Environmental Protection Agency

Region 7

Total Maximum Daily Load

Sphaerotilus natans

Mississippi River Segment (IA 01-NEM-0010_4)

Clinton County, Iowa

Approved by:

for Karen A. Flournoy　　　　　　　　　　1-19-10

William A. Spratlin　　　　　　　　　　Date

Director

Water, Wetlands and Pesticide Division

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Acronyms and Abbreviations

ug/L	Micrograms per liter
ADM	Archer Daniels Midland
avg	Average
CFR	Code of Federal Regulations
CWA	Clean Water Act
d/s	Down Stream
EPGEC	Epiholitic-Periphyton Growth Enhancement Component
EPT	Ephemeroptera, Plecoptera and Trichoptera
HUC	Hydrologic Unit Code
IA	Iowa
IDNR	Iowa Department of Natural Resources
km	Kilometer
LA	Load Allocation
max	Maximum
MGD	Million Gallons per Day
MOS	Margin of Safety
MS4	Municipal Separate Storm Sewer System
n/a	Not available
NPDES	National Pollutant Discharge Elimination System
STP	Sewage Treatment Plant
TMDL	Total Maximum Daily Load
EPA	United States Environmental Protection Agency
u/s	Up Stream
USGS	United States Geological Survey
WLA	Wasteload Allocation
WQS	Water Quality Standards

1 SUMMARY

This Mississippi River Total Maximum Daily Load (TMDL) for *Sphaerotilus natans (addressing an organic enrichment impairment)* is being established in accordance with Section 303(d) of the Clean Water Act (CWA) which requires a TMDL for each water body on a state's Section 303(d) list of impaired waters (303(d) List) and in accordance with requirements of Section 303 of the CWA, Water Quality Planning and Management Regulations (40 Code of Federal Regulations (CFR) Part 130), and United States Environmental Protection Agency (EPA) guidance. To meet the Mississippi Pools milestones of the 2001 Consent Decree, *Sailors, Inc., Mississippi River Revival and Sierra Club v. EPA,* No. 98-134-MJM, EPA is establishing this TMDL. The Mississippi River (IA 01-NEM-0010_4) is included in the Consent Decree because it was on the Iowa 1998 303(d) list due to an organic enrichment impairment that exceeded Iowa's Water Quality Standards (WQS).

This document includes a TMDL for one segment of the Mississippi River assessed as impaired due to "aesthetically objectionable conditions" and "nuisance aquatic life" as defined in the Iowa WQS. The location of this Mississippi River segment is from Wapsipinicon River to Lock & Dam 13 at Clinton (Table 1).

Table 1. Mississippi River Section 303(d) Listed Summary for Segment IA 01-NEM-0010_4

Water body Name	Mississippi River
Water body ID Number	IA 01-NEM-0010_4
Segment Description	From Wapsipinicon R. to Lock & Dam 13 at Clinton
County	Clinton
Use Designation Classes	Aquatic life support (Class B (WW)), primary contact recreation (Class A) and fish consumption
Major River Basin	Copperas-Duck (HUC 07080101)
Pollutant	*Sphaerotilus natans*
Pollutant Sources	Point sources
Impaired Use	Overall Use (narrative criteria); Aquatic life (Class B (WW-1)
2006 303(d) Priority	Low
Watershed Area	55,691 acres
Segment Length	16.1 miles
Load Allocation	Zero *Sphaerotilus natans*
Wasteload Allocation for Point Sources	Zero *Sphaerotilus natans*
Wasteload Allocation for MS4	Zero *Sphaerotilus natans*

The purpose of this TMDL is to assist the EPA with establishing a loading capacity (LC) for this impaired water body. The TMDL quantifies the pollutant loading a water body can assimilate without exceeding the WQS for that pollutant. The TMDL also establishes the pollutant load allocation necessary to meet the WQS based on the relationship between pollutant sources and in-stream conditions. The TMDL consists of a wasteload allocation (WLA), a load allocation (LA), and a margin of safety (MOS). The WLA is the fraction of the total pollutant load apportioned to point sources, while the LA is the fraction apportioned to nonpoint sources.

The MOS is a percentage of the TMDL intended to account for the uncertainty attributed to model assumptions, data limitations, and other sources.

The key elements supporting the development of the *Sphaerotilus natans* TMDL are summarized below:

1. **Name and geographic location of the impaired or threatened water body for which the TMDL is being established:** Mississippi River from Wapsipinicon River (Scott/Clinton County line) to Lock & Dam 13 at Clinton (Clinton County).

2. **Identification of the pollutant and applicable WQS:** The Mississippi River segment IA 01-NEM-0010_4 has been identified as impaired by "nutrients (i.e., some type of slime growth factor)" in Category 5 of the Iowa 2006 Integrated Report, due to resulting aesthetically objectionable conditions and nuisance aquatic life. As discussed in Section 2, the slime has been positively identified as *Sphaerotilus natans.* The *Sphaerotilus natans* growth violates the EPA-approved state of Iowa general use narrative criteria that state:

   - "waters shall be free from materials attributable to wastewater discharges or agricultural practices producing objectionable color, odor, or other aesthetically objectionable conditions," and
   - "waters shall be free from substances, attributable to wastewater discharges or agricultural practices, in quantities which would produce undesirable or nuisance aquatic life."

   Studies of macroinvertebrate communities also suggest that the slime growth causes an additional impairment of the Class B (WW) aquatic life uses of Beaver Slough.

3. **Quantification of the pollutant load that may be present in the water body and still allow attainment and maintenance of the WQS:** The water quality target for this TMDL is no increase in *Sphaerotilus natans* downstream of the Archer Daniels Midland (ADM) facility discharges, compared to upstream conditions. This is a translation of the narrative criteria written to protect against aesthetically objectionable conditions and nuisance aquatic life.

4. **Quantification of the amount by which the current pollutant load in the water body, including the pollutant from upstream sources that is being accounted for as background loading, deviates from the pollutant load needed to attain and maintain WQS:** Nuisance amounts of *Sphaerotilus natans* exist in the impaired water body, violating narrative water quality criteria. The *Sphaerotilus natans* discharge needs to be reduced by essentially 100 percent (%) from existing levels.

5. **Identification of pollution source categories:** Previous studies have shown that nuisance levels of slime *(Sphaerotilus natans*) only occur downstream where the ADM facility discharges and that this facility is the source of the slime impairment (Johnson, 2001 through 2008a).

6. **WLAs for pollutants from point sources:** The only facility with a current permit that specifies slime limits is ADM Corn Processing Facility – Clinton (IA0003620), which discharges to Mississippi River segment IA 01-NEM-0010_4. The WLA for IA-01-NEM-0010_4 is zero *Sphaerotilus natans.*

7. **LA for pollutants from nonpoint sources:** The LA for nonpoint sources is zero *Sphaerotilus natans.*

8. **A MOS:** This TMDL contains an implicit MOS based on the conservative allocation of zero *Sphaerotilus natans* to all sources.

9. **Consideration of seasonal variation:** Nuisance growths of *Sphaerotilus natans* are typically observed in the spring and fall when water temperatures are between 50 and 60 degrees Fahrenheit. This TMDL applies year-round, considering all seasonal variation.

10. **Allowance for reasonably foreseeable increases in pollutant loads:** *Sphaerotilus natans* growths are not acceptable at any noticeable amount, and therefore no allowance for increased future *Sphaerotilus natans* loads is included in this TMDL.

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2 MISSISSIPPI RIVER, DESCRIPTION AND HISTORY

ADM, a commercial agricultural processor, operates a corn-wet-milling facility that discharges to Beaver Slough, a side channel of the impaired Mississippi River segment IA 01-NEM-0010_4. Historical reports indicate slime growth has been a problem in the slough for many years. More recently, there have been complaints that the nets set by commercial fishermen in Beaver Slough were fouled with slime growth. These slime growths are typically observed during the spring and fall, when water temperatures are between 50 and 60 degrees Fahrenheit. The slime growths are the problem that led to the listing of segment IA 01-NEM-0010_4 for violations of the narrative water quality criteria protecting against aesthetically objectionable conditions and nuisance aquatic life.

ADM conducted a study of the slime in 1996, and subsequently has been working to review and improve plant operations to reduce the extent of slime growth in Beaver Slough downstream of this facility. The National Pollutant Discharge Elimination System (NPDES) permit issued for the ADM facility on March 18, 1998, includes a narrative effluent limitation to prohibit the permittee from discharging wastewater that produces an objectionable color, odor, or other aesthetically objectionable condition in the receiving stream. Compliance with this narrative standard is based on a comparison of the extent of slime growths on control and test samplers placed in Beaver Slough.

To comply with the permit conditions, ADM has published two sets of reports annually since 2001: "Slime Study Reports" and "Slime Report Beaver Slough," (ADM, 2001 through 2004; Johnson, 2001 through 2008a). The Slime Study Reports evaluate compliance with the narrative standards for Beaver Slough, by comparing growths of slime on control samplers placed at a location upstream of the ADM discharges and at a location downstream of ADM's outfalls. The second set of reports, Slime Report Beaver Slough, tracks the extent of slime using visual observations to determine presence or absence of slime at a series of stations in the slough. These studies have helped characterize the location of the slime throughout Beaver Slough and downstream in the Mississippi River.

The reports document work that ADM has done to identify and remove waste streams that may contribute to slime growth. Specific projects identified in these reports include work to trace waterlines discharging to specific outfalls and to identify slime growths within the plant. Barometric evaporators and condensers were identified as Epiholitic-Periphyton Growth Enhancement Component (EPGEC) contributors during the 2003 study, and subsequently, several barometric evaporators and condensers were removed (Johnson, 2005). In 2003, ADM initiated work to upgrade wastewater treatment processes, including projects to install two covered equalization basins, add additional aeration and improve hydraulic capacity (Johnson, 2007).

A review of the Slime Study Reports indicates a reduction in the downstream extent of the slime beginning in fall 2004 and continuing through fall 2007. The reports suggest that recent reductions to the extent of the "slime zone" may be related to the extensive modifications to the production processes and waste stream diversion activities that were accomplished within the ADM Facility during 2004, 2005, and 2006 (Johnson, 2007a).

Until recently, the slime had not been positively identified, and the pollutant causing the slime growth was not known. In the state 305(b) report, the pollutant is identified as "nutrients (i.e., some type of slime growth factor)." Sampling conducted during 2008 for this TMDL resulted in the positive identification of the slime as *Sphaerotilus natans*, a heterotrophic bacteria (Appendix B and personal communication, 2008). *Sphaerotilus natans* does not produce its own energy from photosynthesis (which could be controlled through the control of inorganic nutrients), but instead gets its energy from a variety of external carbon and nitrogen sources such as sugars, alcohols, organic acids, and amino acids (Richard, Hao, and Jenkins, 1985; Welch, Jacoby and Lindell, 2004).

2.1 MISSISSIPPI RIVER (IA 01-NEM-0010_4)

The Mississippi River originates in Minnesota and flows 2,320 miles south to the Gulf of Mexico. This river serves as the east-west boundary for several states, including Iowa and Illinois, where this impaired segment is located.

The impaired segment (IA 01-NEM-0010_4) of the Mississippi River is 16.1 miles long, and flows southward (Figure 1). It begins at Lock & Dam 13 at Clinton, Iowa, and ends at the mouth of the Wapsipinicon River (Scott/Clinton County line) (IDNR, 2006). This segment of the river is within the Copperas-Duck watershed in eastern Iowa. The impaired segment borders Clinton County, Iowa to the east, and Rock Island and Whiteside Counties, Illinois to the west.

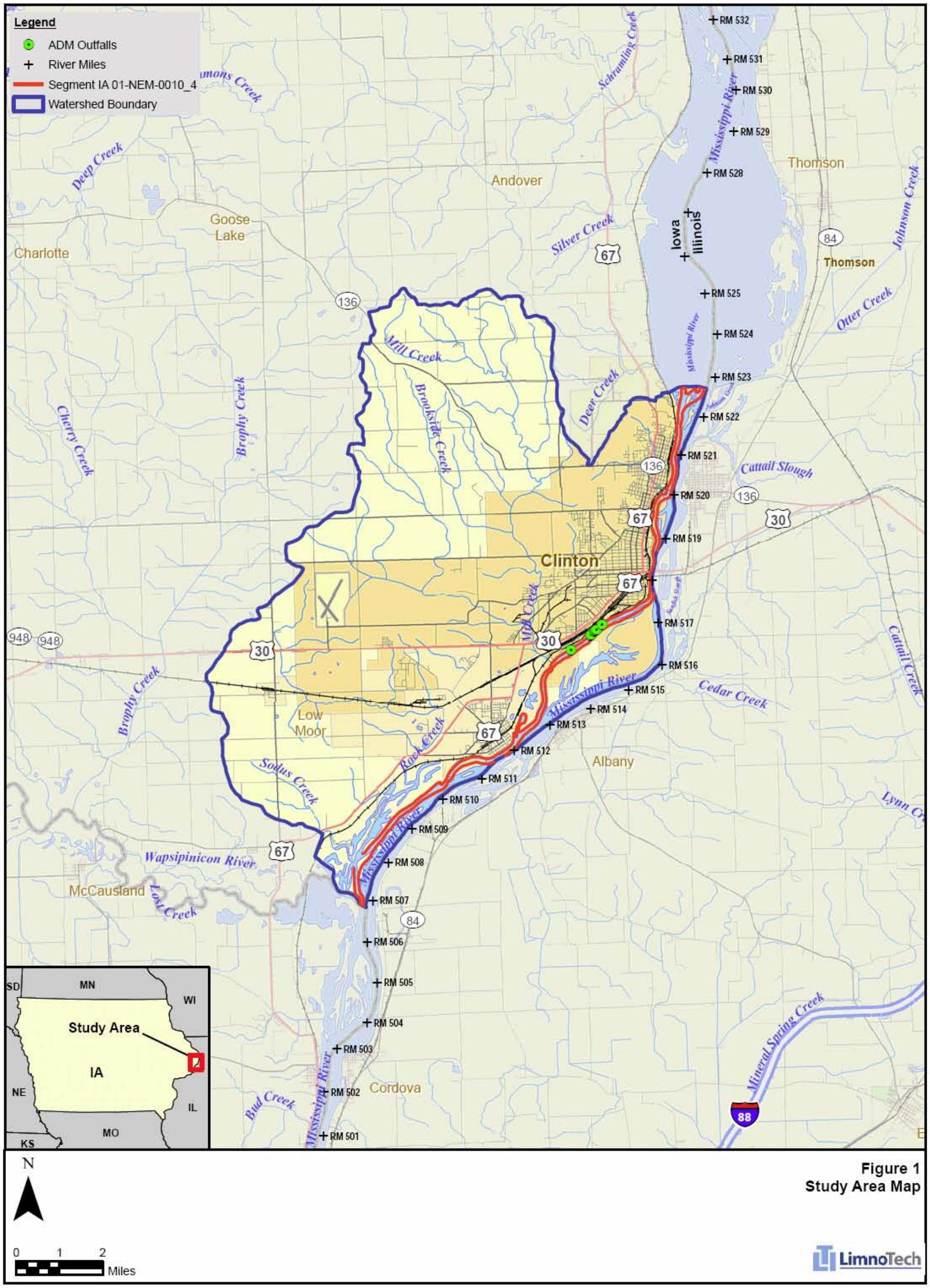

Figure 1. Mississippi River Segment IA 01-NEM-0010_4

2.2 THE WATERSHED (IA 01-NEM-0010_4)

The watershed located in Clinton County, Iowa that drains directly to the Mississippi River segment IA 01-NEM-0010_4 was delineated and used for assessment purposes to support TMDL development. This watershed is 87 square miles in size. Land use in the study area is presented in Table 2 and shown in Figure 2. As shown, the study area watershed is primarily agricultural, with roughly 10% coverage by developed land.

Table 2. Year 2002 Land Use within IA 01-NEM-0010_4 Watershed (Clinton County, Iowa)

Land use	Acres	Percent of total
Cropland	26,137	47%
Grassland	11,606	21%
Forest	4,752	9%
Water or wetland	3,609	6%
Commercial/Industrial	3,549	6%
Grazed grassland	2,421	4%
Residential	2,317	4%
Clouds/Shadow/No Data	1,072	2%
Barren	228	0%

Source: IDNR http://csbweb.igsb.uiowa.edu/imsgate/introduction/home.asp

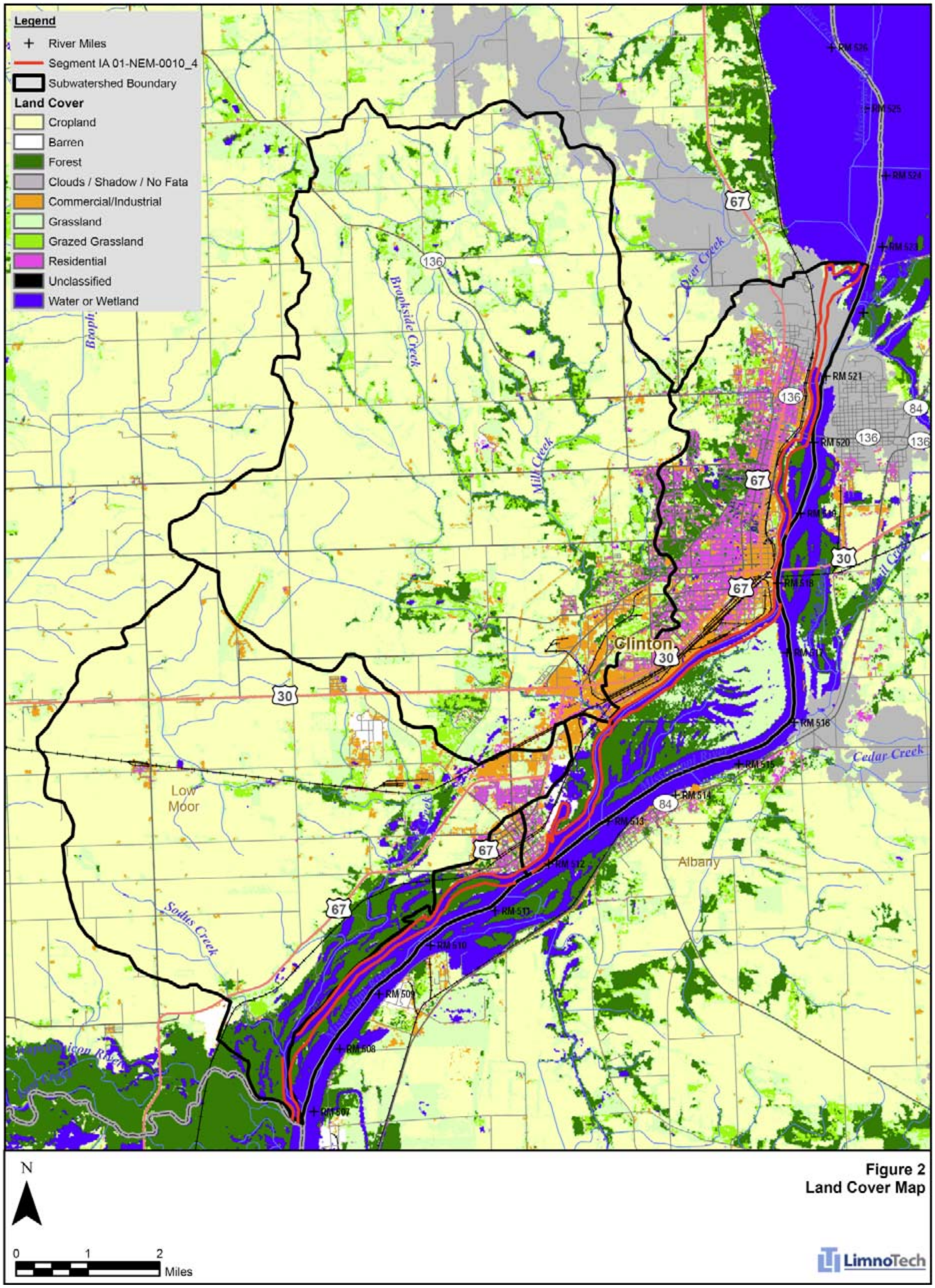

Figure 2. 2002 Land Cover, Clinton County, Iowa

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3 TMDL FOR *SPHAEROTILUS NATANS*

3.1 PROBLEM IDENTIFICATION

3.1.1 Impaired Beneficial Uses and Applicable Water Quality Standards

The Iowa Department of Natural Resources (IDNR) 2006 Water Quality Assessment specifies the following uses for Mississippi River segment IA 01-NEM-0010_4: overall use, primary contact recreation (Class A), aquatic life (Class B (WW)), and fish consumption.

The final Section 305(b) assessment (IDNR, 2006a) identifies Mississippi River segment IA-01-NEM-0010_4 as "partially supporting" general uses due to violations of the narrative water quality criteria protecting against aesthetically objectionable conditions and nuisance aquatic life, and "not supporting" the aquatic life support use. The impairment is due to the continuing problem with growth of slime in the heavily-industrialized Beaver Slough (River miles 517 to 513) portion of this river reach. The presence of slime growth on substrates and objects placed in the river constitutes a violation of Iowa's Class B (WW) aquatic life criteria and the general (narrative) water quality criterion regarding "aesthetically objectionable conditions" and "nuisance aquatic life" as defined in Iowa WQS:

> "(1) all surface waters shall be free from materials attributable to wastewater discharges or agricultural practices producing objectionable color, odor, or other aesthetically objectionable conditions;
>
> (2) all surface waters shall be free from substances from wastewater discharges or agricultural practices in quantities which would produce undesirable or nuisance aquatic life (IDNR, 2006a)."

Studies conducted by ADM have shown that the occurrence of slime is restricted to sample sites downstream from the ADM facility and that slime does not occur at main channel sites or at sites in Beaver Slough upstream from the ADM facility (Johnson, 2001 through 2008a). "Slime studies" were conducted in 2000 through 2007 by ADM (ADM, 2001 through 2004; Johnson, 2001 through 2008a). These studies confirmed that:

- Slime growth is observed in Beaver Slough downstream of ADM discharges,
- Slime growth occurs within the ADM facility, and
- The ADM facility is the major source of the slime and/or growth factors that produce slime growths.

In addition, both bioassessments conducted as part of the 2000 and 2001 studies indicate that the macroinvertebrate community of Beaver Slough may be adversely affected by the discharges from ADM outfalls (Johnson, 2002). Using multiple-plate samplers, petite Ponar dredge, and underwater videotaping, these studies suggested that slime growths are related to decreases in the total number of taxa, and the percentage of ephemeroptera, plecoptera and trichoptera (EPT) (i.e., pollution-intolerant) taxa downstream from ADM outfalls. Also, the population density of zebra mussels (*Driessena* sp.) was found to decrease downstream from the ADM outfalls. Thus, in addition to the impacts on the warm water commercial fishery in this river reach, information on macroinvertebrate communities suggests an additional impairment of the Class B(WW) aquatic life uses of Beaver Slough (IDNR, 2006b). Based on the information in the studies by Johnson (2001, 2002, and 2003), the cause and source of impairment listed in previous Section 305(b) reports (organic enrichment/low dissolved oxygen due to unknown source) was changed to an impairment caused by nutrients (i.e., some type of slime growth factor) with industry as the primary source of this impairment.

3.1.1.1 Interpreting Mississippi River Impaired Segment Water Quality Data

The 2006 305(b) assessment (IDNR, 2006a) indicates that, "Despite the assessment as "impaired" for aquatic life uses due to slime growth in Beaver Slough, results of water quality monitoring at the United States Geological Survey (USGS) National Stream-Quality Accounting Network near Clinton at the lower end of Beaver Slough (station 05420500) continue to show relatively good water quality in this reach of the Upper Mississippi River and suggest "full support" of the Class B(WW) aquatic life uses. Monitoring data from this station for the period February 2002 through September 2004 show 1) no violations of Class B(WW) water quality criteria for dissolved oxygen, pH, and ammonia-nitrogen in the 35 samples analyzed, 2) no violations of Class B(WW) chronic criteria for toxic metals in the 11 samples analyzed, and 3) no violations of Class B(WW) criteria for the 35 samples analyzed for pesticides." This implies that the observed biotic impairment discussed above is not being caused by traditional water quality parameters and can instead be addressed by control of the slime.

3.1.2 Key Sources of Data

The project schedule allowed for the compilation of existing data as well as the collection of additional water quality data during spring and fall 2008, to assess water quality in the impaired segment and to identify the slime.

The following data were acquired and assessed to support TMDL development:

- Slime studies from 2000-2007 published by Johnson for ADM
- ADM intake and effluent monitoring data from April 2007 through March 2008
- Existing IDNR Assessment Reports for 1998-1999, and 2002-2004
- USGS stream flow data
- Permit information for NPDES-permitted facilities
- Land use and other geographic information data in digital format for Iowa.

The slime reports described in Section 2 provide historical context for the slime problem, but do not describe water quality in Beaver Slough or specifically identify the slime. Water quality monitoring was conducted in support of this TMDL in spring and fall 2008, following an EPA-approved Quality Assurance Project Plan, to characterize water quality conditions in Beaver Slough and to positively identify the slime. The following data were used to support the development of this TMDL:

- Water quality surveys conducted in May and October 2008 (Appendix A), and
- Slime collection and identification conducted in November 2008 (Appendix B).

3.2 TMDL TARGET

A TMDL is required for the impaired Mississippi River segment to restore and maintain the general and aquatic life uses. Nuisance levels of *Sphaerotilus natans* have been observed downstream of the ADM discharges. *Sphaerotilus natans* has also been identified in a sample collected from a location upstream of the ADM discharges, but only at very low, non-nuisance levels. Extensive sampling and analysis performed from 2006 to 2009 reveals that the upstream non-nuisance levels do not impact this TMDL. Intake water to the ADM facility likely contains low concentrations of *Sphaerotilus natans.* Some growth factor then feeds growth of the *Sphaerotilus natans* in the facility, and results in the discharge of high concentrations of *Sphaerotilus natans* into Beaver Slough.

The specific compound(s) that cause(s) the *Sphaerotilus natans* growth in this impaired reach is unknown; it has been given the generic name "epiholitic-periphyton growth enhancement component" or EPGEC (Johnson, 2007a). *Sphaerotilus natans* has been documented as having a remarkable nutritional versatility (Spring, 2006), using a variety of carbon and nitrogen sources (Pellegrin, Juretschko, Wagner, and Cottenceau, 1998; Richard et al., 1985; Van Veen, Mulder and Deinema, 1978). Because the exact makeup of EPGEC in Beaver Slough is unknown, the TMDL target cannot be based on EPGEC and must therefore be based on allowable levels of *Sphaerotilus natans*.

The target for this TMDL is Iowa's narrative criteria contained in the existing NPDES permit for ADM. The permit requires a comparison of the extent of slime growths on control and test samplers placed in Beaver Slough, with the requirement that slime concentrations on samplers near the ADM discharge not be greater than levels at background control sites.

Specifically the TMDL target is defined as no increase in *Sphaerotilus natans* above natural background levels observed upstream of all ADM discharges.

3.3 POLLUTION SOURCE ASSESSMENT

To support TMDL development, a pollutant source assessment is designed to characterize known and suspected sources of pollutant loading to the impaired water body. Pollutant sources within a watershed are characterized and quantified to the extent that information is available. *Sphaerotilus natans* sources that could contribute to the impairment include point source discharges. *Sphaerotilus natans* has been identified in very low concentrations at a location upstream of the ADM facility.

3.3.1 Identification of Pollution Sources

3.3.1.1 Point Sources

Under 40 CFR, Section 122.2, a point source is described as a discernable, confined, and discrete conveyance from which pollutants are or may be discharged to surface waters. Only one facility in this watershed, the ADM Corn Processing Facility (Table 3), has a permit limit for slime. The research and data used to generate this TMDL (found in Appendices A and B) reveals that no slime is being discharged from other point sources. The location of the ADM Corn Processing Facility is shown on Figure 1.

Other permitted dischargers that are located in the watershed, which do not have permit limits for slime, are listed in Table 4. *Sphaerotilus natans* can be associated with poorly treated sewage and so wastewater treatment facilities in this table may be potential sources of *Sphaerotilus natans.* The location of the *Sphaerotilus natans* in Beaver Slough downstream of the ADM facility and the decreasing extent of the problem concurrent with ADM facility improvements, indicates the Table 4 dischargers are not significant contributors to the problem.

Table 3. NPDES Permitted Facility with Slime Limits

NPDES ID	Facility Name	County	State	Receiving stream	Permitted Flow (MGD)	Facility Type
IA0003620	ADM Corn Processing Facility	Clinton	IA	Mississippi River	0.9	Wet corn milling

MGD=Million Gallons per Day

Table 4. Other NPDES Permitted Facilities

NPDES	Facility Name	County	State	Receiving Water	Permitted Flow (MGD)	Facility Type
IA0035947	City of Clinton Sewage Treatment Plant (STP)	Clinton	IA	Mississippi River	10 (avg) 16 (max)	Sewerage systems
IA0080543	ADM Clinton Cogeneration Plant	Clinton	IA	n/a	n/a	Electric services
IAU000285	Economy Coating Systems	Clinton	IA	n/a	n/a	Autobody repair Hwy paint, street construction paint shop
IA0068101	Vertex Chemical Corporation	Clinton	IA	Mississippi River	n/a	Industrial inorganic chemicals, not elsewhere classified
IAG140337	Wendling Quarries Inc - Camanche	Clinton	IA	n/a	n/a	Crushed and broken limestone
IA0001759	ML Kapp Generating Station - Alliant/Interstate Power Company	Clinton	IA	Mississippi River	28.8	Electric services
IA0003522	PCS Nitrogen Fertilizer	Clinton	IA	Mississippi River	0.97	Nitrogen fertilizer
IA0073407	ACC Chemical Company & Getty Chemical Company	Clinton	IA	Mississippi River	0.72	Sewerage systems
IA0000183	Sethness Products Company	Clinton	IA	Mississippi River	n/a	Cane sugar refining
IA0000914	National By-products, Inc./Darling National	Clinton	IA	Mississippi River	n/a	Animal and marine fats and oils
IA0001066	Bemis Clysar (E.I. Dupont de Nemours)	Clinton	IA	Mississippi River	7.59 (total of all outfalls)	Plastic materials, synthetic resins & nonvulcanizable elastomers
IA0000752	Collins Inc./SSW Holding Co.	Clinton	IA	Mill Creek	n/a	Misc. fabricated wire products
IA0000191	US Filter Operating Services/Equistar Chemicals	Clinton	IA	Mississippi River	0.67	Plastic materials, synthetic resins & nonvulcanizable elastomers
IA0021261	City of Camanche STP	Clinton	IA	Mississippi River	0.9	Sewerage systems
IA0040100	City of Low Moor STP	Clinton	IA	Rock Creek	0.064	Sewerage systems
IA0071391	Royal Pines Village/Pine Ridge Mobile Home Park	Clinton	IA	Rock Creek	0.039	Residential mobile home sites

n/a = not available   avg= average   max= maximum

The narrative effluent limitation for the ADM Corn Processing Facility is written as follows: "The permittee is prohibited from discharging wastewater that produces an objectionable color, odor, or other aesthetically objectionable condition in the receiving stream. Compliance with this narrative standard shall be based on a comparison of the extent of slime growths on control and test samplers placed in Beaver Slough."

3.3.1.2 Regulated Stormwater: MS4 Contributions

The City of Clinton (EPA Permit No. IA0078956) has a Phase II Municipal Separate Storm Sewer System (MS4) permit. This permit was issued on March 7, 2007, and expires March 6, 2010. Stormwater runoff from the City of Clinton is not believed to be contributing to the current impairment.

3.3.1.3 Nonpoint Sources

Nonpoint sources are comprised of runoff from different land uses in the watershed (see Table 4). As discussed previously, nuisance levels of *Sphaerotilus natans* are confined to Beaver Slough downstream from the ADM facility and the ADM facility is identified as the major source of the slime and growth factors that produce slime growths (IDNR, 2006a). For this reason, nonpoint sources are not believed to be contributing to the current impairment.

3.3.2 Linkage of Sources to Target

The approach for linking allowable loads to the TMDL target was documented in the "Beaver Slough TMDL Modeling Plan" (LimnoTech, 2008). As described in Section 3.2, the specific compound(s) that cause(s) *Sphaerotilus natans* growth in this reach is unknown and has been generically named EPGEC. The most direct approach to defining necessary *Sphaerotilus natans* controls would be to model EPGEC directly, to determine EPGEC reductions needed to prevent its growth. This is not feasible at this time because the exact makeup of EPGEC is unknown. Two potential approaches were considered for controlling *Sphaerotilus natans* in Beaver Slough:

- Prohibit *Sphaerotilus natans* discharge from point sources,
- Control the concentration of the parameter(s) controlling *Sphaerotilus natans* growth.

The first option recognizes that the impairing *Sphaerotilus natans* growth originates within the ADM facility itself, and that stopping the facility from discharging these bacteria is the primary mechanism for preventing its proliferation in the slough. The second option is designed to ensure that conditions in the slough are not amenable to the growth of nuisance levels of *Sphaerotilus natans*. Because *Sphaerotilus natans* can utilize a variety of carbon and nitrogen sources (i.e., EPGEC), a surrogate such as total organic carbon could theoretically be used as a surrogate parameter for the TMDL. This is not a desirable option, primarily because the exact composition of EPGEC in Beaver Slough is unknown. Furthermore, establishing a threshold concentration of traditional water quality parameters such as organic carbon to prevent outbreaks of *Sphaerotilus* has been documented as being difficult (Welch et al., 2004).

Because nuisance levels of *Sphaerotilus natans* are believed to originate from the ADM facility, the TMDL will consist of specifying a maximum *Sphaerotilus natans* load from this source. Therefore, this approach will not require application of a water quality model.

3.4 POLLUTANT ALLOCATIONS

The pollutant allocations described below apply year-round and are not flow dependent.

3.4.1 Wasteload Allocations

A point source can be either a wastewater (continuous) or stormwater MS4 discharge. Stormwater point sources are typically associated with urban and industrialized areas, and recent EPA guidance includes NPDES-permitted stormwater discharges as point source discharges and, therefore, part of the WLA.

A WLA of zero *Sphaerotilus natans* is set for this TMDL. This approach recognizes that the source of the *Sphaerotilus natans* is a point source discharger, the ADM Corn Processing Facility, but also applies to other permitted dischargers, recognizing that *Sphaerotilus natans* can be associated with wastewater discharges.

The WLA is consistent with maintaining the TMDL target and with the current NPDES narrative effluent limitations for the ADM facility, which specify:

> "The permittee is prohibited from discharging wastewater that produces an objectionable color, odor, or other aesthetically objectionable condition in the receiving stream. Compliance with this narrative standard shall be based on a comparison of the extent of slime growths on control and test samplers placed in Beaver Slough."

3.4.2 Load Allocations

A LA of zero *Sphaerotilus natans* is set for this TMDL. This applies to all sources in the watershed. No load reductions are required to achieve this allocation, because existing sources are not believed to be significantly contributing to the impairment.

3.4.3 Margin of Safety

The TMDL contains an implicit MOS, due to the use of conservative assumptions used in the development of the TMDL. The TMDL specifies WLA and LA of zero, so that no increase in *Sphaerotilus natans* above that observed upstream of the ADM discharge will occur. These allocations apply during all flow conditions and all seasons.

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4 MONITORING

The IDNR should continue to require water quality monitoring and reporting, as described within the existing ADM permit, to assess the presence and extent of the slime growths in Beaver Slough. Monitoring should take place during periods of expected slime growth (Sec. 2 Page 5). At least one sampling location should be upstream of all ADM wastewater and storm water discharges, and at least one sampling location should be located downstream of all ADM wastewater and storm water discharges.

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5 PUBLIC PARTICIPATION

EPA regulations require that TMDLs be subject to public review (40 CFR 130.7). EPA provided public notice of this TMDL for Mississippi River on the EPA, Region 7, TMDL website: http://www.epa.gov.region07/water/tmdl public_notice_htm from September 8 through November 22, 2009. IDNR also provided a link to the public notice on the Iowa TMDL Public Notice webpage at: http://www.iowadnr.gov/water/watershed/pubs.html. During the comment period five comments were received. The Summary of Comments and Responses and the final TMDL is available at: http://www.epa.gov/region07/water/apprtmdl.htm#Iowa.

This water quality limited segment of Mississippi River in Clinton County, Iowa, is included on the EPA approved 1998 Section 303(d) list for Iowa. This TMDL is being produced by EPA to meet the requirements of the 2001 Consent Decree, *Sailors, Inc., Mississippi River Revival and Sierra Club v. EPA, No. 98-134-MJM.* EPA is establishing this TMDL to fulfill the *Sailors* consent decree obligations. Iowa may submit and EPA may approve another TMDL for this water at a later time.

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6 REFERENCES

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Appendix A

2008 Water Quality Survey Data

Includes:

- Sampling Descriptions
- Map of Sampling Locations
- Data from May and October 2008 surveys

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Sampling Descriptions

Sample location	Location description
1	Mississippi R. at USGS gage (u/s of Beaver Slough)
2	Beaver Slough u/s of ADM discharge (ADM control site)
3	Beaver Slough u/s of ADM discharge (ADM slime sampling site)
4	ADM outfall 005
5	Beaver Slough between ADM and National By Products outfalls
6	NBP outfall
7	Beaver Slough between NBP outfall and Mill Creek
8	Mill Creek near mouth
9	Beaver Slough 1 km d/s of Mill Creek
10	Beaver Slough – d/s end of Slough
11	Mississippi R. at USGS gage (d/s of Beaver Slough)

u/s= Up stream
d/s= Down stream
km= Kilometer

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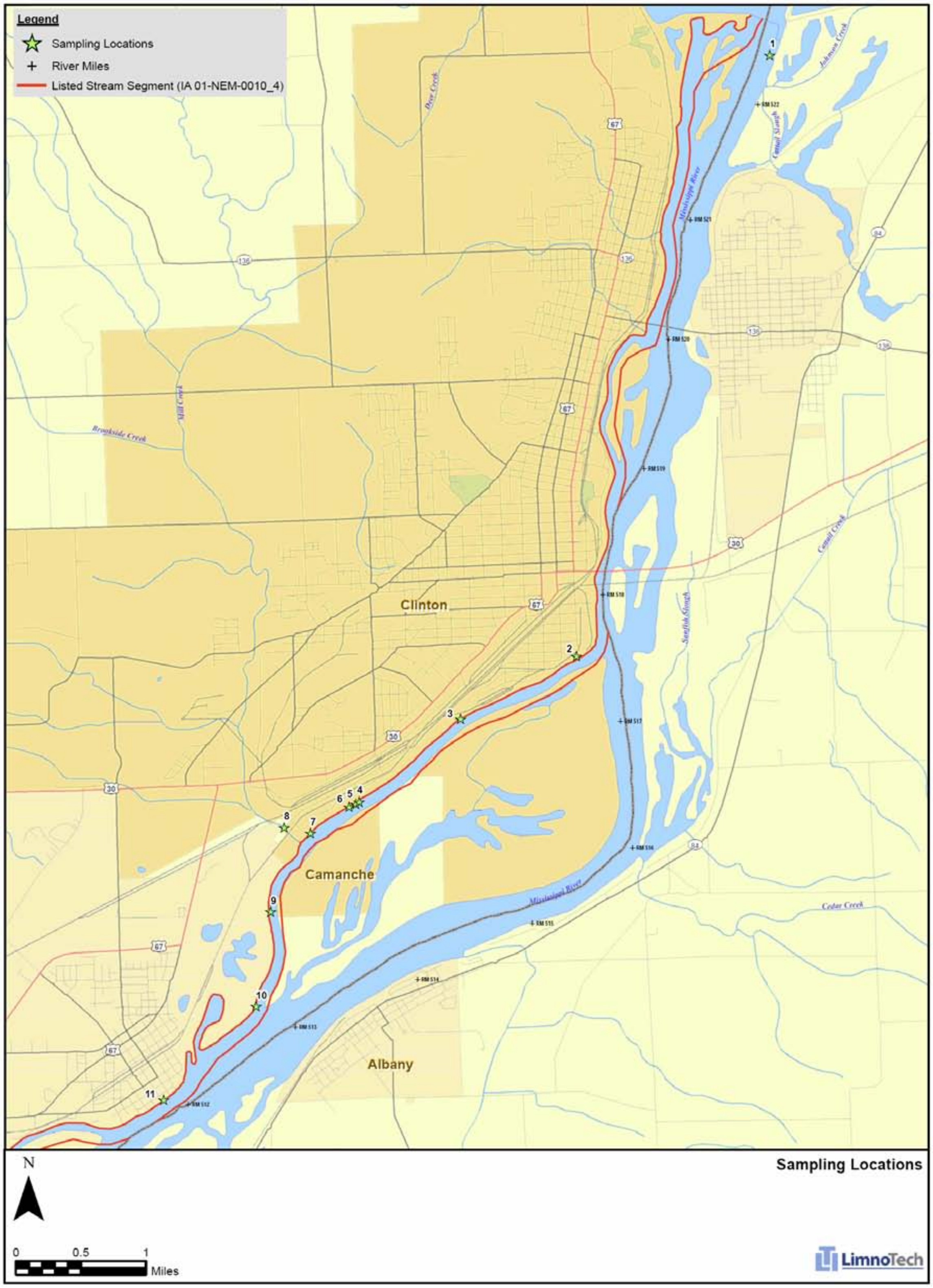

Figure A-1: Sampling Locations

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Sample Date	Sample location	Parameter	Results	Units	DL	Qualifier	Notes
5/20/2008	1	Ammonia	10	ug/l	10	U	Less than detection
5/20/2008	1	Nitrate	**2200**	ug/l	10		
5/20/2008	1	Nitrite	10	ug/l	10	U	Less than detection
5/20/2008	1	Nitrogen (Kjeldahl)	**1200**	ug/l	100		
5/20/2008	1	Phosphorus (ortho)	**20**	ug/l	10		
5/20/2008	1	Phosphorus (total)	**100**	ug/l	10		
5/20/2008	1	Total Alkalinity	**140000**	ug/l	5000		
5/20/2008	1	Total Suspended Solids	**74000**	ug/l	1000		
5/20/2008	1	Total Volatile Residue	**80000**	ug/l	10000		
5/20/2008	1	Carbonaceous BOD	2000	ug/l	2000	U	Less than detection
5/20/2008	1	Total Organic Carbon	**8500**	ug/l	500		
5/20/2008	2	Ammonia	10	ug/l	10	U	Less than detection
5/20/2008	2	Nitrate	**2700**	ug/l	10		
5/20/2008	2	Nitrite	10	ug/l	10	U	Less than detection
5/20/2008	2	Nitrogen (Kjeldahl)	**1300**	ug/l	100		
5/20/2008	2	Phosphorus (ortho)	**20**	ug/l	10		
5/20/2008	2	Phosphorus (total)	**100**	ug/l	10		
5/20/2008	2	Total Alkalinity	**150000**	ug/l	5000		
5/20/2008	2	Total Suspended Solids	**56000**	ug/l	1000		
5/20/2008	2	Total Volatile Residue	**100000**	ug/l	10000		
5/20/2008	2	Carbonaceous BOD	2000	ug/l	2000	U	Less than detection
5/20/2008	2	Total Organic Carbon	**8400**	ug/l	500		
5/20/2008	3	Ammonia	10	ug/l	10	U	Less than detection
5/20/2008	3	Nitrate	**2700**	ug/l	10		
5/20/2008	3	Nitrite	10	ug/l	10	U	Less than detection
5/20/2008	3	Nitrogen (Kjeldahl)	**1300**	ug/l	100		
5/20/2008	3	Phosphorus (ortho)	**20**	ug/l	10		
5/20/2008	3	Phosphorus (total)	**100**	ug/l	10		
5/20/2008	3	Total Alkalinity	**140000**	ug/l	5000		
5/20/2008	3	Total Suspended Solids	**62000**	ug/l	1000		
5/20/2008	3	Total Volatile Residue	**88000**	ug/l	10000		
5/20/2008	3	Carbonaceous BOD	2000	ug/l	2000	U	Less than detection
5/20/2008	3	Total Organic Carbon	**8000**	ug/l	500		
5/20/2008	4	Ammonia	10	ug/l	10	U	Less than detection
5/20/2008	4	Nitrate	**2700**	ug/l	10		
5/20/2008	4	Nitrite	10	ug/l	10	U	Less than detection
5/20/2008	4	Nitrogen (Kjeldahl)	**1500**	ug/l	100		
5/20/2008	4	Phosphorus (ortho)	**30**	ug/l	10		
5/20/2008	4	Phosphorus (total)	**130**	ug/l	10		
5/20/2008	4	Total Alkalinity	**150000**	ug/l	5000		
5/20/2008	4	Total Suspended Solids	**72000**	ug/l	1000		
5/20/2008	4	Total Volatile Residue	**100000**	ug/l	10000		
5/20/2008	4	Carbonaceous BOD	2000	ug/l	2000	U	Less than detection
5/20/2008	4	Total Organic Carbon	**8100**	ug/l	500		
5/20/2008	5	Ammonia	10	ug/l	10	U	Less than detection
5/20/2008	5	Nitrate	**2700**	ug/l	10		
5/20/2008	5	Nitrite	10	ug/l	10	U	Less than detection
5/20/2008	5	Nitrogen (Kjeldahl)	**1500**	ug/l	100		
5/20/2008	5	Phosphorus (ortho)	**20**	ug/l	10		
5/20/2008	5	Phosphorus (total)	**80**	ug/l	10		
5/20/2008	5	Total Alkalinity	**150000**	ug/l	5000		

Sample Date	Sample location	Parameter	Results	Units	DL	Qualifier	Notes
5/20/2008	5	Total Suspended Solids	**70000**	ug/l	1000		
5/20/2008	5	Total Volatile Residue	**96000**	ug/l	10000		
5/20/2008	5	Carbonaceous BOD	2000	ug/l	2000	U	Less than detection
5/20/2008	5	Total Organic Carbon	**8100**	ug/l	500		
5/20/2008	6	Ammonia	10	ug/l	10	U	Less than detection
5/20/2008	6	Nitrate	**2700**	ug/l	10		
5/20/2008	6	Nitrite	10	ug/l	10	U	Less than detection
5/20/2008	6	Nitrogen (Kjeldahl)	**1600**	ug/l	100		
5/20/2008	6	Phosphorus (ortho)	**20**	ug/l	10		
5/20/2008	6	Phosphorus (total)	**80**	ug/l	10		
5/20/2008	6	Total Alkalinity	**150000**	ug/l	5000		
5/20/2008	6	Total Suspended Solids	**68000**	ug/l	1000		
5/20/2008	6	Total Volatile Residue	**84000**	ug/l	10000		
5/20/2008	6	Carbonaceous BOD	2000	ug/l	2000	U	Less than detection
5/20/2008	6	Total Organic Carbon	**7900**	ug/l	500		
5/20/2008	7	Ammonia	10	ug/l	10	U	Less than detection
5/20/2008	7	Nitrate	**2700**	ug/l	10		
5/20/2008	7	Nitrite	10	ug/l	10	U	Less than detection
5/20/2008	7	Nitrogen (Kjeldahl)	**1100**	ug/l	100		
5/20/2008	7	Phosphorus (ortho)	**20**	ug/l	10		
5/20/2008	7	Phosphorus (total)	**130**	ug/l	10		
5/20/2008	7	Total Alkalinity	**150000**	ug/l	5000		
5/20/2008	7	Total Suspended Solids	**74000**	ug/l	1000		
5/20/2008	7	Total Volatile Residue	**100000**	ug/l	10000		
5/20/2008	7	Carbonaceous BOD	2000	ug/l	2000	U	Less than detection
5/20/2008	7	Total Organic Carbon	**8000**	ug/l	500		
5/20/2008	8	Ammonia	**90**	ug/l	10		
5/20/2008	8	Nitrate	**4200**	ug/l	10		
5/20/2008	8	Nitrite	**110**	ug/l	10		
5/20/2008	8	Nitrogen (Kjeldahl)	**670**	ug/l	100		
5/20/2008	8	Phosphorus (ortho)	**110**	ug/l	10		
5/20/2008	8	Phosphorus (total)	**100**	ug/l	10		
5/20/2008	8	Total Alkalinity	**2500000**	ug/l	5000		
5/20/2008	8	Total Suspended Solids	**12000**	ug/l	1000		
5/20/2008	8	Total Volatile Residue	**130000**	ug/l	10000		
5/20/2008	8	Carbonaceous BOD	2000	ug/l	2000	U	Less than detection
5/20/2008	8	Total Organic Carbon	**5100**	ug/l	500		
5/20/2008	9	Ammonia	10	ug/l	10	U	Less than detection
5/20/2008	9	Nitrate	**2700**	ug/l	10		
5/20/2008	9	Nitrite	10	ug/l	10	U	Less than detection
5/20/2008	9	Nitrogen (Kjeldahl)	**1700**	ug/l	100		
5/20/2008	9	Phosphorus (ortho)	**80**	ug/l	10		
5/20/2008	9	Phosphorus (total)	**110**	ug/l	10		
5/20/2008	9	Total Alkalinity	**140000**	ug/l	5000		
5/20/2008	9	Total Suspended Solids	**51000**	ug/l	1000		
5/20/2008	9	Total Volatile Residue	**100000**	ug/l	10000		
5/20/2008	9	Carbonaceous BOD	2000	ug/l	2000	U	Less than detection
5/20/2008	9	Total Organic Carbon	**800**	ug/l	500		
5/20/2008	10	Ammonia	10	ug/l	10	U	Less than detection
5/20/2008	10	Nitrate	**2800**	ug/l	10		
5/20/2008	10	Nitrite	10	ug/l	10	U	Less than detection

Sample Date	Sample location	Parameter	Results	Units	DL	Qualifier	Notes
5/20/2008	10	Nitrogen (Kjeldahl)	**1700**	ug/l	100		
5/20/2008	10	Phosphorus (ortho)	**30**	ug/l	10		
5/20/2008	10	Phosphorus (total)	**140**	ug/l	10		
5/20/2008	10	Total Alkalinity	**150000**	ug/l	5000		
5/20/2008	10	Total Suspended Solids	**40000**	ug/l	1000		
5/20/2008	10	Total Volatile Residue	**88000**	ug/l	10000		
5/20/2008	10	Carbonaceous BOD	2000	ug/l	2000	U	Less than detection
5/20/2008	10	Total Organic Carbon	**8700**	ug/l	500		
5/20/2008	11	Ammonia	10	ug/l	10	U	Less than detection
5/20/2008	11	Nitrate	**2400**	ug/l	10	J	May be biased low
5/20/2008	11	Nitrite	10	ug/l	10	U	Less than detection
5/20/2008	11	Nitrogen (Kjeldahl)	**2500**	ug/l	100		
5/20/2008	11	Phosphorus (ortho)	**20**	ug/l	10		
5/20/2008	11	Phosphorus (total)	**160**	ug/l	10		
5/20/2008	11	Total Alkalinity	**140000**	ug/l	5000		
5/20/2008	11	Total Suspended Solids	**66000**	ug/l	1000		
5/20/2008	11	Total Volatile Residue	**72000**	ug/l	10000		
5/20/2008	11	Carbonaceous BOD	**2000**	ug/l	2000		
5/20/2008	11	Total Organic Carbon	**9200**	ug/l	500		

ug/L= microgram per liter

Sample Date	Sample location	Parameter	Results	Units	DL	Qualifier	Notes
10/18/2008	1	Ammonia	**100**	ug/l	10		
10/18/2008	1	Nitrate	**1100**	ug/l	10		
10/18/2008	1	Nitrite	10	ug/l	10	U	Less than detection
10/18/2008	1	Nitrogen (Kjeldahl)	**650**	ug/l	100		
10/18/2008	1	Phosphorus (ortho)	**80**	ug/l	10		
10/18/2008	1	Phosphorus (total)	**90**	ug/l	10		
10/18/2008	1	Total Alkalinity	**190000**	ug/l	5000		
10/18/2008	1	Total Suspended Solids	**22000**	ug/l	1000		
10/18/2008	1	Total Volatile Residue	**120000**	ug/l	10000		
10/18/2008	1	Carbonaceous BOD	1000	ug/l	1000	U	Less than detection
10/18/2008	1	Total Organic Carbon	**4400**	ug/l	500		
10/18/2008	2	Ammonia	**120**	ug/l	10		
10/18/2008	2	Nitrate	**1100**	ug/l	10		
10/18/2008	2	Nitrite	**50**	ug/l	10		
10/18/2008	2	Nitrogen (Kjeldahl)	**470**	ug/l	100		
10/18/2008	2	Phosphorus (ortho)	**80**	ug/l	10		
10/18/2008	2	Phosphorus (total)	**120**	ug/l	10		
10/18/2008	2	Total Alkalinity	**190000**	ug/l	5000		
10/18/2008	2	Total Suspended Solids	**17000**	ug/l	1000		
10/18/2008	2	Total Volatile Residue	**98000**	ug/l	10000		
10/18/2008	2	Carbonaceous BOD	**2000**	ug/l	1000		
10/18/2008	2	Total Organic Carbon	**4500**	ug/l	500		
10/18/2008	3	Ammonia	**90**	ug/l	10		
10/18/2008	3	Nitrate	**1200**	ug/l	10		
10/18/2008	3	Nitrite	**40**	ug/l	10		
10/18/2008	3	Nitrogen (Kjeldahl)	**670**	ug/l	100		
10/18/2008	3	Phosphorus (ortho)	**80**	ug/l	10		
10/18/2008	3	Phosphorus (total)	**60**	ug/l	10		
10/18/2008	3	Total Alkalinity	**190000**	ug/l	5000		
10/18/2008	3	Total Suspended Solids	**18000**	ug/l	1000		
10/18/2008	3	Total Volatile Residue	**77000**	ug/l	10000		
10/18/2008	3	Carbonaceous BOD	**1000**	ug/l	1000		
10/18/2008	3	Total Organic Carbon	**5000**	ug/l	500		
10/18/2008	4	Ammonia	**100**	ug/l	10		
10/18/2008	4	Nitrate	**1100**	ug/l	10		
10/18/2008	4	Nitrite	10	ug/l	10	U	Less than detection
10/18/2008	4	Nitrogen (Kjeldahl)	**730**	ug/l	100		
10/18/2008	4	Phosphorus (ortho)	**80**	ug/l	10		
10/18/2008	4	Phosphorus (total)	**80**	ug/l	10		
10/18/2008	4	Total Alkalinity	**190000**	ug/l	5000		
10/18/2008	4	Total Suspended Solids	**12000**	ug/l	1000		
10/18/2008	4	Total Volatile Residue	**88000**	ug/l	10000		
10/18/2008	4	Carbonaceous BOD	1000	ug/l	1000	U	Less than detection
10/18/2008	4	Total Organic Carbon	**4900**	ug/l	500		
10/18/2008	5	Ammonia	**90**	ug/l	10		
10/18/2008	5	Nitrate	**1100**	ug/l	10		
10/18/2008	5	Nitrite	10	ug/l	10	U	Less than detection
10/18/2008	5	Nitrogen (Kjeldahl)	**660**	ug/l	100		
10/18/2008	5	Phosphorus (ortho)	**80**	ug/l	10		
10/18/2008	5	Phosphorus (total)	**100**	ug/l	10		

Sample Date	Sample location	Parameter	Results	Units	DL	Qualifier	Notes
10/18/2008	5	Total Alkalinity	**190000**	ug/l	5000		
10/18/2008	5	Total Suspended Solids	**4000**	ug/l	1000		
10/18/2008	5	Total Volatile Residue	**110000**	ug/l	10000		
10/18/2008	5	Carbonaceous BOD	**2000**	ug/l	1000		
10/18/2008	5	Total Organic Carbon	**5300**	ug/l	500		
10/18/2008	6	Ammonia	**90**	ug/l	10		
10/18/2008	6	Nitrate	**1200**	ug/l	10		
10/18/2008	6	Nitrite	10	ug/l	10	U	Less than detection
10/18/2008	6	Nitrogen (Kjeldahl)	**680**	ug/l	100		
10/18/2008	6	Phosphorus (ortho)	**300**	ug/l	10		
10/18/2008	6	Phosphorus (total)	**90**	ug/l	10		
10/18/2008	6	Total Alkalinity	**190000**	ug/l	5000		
10/18/2008	6	Total Suspended Solids	**11000**	ug/l	1000		
10/18/2008	6	Total Volatile Residue	**100000**	ug/l	10000		
10/18/2008	6	Carbonaceous BOD	1000	ug/l	1000	U	Less than detection
10/18/2008	6	Total Organic Carbon	**4900**	ug/l	500		
10/18/2008	7	Ammonia	**80**	ug/l	10		
10/18/2008	7	Nitrate	**1100**	ug/l	10		
10/18/2008	7	Nitrite	10	ug/l	10	U	Less than detection
10/18/2008	7	Nitrogen (Kjeldahl)	**630**	ug/l	100		
10/18/2008	7	Phosphorus (ortho)	**80**	ug/l	10		
10/18/2008	7	Phosphorus (total)	**60**	ug/l	10		
10/18/2008	7	Total Alkalinity	**190000**	ug/l	5000		
10/18/2008	7	Total Suspended Solids	**25000**	ug/l	1000		
10/18/2008	7	Total Volatile Residue	**59000**	ug/l	10000		
10/18/2008	7	Carbonaceous BOD	**1000**	ug/l	1000		
10/18/2008	7	Total Organic Carbon	**5500**	ug/l	500		
10/18/2008	8	Ammonia	**90**	ug/l	10		
10/18/2008	8	Nitrate	**1100**	ug/l	10		
10/18/2008	8	Nitrite	10	ug/l	10	U	Less than detection
10/18/2008	8	Nitrogen (Kjeldahl)	**680**	ug/l	100		
10/18/2008	8	Phosphorus (ortho)	**80**	ug/l	10		
10/18/2008	8	Phosphorus (total)	**90**	ug/l	10		
10/18/2008	8	Total Alkalinity	**190000**	ug/l	5000		
10/18/2008	8	Total Suspended Solids	**18000**	ug/l	1000		
10/18/2008	8	Total Volatile Residue	**110000**	ug/l	10000		
10/18/2008	8	Carbonaceous BOD	**3000**	ug/l	1000		
10/18/2008	8	Total Organic Carbon	**4700**	ug/l	500		
10/18/2008	9	Ammonia	**80**	ug/l	10		
10/18/2008	9	Nitrate	**4400**	ug/l	10		
10/18/2008	9	Nitrite	**70**	ug/l	10		
10/18/2008	9	Nitrogen (Kjeldahl)	100	ug/l	100	U	Less than detection
10/18/2008	9	Phosphorus (ortho)	**80**	ug/l	10		
10/18/2008	9	Phosphorus (total)	**90**	ug/l	10		
10/18/2008	9	Total Alkalinity	**350000**	ug/l	5000		
10/18/2008	9	Total Suspended Solids	**13000**	ug/l	1000		
10/18/2008	9	Total Volatile Residue	**130000**	ug/l	10000		
10/18/2008	9	Carbonaceous BOD	1000	ug/l	1000	U	Less than detection
10/18/2008	9	Total Organic Carbon	**2500**	ug/l	500		
10/18/2008	10	Ammonia	**90**	ug/l	10		

Sample Date	Sample location	Parameter	Results	Units	DL	Qualifier	Notes
10/18/2008	10	Nitrate	**1200**	ug/l	10		
10/18/2008	10	Nitrite	10	ug/l	10	U	Less than detection
10/18/2008	10	Nitrogen (Kjeldahl)	**610**	ug/l	100		
10/18/2008	10	Phosphorus (ortho)	**100**	ug/l	10		
10/18/2008	10	Phosphorus (total)	**110**	ug/l	10		
10/18/2008	10	Total Alkalinity	**190000**	ug/l	5000		
10/18/2008	10	Total Suspended Solids	**19000**	ug/l	1000		
10/18/2008	10	Total Volatile Residue	**120000**	ug/l	10000		
10/18/2008	10	Carbonaceous BOD	**1000**	ug/l	1000		
10/18/2008	10	Total Organic Carbon	**6300**	ug/l	500		
10/18/2008	11	Ammonia	**90**	ug/l	10		
10/18/2008	11	Nitrate	**1100**	ug/l	10		
10/18/2008	11	Nitrite	10	ug/l	10	U	Less than detection
10/18/2008	11	Nitrogen (Kjeldahl)	**920**	ug/l	100		
10/18/2008	11	Phosphorus (ortho)	**90**	ug/l	10		
10/18/2008	11	Phosphorus (total)	**70**	ug/l	10		
10/18/2008	11	Total Alkalinity	**190000**	ug/l	5000		
10/18/2008	11	Total Suspended Solids	**18000**	ug/l	1000		
10/18/2008	11	Total Volatile Residue	**48000**	ug/l	10000		
10/18/2008	11	Carbonaceous BOD	1000	ug/l	1000	U	Less than detection
10/18/2008	11	Total Organic Carbon	**4600**	ug/l	500		

ug/L= microgram per liter

Appendix B

2008 Slime Identification Report

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Scott W. Tighe Microbiologist – Myclogist- Geneticist
320 Stowebury Properties Waterbury Center Vermont 05677
Tele: [802]-999-6666 Email: Mycology-lab@lycos.com

I. Sampling Information

Date Samples Received:	11/0608 10:56 am	**Project ID:**	Beaver Slough
Date Samples Collected:	11/5/08	**Requested Assay:**	ID Slime
Submitting Firm:	LTI-Limno-Tech, Inc.	**Analyst:**	S. Tighe
Collected by:	Kent Johnson		

II. Microbiological Data

ID	*Sample Date*	*Client Sample Location*	*Results*
2225	11/5/08 13:17	In plant E4#2 Waste stream-vacuum pump to wwtp via recirc	Moderate concentrations of Sphearotilus spp.
2264	11/5/08 13:18	In plant E4#3 Waste stream-vacuum pump to wwtp via recirc	Heavy concentrations of Sphearotilus spp
2272	11/5/08 15:21	On River-up river Periphyton on riprap	Primarily miscellanious debris with abundant diatoms with Very low concentrations of Sphearotilus spp
2283	11/5/08 15:48	On river-ADM002M2 On array mash in mixing zone	Heavy concentrations of Sphearotilus spp

Methods

MICROSCOPIC EXAM:

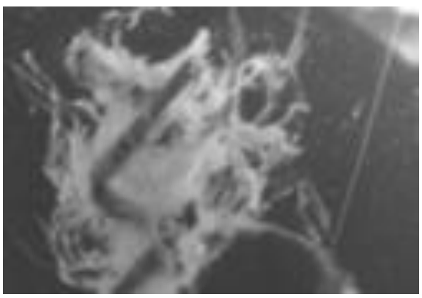
10 to 50 ul of slime or target debris was transferred to a microscope slide and examined at 100, 400, and 1000x magnification. Pictures were taken with a Sony 7.2MP Cyber-shot digital camera. Slime from mesh was aseptically transferred to microscope slide and examined.

DNA Identification Report

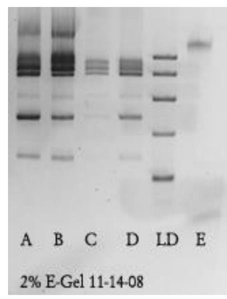
Methods

DNA AMPLIFICATION OF 16s rDNA AND BLAST SEQUNCE:

One sample representing the organism of interest for all samples was micro-dissectted, washed in PBS 3x, and resuspended in AL lysis buffer along with DNase and RNase free ALO3 microabrasive. Lysis was performed using a FastPrep-24 high speed automated homogenizer at 6k revs for 20 seconds. DNA was extracted using a Qiagen QiaAmp kit. The resulting DNA was eluted in TRIS buffer and quantified using a Qubit spectrofluorometer. Amplification of a 1301 base pair fragment of the 16s rDNA gene was achieved using the primers described by White et al in PCR Protocol 1989. PCR reagents consisted of Takara XL titanium hot start taq system and amplified in an MJ thermocycler using 30 cycles of a 94-51-68 program. PCR amplicons were visualized on a 2% E-Gel and treated with exonuclease and shrimp alkaline phosphatase prior to DNA sequencing. DNA sequencing was performed using Big Dye V3 chemistry for PCR products and analyzed on a Applied Biosystems 3130 genetic analyzer. The resulting DNA sequence was compared to the NIH-NCBI Blast NR database.



-Microdissected Slime-



A 2264 PCR1
B 2264 PCR2
C 2264 PCR3
D 2264 PCR 4
LD ladder 100
250
500
1K
2K
E 2264 gDNA

-Image of DNA Gel and lane designations-

Limno-Tech 2264 slime **DNA Match: Sphaerotilus sp. FSK08** 16S ribosomal RNA gene, partial sequence. Grouping>>Bacteria>>Proteobacteria>> Betaproteobacteria>>Burkholderiales>> Sphaerotilus. **Origin of Closest match:** Submitted (19-MAY-2008) College of Life Science, Fujian Normal University (New Campus), Shangjie Town, Minhou District, Fuzhou, Fujian 350108, China

Limnotech sequence 2264-**lclj28197**

**CGACCGGTTTTCTGGGAtTGGCTCCCCCTCGCGGGTTGGCAGCCCTCTGTACCGGCCATTGTATGACGTGTGTAGCCCTACCCATAAGGGCCATGAGGAC
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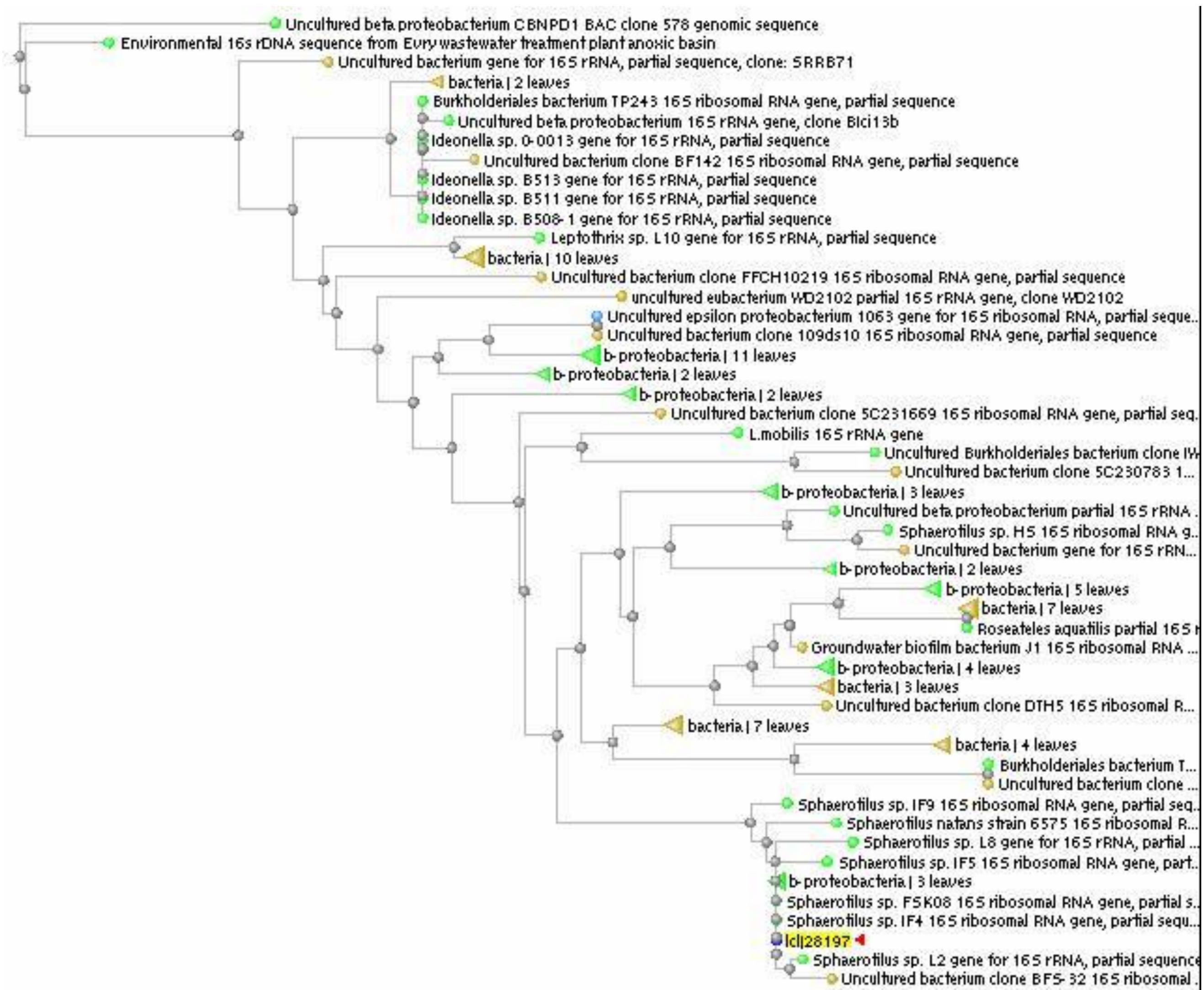
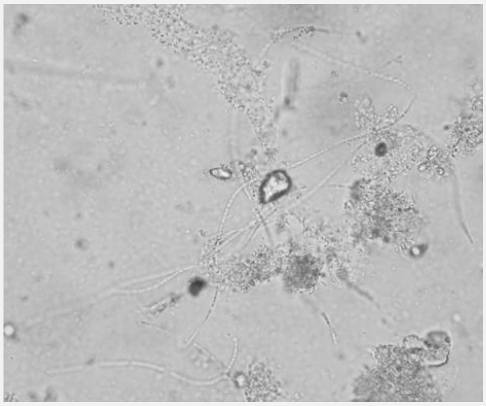
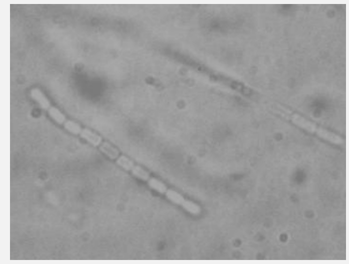
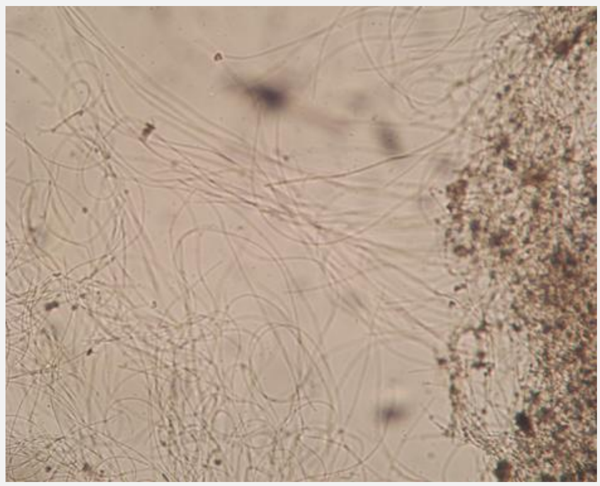
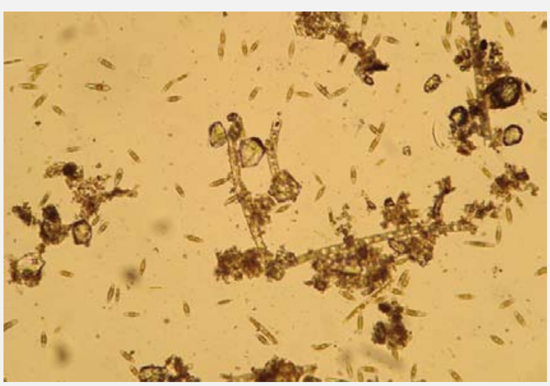
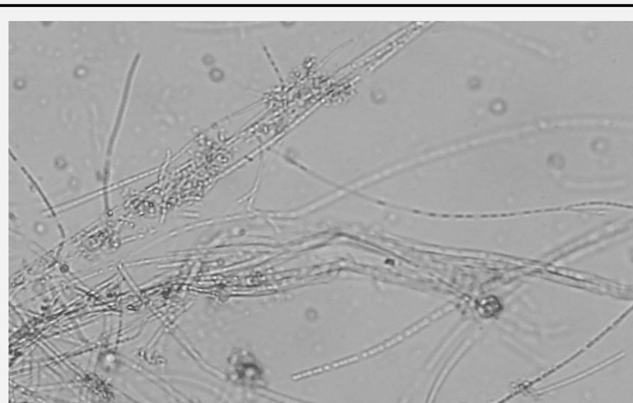
ACTGCTACACGCGGAATTCCATCCCCCTCTACCGTACTCTAGCTCCACAGTCACAAATGCAGTTCCCAGGTTGAGCCCGGGGATTTCACATCTGTCTTAT
GGAACCGCCTGCGCACGCTTTACGCCCAGTAATTCCGATTAACGCTTGCACCCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGGTGCTTATTCT
TCagtACCGTCATCCTCCCGAGGTA**

Phylogenetic Position of LimnoTech *Sphaerotilus* 2264 (lclj28197)



2225	 
2264	
2272	 
2283	

DNA Sequence of 2264