitrogen in the Caloosahatchee River and Estuary appears to be to develop a theoretical process to convert BDON to RDON and to conduct basic research concerning this transformation. The proposed Atmospheric Pressure Photoionization-Electrospray Ionization – Mass Spectrometry (APPI-ESI-MS) fractional methods and the bioassay methods are in early stages of research/development and are experimental. With as many as 3,000 organic nitrogen compounds to evaluate using these methods, there is little possibility of cost effective and timely implementation of a treatment process to solve a long-standing problem of eutrophication in the Caloosahatchee River and Estuary. Loh (2008) concludes that DON released from the S-79 to the Caloosahatche Estuary is not susceptible to degradation by estuarine bacteria and that DIN is the most bioavailable form. The priority of this project and this review should be on determining which alternative technologies for TN removal are feasible, effective, and ready for implementation. Theoretical processes that will take considerable time and monetary investment to prove do not offer a realistic solution for the current problems in this aquatic ecosystem.

The nitrogen loading graphs provided by the Consultant should be better explained. Loading rate vs. removal rate graphs (Exhibits 10-12 in the TM) typically show high correlations due to the autocorrelation of the variables on the x and y-axes. The Consultant should emphasize the difference between the loading vs. concentration graphs presented in Exhibits 13, 14, and 18 and the three preceding graphs in the TM. The latter three graphs are more valuable as an empirical tool for assessing treatment wetland performance (Kadlec and Wallace 2008). It should also be noted that both axes in these loading vs. concentration graphs are plotted with logarithmic scales, de-emphasizing the actual variability of data between different treatment wetlands with highly different designs and antecedent soil conditions.
The point of this explanation is that the focus for this analysis should be on why some treatment wetlands attain very low organic N outflow concentrations compared to others that have higher C* values. Specific attention should be drawn to Exhibit 17 in the TM that shows the effluent organic N from STA 5 being between about 1 and 1.5 mg/L, compared to the more northern STAs that have outflow organic N concentrations above 2 mg/L, probably due to their differing antecedent soil properties. The discussion of the data presented in Exhibit 18 (North American Treatment Wetland Database v. 2) should focus on what is different/special about the wetlands with data points below 1 mg/L of organic nitrogen.

**Exhibit 2** provides a more detailed look at organic nitrogen loading vs. outflow concentration data from large-scale Florida wetland and aquatic systems. Of particular importance are the data from systems such as Orlando Iron Bridge, Titusville, and STAs 5 and 6 that indicate that wetlands constructed on sandy or clayey soils and dominated by emergent marsh vegetation consistently achieve TON concentrations less than 1.5 mg/L. The Consultant emphasizes the data collected from the Wellington Pilot study. The data collection period for this system only lasted about 15 months and ammonia nitrogen was not measured so it was not possible to calculate organic nitrogen by difference between total kjeldahl nitrogen (TKN) and NH₃. The Wellington cells were very small, flows were difficult to measure accurately, and the system data are probably more representative of start-up performance than long-term sustainable operation. While the Consultant’s conclusions concerning the effectiveness of constructed wetlands dominated by emergent and submerged aquatic plants are based on a very large data set derived from numerous projects many of which have long-term data sets, the use of the Wellington data set to summarily discard a periphyton-based stormwater treatment area technology (PSTA) and to promote the use of a Floating Aquatic Vegetation (FAV) technology is not sufficient to justify the investment of millions of dollars for additional research and demonstration. The Consultant is very aware of the much larger PSTA data set from the District’s own Advanced Treatment Technologies (ATT) project (CH2M HILL 2003). There are also extensive data sets available for FAV systems (e.g., the 30-acre Orlando Iron Bridge water hyacinth treatment system and more recent Hydromentia systems).

The three-year data set from the Everglades Nutrient Removal (ENR) project buffer cell that was dominated by water hyacinths is probably more useful than the Wellington data set, but it was still of comparatively short duration for a pond system. Unharvested water hyacinth ponds can accumulate considerable organic solids that result in an eventual feedback of DON to the water column following the first few years of operation.

**Exhibit 3** illustrates the percentile ranking of average monthly TON outflow concentrations reported for Florida treatment wetland and aquatic systems dominated by various plant communities and open water. This data analysis indicates that FAV systems do not always have the lowest TON outflow concentrations. In fact, a comparison of data from 1987 to 1999 for Lakeland Cell 4 (cattail-dominated) and Cell 7 (dominated by FAV for about 10 years during this period) indicate that a FAV-dominated cell may actually increase TN concentrations following emergent wetland cells (Cell 4 – TN = 1.33 mg/L and Cell 7 – TN = 1.71 mg/L). The point of this paragraph is that while the data analysis for emergent wetlands is fairly sound as a basis for recommending that technology, the basis for
recommending FAV as the most significant component of a treatment train is less substantiated.
There are additional concerns with a recommendation for an FAV system that limit the feasibility of this technology for consistent and cost effective removal of biologically available nitrogen. Inherent limitations for full-scale FAV treatment systems include the following:

- Water hyacinths are the most commonly used plant species in FAV systems and have a very long history of use (Kadlec and Knight 1996). This history indicates that they need to be harvested regularly to successfully function for consistent nutrient removal and to keep the plants healthy. Harvesting and disposal of water hyacinths is impractical and problematical on any but the smallest scale due to their very high moisture content and fast growth rates. Water hyacinth systems typically have highly anaerobic water conditions that are attractive for breeding of adapted nuisance mosquito species in the genus *Mansonii*. Water hyacinths are not tolerant of any frost and they are susceptible to failure due to cold weather. Water hyacinth systems are subject to herbivory by a suite of biological control agents introduced in Florida to eradicate the species and must be sprayed with insecticides to maintain high growth rates. Some water hyacinth systems have required fertilization with nitrogen and iron to achieve high growth rates in nutrient-poor environments. Water hyacinths are difficult to control in large open cells that are subject to appreciable wind fetch.

- FAV systems dominated by pennywort and water lettuce are possible but have some of the same limitations as those listed above for water hyacinths and have never been used on a large scale.

- Duckweed systems have been used for water quality treatment, but they are even more sensitive to wind effects than water hyacinths and water lettuce and require an expensive floating grid system when used in large ponds, and they have only been used effectively at relatively high nitrogen concentrations.

- The nitrogen-fixing water fern *Azolla* is likely to invade any FAV system with low available nitrogen, thus negating the perceived benefit of these systems as an alternative to nitrogen-fixing algal based treatment technologies.

The merits and applicability for using tussock, Soil Aquifer Treatment (SAT), and Riverbank Filtration (RBF) systems are not very convincing. For instance, there are no full-scale projects in Florida with long-term operational data for either of these “technologies”. Existing engineered “tussock” systems (e.g., beemats) are extremely small and have no relevant applications with long-term operational data sets. There are full-scale emergent treatment wetland cells that have been dominated by tussocks (e.g., ENR Cell 3 and Lakeland Cells 3 and 4), but data from these cells have not been specifically analyzed to defend the pursuit of this idea. One practical problem with a tussock vegetation community observed in both the ENR and at Lakeland is that it is highly unstable due to changing buoyancy of the supporting vegetation, which can cause a catastrophic failure with loss of the floating plant community and an inevitable shift to open water during and following storm events.

Soil Aquifer Treatment systems have been used throughout the western U.S. but more for general wastewater reclamation and groundwater recharge, rather than as high performance nitrogen removal systems. SAT systems have generally been judged to be
successful if they can lower nitrate in groundwater to the drinking water standard of 10 mg/L, which is about 200 times higher than the natural background in Florida’s ground and surface waters. I am not aware of any SAT systems specifically designed or optimized for removal of DON. There are no operating Riverbank Filtration systems in Florida that indicate either feasibility or cost effectiveness of this proposed technology.

There are some surface water-ground water studies previously conducted in Florida that might shed light on the effectiveness of filtering surface water with elevated organic nitrogen concentrations through natural soils (e.g., the C-43 and C-44 Storage Reservoir Test Cell projects funded by the District). Water quality studies conducted at the Storage Reservoir Test Cells constructed at C-43 indicate that the TN declined from 1.05 to 0.76 mg/L and TON declined from 0.91 to 0.67 mg/L from the Test Cell to the adjacent Seepage Canal (WSI 2007a). At the C-44 Reservoir Test Cells, TN declined from 0.87 to 0.61 mg/L and TON declined from 0.76 to 0.48 mg/L from the Test Cells to the adjacent Seepage Canals (WSI 2007b). Although promising from a concentration standpoint, Soil Aquifer Treatment or Riverbank Filtration technologies are likely to be hydraulically limited and impractical on a large scale.

While additional data analysis and testing of tussocks, SAT, and RBF systems may be warranted, it is the opinion of this Reviewer that such testing may not be able to provide a defensible alternative for solving the nutrient problems in the Caloosahatchee River and Estuary within a realistic time frame or a reasonable research & development cost.

Summary and Conclusions

It is the Reviewer’s conclusion that some of findings and recommendations provided in this TM are not supported by adequate evidence:

- The Consultant concludes that particulate organic nitrogen is not important for project planning and design. The proven ability of emergent treatment wetlands to capture particulate nitrogen (especially algal solids) provides an opportunity to utilize their enzymatic systems to trap otherwise unavailable nitrogen before it can move downstream to the estuary;

- The Consultant concludes that the use of experimental and expensive methods for assessing nitrogen recalcitrance is justified for this project due to the perceived need to open the “green box” of these natural treatment systems so we can better manipulate their internal processes. Based on the immediate need to develop an applied solution to a real water quality problem, dependence on highly theoretical and experimental technologies will not be the most cost effective approach to success. For the timely success of this project it is important to focus on technologies and knowledge that are well established and have been proven elsewhere. Research and experimentation can continue in parallel with implementation of the best available technology but should not become a bottleneck that slows restoration of the water quality in the Caloosahatchee River;

- The Consultant concludes that PSTA and emergent wetland systems add DON and that only floating aquatic plant systems remove it. These conclusions do not appear to be supported by data;
• The Consultant concludes that only treatment systems that have a light limited water column (shaded) can remove significant DON concentrations. This conclusion is not supported by an adequate review of existing data.

• The Consultant concludes that FAV and tussock-dominated treatment technologies should be highly ranked as being proven and cost effective. These alternative natural treatment systems are based on plant groups that do not have a long track record or have not been shown to be feasible on a large scale;

• The Consultant concludes that SAT and RBF systems may be an important component of a test and demonstration project. There does not appear to be data provided from comparable systems that support this conclusion;

The following important considerations were omitted in the Consultant’s report:

• The conceptual model for organic nitrogen processing in the proposed treatment train appears to not consider the important feedbacks from site soil and nutrient release due to antecedent soil conditions;

• The Consultant’s TM should provide a preliminary estimate of the size and cost of a WQTA needed to meet the TMDL requirements for the Caloosahatchee Estuary. There will be significant costs associated with the TMDL goal of removing up to 23% of the total upstream nitrogen load at S-79 considering the very high seasonal flows in the C-43 Canal. For example, the average C-43 flow at S-78 is about 940 cfs (607 MGD) and maximum recorded flows are about 9,720 cfs (6,300 MGD). It is important at this preliminary stage in the implementation process to have some idea of the approximate land area requirements and present worth costs.

Based on this review and the Reviewer’s experience, the supportable conclusions from the Consultant’s report are:

• Emergent wetlands are highly reliable and relatively cost effective at reducing all forms of biologically available nitrogen in surface waters to levels that appear to be highly recalcitrant and do not stimulate further biological activity over a reasonable half life; and

• Conventional technologies that are technically proven for high-level nitrogen removal are not as cost effective as an emergent wetland technology operating with the benefit of natural energies.

This Reviewer presents these additional conclusions based on prior experience with a number of similar evaluations of nutrient-removal technologies in Florida:

• Existing relevant information and a careful consideration of the need for an effective and rapid implementation of nitrogen removal in C-43 justifies installation of a large-scale demonstration project with a variety of natural treatment alternatives (emergent wetlands, submerged aquatic plants, floating aquatic vegetation, and algal-dominated treatment systems). No replication is suggested due to the size of each cell in the demonstration project (about 10 to 40 acres each) and these cells should be designed with the flexibility to incorporate them into the eventual full-scale WQTA facility;
Theoretical/experimental approaches to removal of nitrogen and speciation of BDON and RDON will not be productive if they are in the critical path for project implementation. The focus of this report should be on the demonstrated effective technologies and not on approaches that have not been proven effective under similar circumstances. The primary goal of the C-43 WQTA Project should be on optimization of design parameters for constructed wetlands. This conclusion is discussed in more detail under a latter section of this report, but a preliminary list of design issues that might benefit from optimization studies are: the effectiveness of various mixes of wetland/aquatic plants, the effects of water depth on these plant communities and their nitrogen removal kinetics, the effects of antecedent soil conditions on the C* for DON, and the effects of hydraulic residence time on system performance.

Organic Nitrogen Methodology Screening Analysis (Deliverable 3.2.1 Draft, November 2009)

General Comments
The results of the organic nitrogen screening analyses are interesting from a scientific viewpoint and could generate considerable discussion. From a practical standpoint of implementing nitrogen control in the Caloosahatchee Estuary, these findings are not likely to provide a timely solution or a ‘silver bullet’ approach to solve an existing water quality crisis. For these reasons, this review does not include a detailed critique of the methods and findings of these analyses.

The Consultant continues with the assumption that “…treatment of DON must transform BDON to RDON…” and that “…the DON removal process needs to be based on an understanding of the compounds to be treated…” The Reviewer does not agree with these statements. There are many examples of limited scientific understanding of processes used every day in the pollution control industry. Examples include hundreds of constructed treatment wetlands used by municipalities, industries, agricultural and industrial interests for reduction of nutrient levels to meet permit limits. The Everglades STAs are an example of how the use of wetlands for phosphorus removal has advanced without a clear understanding of many of the detailed process-level transformations of inorganic and organic phosphorus compounds. It is this Reviewer’s opinion that not all of the internal workings of natural treatment systems (and for that matter, conventional treatment systems that rely on biological communities of microbes) will ever be completely understood nor need to be understood to use these systems for highly reliable pollution control. This does not preclude detailed research of the processes to help optimize the performance of these systems. The point is that a “green box” can be relied upon for consistent nutrient removal as long as we have an adequate understanding of how to control the major external forcing functions that affect its performance.

Based on data presented in this report and by others (Knight and Steele 2005), it is clear that organic nitrogen can be degraded/assimilated in the C-43 Canal environment. This is a notable but unmentioned conclusion from the Consultant’s observation that more of the DON is recalcitrant in C-43 during the dry season than in the wet season. Based on the existing seasonal comparisons it appears that this in situ degradation is significant and should be better understood to optimize the natural attenuation occurring in the canal.
environment. This may well be the most cost effective nitrogen removal that will occur in this basin other than source control (the ultimate solution for much of the problem).

The Virginia Institute of Marine Science (VIMS) team states categorically that the high variability in the bioassay results is due to variation in the field duplicates rather than to variability in the analytical methods. Given a personal knowledge of collecting field duplicates in the C-43 and tributary canals, this statement does not seem probable. Perhaps the Consultant should better explain why this conclusion was accepted and repeated elsewhere in this series of reports.

The VIMS bioassay method had other problems as well. For example there was only 8 to 10% removal of DON in the assays at 120 hours incubation time. This finding does not indicate that there is a highly significant effect of recalcitrant nitrogen forms in a saline estuarine environment. The other apparent problem with the bioassay procedure was the observation that the total amount of DON increased in many of the tests. It was stated in the report that these increases were likely due to nitrogen fixation by the inoculums or death and lysis of the algal/bacterial cells. Both of these possible complications can be expected to result in additional cell growth that is not based on the DON in the original water sample. Perhaps this is a partial explanation of the relatively high variability observed in the results.

The bioassays only measure chlorophyll and its degradation products (total pigments), rather than the entire inoculated community of algae and bacteria. Perhaps it would be better to use total biomass rather than total pigments. A fundamental question is why didn’t the Consultant use the readily available Algal Growth Potential (AGP) test for measuring nitrogen bioavailability in these samples? This test is widely used and while not very useful for accurately predicting the stimulatory effects of nutrients on complex natural algal systems, it is already developed and has over 40 years of data to aid with interpretation of results. At a minimum the Consultant and their VIMS team should have compared the results of the two tests on these samples.

This memo offers the statement that “…shallow STAs increase DON because they are ideal for nitrogen fixing cyanophytes…” Based on this Reviewer’s experience most wetland treatment systems increase the concentration of RDON to some extent due to releases from organic soils and plant detritus. Certainly nitrogen fixation is also possible but emergent wetlands generally have relatively small plant biomass in the form of blue green algae. On the other hand, emergent wetlands are extraordinarily rich in aerobic and anaerobic bacteria.

Summary and Conclusions

It is this Reviewer’s conclusion that some of the findings and recommendations provided in this TM are not supported by adequate evidence:

- The Consultant concludes that based on the literature the removal of DON on a concentration basis is not possible. On the contrary, evidence from dozens of full-scale treatment wetlands shows that various fractions of DON are highly susceptible to removal in emergent wetlands;

- The Consultant concludes that the transformation of BDON to RDON is the only way to reduce the DON that is stimulating red tide blooms. While I personally agree
that red tide blooms are increasing as a result of anthropogenic nitrogen loads, that conclusion is controversial (K.A. Steidenger, personal communication). More importantly for this evaluation, this Reviewer does not agree with this statement since it ignores the array of BON compounds that are mineralized to ammonia in natural treatment systems and in the Caloosahatchee River and subsequently used by the ecosystem.

- The Consultant makes the assumptions that an effective natural treatment system for conversion of BDON to RDON will have to be aerobic and require dominance by floating aquatic vegetation. However, conversion of DON to inorganic nitrogen forms occurs under both aerobic and anaerobic conditions (Kadlec and Wallace 2008). FAV dominated systems do not have an adequate track record of successful management and performance to justify this proposed reliance. FAV systems also typically overlie anaerobic waters due to the very high organic decomposition that they create through high plant productivity. Experience to-date from a variety of natural treatment systems indicates that this low oxygen environment cannot be aerated effectively either by upstream high oxygen pond environments or by artificial reaeration on a practical scale.

This Reviewer concludes that other more generally available, affordable, and consistent surrogates of DON bioavailability/recalcitrance are needed. Examples include: the use of color measurements and the use of analytical procedures that hydrolyze urea and other easily degraded forms of organic nitrogen. The Consultant’s suggested reliance on highly theoretical/experimental and costly analytical procedures for measuring the quantity and effects of DON appear to this Reviewer to be impractical.

**Findings Memorandum (Deliverable 3.1.2 Final, December 2009)**

**General Comments**

It is interesting to note that the FAV sampling stations have some of the highest TN and DON concentrations observed. This finding seems to contradict the Consultant’s conclusion that FAV systems are the most capable of reducing DON concentrations.

This document states that at the C-43 Canal sampling stations nearly all of the nitrogen is in the organic form and that the levels of DIN and NH₃ are an order of magnitude lower than the DON and therefore negligible. This conclusion is a slight exaggeration of the data reported. The maximum DIN concentration in each data set varied between 0.35 and 0.65 mg/L. The mean DIN concentrations varied between 0.186 and 0.294 mg/L. The mean DIN in these samples ranges up to more than 20% of the TN at the C-43 Canal stations. Between the removal of DIN and particulate N in these samples it may be possible to approach the TMDL requirement of 23% reduction in TN, especially during those times when a significant fraction of the DON is susceptible to ammonification.

**Water Quality Treatment Area Test Facility Parameter Plan (January 2010)**

**General Comments**

This document is intended to provide the details of the proposed two-year sampling plan for the WQTA test facility. It focuses attention on the DON compound-specific and bioassay
procedures and provides little discussion of the rest of the parameters. The plan should provide a summary of the total number of samples to be collected and the associated costs of the plan.

The plan recommends a significant use of resources for monitoring chlorophyll in situ in the treatment cells by use of fluorometers. This sampling component may not be of much use since most of these cells are supposed to be covered by some form of floating or emergent vegetation that will shade out planktonic algae.

**Reviewer Conclusions**

This parameter plan is insufficient to provide a basis for sampling at the proposed WQTA test facility. Recommended changes include the following:

- This plan should describe the justification for all recommended sample parameters as well as for the sampling frequency and locations of sampling stations.
- A detailed list of parameters, numbers of samples collected, and estimated costs associated with labor, equipment, and laboratory analysis should be included.

**C-43 Water Quality Treatment Area Draft Conceptual Plan Technical Memorandum (Deliverable 4.2.8 Draft, March 2010)**

**General Comments**

Many of the comments provided above are relevant to this TM also. A brief list of relevant comments follows:

- There is no “rationale” for the design and components of the Test Facility as required by the Consultant’s scope of work;
- The focus of the proposed WQTA Test Facility should be on optimizing the use of natural treatment technologies for reduction of bioavailable nitrogen and not on TN or organic nitrogen;
- If DON is at C*, then BDON must convert to RDON without going through an inorganic nitrogen form. There is no known process that converts biologically available organic nitrogen directly to recalcitrant organic nitrogen;
- The proposed test facility should include an evaluation of the effects of soil type and nutrient-loading history on NTS nitrogen removal performance. The proposed mesocosms should also include soils or they will not be representative of any full-scale NTS project. The proposed Test Cells and Demonstration cell should include an evaluation of legacy soil nutrient conditions to be able to accurately evaluate start up and long-term release/sequestration of nitrogen and phosphorus;
- Data from existing full-scale constructed wetlands that are able to achieve low TN and organic N concentrations should be completely evaluated to provide design criteria needed for success of this Test Cell project. Phosphorus removal data from NTS should be thoroughly evaluated and considered in design and operation of this facility;
Will the operators of the Test Facility need to be concerned about controlling populations of nitrogen fixers? The water fern *Azolla* is likely to invade the proposed FAV and emergent wetland cells;

The Consultant states that an “open slough-type cell” will provide passive aeration to elevate dissolved oxygen concentrations in downstream cells. This is not likely to work (consider low DO concentrations in Everglades slough plant communities receiving elevated nutrient levels);

This plan needs a better description of the methods that will be used for data analysis. For example, will tracer tests be conducted to provide an understanding of the actual cell hydraulics? Will $k$, $C^*$, and theta values be estimated from the data?

Section 8 should provide a justification for the need for additional geotechnical work or for the use of FSU and VIMS for analytical services;

This plan should provide preliminary estimates of the operation and monitoring costs for the project and undefined “research into nitrogen removal processes”.

**Task 2 – Guidance on C-43 WQTA Test Facility Design**

**Promising Approaches to Removal of Total Nitrogen**

This Reviewer has considerable experience with the use of natural treatment systems for nitrogen control, including all major types of engineered wetlands and ponds. This experience indicates that wetland treatment systems are generally highly superior to pond-based systems. The diversity and rates of processes in shallow-water wetland environments are significantly greater than similar processes in pond systems. Nitrogen removal rates are typically several times greater in vegetated treatment systems than in algal dominated ponds.

The Reviewer’s experience also indicates that not all vegetated wetland systems are equal in their effectiveness for nitrogen removal. Some wetland systems have faster nitrogen removal kinetics than others. Some wetland plant communities require considerably less management than others and as a direct result are more cost effective for large-scale nitrogen removal project implementation. Some wetland and algal-based nutrient removal technologies are more dependent than others on the use of fossil fuel energies and complex engineering, construction, and operations. These differences all factor into the comparison of present worth cost of different alternatives.

The considerations described above result in a range of cost/benefit ratios for different natural treatment system alternatives. The preferred method for ranking alternatives from most preferred to least preferred is to provide realistic estimates of performance and present worth cost and to compare the ratio of the estimated pounds of nitrogen removed per dollar. Detailed spreadsheets have been prepared previously to evaluate nutrient treatment alternatives throughout the District (e.g., the Water Quality Treatment Technology Ranking method, WSI 2006). The design of the C-43 WQTA Test Facility and the full-scale project design should be based on such an analysis.
In general, this Reviewer’s experience indicates the following ranking (from most cost effective to least) of natural treatment system alternatives in terms of the amount of nitrogen that can be consistently removed over an extended project life (e.g., 50 years):

- Emergent macrophyte dominated constructed wetland cells;
- Ponds dominated by a mix of floating and submerged aquatic vegetation without harvesting;
- Ponds dominated by algae;
- Harvested FAV ponds;
- Algal turf scrubber systems; and
- Subsurface flow wetlands consisting of vertical and horizontal flow through gravel substrates.

Of these potential natural treatment technologies this Reviewer concludes that only the first two are promising enough to warrant study/demonstration at the C-43 WQTA Test Facility. This conclusion is essentially in agreement with the Consultant’s recommendations.

In addition to these natural treatment technologies, this Reviewer is also aware of more highly engineered technologies for nitrogen removal that are more dependent upon external inputs of energy and chemicals. Examples include extended aeration activated sludge processes, biological nutrient removal, coagulation technologies, and reverse osmosis. This Reviewer agrees with the District’s Consultant that none of these “conventional” technologies are viable for the C-43 project due to cost considerations.

This Reviewer is also peripherally aware of the SAT and RBF technologies proposed by the District’s Consultant for the C-43 Project. It is this Reviewer’s understanding that these technologies are used primarily for groundwater recharge. Nitrogen removal is secondary and is usually based on meeting the nitrate drinking water criterion. It is this Reviewer’s conclusion that the design basis, hydraulics, water quality performance, ancillary benefits, and costs of these technologies are not currently developed to the point of consideration needed for implementation of the C-43 Project.

**Recommended Test Facility Changes to Evaluate Most Promising Approaches**

The following recommendations for the C-43 WQTA Test Facility are offered:

- The focus of the Test Facility should be the development of the most optimal design criteria for full-scale implementation of a natural treatment system for reduction of TN on the C-43 Canal;
- The only natural treatment technologies that are currently developed to the point of serious consideration at the Test Facility are constructed wetlands dominated by emergent, submerged, and/or floating plants;
- Testing/demonstration at the proposed C-43 Test Facility is most needed for developing a more precise understanding of the benefits of various plant
combinations, effects of water depth, hydraulic loading rates, and antecedent soil conditions on nitrogen removal;

- The Test Facility should be designed in such a way that it can be flexible in controlling the above-listed variables of water depth, loading rates, and substrates and ultimately be integrated into the full-scale project implementation at this site;

- Mesocosms, if used at all, should only be used for looking at processes related to substrate and plant community effects on release and uptake of nitrogen but not for assessing full-scale design criteria such as hydraulic loading rates, vegetation establishment techniques, or expected system performance;

- Test cells should be large enough (about 10 to 40 acres each) to eliminate edge effects and to provide a realistic plant establishment experience, but should not be used for replicated experiments. They should be used for demonstration of the effects of differing water depths, hydraulic loading rates, plant communities, and cell-in-series effects on sustainable nitrogen and phosphorus removal. The number of Test Cells should be based on the number of distinctly different natural treatment technologies supported by an updated summary of feasible alternatives.

- A larger Demonstration Cell/Treatment Train should not be put into operation until preliminary design criteria optimization is complete from the proposed Test Cells. The Demonstration Cell(s) or Treatment Train should be constructed in parallel with the monitoring/optimization work in the Test Cells. The Demonstration Cell(s) will be comparable to the ENR in STA-1W and will provide lessons in full-scale project implementation.

- Monitoring should be limited to well understood parameters and should not be dependent upon experimental techniques that are not fully developed or costly;

- A complete Monitoring and Sampling Plan should be prepared that fully describes the work to be accomplished, the schedule for that work, and the estimated cost for implementation.

**Task 3 - Recommendations for Parallel Work Efforts to Improve the Information Derived from the Test Facility**

The following recommendations are offered for parallel work efforts while the Test Facility Design and Monitoring Plan are completed:

- Review additional and updated relevant data sets for nitrogen dynamics in Florida wetland and reservoir systems (e.g., Iron Bridge, Lakeland, C-43 and C-44 Reservoir Test Cells, Lake Apopka, Lake Griffin Flow Way, Taylor Creek STA, Ten Mile Creek Reservoir and STA, Everglades STAs, PSTA systems, ENR Test Cells, etc.). Focus analysis on systems with low organic nitrogen in outflows and on the range of vegetation, water depth, and antecedent substrate effects. Calibrate P-k-C* model for each system and for each substrate and vegetation type and/or develop a DMSTA-type model for predicting nitrogen transformations and removal under dynamic operating conditions;
• Continue work to develop reliable and cost effective surrogates for fractionation of DON into biologically available and unavailable forms. Candidate tests include the AGP test, dissolved color, and measurement of hydrolysable DON;

• Based on existing treatment wetland calibration data prepare a preliminary conceptual performance model and design for a full-scale WQTA facility to meet the TMDL for the Caloosahatchee Estuary. Utilize the model to optimize the costs and benefits of load reduction in the watershed vs. end of pipe treatment with constructed wetlands;

• Coordinate the functionality of the proposed C-43 West Storage Reservoir and the C-43 WQTA and consider a trade-off in area of the reservoir and the ultimate full-scale nitrogen treatment wetland. This concept of coordinating the projects is worthy since relatively small gains in N removal that could be achieved in the WQTA could be undone if the Storage Reservoir exports N. The test cell data set was too short to state that N removal is consistently positive in deep reservoirs. During the one-year study period, there was no natural development of a floating plant cover, algal solids increased, but associated organic N and TN decreased slightly (about 10%) (WSI 2007a).

References


Appendix D

C-43 Water Quality Treatment Area – Expert Panel Power Point Presentations, July 12, 2010
C-43 Water Quality Treatment Technical Review Panel Workshop

Robert L. Knight, Ph.D.
Wetland Solutions, Inc.

July 12, 2010

C-43 WQTA Review Panel Workshop

Tasks

- Review and Evaluate Consultant Deliverables
- Provide Guidance on C-43 WQTA Test Facility Design and Operations
- Participate at Workshop with District and County Staff and prepare final report
Total N Reduction Technologies (April 2008)

- Proposed project should include both N and P
- Focus should be shifted to “biologically available N” (e.g., total inorganic and organic N that will degrade in the water environment in 30 days)

Organic N model needs to consider the important contributions from antecedent soil conditions and translocation by rooted plants

Treatment wetlands with organic soils have a higher $C^{*}_{TN}$ than sites with sandy and clayey soils
Effect of Soil Type on Organic N

Based on data from 26 Florida sites

<table>
<thead>
<tr>
<th>Soil Type</th>
<th>10%</th>
<th>25%</th>
<th>50%</th>
<th>75%</th>
<th>90%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clay</td>
<td>0.62</td>
<td>0.25</td>
<td>0.47</td>
<td>0.75</td>
<td>0.90</td>
</tr>
<tr>
<td>Peat</td>
<td>1.43</td>
<td>1.72</td>
<td>2.04</td>
<td>2.41</td>
<td>2.78</td>
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<td>Sand</td>
<td>0.58</td>
<td>0.67</td>
<td>0.85</td>
<td>1.38</td>
<td>1.85</td>
</tr>
</tbody>
</table>

Total N Reduction Technologies (April 2008)

- Review of emergent wetland data is good but not comprehensive for Florida projects
- Analysis should focus on systems with low organic N outflow concentrations
Focus on Low Org N Outflow Data

Wellington Pilot project data may be misleading due to small scale and short duration

Need to evaluate long-term FAV datasets

Florida data do not support Consultant’s conclusions about FAV
Lower TON from Emergent and Open Water

- FAV systems are difficult to manage:
  - Require harvesting and disposal for high N removal
  - Have low DO and high TSS
  - Susceptible to pests and frost
  - Easily moved by wind and high flows
- No published information on FAV-tussock combination systems
Total N Reduction Technologies (April 2008)

- Soil Aquifer Treatment and Riverbank Infiltration are not proven
- There are some applicable data sets that could be evaluated to indicate if SAT/RBF is feasible
- Would have hydraulic limitations

<table>
<thead>
<tr>
<th>Study Site</th>
<th>TN (mg/L)</th>
<th>NOx-N (mg/L)</th>
<th>TKN (mg/L)</th>
<th>NH3-N (mg/L)</th>
<th>TON (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head Cell</td>
<td>1.22</td>
<td>0.19</td>
<td>1.03</td>
<td>0.05</td>
<td>0.98</td>
</tr>
<tr>
<td>Test Cell</td>
<td>1.05</td>
<td>0.11</td>
<td>0.94</td>
<td>0.03</td>
<td>0.91</td>
</tr>
<tr>
<td>Seepage Canal</td>
<td>0.76</td>
<td>0.07</td>
<td>0.70</td>
<td>0.02</td>
<td>0.67</td>
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<tr>
<td>Head Cell</td>
<td>0.90</td>
<td>0.03</td>
<td>0.88</td>
<td>0.12</td>
<td>0.76</td>
</tr>
<tr>
<td>Test Cell</td>
<td>0.87</td>
<td>0.01</td>
<td>0.86</td>
<td>0.11</td>
<td>0.76</td>
</tr>
<tr>
<td>Seepage Canal</td>
<td>0.61</td>
<td>0.02</td>
<td>0.58</td>
<td>0.11</td>
<td>0.48</td>
</tr>
</tbody>
</table>
Complex and experimental methods for characterization and bioassay are not expedient or cost effective.
Should emphasize existing methods (chemical analysis and AGP tests).
Can rely on “green box” approach.

Note that FAV sampling stations have some of the highest TN and DON concentrations.
A significant fraction (up to 20%) of the TN in C-43 is biologically available.
Organic N concentration decreases downstream in C-43.
C-43 WQTA Review Panel Workshop


Test Facility Parameter Plan
(January 2010)

- Need better justification for selection of parameters, frequency, and locations
- Summary of sampling plan is needed as well as estimated costs
WQTA Test Facility Plan (March 2010)

- Rationale for components and design of the Test Facility is insufficient
- Test Facility should evaluate the effects of antecedent soil conditions on NTS performance

Need to better describe proposed methods for data analysis
- Will tracer tests be conducted to evaluate hydraulic efficiencies?
- Should provide estimated costs for monitoring and data analysis
Guidance on WQTA Test Facility Design

- In general, wetland treatment systems are superior to pond systems
- SAT and RBF are not demonstrated technologies for achieving low TN levels
- NTS are likely to be superior to conventional technologies in terms of reliable performance and cost

Reviewer's ranking for NTS for TN reduction:
1. Emergent macrophyte wetlands
2. Ponds dominated by floating and submerged vegetation (no harvesting)

Optimal configuration to be demonstrated
Other proposals are not realistic
Test Facility Design Changes

- Test Cells - four at 20 acres each
  - EMG wetland shallow (15 – 30 cm)
  - EMG wetland deep (30 – 60 cm)
  - FAV/SAV pond (90 – 150 cm)
  - Pond/marsh combination
- Operate over a range of HLRs from 1 – 6 cm/ d

Mesocosms should only be used for process-level evaluation:
- Effect of soil type and antecedent fertilization practices
- Release and fractionation of DON from a variety of plant communities
Test Facility Design Changes

- Demonstration Facility should be designed based on results of Test Cell monitoring (minimum of one to two years of post-startup data)
- Comparable to ENR - should include only the optimal design and should be large enough to provide full-scale verification

Suggested Parallel Work Efforts

- Update and expand review of existing NTS facility data with focus on systems with low organic N in outflows
- Calibrate wetland P-k-C* model for N and develop dynamic N transformation model
Suggested Parallel Work Efforts

- Continue work to develop a reliable and cost effective test for biologically available organic N
- Prepare a preliminary design for full-scale project implementation to estimate land area requirements, cost, and coordination with C-43 West Storage Reservoir project
Review of wetlands-based Total Nitrogen removal techniques for the South Florida Water Management District

Alex Horne
Professor Emeritus, Ecological Engineering
Dept. Civil & Environmental Engineering
University of California, Berkeley
Summary

- CH2M & other consultants have done a good job of:
  - Defining a problem of degradation of DON in the regional context
  - Suggesting solutions using NTS
Concerns remaining are:

• Only 30% maximum average TN reduction with FAV as the main NTS
• N-limitation assumptions leading to a likely average of ~ 20% TN reduction
• Lack of role of UV light, so need for dark (to prevent N$_2$-fixation by BGA) leading to FAV as the main NTS recommendation
• Bioassay for DON availability
Possible solutions

• Reconsider N-limitation in District’s waters versus target Caloosahatchee estuarine waters
• If needed design a denitrification wetland to proceed proposed NTS wetland
• Graph bioassay in more conventional way?
Details: The Problem

- The complex chemistry & bioavailability of DON in aquatic ecosystems is not reflected in the TMDL regulations faced by the District because all N-species are lumped as TN.
- A lesser level of knowledge may suffice to solve the District’s TMDL concerns.
- Reports from CH2M & others have made a commendable job of summarizing the old information & creating new knowledge about DON that is an essential step to removing it in Natural Treatment Systems.
CH2M solutions 1

• Convert a fraction (15-30%) of wet season labile DON to TIN (where TIN $\rightarrow N_2$) since most RDON not bioavailable over the short time periods of algal blooms.

• Current TMDL assumes all TN is bioavailable. That may be true, at least in part, for the different chemical conditions of the Caloosahatchee Estuary.
### N-limitation: District vs Estuary

<table>
<thead>
<tr>
<th>Site</th>
<th>DON</th>
<th>DIN</th>
<th>NO3 + NO2</th>
<th>NH4</th>
</tr>
</thead>
<tbody>
<tr>
<td>District, dry (April-May 2008)</td>
<td>2,100</td>
<td>242</td>
<td>113</td>
<td>128</td>
</tr>
<tr>
<td>District, wet (June-July, 2008)</td>
<td>1,850</td>
<td>205</td>
<td>85</td>
<td>120</td>
</tr>
<tr>
<td>Caloosahatchee Estuary, means for growth season (May-August 2007)</td>
<td>80</td>
<td>49</td>
<td>45</td>
<td>3</td>
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<tr>
<td>Caloosahatchee Estuary, uppermost station #1 mean for growth season (May-August 2007)</td>
<td>131</td>
<td>88</td>
<td>82</td>
<td>3.5</td>
</tr>
<tr>
<td>Caloosahatchee Estuary, seaward station #4 mean for growth season (May-August 2007)</td>
<td>50</td>
<td>23</td>
<td>21</td>
<td>2</td>
</tr>
</tbody>
</table>
N-limitation in the Caloosahatchee Estuary but not the District’s Waters

- Inorganic-N (TIN or DIN) in the CH2M samples never fell to what I consider N-limiting levels (< 150 ug/L). There was no pressure for the wetland bacteria to break down DON for its N-content.
- In contrast, phytoplankton in the target area the Caloosahatchee Estuary is (potentially) growth-limited by N as shown in the recent experimental work by Loh. The TIN concentrations also reported by Loh during the estuary growth season are less (mean ~ 70 ug/L) and sometimes very much less (< 5 ug/L) than those I used mentioned above as likely N-limiting in the natural environment (< 150 ug/L) or that were present in the District’s waters.
- In the turbid waters of the Caloosahatchee Estuary, light may be the normal growth-limiting factor rather than nutrients. This might be checked before assuming N is the driving force for eutrophication in the estuary.
Assays

• **Question # 2 Task 2.2.** Did the assays (salinity release, photolysis, bioavailability) presented in the Organic Nitrogen Methodology Screening Analysis adequately quantify the fraction of the DON pool that could become available to bacteria and algae?

• **Answer # 2.** I think so but I am not sure. I would have expected a conventional bioassay result presentation. It appears from Exhibit # 14 that there were 200% increases in chlorophyll in the wet season with most site and smaller increases in the dry season. However, no control data was shown and linking the individual data points from all the sites as if they were a continuum on the x-axis seems non standard practice.
Better bioassay diagram?

- Typical bioassay for single stations used in most biology
- Easier to read actual results than Exhibit 14 which suggests incorrectly (?) that the stations are a continuous array
Use control charts?

Percent Over Control

Treatment
CH2M solutions 2

• Experiments made by CH2M also show that the proposed FAV solution will at best reduce TN by 30% and require the use of a new type of floating treatment wetland.
• Floating wetlands have been used for many years but have a shaky record for pollution control and have not been used for DON removal.
• Overall reduction in TN in full scale wetlands may be lower than that measured in the small test cells where conditions are easier to impose.
CH2M solutions 3

• The CH2M position is that if wetlands treatment releases only refractory DON the eutrophication problem is resolved.
• Although true, the concept will be a difficult but not impossible sell to regulators who worry that what is refractory to one alga may be less so to another.
## My attempt at wetland comparisons

<table>
<thead>
<tr>
<th>Wetland</th>
<th>% DON removal</th>
<th>TIN removal</th>
<th>Average 1= best</th>
<th>Overall rank</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Single unit cells</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FAV normal</td>
<td>47, 12 x=30 (1)</td>
<td>2</td>
<td>1.5</td>
<td>3</td>
</tr>
<tr>
<td>Tall, dense EAV</td>
<td>24, 1.5 x=13 (1.5)</td>
<td>1</td>
<td>1.25</td>
<td>2</td>
</tr>
<tr>
<td>SAV</td>
<td>29 (partial) (1)</td>
<td>1.5</td>
<td>1.25</td>
<td>2</td>
</tr>
<tr>
<td>Sum % removal</td>
<td>47,22 x=37 (1)</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><strong>Combinations</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FAV + EAV</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>FAV + SAV</td>
<td>1</td>
<td>2</td>
<td>1.5</td>
<td>3</td>
</tr>
<tr>
<td>EAV + SAV</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>
ΣDON breakdown

• The ideal solution is reducing most TN by breaking down refractory DON. This will be easier for regulators to accept than a change in chemical state from labile to refractory DON.

• There is still some room to pursue the Holy Grail for direct TN reduction in wetlands by testing to see how to speed up the reaction RDON \( \rightarrow \) LDON \( \rightarrow \) DIN \( \rightarrow \) N\(_2\).

• The reaction may involve light & initial TIN scrubbing before FAV which is not part of the current plan for C-43. I recommend that this be followed up before committing all efforts to the floating wetlands concept, good idea though that may eventually prove to be.
UV experiments

• A surprising finding was the lack of effect of UV light on DON breakdown. The short, single experiment may not have covered all the conditions typical of DON in the District’s waters or the Caloosahatchee Estuary.

• Degradation time of DON-DOC was reported at days to weeks in the Estuary (Loh) so one would expect some breakdown of RDON in the wetlands with intense UV found on sunny days in Florida.

• Different kinds of UV experiments should be repeated, with a different set of circumstances to determine their applicability. The normal path of breakdown of RDON is likely to involve photo-degradation of some form so this avenue is worth pursuing in the C-43 experiments as a route to attacking the 70% of DON that apparently resists degradation by FAV or the tested design of an EAV.
RDON breakdown: wetlands v estuary 1

• Loh assumes but does not fully prove cycling rates of overall DON-DOC in the Estuary are days to months and more precisely 1-2 weeks. If so then the same processes should be replicable in the C-43 tests.

• The lesson for the District is that DIN may need reduction to lower levels before RDON degradation with a FAV cell can be contemplated.

• Use of a good denitrifying EAV cell with ample labile organic-C in the vegetation (e.g. cattails- *Typha*) as the first cell in the treatment train should be tested in the C-43 pilot work.
RDON breakdown: wetlands v estuary 2

- A TIN goal of < 70 ug/L, mostly as nitrate is suggested. How to convert the large ammonia fraction of the District’s water TIN to nitrate with the time and space available is not clear.
- In addition, if the bacteria were breaking down DON for its carbon energy, rather than N, then C-limitation would provide the driver to getting more RDON removed.
- Bacterial metabolism in wetlands is usually C-limited in the warm season and temperature limited otherwise. Given the rainy season in the summer as in Florida it is not clear to me which season should be C-limited.
Reduced N concentrations

• There is a surprising (to me) amount of reduced or semi-reduced compounds (ammonia, nitrite & perhaps some DON) in the District’s waters. In contrast water in the Caloosahatchee Estuary is dominated by nitrate, the most oxidized form of N. The TIN in these Florida samples was made up of an unusually high amount of ammonia and nitrite relative to ammonia - a situation I have not tested for N-limitation. In most open waters with reasonably high algal biomass, the oxygen produced by photosynthesis keeps most DIN as nitrate. The large amount of reduced soils in the STA wetlands, canal bottoms and perhaps BOD in the Lake Okeechobee outflow may account for this problem.
RDON breakdown: wetlands v estuary 3

• The every-present humic acids must reduce photosynthetic oxygen production and may be the reason for the persistence of these reduced TIN species.

• I think DIN reduction is needed to spur degradation of RDON in the FAV or other wetland. It is not clear how to increase oxygenated forms of N for such a large scale or at the C-43 site scale. Options are discussed in the response to question Task 3
The main concern

• The CH2M route leads to FAV by a logical progression that RDON degradation requires darkness (= absence of N₂-fixing algae). The net reduction of ΣDON (= most TN) is so far lowish (max. 30%).

• My examination of the data now available suggests that the alternative route via photodegradation &/or RDON use at low TIN levels (> ~70 ug/L) requires light.

• Both FAV, EAV & SAV may be used to give both light and dark reactions but both conditions needs clarification before the tests at C-43 are carried out.
Total Nitrogen treatment has been most successful for inorganic N and particulate species.

DON for Nitrogen, much as DOP is for Phosphorus, is the more difficult fraction to remove.

In simple amino acids, the N is available to a simple de-amination but for ring structures, double bonds, the removal of N is more energetically difficult to remove.
Focus is on the evaluation of the consultants report and approach

Debate on whether DON or recalcitrant DON will be an adopted water quality criteria

Are Major Conclusions of the Total Nitrogen Reduction Technologies valid?
There is a baseline value of DON treatment capability

Assuming the North American Treatment Wetlands Database values will provide the ultimate level of Treatment is incorrect as most, if not all, treatments Systems are not designed or maintained for DON removal

There is no easy way to identify all DON compounds – bioavailability assay in the most useful

Thousands of compounds comprise DON Compound specific or compound class identification is not well established to determine the bioavailability of the DON pool, an easily replicated bioassay is need if DON conversion is the goal
FAV systems appear to be the best candidates NTS for bioavailable DON removal followed by EAV

Goal: bacterial degradation and minimal N fixation

This design has drawbacks
- wind
- water flow
- high TSS
- unproven for DON

SAT may be effective at DON removal

This has not been evaluated for DON removal
However, inorganic N removal is successful in some systems, in particular Nitrate, which suggested bacterial Films on the surface of the porous media allows for the Reduction or uptake of the bioavailable N.

This technology could be tested as a potential for removal in the upper watershed (potential for P removal)

Vertical flow systems may reduce DON and could be investigated as a polishing cell, providing the flow rate of the treatment facility does not overwhelm percolation.
FAV – EAV – SAV  possible SAT

The FAV cell would produce TSS of which TN would be a component.

EAV cells could remove the TSS

SAV cells, with little bioavailable N would have N fixers which was a major concern in any STA design.

SAT – though interesting does not appear to be a valid Technology for this scale system and might be more valuable in the watershed to reduce total loads to the River.

Is the Area for each component adequate

Having mesocosms and test cells makes a lot of sense and builds on the successful model the District has used for TP reductions

However, design of the large scale treatment single concept (bioavailable to recalcitrant DON) seems unwise given concerns over acceptable limits TN vs DON and that no mesocosm and test cells have provided the data needed to justify this approach at this time
Conventional Treatment is not good, averaging 3 mg/L, twice the concentration of the Caloosahatchee River – costs are high for drinking water treatment

Conventional treatment systems are not attempting to maximize TN or DON removal. Treatment may not be low enough since there is no push to go any lower in a particular application. Therefore, a full review of conventional treatment should not be overlooked based on this. It may be too costly and impractical for this application, but there may be an aspect of treatment which might provide some opportunity. Also important for the District to show in any adoptions, that it has turned over every rock

Chemical treatment for P was still investigated, for example

Did the assays (salinity, photolysis, bioavailability) quantify the fraction of the DON pool that could be bioavailable?

Salinity - what mechanisms breaks down DON potential for desorption from particles
Was the assay sterilized or meant to be bacterial?

Photolysis – An 8 hour incubation, sterile samples?
What about UV light? Test this concept
Cost would need to be determined (replacement, pumps).
Bioassays duplicates produced variable results. Why wasn’t the inoculum investigated? Look under a microscope, live cells.

Much of these results were inconclusive and experiments seemed preliminary.

Are seasonal shifts in the DON bioavailability/recalcitrance supported by the chemical and biological evidence?

Wet vs dry Season
- source of material
  - fresh upland material
  - degraded DON

Mass Spec data is interesting, novel and in it’s infancy

There was some difference in spectra
How much of the spectra can be seen?
Are pools moving from seem to unseen
Back to an easily replicated bioassay
Is the preliminary surrogate method for determination of biologically available DON reasonable and supported by the evidence?

This method can document the change in active algal biomass but the results can be related to any number of limiting factors and therefore, unless macro and micro nutrients were added, (except for N) this approach has pitfalls. This method also does not account for algae that produce enzymes to break down DON and there may need to be a priming effect for this.

Are the Conclusions of the Findings Memorandum supported by the data and its analysis?

Changes in concentration in DON from wet to dry Dilution could play a major role

Why was the range of the DON so variable by season?

If the DON is bioavailable only in the wet season, then treatment would not be likely required in the dry season.
Are the Conclusions of the Parameter Plan supported by the data and its analysis?

“there is a reproducible pattern of change in compound-level analyses and total pigment change in the bioavailability assay as DON is transformed from bioavailable to recalcitrant forms”

This is not quantitatively supported.

Fully embracing the idea of bioavailable DON being the standard and not DON or even TN, is a risky proposition and the large scale system as well as the mesocosms are all designed along these lines.

Should be a focus on TN concentration

Overall Comment on the Scientific Approach

Treatment of this TN (mostly DON) pool is going to be challenging

Using very new, unproven analytical methods should not be used to drive a wetland treatment design.

Assumption is that algal blooms are triggered by the wet season DON – DON is more bioavailable but are conditions are more favorable for blooms?
**Mesocosm Approach**

Try Many Different approaches  
Not linked in series, initially, but sequential treatment can be evaluated later.

How will treatment vary on non organic soils?  
May work well initially but fail as organic matter builds up.

Data on DON removal in STAs is likely useless as organic soils produce/release dissolved organic compounds, does not mean approach won’t work, just needs to be evaluated.

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**Test Cell Approach**

Using Test cells as scaled up version of the mesocosms would be advisable, in particular because mesocosms are easier to control and this approach allows testing of technologies, closer to full scale.
**Large-Scale Facility**

The advantage of this system will be the ability to test and elucidate the challenges to any sort of floating system.

The disadvantages include – limited to one design
  The TSS problem, wind

Larger system could be subdivided to test several approaches.

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**SAT – bank infiltration**

The site provides the ability to test this on some small scale

This approach would be more beneficial further up in the watershed if it was proven to work.
**Conclusion on Design Approach**

Little is known about the DON pool characterization bioavailability.

Methods provided do not appear to be developed to a sufficient point to provide an effective tool.

Other technologies should be evaluated.

Focus should be initially on TN, specifically the entire DON pool in this case.

Similar to the treatment of P, a low concentration level of DON might allow further treatment.

SAT may be promising in the upper watershed.

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**Alternative Treatment Technologies**

Look at the traditional EAV systems on non organic soils.

Allowing drawdown has shown that DON spikes are released — on organic soils.

Limerock vertical flow – crushed limerock berm.

Material exists on-site?

Can it work on this scale?
Conclusion

Focus should be initially on TN

For the river water, that means DON primarily

Scrubber technologies including UV lights, rock filtration might provide an effective method for removing some percentage of the difficult DON pool.

Traditional natural treatment can deal with particulate N, as well as ammonium and nitrate such that the focus should be on removing DON to reduce the overall TN load.

Additional Comments

If Algal blooms are the primary driver of treatment

1) What times of the year are the blooms present?

2) Are there times where treatment would not be required because blooms do not persist?

Coastal Louisiana hypoxia, for example, is in part, due to high nitrate loads in the spring, driving algal blooms followed by massive dieoffs leading to low dissolved oxygen.

River Diversion removals of N during the Spring
21 – 28 July 2007 Bottom-Water Hypoxia

- up to 22,000 km²
- 4 - 5 m nearshore to 35 - 45 m offshore
- 0.5 km nearshore to 100+ km offshore
Mississippi River – Peak Flow 1.2 M cfs

<table>
<thead>
<tr>
<th>Diversion</th>
<th>Peak Flow</th>
<th>% of MR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Davis Pond Diversion</td>
<td>10,000 cfs</td>
<td>0.83 %</td>
</tr>
<tr>
<td>Caernarvon Diversion</td>
<td>8,000 cfs</td>
<td>0.67 %</td>
</tr>
<tr>
<td>Bonnet Carré Spillway</td>
<td>250,000 cfs</td>
<td>20.8 %</td>
</tr>
</tbody>
</table>
During the month long diversion, ~8 % of the N load during the month was removed from the Mississippi River.

The Area of hypoxia was ~8 % < predicted from the N load of the Mississippi River - The annual expression of eutrophication in coastal Lousiana.
This result suggests that eutrophication is time dependent -seasonal

Does the River water need to be treated Year round for Nitrogen removal

Ocean provides for mixing and dilution

Organic vs Mineral Soils

City of Orlando’s Easterly Wetland Treatment Facility in Christmas, Florida
In July of 1987 the Orlando Easterly Wetlands began receiving flow from Iron Bridge.

Aerial Photograph taken: October 1999

Organic matter builds up
Short circuiting of flow
Release of previously stored nutrients
The concentrations of nutrients were over an order of magnitude lower than before the modification.

The concentrations of inorganic and organic nutrients increased as the water flowed into the downstream organic soil cells.

The internal nutrient load is much lower for the mineral soils – longevity?
The potential for the last/scrubber cell

Low nutrients should limit plant productivity

Decrease the rate of organic matter accretion

Maintenance – seasonality