EERC
Energy & Environmental Research Center

UNIVERSITY OF NORTH DAKOTA
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July 1, 2011

Ms. Karlene Fine
Executive Director
North Dakota Industrial Commission
State Capitol – 14th Floor
600 East Boulevard Avenue, Department 405
Bismarck, ND 58505-0840

Dear Ms. Fine:

Subject: EERC Proposal No. 2011-0291 Entitled "Improving the Profitability of North Dakota Ethanol Plants with Algae"

The Energy & Environmental Research Center (EERC) of the University of North Dakota is pleased to submit the subject proposal to the North Dakota Industrial Commission Renewable Energy Program. The EERC is committed to completing the project as described in this proposal if the Commission makes the requested grant.

Enclosed please find an original and one copy of the proposal entitled "Improving the Profitability of North Dakota Ethanol Plants with Algae." Also enclosed is the $100 application fee. The EERC, a research organization within the University of North Dakota, an institution of higher education within the state of North Dakota, is not a taxable entity; therefore, it has no tax liability.

Initiation of the proposed work is contingent upon the execution of mutually negotiated agreements or modifications to existing agreements between all participating sponsors.

If you have any questions, please contact me by telephone at (701) 777-5040 or by e-mail at pletvin@undeerc.org.

Sincerely,

Peter A. Letvin
Research Engineer

Approved by:

Dr. Gerald H. Groenewold, Director
Energy & Environmental Research Center

PAL/kmd
Enclosures

Renewable Energy Program

North Dakota Industrial Commission

Application

Project Title: Improving the Profitability of North Dakota Ethanol Plants with Algae

Applicant: University of North Dakota Energy & Environmental Research Center

Principal Investigator: Peter A. Letvin

Date of Application: July 1, 2011

Amount of Request: $200,000

Total Amount of Proposed Project: $426,550

Duration of Project: 12 months

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ABSTRACT

Objective:

The Energy & Environmental Research Center (EERC) proposes to perform heterotrophic growth of omega-3-rich algae on thin stillage, syrup, evaporator condensate, and/or methanator effluent from ethanol refineries in North Dakota. Bench-scale research is required to determine if a field demonstration and, finally, implementation are warranted. A report will be generated that discusses the technical and economic feasibility of growing algae on ethanol side streams. This report will estimate the revenue and capital and operating costs to produce omega-3-rich algae grown on ethanol side streams.

Expected Results:

It is expected that this research will show that a 50-million-gallon-per-year (MGY) ethanol refinery could generate up to 19,000 pounds per day of algae grown on thin stillage. Anaerobic digesters utilizing thin stillage (50-MGY-ethanol basis) produce methane valued between $2400 and $10,000 per day. Algae have an existing value of $1/lb (for kelp meal) to $9/lb (for bulk spirulina) for livestock feed additives. At that value, the EERC believes that algae grown on thin stillage have the potential to increase the revenues of North Dakota ethanol producers and, as a result, create jobs in North Dakota.

Duration:

The project duration is 1 year.

Total Project Cost and Partners:

The total project cost is $426,550. The EERC is requesting $200,000 from the North Dakota Industrial Commission Renewable Energy Program along with an additional $200,000 to be requested from the U.S. Department of Energy-funded Center for Biomass Utilization® at the EERC. Blue Flint Ethanol will contribute $15,000, Red Trail Energy $4800, and Chem E Inc. $6750, all as noncash cost share in the form of labor. Other participants are Great River Energy and the North Dakota Ethanol Producers Association.

PROJECT DESCRIPTION

Objectives:

The objective of this proposed Energy & Environmental Research Center (EERC) project is to increase revenues and reduce costs to North Dakota ethanol refineries with algae, thus making North Dakota ethanol plants more competitive, protecting against the effects of lost government subsidies, and protecting against the future cost competition from the proposed ethanol pipeline from South Dakota to East Coast markets. Specifically, the objective is to grow algae on thin stillage, syrup, evaporator condensate, or methanator effluent and sell it as a feed additive. These side streams are highlighted in Figure 1.

The proposed research is the first of several planned phases to actually grow algae at an ethanol facility. The first phase involves bench-scale work: growing algae in a lab, collecting preliminary data, and performing preliminary estimates of capital costs, production costs, and valuation of algae. Any changes in costs or revenues will be considered in relation to an existing ethanol plant's process. This first phase of research will provide direction for further phases such as a field demonstration on a slipstream.

Bench-scale work is necessary because of the unique issues anticipated when growing algae cultures on industrial side streams. There is little research showing algae can be grown on ethanol thin stillage; however, heterotrophic algae and diatoms have been reported to grow on carbon sources such as glucose, fructose, glycerol, starch, oleic acid, lactose, saccharose, linseed oil, and nutrient sources such as

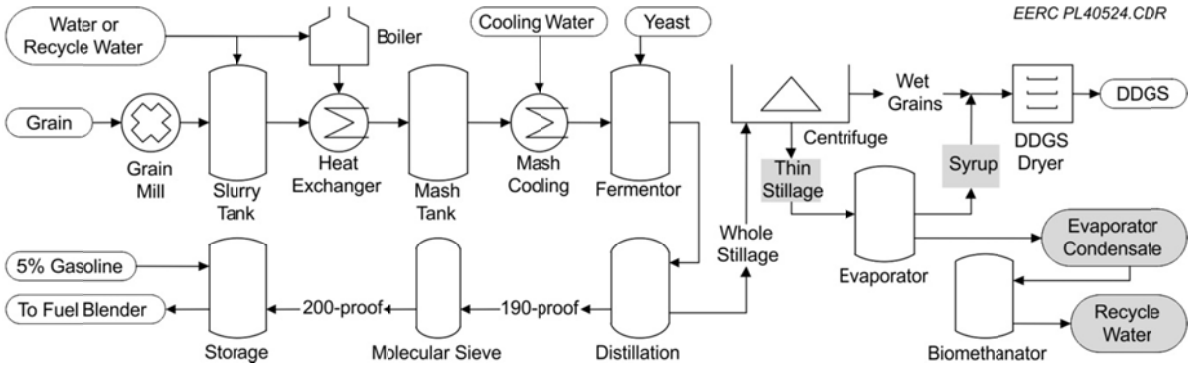

Figure 1. Generic ethanol plant schematic.

yeast extract, corn steep liquor, ammonium acetate, urea, polypepton, and tryptone.[1–5] These same or similar components are found in the side streams of ethanol production including thin stillage (sugars, lipids, and starches).[2, 3, 6, 7] The heterotrophic species identified in the literature may have special needs to grow well on ethanol side streams because of pH ranges, toxins, or contaminant competition. These issues will be determined and documented during the bench-scale work.

Methodology:

Overall, this research is the first phase in a path to production. Phase 1, which is currently proposed, is bench-scale work including the following tasks: 1) Sample Collection, Culture Management, and Targeted Species Screening; 2) Further Algae Experiments; 3) Data Reduction and Modeling; 4) Conceptual Engineering Design and Economics; and 5) Project Management (this task is discussed under the Project Management section).

1) Sample Collection, Culture Management, and Targeted Species Screening

Algae will be grown heterotrophically using the organic carbon in the selected ethanol refinery side streams as the carbon and energy for growth. Stream samples will be collected as needed from two partner refineries. Collected cultures will be grown in laboratory incubators to prepare them for experiments and to propagate enough culture for the experiments. Initially, up to eight cultures of commercial interest are planned to be tested, but more cultures may be tested because of the complex nature of the substrate. The screening experiments will be performed in the Environmental Microbiology Laboratory at the EERC using an automated respirometer, because it allows the simultaneous collection of continuous oxygen uptake data from multiple reactors. These data are of very high quality and are used along with analytical measurements of initial and final samples from each experiment to determine the kinetics and stoichiometry of heterotrophic growth. Because of the high fidelity of the data collected and the fact that they are collected on a continuous basis, the respirometric data can also reveal growth associated with multiple substrates and the presence of nutrient limitations during screening. Respirometric data can also be used to study effects due to substrate inhibition and the presence of nonsubstrate inhibitors. Appendix A contains a book chapter[8] (used with permission) that illustrates some

of the ways respirometric measurements can be used to study the kinetics and stoichiometry of heterotrophic growth.

2) Further Algae Experiments

A second set of experiments in the respirometer will follow the screening experiments to adjust for any media deficiencies or issues to maximize growth. Data will be collected during these experiments, which will be used in engineering and economic order-of-magnitude calculations. Table 1 shows the data to be collected and their intended use for this research.

3) Data Reduction and Modeling

Data interpretation will include numerical regression analysis (using Dr. Cowan's existing spreadsheet programs) to obtain best fit estimates of the stoichiometric and kinetic parameters that describe heterotrophic growth. These parameters will be used to calibrate numerical models useful in sizing the processing equipment and predicting process performance, including chemical oxygen demand (COD) and nutrient removal and algal biomass yield. This is further explained in Appendix A.

4) Conceptual Engineering Design and Economics

The reduced data from the bench-scale work will be used with partner input to create a conceptual process flow diagram, stream tables, and conceptual designs for an algae facility addition to a North Dakota ethanol plant. The process flow diagrams and stream tables will define the engineering order-of-magnitude specifications needed to calculate the rough costs of growing algae at an ethanol plant. The

Table 1. Data Uses and Calculations

Data	Uses/Calculations
Carbon Conversion	Potential algae output, media recycle
COD (before/after)	Used in regression of kinetics and stoichiometry, water recycle
O_2 Uptake Rate	Growth kinetics and stoichiometry, use for engineering calculations such as reactor sizing
Omega-3 Fatty Acids	Quality of produced algae as a high-value feed
Sugars and Volatile Fatty Acids Analysis	Water quality related to water recycle
Nutrients (e.g., N, P)	Nutrient limitations, nutrient supply cost, influence on algae quality
Settleability, Filterability	Preliminary evaluation of algae-harvesting methods, use for engineering and economic calculations

system process flow diagram will include everything from culturing and labor to the harvesting and processing of algal biomass.

Marketing research will help determine the value of the algae as an animal feed or nutritional additive. Lipid, protein, carbohydrate, and mineral data from analysis of algae biomass samples harvested from the experiments will be used in the valuation of the algae. As stated earlier, it is expected that these algae will be at least as valuable as kelp meal but potentially more valuable. The fatty acid analysis of the algae cultures will be used to determine the potential level of benefit to livestock fed algae feed additives. Industry input as well as nutritional measurements will be used to justify a potential value that can be expected of this feed product. Animal feed studies are not part of this phase, but will likely be included in future phases of this research in North Dakota.

An economic summary, including all of the above information and considerations, will be discussed in a final report. Included in the report will be discussions of the overall feasibility of this project, the estimated capital and operating costs, algal production data, production inhibitors, the potential market value of algae livestock feed, and the overall cost and benefit to an existing North Dakota facility. This analysis will be completed by the EERC, with strong industry input.

Anticipated Results:

We anticipate finding one or more species of algae that will grow well on thin stillage and may grow on the other side streams. However, the composition of the side streams may not contain all of the components necessary to maximize algal production and will likely have to be adjusted through nutrient or carbon supplementation (e.g., addition of syrup from the thin stillage evaporator to the evaporator condensate of biomethanator effluent to boost algae production or nutrient supplementation). This is where the respirometer will be of great value, saving significant costs in analytical and labor during the screening process. These experiments will be covered under the Further Algae Experiments task.

An additional issue that will be monitored is inhibition—contamination of the algae cultures by other microbes that will compete for substrates and nutrients. Avoiding growth of competing species and control and/or recovery from contamination events will be a point of consideration that may influence the

ability to run the algae growth process in a continuous or batch mode. The details of these issues are unknown at this time, but will be part of the final report.

Based on thin stillage measurements in the literature, [3, 4, 6] and the potential carbon conversion of these species,[1, 4] we believe that a 50-million-gallon-per-year (MGY) ethanol plant in North Dakota may be able to produce as much as 19,000 lb of dry algae biomass. Algae have an existing value of $1/lb (for kelp meal) to $9/lb (for bulk spirulina) for livestock feed additives. Comparatively, anaerobic digesters utilizing thin stillage (50-MGY ethanol basis) produce methane valued between $2400 per day[3] and $10,000 per day.[7] If algae are grown on evaporator condensate, biomass production will be much lower, but the production costs will also be much less since the water will contain fewer biological contaminants.

Ethanol plants in North Dakota are in a strategic position to capitalize on this idea. The special advantage they have is an existing customer base to immediately market algae as a stand-alone feed additive or an additive to the existing livestock feed commodity, dried distiller's grains (DDGs). Provided a favorable economic assessment, this technology could be further advanced by a demonstration facility in North Dakota to produce algae that will be used in livestock feed research, also in North Dakota.

Facilities:

Heterotrophic growth of algae will be performed efficiently in an automated respirometer[8] at the EERC (also see Appendix A). Data collection will include characterization of the initial and final conditions of each reactor, including COD, nitrogen, phosphorus, pH, total and volatile suspended solids (TSS and VSS), and analysis of lipid content. These measurements will be performed in the EERC Analytical Research Laboratory (ARL). Continuous monitoring of oxygen uptake will be performed using an automated respirometer (such as the BI-2000® laboratory respirometer from Bioscience, Inc.; AER-200 respirometer from Challenge Technology; or Respirometer Systems and Applications [RSA] PF-8000). Funds are requested for purchase of this equipment at an estimated cost of $25,000, based on a quote from RSA (justification provided in Appendix B).

Resources:

In addition, the EERC has a wide range of analytical capabilities that have been tailored to fuels, ash, and other materials associated with energy and environmental issues; these techniques include a full range of organic, inorganic, surface and mineralogical, thermal, and physical analyses. Access to this caliber of analytical expertise will ensure the success of this project.

Techniques to Be Used, Their Availability, and Capability:

The design of the respirometer experiments and interpretation of the results will be performed by Dr. Robert Cowan, an EERC Research Engineer who is a recognized expert in the area of respirometric methods for environmental engineering and science applications. For more information, see Appendix A.

Environmental and Economic Impacts While Project Is Under Way:

This project will provide engineers and scientists in North Dakota the funding to complete valuable research that will benefit North Dakota ethanol producers. The method described here to produce high-quality livestock feed from algae and ethanol refineries is unique.

Ultimate Technological and Economic Impacts:

Following the successful completion of this project, a second phase of research will involve a pilot plant to continue technical research. Operation of the pilot facility will provide verification of all cost estimates from Phase 1 research. Currently, there is a volume of research[9–17] showing the benefits of feeding kelp meal (macroalgae) and microalgae to livestock; dairy, egg, and meat production volume are all increased with these practices. Microalgae used as a feed additive may also enrich the omega-3 content of livestock produce. Ethanol plants can reduce side stream recycle costs and generate a profit from feed-grade algae grown on side streams from ethanol production. Algae can also utilize glycerol from biodiesel production before purification to glycerin, decreasing processing costs. These increased revenue streams will provide North Dakota renewable energy producers the ability to create jobs to operate these facility additions.

North Dakota farmers can add value to their livestock produce, giving them a disruptive, competitive advantage over farmers in other states. Dairy, egg, and meat producers in North Dakota will have algae-based high-quality feed additive that will increase their revenues, marketability, and

competitive edge as producers of omega-3-enriched products. More information on the value of algae as a feed additive is contained in Appendix C.

Why the Project Is Needed:

Ethanol producers in our state are facing economic pressures from several fronts. One is that the current federal government is strongly considering the expiration of ethanol subsidies or tax credits. The other regional pressure that North Dakota ethanol producers may soon face is the proposed pipeline from South Dakota to the East Coast. This pipeline is estimated to give ethanol producers in those areas a $0.20/gallon advantage by reducing transportation costs to get their fuel to market.

There is a huge potential that algae for feed could be a very big industry, and many people are beginning to recognize that fact. A developer from Oklahoma City said, “It’s a business that could be worth $50 billion ... and won’t need carbon credits to be viable commercially.”[18] A market research paper by Frost & Sullivan discussed “a projected ten-percent compound annual growth rate from 2008 to 2013 in the marine and algae oil omega-3 ingredient market.”[19] These statements have also been made in a new report from Packaged Facts (a division of MarketResearch.com) “With its United States market value swelling from approximately $100M to more than $2B in four years, omega-3 enriched foods make up the strongest sector of the functional foods market—and there is still room for significant growth.”[20]

STANDARDS OF SUCCESS

This research could lead to millions of dollars in increased revenue for North Dakota ethanol producers. It is expected that there will be a very reasonable payback period for the expense of algae equipment at an ethanol refinery and that an algae process can be successfully integrated at the facility. An algae feed commodity would have an immediate salable value because of the existing customer base for DDGs as livestock feed. If this research shows that heterotrophic algae can be integrated into ethanol refineries in North Dakota to supplement some costs to produce ethanol, then this project will be a success. If this is the case, a second-phase project should follow this first-phase research to build a demonstration plant in North Dakota.

Once all phases are complete, this research could result in high-tech jobs for North Dakotans, and ethanol refineries in the state will be economically competitive to meet the challenges of lost government subsidies and transportation cost competition.

BACKGROUND/QUALIFICATIONS

The EERC is one of the world's major energy and environmental research organizations. Since its founding in 1949, the EERC has conducted research, testing, and evaluation of fuels, combustion and gasification technologies, emission control technologies, ash use and disposal, analytical methods, groundwater, waste-to- energy systems, and advanced environmental control systems. The EERC is committed to a partnership team approach for energy and environmental technologies.

The Centers for Renewable Energy and Biomass Utilization are a designated Center of Excellence located at the EERC. The centers conduct critical research, development, demonstration, and commercial deployment of technologies utilizing biomass, wind, solar, geothermal, and hydroelectric energy sources. Under the Center for Biomass Utilization® (CBU®), the EERC offers the most comprehensive approach to biomass conversion research.

Blue Flint Ethanol, LLC, which is supporting this project, is a joint venture between Great River Energy (GRE) and Headwaters Incorporated. Utilizing primarily waste heat from the adjacent Coal Creek Station, Blue Flint is the first collocated, directly integrated ethanol plant in the world. Blue Flint continues to pursue innovative projects to drive down production costs, diversify its revenue, achieve greater value for its coproducts, and reduce its carbon footprint. Blue Flint will provide technical support and advice for this project. Chem E Inc., a process engineering company operating out of Fargo, North Dakota, for the past 10 years, provides processing engineering consultancy services to manufacturing facilities around the globe but has a special interest in local North Dakota industries. Chem E specializes in the sugar, food, and bioenergy industries such as biodiesel and ethanol production. Chem E is very supportive of innovative energy efforts and hopes to support North Dakota as it continues to expand the renewable energy efforts begun by local providers. Red Trail Energy, LLC (RTE), is a North Dakota-based investor group formed to finance, construct, and operate a corn-based ethanol production facility

located near Richardton, North Dakota. As one of the first coal-fired ethanol plants in the nation, RTE produces 50 million gallons of ethanol, using 18–20 million bushels of corn and ~100,000 tons of coal annually. RTE now employs 41 personnel with an annual payroll of $1.8 million. Letters of commitment from participating organizations are contained in Appendix D.

The project will be managed by Mr. Peter Letvin, a Research Engineer at the EERC. Mr. Letvin's work focuses on algae energy, wastewater cleanup, hydrogen production, and emission control. Prior to his position at the EERC, he served as Director of Operations for Solix Biofuels in Fort Collins, Colorado. Mr. Letvin's principal areas of expertise include algae growth and culturing, large-scale algae cultivation, low-cost algae photobioreactors, and low-cost algae-processing equipment. Mr. Letvin is named as inventor on four patents and patent applications resulting from his work in this area. Mr. Letvin holds an M.S. degree in Mechanical Engineering from Colorado State University and a B.S. degree in Mechanical Engineering from the University of North Dakota.

Dr. Robert Cowan will be a principal investigator and will lead culturing tasks for the EERC. Dr. Cowan has 18 years of academic, research, and consulting experience in environmental engineering focused on biodegradation kinetics, bioremediation, and biological wastewater treatment. He is an author of over 100 publications and presentations at national and international conferences. Dr. Cowan's work with respirometers has concentrated on methods for measuring kinetic parameters and evaluating factors affecting the application of the measurements to wastewater treatment plant environments.

Dr. Steven Schlasner will lead tasks related to engineering design and economic evaluation. Dr. Schlasner has more than 25 years of experience in chemical and microbial bioprocess engineering, which includes supervision of an industrial R&D biopharmaceutical pilot plant as well as a petroleum refinery wastewater biotreater. Dr. Schlasner has also served as an internal consultant on microalgae-based biofuels for a major oil company, has consulted with the U.S. Air Force Research Laboratory on bioremediation of hazardous aircraft paint waste, and has served as consultant and reviewer of biologically derived hydrogen projects for the U.S. Department of Energy (DOE).

Dr. Laura Raymond will lead the omega-3 analysis of algae cultures. Dr. Raymond has a Ph.D. in Biochemistry and Molecular Biology, with a cognate emphasis in nutritional metabolism, and an undergraduate degree in Microbiology. As the Research Manager of the Health and Analytical Research Group, she oversees the Natural Materials Analytical Research Laboratory (NMARL), the ARL, and the Cell and Tissue Culture Laboratory at the EERC. Pertinent to this project is her expertise in the physiological processes involved in risks and the benefits associated with fish consumption, specifically the omega-3 fatty acids DHA (docosahexaenoic acid) and EPA (eicosupentaenoic acid).

MANAGEMENT

Mr. Peter Letvin will be responsible for overall project management. Dr. Cowan will lead culturing and respirometry tasks, Dr. Schlasner will lead engineering tasks, and Dr. Raymond will lead the omega-3 analysis and marketing.

In order to manage the schedule, task managers and others as necessary will meet biweekly with the project manager to discuss current technical findings, future plans, and schedule updates. Because of the rapid nature of the respirometry and screening experiments, the project manager will be assisting in the experiments in order to remain fully aware of the effects to the project schedule. The project manager will also be involved in all of the scheduled tasks as a researcher to remain aware of the schedule impacts and technical results.

Project presentations will be made to the North Dakota Industrial Commission and DOE at the completion of this project. Interim presentations will be made at larger meetings with project partners. These meetings will focus on unity between project participants and agreement on common engineering and economic information. In order for the project to be effectively managed, previously discussed tasks with the accompanying dates are shown in Table 2. Timing of interim reports summarizing task activities and accomplishments will follow the guideline specified by NDIC requirements and in accordance with the contracted agreement. Any delays in the schedule will also be noted, along with corrective action to ensure timely completion of the project. Resumes for key personnel can be found in Appendix E.

TIMETABLE

Table 2. Project Tasks and Time Line

Tasks	Event	Start	Completion
1	Sample Collection, Culture Management, and Targeted Species Screening	Month 1	Month 4
2	Further Algae Experiments	Month 4	Month 6
3	Data Reduction and Modeling	Month 2	Month 9
4	Conceptual Engineering Design and Economics	Month 3	Month 9
5	Project Management	Month 1	Month 12

A final report will be submitted at the end of Month 12.

BUDGET

Project Associated Expense	NDIC Share	Blue Flint Ethanol (In-Kind)	Chem E Inc. (In-Kind)	Red Trail Energy (In-Kind)	CBU Share (Cash)
Total Direct Salaries	$52,861				$57,522
Total Fringe	$29,074				$31,637
Total Labor	$81,935				$89,159
Travel	$2,583				$6,585
Equipment > $5000	–				$25,000
Supplies	$12,512				$9,488
Communication	$250				$250
Printing & Duplicating	$100				$136
Food	–				$500
Operating Fees and Services	$27,620				$11,332
Total Direct Costs	$125,000				$142,450
Total Indirect Costs (F&A)	$75,000				$57,550
Noncash Cost Share	–	$15,000	$6,750	$4,800	–
Total Project Cost	$200,000	$15,000	$6,750	$4,800	$200,000

CONFIDENTIAL INFORMATION

There is no confidential information being claimed by the EERC in this proposal.

PATENTS/RIGHTS TO TECHNICAL DATA

There are no patents or rights that are reserved by the EERC in this proposal.

REFERENCES

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IMPROVING THE PROFITABILITY OF NORTH DAKOTA ETHANOL PLANTS WITH ALGAE
NDIC - RENEWABLE ENERGY COUNCIL
PROPOSED PROJECT START DATE: 11/1/11
EERC PROPOSAL #2011-0291 (REVISION TO 2011-0227)

BUDGET

CATEGORY		TOTAL			NDIC - REC SHARE			DOE - CBU SHARE		
LABOR		Rate	Hrs	$ Cost	Hrs	$ Cost		Hrs	$ Cost	
Letvin, P.	Project Manager	$ 31.80	610	$ 19,398	300	$ 9,540		310	$ 9,858	
Cowan, R.	Principal Investigator	$ 46.80	370	$ 17,316	180	$ 8,424		190	$ 8,892	
Schlasner, S.	Research Scientist/Engineer	$ 48.06	180	$ 8,651	100	$ 4,806		80	$ 3,845	
Raymond, L.	Research Scientist/Engineer	$ 41.17	150	$ 6,176	98	$ 4,035		52	$ 2,141	
----------	Senior Management	$ 74.19	96	$ 7,122	-	$ -		96	$ 7,122	
----------	Research Scientists/Engineers	$ 39.47	1,033	$ 40,773	573	$ 22,616		460	$ 18,157	
----------	Research Technicians	$ 25.94	148	$ 3,839	9	$ 233		139	$ 3,606	
----------	Technical Support Services	$ 21.50	40	$ 860	10	$ 215		30	$ 645	
				$ 104,135		$ 49,869			$ 54,266	
Escalation Above Base		6%		$ 6,248		$ 2,992			$ 3,256	
TOTAL DIRECT HRS/SALARIES			2,627	$ 110,383	1,270	$ 52,861		1,357	$ 57,522	
Fringe Benefits - % of Direct Labor - Staff		55.0%		$ 60,711		$ 29,074			$ 31,637	
TOTAL FRINGE BENEFITS				$ 60,711		$ 29,074			$ 31,637	
TOTAL LABOR				$ 171,094		$ 81,935			$ 89,159	
OTHER DIRECT COSTS										
TRAVEL				$ 9,168		$ 2,583			$ 6,585	
EQUIPMENT > $5000				$ 25,000		$ -			$ 25,000	
SUPPLIES				$ 22,000		$ 12,512			$ 9,488	
COMMUNICATION - LONG DISTANCE & POSTAGE				$ 500		$ 250			$ 250	
PRINTING & DUPLICATING				$ 236		$ 100			$ 136	
FOOD				$ 500		$ -			$ 500	
OPERATING FEES & SVCS										
Analytical Research Lab.				$ 37,784		$ 27,120			$ 10,664	
Graphics Support				$ 668		$ -			$ 668	
Freight				$ 500		$ 500			$ -	
TOTAL DIRECT COST				$ 267,450		$ 125,000			$ 142,450	
FACILITIES & ADMIN. RATE - % OF MTDC			VAR	$ 132,550	60%	$ 75,000		49%	$ 57,550	
NON-CASH COST SHARE - BLUE FLINT ETHANOL				$ 15,000		$ -			$ -	
NON-CASH COST SHARE - CHEM E INC.				$ 6,750		$ -			$ -	
NON-CASH COST SHARE - RED TRAIL ENERGY, LLC				$ 4,800		$ -			$ -	
TOTAL PROJECT COST - US DOLLARS				$ 426,550		$ 200,000			$ 200,000	

Due to limitations within the University's accounting system, bolded budget line items represent how the University proposes, reports and accounts for expenses. Supplementary budget information, if provided, is for proposal evaluation.

IMPROVING THE PROFITABILITY OF NORTH DAKOTA ETHANOL PLANTS WITH ALGAE
EERC PROPOSAL #2011-0291 (REVISION TO 2011-0227)

BUDGET - TRAVEL

SITE VISIT - Blue Flint Ethanol (Underwood, ND)

					AIRFARE		PER DIEM			CAR		
					RND TRP	1-WAY	HOTEL	MEALS	MISC	RENTAL	MILEAGE	
NAME	# PERSONS	# DAYS	# NIGHTS	# MILES								TOTAL
Person 1	1	1	-	600	$ -	$ -	$ -	$ 25	$ 10	$ -	$ 510	$ 545
Person 2	1	1	-	-	$ -	$ -	$ -	$ 25	$ 10	$ -	$ -	$ 35
Person 3	1	1	-	-	$ -	$ -	$ -	$ 25	$ 10	$ -	$ -	$ 35
Person 4	1	1	-	-	$ -	$ -	$ -	$ 25	$ 10	$ -	$ -	$ 35
Person 5	1	1	-	-	$ -	$ -	$ -	$ 25	$ 10	$ -	$ -	$ 35
Total - Site Visit												$ 685

SAMPLING - (Hankinson, ND)

					AIRFARE		PER DIEM			CAR		
					RND TRP	1-WAY	HOTEL	MEALS	MISC	RENTAL	MILEAGE	
NAME	# PERSONS	# DAYS	# NIGHTS	# MILES								TOTAL
Person 1 - Sampling	1	1	-	350	$ -	$ -	$ 130	$ 25	$ 10	$ -	$ 298	$ 333
Person 2 - Sampling	1	1	-	-	$ -	$ -	$ -	$ 25	$ 10	$ -	$ -	$ 35
Total - Sampling												$ 368

SAMPLING - ADM Biodiesel (Velva, MN)

					AIRFARE		PER DIEM			CAR		
					RND TRP	1-WAY	HOTEL	MEALS	MISC	RENTAL	MILEAGE	
NAME	# PERSONS	# DAYS	# NIGHTS	# MILES								TOTAL
Person 1 - Sampling	1	1	-	500	$ -	$ -	$ 140	$ 25	$ 10	$ -	$ 425	$ 460
Person 2 - Sampling	1	1	-	-	$ -	$ -	$ -	$ 25	$ 10	$ -	$ -	$ 35
Total - Sampling												$ 495

SAMPLING - (Unknown)

					AIRFARE		PER DIEM			CAR		
					RND TRP	1-WAY	HOTEL	MEALS	MISC	RENTAL	MILEAGE	
NAME	# PERSONS	# DAYS	# NIGHTS	# MILES								TOTAL
Person 1 - Sampling	1	1	-	350	$ -	$ -	$ 125	$ 25	$ 10	$ -	$ 298	$ 333
Person 2 - Sampling	1	1	-	-	$ -	$ -	$ -	$ 25	$ 10	$ -	$ -	$ 35
Total - Sampling												$ 368

OTHER TRAVEL

					AIRFARE		PER DIEM			CAR			
					RND TRP	1-WAY	HOTEL	MEALS	MISC	RENTAL	MILEAGE	REGISTR.	
NAME – PURPOSE	# PERSONS	# DAYS	# NIGHTS	# MILES									TOTAL
Person 1 - Conference	1	4	3	-	$ 950	$ -	$ 525	$ 284	$ 80	$ 300	$ -	$ 575	$ 2,714
Person 2 - Conference	1	4	3	-	$ 950	$ -	$ 525	$ 284	$ 80	$ -	$ -	$ 575	$ 2,414
Person 1 - DOE Review Meeting	1	2	1	-	$ 950	$ -	$ 175	$ 142	$ 40	$ 150	$ -	$ -	$ 1,457
Person 1 - Meetings	1	2	1	500	$ -	$ -	$ 175	$ 142	$ 40	$ -	$ 310	$ -	$ 667
Total - Other Travel													$ 7,252

TOTAL ESTIMATED TRAVEL $ 9,168

DETAILED BUDGET - EQUIPMENT

Other Equipment	$ COST
PF-8000 Respirometer	$ 25,000
	$ 25,000
Total Equipment	$ 25,000

IMPROVING THE PROFITABILITY OF NORTH DAKOTA ETHANOL PLANTS WITH ALGAE
EERC PROPOSAL #2011-0291 (REVISION TO 2011-0227)

DETAILED BUDGET - EERC RECHARGE CENTERS

Analytical Research Lab.	Rate	#	$ Cost
Alkalinity	$ 27	10	$ 270
COD	$ 16	378	$ 6,048
IC	$ 31	401	$ 12,431
ICP	$ 36	10	$ 360
Trace Element Digestion	$ 60	10	$ 600
NH3	$ 34	122	$ 4,148
pH	$ 16	10	$ 160
TKN	$ 60	10	$ 600
TSS	$ 9	734	$ 6,606
TVS	$ 33	134	$ 4,422
Subtotal			$ 35,645
Escalation		6%	$ 2,139
Total Analytical Research Lab.			$ 37,784

Graphics Support	Rate	#	$ Cost
Graphics (hourly)	$ 63	10	$ 630
Subtotal			$ 630
Escalation		6%	$ 38
Total Graphics Support			$ 668

BUDGET NOTES

ENERGY & ENVIRONMENTAL RESEARCH CENTER (EERC)

BACKGROUND

The EERC is an independently organized multidisciplinary research center within the University of North Dakota (UND). The EERC receives no appropriated funding from the state of North Dakota and is funded through federal and nonfederal grants, contracts, and other agreements. Although the EERC is not affiliated with any one academic department, university faculty may participate in a project, depending on the scope of work and expertise required to perform the project.

INTELLECTUAL PROPERTY

If federal funding is proposed as part of this project, the applicable federal intellectual property (IP) regulations may govern any resulting research agreement. In addition, in the event that IP with the potential to generate revenue to which the EERC is entitled is developed under this agreement, such IP, including rights, title, interest, and obligations, may be transferred to the EERC Foundation, a separate legal entity.

BUDGET INFORMATION

The proposed work will be done on a cost-reimbursable basis. The distribution of costs between budget categories (labor, travel, supplies, equipment, etc.) is for planning purposes only. The project manager may, as dictated by the needs of the work, incur costs in accordance with Office of Management and Budget (OMB) Circular A-21 found at www.whitehouse.gov/omb/circulars. If the Scope of Work (by task, if applicable) encompasses research activities which may be funded by one or more sponsors, then allowable project costs may be allocated at the Scope of Work or task level, as appropriate, to any or all of the funding sources. Financial reporting will be at the total-agreement level.

Escalation of labor and EERC recharge center rates is incorporated into the budget when a project's duration extends beyond the current fiscal year. Escalation is calculated by prorating an average annual increase over the anticipated life of the project.

The cost of this project is based on a specific start date indicated at the top of the EERC budget. Any delay in the start of this project may result in a budget increase. Budget category descriptions presented below are for informational purposes; some categories may not appear in the budget.

Salaries: The EERC employs administrative staff to provide required services for various direct and indirect support functions. Salary estimates are based on the scope of work and prior experience on projects of similar scope. The labor rate used for specifically identified personnel is the current hourly rate for that individual. The labor category rate is the current average rate of a personnel group with a similar job description. Salary costs incurred are based on direct hourly effort on the project. Faculty who work on this project will be paid an amount over their normal base salary, creating an overload which is subject to limitation in accordance with university policy. Costs for general support services such as contracts and intellectual property, accounting, human resources, purchasing, shipping/receiving, and clerical support of these functions are included in the EERC facilities and administrative cost rate.

Fringe Benefits: Fringe benefits consist of two components which are budgeted as a percentage of direct labor. The first component is a fixed percentage approved annually by the UND cognizant audit agency, the Department of Health and Human Services. This portion of the rate covers vacation, holiday, and sick leave (VSL) and is applied to direct labor for permanent staff eligible for VSL benefits. Only the actual approved rate will be charged to the project. The second component is estimated on the basis of historical data and is charged as actual expenses for items such as health, life, and unemployment insurance; social security; worker's compensation; and UND retirement contributions.

Travel: Travel is estimated on the basis of UND travel policies which can be found at www.und.edu/dept/accounts/policiesandprocedures.html. Estimates include General Services Administration

(GSA) daily meal rates. Travel may include site visits, field work, meetings, and conference participation as indicated by the scope of work and/or budget.

Equipment: If equipment (value of $5000 or more) is budgeted, it is discussed in the text of the proposal and/or identified more specifically in the accompanying budget detail.

Supplies – Professional, Information Technology, and Miscellaneous: Supply and material estimates are based on prior experience and may include chemicals, gases, glassware, nuts, bolts, and piping. Computer supplies may include data storage, paper, memory, software, and toner cartridges. Maps, sample containers, minor equipment (value less than $5000), signage, and safety supplies may be necessary as well as other organizational materials such as subscriptions, books, and reference materials. General purpose office supplies (pencils, pens, paper clips, staples, Post-it notes, etc.) are included in the facilities and administrative cost.

Subcontracts/Subrecipients: Not applicable.

Professional Fees/Services (consultants): Not applicable.

Other Direct Costs

Communications and Postage: Telephone, cell phone, and fax line charges are generally included in the facilities and administrative cost. Direct project costs may include line charges at remote locations, long-distance telephone, postage, and other data or document transportation costs.

Printing and Duplicating: Photocopy estimates are based on prior experience with similar projects. Page rates for various photocopiers are established annually by the university's duplicating center.

Food: Food expenditures for project meetings, workshops, and conferences where the primary purpose is dissemination of technical information may include costs of food, some of which may exceed the institutional limit.

Professional Development: Fees are for memberships in technical areas directly related to work on this project. Technical journals and newsletters received as a result of a membership are used throughout development and execution of the project by the research team.

Fees and Services – EERC Recharge Centers, Outside Labs, Freight: EERC recharge center rates for laboratory, analytical, graphics, and shop/operation fees are established and approved at the beginning of the university's fiscal year.

Laboratory and analytical fees are charged on a per sample, hourly, or daily rate, depending on the analytical services performed. Additionally, laboratory analyses may be performed outside the university when necessary.

Graphics fees are based on an established per hour rate for production of such items as report figures, posters, and/or PowerPoint images for presentations, maps, schematics, Web site design, professional brochures, and photographs.

Shop and operation fees are for expenses directly associated with the operation of the pilot plant facility. These fees cover such items as training, personal safety (protective eyeglasses, boots, gloves), and physicals for pilot plant and shop personnel.

Freight expenditures generally occur for outgoing items and field sample shipments.

Facilities and Administrative Cost: Facilities and administrative (F&A) cost is calculated on modified total direct costs (MTDC). MTDC is defined as total direct costs less individual capital expenditures, such as equipment or software costing $5000 or more with a useful life of greater than one year, as well as subawards in excess of the first $25,000 for each award. The F&A rate for nonfederal sponsors is 60%. This rate is based on costs that are not included in the federally approved rate, such as administrative costs that exceed the 26% federal cap and depreciation/use allowance on buildings and equipment purchased with federal dollars.

APPENDIX A

CHAPTER 5 OF "RESPIROMETRY FOR ENVIRONMENTAL SCIENCE AND ENGINEERING" BY YOUNG AND COWAN, 2004

Biodegradation Kinetics

by

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JCY Environmental

Springdale, Arkansas USA

and

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March 2004

The material presented herein represents Chapter 5 of a book entitled "Respirometers for Environmental Science and Engineering Applications" by James C. Young and Robert M. Cowan

Chapter 5

Biodegradation Kinetics

5.1 Introduction

An understanding of biodegradation rates, or kinetics, of specific industrial chemicals is useful when evaluating their treatability or their impact on treatment processes. The objective of kinetic assessments usually is to model the biodegradation reactions for specific chemicals using either pure cultures or defined mixed cultures, or natural mixed culture systems such as those that occur in wastewater treatment processes. Modeling biodegradation reactions is either complex or simple depending on one's point of view or objective of the tests. Collection of data for assessing biodegradation kinetics of organic chemicals usually is accomplished respirometrically by dosing a microbial culture with a defined amount of organic chemical or wastewater followed by monitoring the reactions through measurement of oxygen uptake in aerobic tests or gas production in anoxic and methanogenic tests. Batch tests are therefore transient, non-steady-state reactions in which both substrate and biomass concentrations change throughout the biodegradation reaction. An example of oxygen uptake measurements for assessing biodegradation kinetics for four chemicals is shown in Figure 5.1.

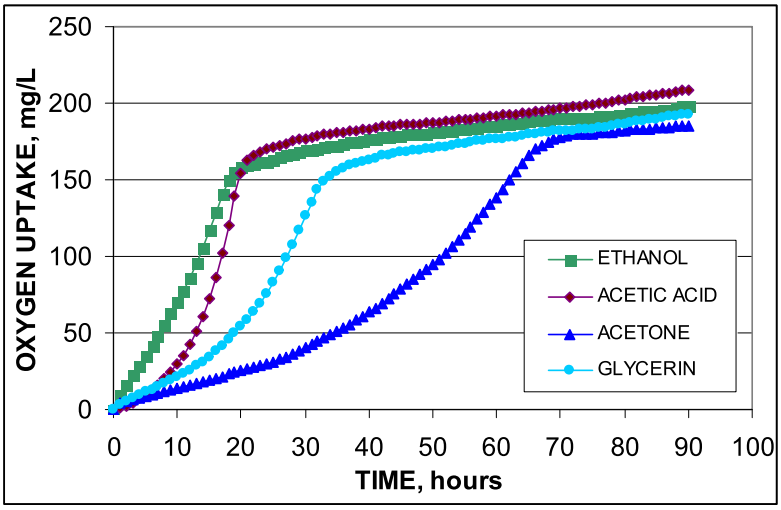


Figure 5.1

Example oxygen uptake data collected for kinetic modeling (Young, 2000).

Because of the multitude of factors affecting kinetic measurements, Smets *et al.* (1996) recommended a basis for categorizing kinetic tests as either intrinsic or extant depending on the culture and substrate environment. Intrinsic kinetic assessments are designed to measure biodegradation parameters for single compounds in the presence of pure or enriched cultures. Ideally, intrinsic kinetic parameters would be identical among tests conducted by different analysts and in different laboratories and therefore would not be system dependent. Even with such standardization and when conducting tests within a well-defined culture environment,

intrinsic kinetic parameters have shown variations of $^{\pm}$50 % of average (Grady and Magbanua, 1998).

Extant kinetic tests are conducted using the natural culture and substrate environment. For activated sludge processes this means high mixed liquor volatile solids concentrations and low concentrations of individual chemical constituents. In this manner, the coefficients can be used to model interactions and make predictions of the impact of operating changes on full-scale treatment processes. Extant kinetic parameters are affected by so many variables that they are not transferable between systems and must be determined for each application (Ellis *et al.*, 1996a,b; Grady and Magbanua, 1998; Eliosov *et al.*, 2001).

The use of batch tests for the measurement of either intrinsic or extant kinetic parameters must be accompanied by good experimental design together with appropriate mathematical techniques for analyzing the data. Among other things, the experimental design must consider 1) proper balance between the initial substrate and biomass concentrations, 2) an adequate number of data points must be collected throughout the critical phases of the biological reactions, and 3) the influence of decay must be considered if long time periods are required for substrate utilization.

5.2 Intrinsic Kinetic Tests

Grady and co-workers presented recommended guidelines for conducting intrinsic kinetic tests when using respirometric oxygen uptake as a test variable (Brown *et al.*, 1989; Smets *et al.*, 1996; Grady and Magbanua, 1998). In this case, a substrate to biomass ratio (mg COD/mg VSS) of at least 20:1 was recommended so that the microbial reaction progresses from lag through maximum growth rate through decay. An example of an intrinsic kinetic assessment of oxygen uptake data for aniline is shown in Figure 5.2 along with the resulting fit of the kinetic model described by Eq. 2-22. In this case, $\mathbf{Y_g}$ = 0.42 g VSS/g COD_r, $\mathbf{q_m}$ = 0.42 g COD/g VSS/hr and $\mathbf{K_s}$ = 15 mg/L.

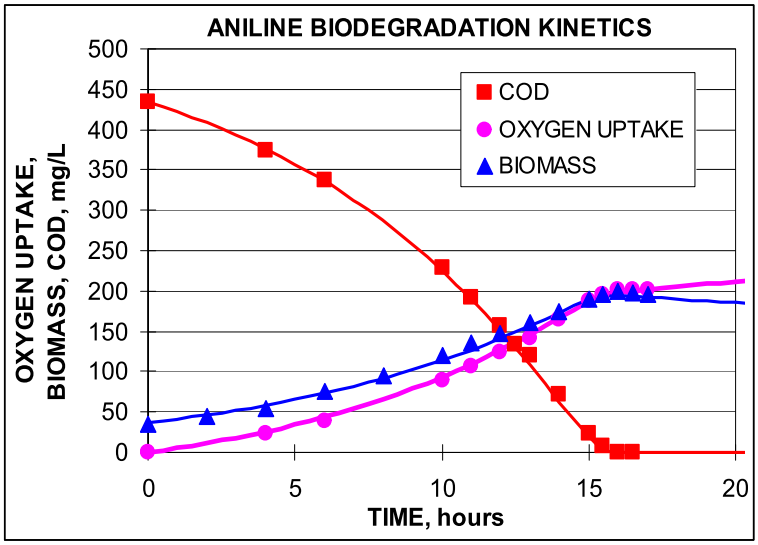


Figure 5.2

Kinetics of aniline biodegradation including modeling of oxygen uptake, biomass concentration, and aniline COD. Symbols represent measured values, lines are model values (Data from Zissi *et al.*, 1999).

Modeling of biodegradation kinetics begins with the Monod relationship expressed by Eq. 2-2. Mathematically, the Monod equation is given as the specific growth rate or specific substrate removal rate as a function of the substrate concentration:

$$\mu = \frac{\mu_m S}{K_S + S} \qquad \textbf{(5-1)}$$

or

$$q = \frac{q_m S}{K_S + S} \qquad \textbf{(5-2)}$$

where:

μ	=	specific growth rate, hr^{-1}
μ_m	=	maximum specific growth rate, hr^{-1}
q	=	specific substrate removal rate, mg COD/mg VSS-hr
q_m	=	maximum specific substrate removal rate, mg COD/mg VSS-hr
S	=	Substrate concentration, mg COD/L
K_s	=	half-saturation coefficient, mg COD/L

The relationship between specific substrate removal rate and substrate concentration is illustrated graphically in Figure 5.3.

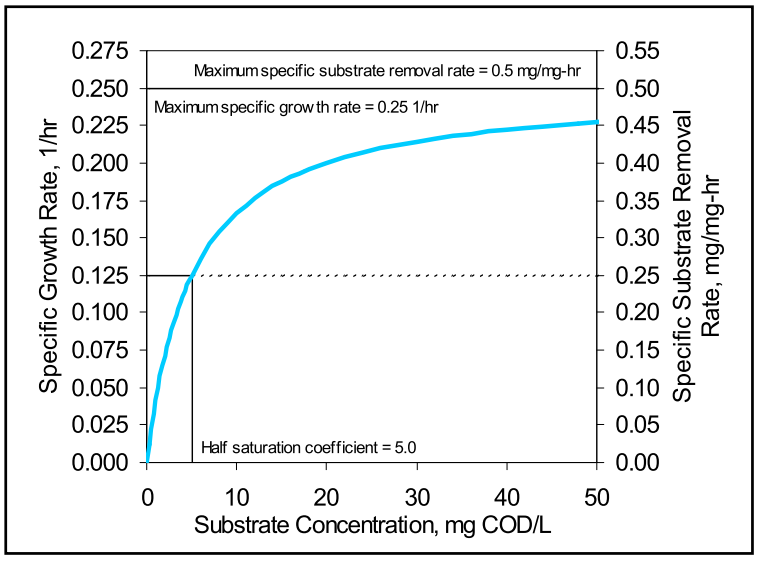


Figure 5.3

The Monod plot for μ_m = 0.25 1/hr, K_S = 5.0 mg COD/L.

The parameter μ_m is a measure of the highest rate at which a single unit of microbial population can grow. The parameter q_m is a measure of the highest rate at which a unit of the microbial population can consume substrate (contaminant). K_S is a measure of the substrate concentration at which the specific rates are one-half their maximums. Note that μ_m and q_m

are not independent parameters, just different ways of expressing the same phenomena. Mathematically they are related as follows:

$$\mu_m = Y_g \, q_m \qquad \textbf{(5-3)}$$

Care must be taken to define the units for each of these parameters. Biomass is usually measured as volatile suspended solids but can be expressed in COD units. Substrate is best given in THOD or COD units but sometimes is expressed as mass of a specific compound. If biomass is given in units of VSS the yield coefficient can be converted to COD units by multiplying by the conversion factor β = 1.42 mg biomass COD/g biomass as VSS. If the substrate is given as the mass of chemical compound, conversion to COD units can be accomplished by multiplying by the mass of oxygen required for complete oxidation of the given chemical mass (See Eq. 1-1 and Table 1.1).

Figure 5.4 shows that as K_S increases, higher concentrations of the substrate (contaminant) are needed for the specific growth rate to approach its maximum, and as K_S decreases, μ approaches μ_m more quickly. Proportional changes occur with q and q_m.

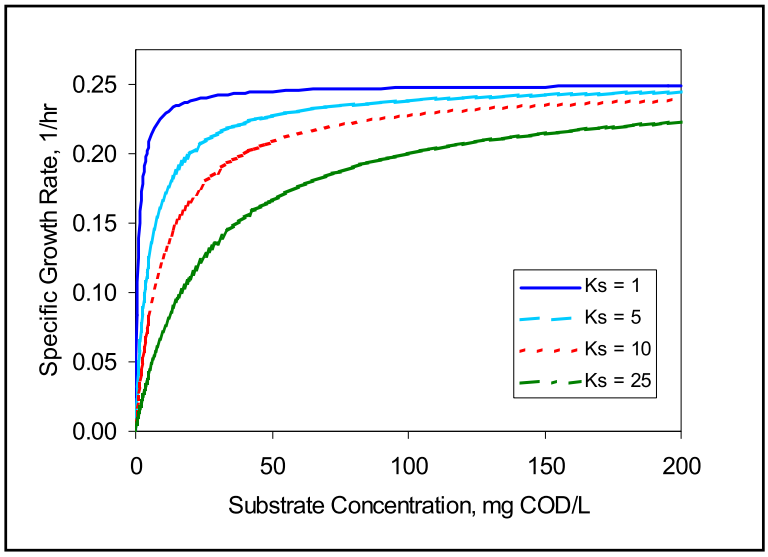


Figure 5.4

Effect of K_S on Monod equation plot for μ_m = 0.25/hr.

When batch reactors are used to measure oxygen uptake for kinetic parameter assessment, we typically write a set of equations describing the rate of biomass growth and the rate of substrate (contaminant) removal over time (see Section 2.3). These can be written as:

$$R_g = Y_g R_s - b_a X_a \quad , \qquad \text{mg/L-hr} \qquad \textbf{(5-4)}$$

and

$$R_S = \frac{q_m S X_a}{K_S + S} = \frac{(\mu_m / Y_g) S X_a}{K_S + S} \quad , \qquad \text{mg/L-hr} \qquad \textbf{(5-5)}$$

where $\mathbf{b_a}$ is the rate of active biomass decay and $\mathbf{X_a}$ is the concentration of biomass that is active for degrading **S**.

One more equation that is needed to make a complete set is a mass balance on the oxygen demand. We can write the following equation for oxygen uptake at any time, **t**, after test initiation:

$$\mathbf{O_{u\text{-}t} = (S_0 - S_t) - \beta\,(X_t - X_0) - X_{p,t}} \quad , \quad \mathbf{mg\ O_2/L} \qquad \mathbf{(5\text{-}6)}$$

where:

$\mathbf{O_u}$ = oxygen uptake at any given time, mg O_2/L
$\mathbf{S_0}$ = initial substrate concentration, mg COD/L
S_t = residual substrate concentration at time t, mg COD/L
$\mathbf{X_0}$ = initial biomass concentration, mg VSS/L
X_t = residual biomass concentration at time, t, mg VSS/L
$X_{p,t}$ = soluble metabolic product, mg COD/L

An equation for the oxygen uptake rate, OUR, can be written as (also see Chapters 2 and 6):

$$\mathbf{OUR = (1 - \beta\,Y_g - Y_p)\,R_S + \beta\,b\,X_a} \quad , \quad \mathbf{mg\ O_2/L\text{-}hr} \qquad \mathbf{(5\text{-}7)}$$

Fitting Eqs. 5-4 through 5-7 to oxygen uptake data from a batch respirometer test, and factoring in the initial conditions, will produce estimates of the Monod kinetic parameters. Numerous mathematical approaches have been used for estimating the biological growth and biodegradation kinetic parameters – $\mathbf{Y_g}$, $\mathbf{\mu_m}$, $\mathbf{q_m}$, and $\mathbf{K_S}$ – from batch tests (Knowles *et al.*, 1965; Gates and Marlar, 1968; Ong, 1983; Corman and Pave, 1983; Robinson and Tiedje, 1983; Montgomery, 1984; Rozich and Gaudy, 1986; Han and Levenspiel, 1988; Mulchandani and Luong, 1989; Brown *et al.*, 1989; Davies-Venn, 1989; Kim, 1991; Grady and Magbanua, 1998). These methods generally have used non-linear techniques to fit Eqs. 5-4 through 5-7 to oxygen uptake or gas production data. The fit of the equations to the data can be accomplished using dedicated computer programs or spreadsheets. All the methods produce reasonably good mathematical fit of the measured data. Consequently, no single method is considered best, and the reader is referred to the original publications for a detailed description of the modeling process.

Sensitivity analyses have indicated that the accuracy of estimating $\mathbf{\mu_m}$ or $\mathbf{q_m}$ is critically related to the duration of the zero-order or high-rate part of the reaction when $\mathbf{S >> K_S}$ (see Figure 4.1A). The ability to obtain accurate measures of $\mathbf{K_S}$ is more sensitive to the accuracy of data collected at low substrate concentrations especially near the break in the curve (see Figure 4.1A) (Robinson and Tiedje, 1984; Davies-Venn, 1989).

Figure 5.5 shows a theoretical representation of these relationships in terms of 1) cumulative oxygen uptake data that would be collected using a respirometer, 2) residual

substrate concentrations, 3) biomass concentrations associated with oxygen uptake, and 4) the oxygen uptake rate, OUR. The kinetic parameters used to perform these simulations were: μ_m = 0.25/hr, K_S = 5 mg COD/L, Y_g = 0.35 mg VSS/mg COD_r, and b_a = 0.008/hr. (S_o = 100 mg COD/L, X_o = 5 mg COD/L or 3.5 mg VSS/L, Y_p was assumed to be 0 mg COD_p/mg COD_r).

Smets *et al.* (1996) discussed a number of methods for transforming oxygen uptake data to help evaluate data quality and decide if the Monod equation is the appropriate mathematical function to use in representing the kinetic behavior. Two of these methods involve plotting oxygen uptake rate, **OUR**, and specific growth rate, μ, or specific substrate removal rate, **q**, versus cumulative oxygen uptake, O_u (Figure 5.6). The specific growth rate is calculated from the oxygen uptake data, the estimated yield value, Y_g, and the initial biomass concentration, X_o, using Eq. 5-8. These transformed data plots will be discussed in more detail later.

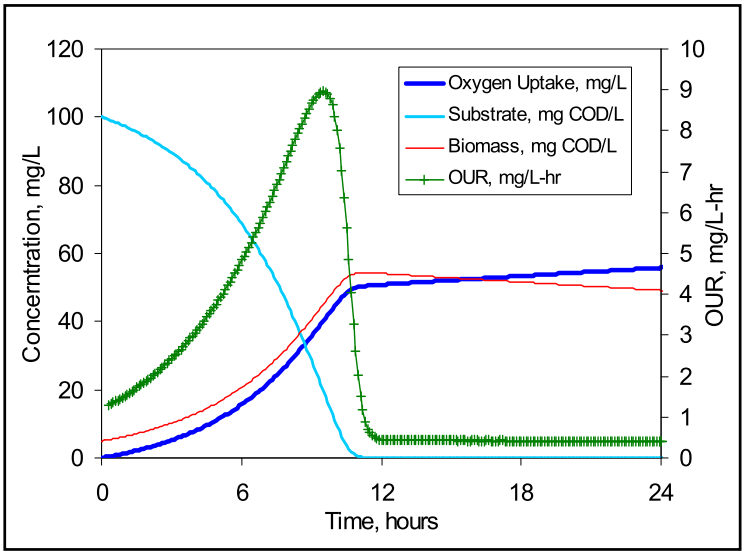


Figure 5.5

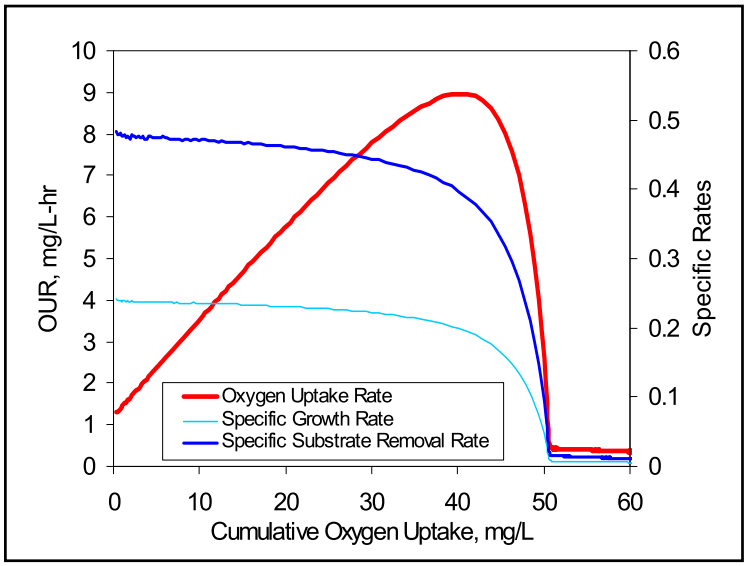
Batch biodegradation test simulation showing oxygen uptake, substrate concentration, biomass concentration, and OUR versus time.

$$\mu_t = \frac{OUR_t}{(X_o/Y_g)\,(1 - \beta\, Y_g - Y_p) + O_{u,t}} \qquad \textbf{(5-8a)}$$

$$q_t = \frac{OUR_t}{(X_o)\,(1 - \beta\, Y_g - Y_p) + O_{u,t}} \qquad \textbf{(5-8b)}$$

Figure 5.6

Batch biodegradation simulation showing oxygen uptake rate, OUR, specific growth rate, μ, and specific substrate removal rate, q, versus oxygen uptake, O_u.



5.3 Relationship Between Kinetics and Reactor Performance

As mentioned above, kinetic parameter values are used in mathematical models to help predict reactor performance and to study the effect that changes in operating parameters might have on effluent quality. This relationship is of particular importance when discharge limits are set for specific wastewater contaminants such as ammonia or specific organics. For a continuous-flow, complete-mixed activated sludge (CMAS) process operating under steady-state conditions, the effective growth rate of the microbial population is directly related to the solids residence time (**SRT**) as follows:

$$1/SRT = \mu - b_a = q\,Y_g - b_a \qquad (5\text{-}9)$$

Substituting the Monod equation (Eq. 5-1 or 5-2) for **μ** or **q** and rearranging produces an expression that relates the effluent substrate (contaminant) concentration, S_e, to kinetic parameters and solids retention time, or:

$$S_e = \frac{K_S\,(1 + b_a\,SRT)}{SRT\,(Y_g\,q_m - b_a) - 1} \qquad (5\text{-}10)$$

Figure 5.7 illustrates the effect of the value of K_S on the effluent substrate (contaminant) concentration achieved by a CMAS operated at the given solids residence times where biodegradation follows Monod kinetics with μ_m = 0.25/hr, Y_g = 0.35 mg VSS biomass formed/mg COD substrate consumed, and b_a = 0.008/hr. It is clear from Figure 5.7 that much longer SRTs are needed to achieve very low effluent substrate concentrations when K_S is large.

Figure 5.7

Effect of K_S on the relationship between effluent substrate concentrations and solids residence time, SRT, for a steady state CMAS.

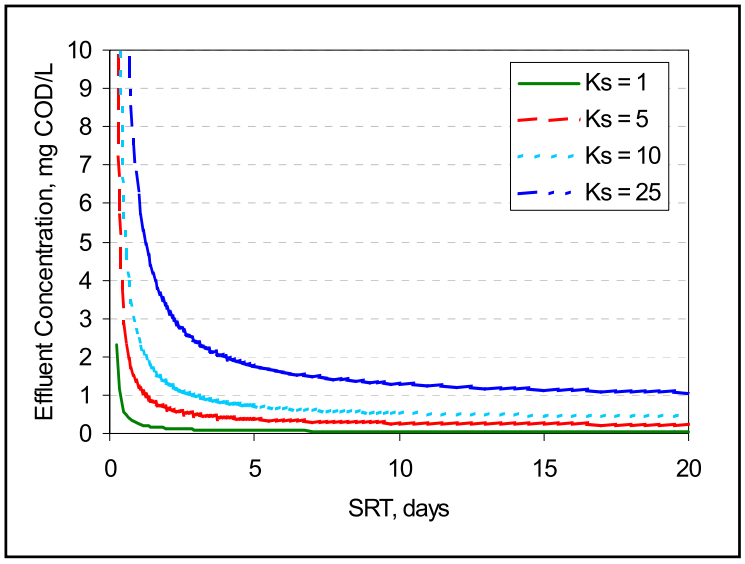
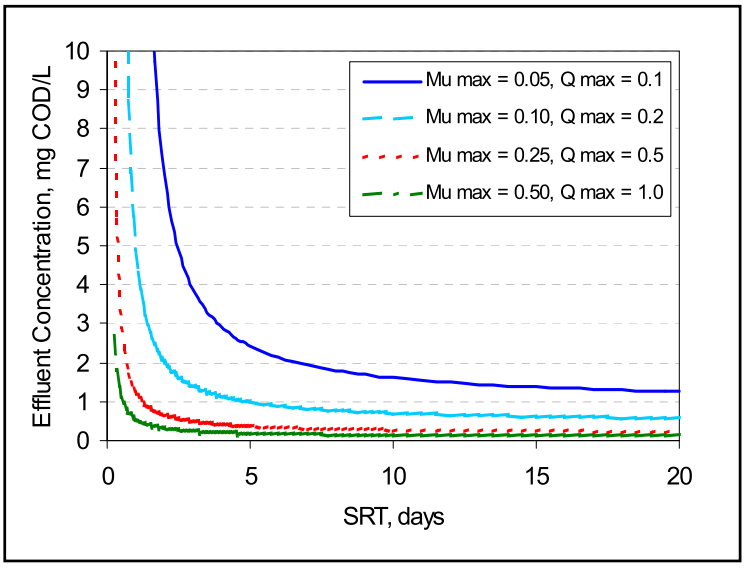


Figure 5.8 illustrates the effect of changes in μ_m or q_m on S_e for a steady-state CMAS where biodegradation follows Monod kinetics when K_S = 5 mg COD/L, Y_g = 0.35 mg VSS/mg COD_r substrate consumed, and b_a = 0.008/hr. Figure 5.8 shows clearly that high maximum specific growth rates make it easier to achieve lower effluent concentrations at shorter solids retention times (SRT).

The above relationships show that influent substrate concentration, S_o, does not affect effluent substrate concentrations, S_e, for a steady-state CMAS when biodegradation follows Monod kinetics.

Figure 5.8

Effect of μ_m and q_m on the relationship between effluent substrate concentration and solids retention time, SRT.



Rather than extend the SRT to extreme values, we can calculate the lowest achievable S_e value, or $S_{e,min}$, at a theoretically infinite solids retention time by substituting an infinite SRT into Eq. 5-10 and rearranging to produce the following relationship:

$$S_{e,min} = K_S b_a/(1-b_a) \quad (5\text{-}11)$$

$S_{e,min}$ is then directly proportional to K_S and is quite sensitive to the value of b_a. When conducting intrinsic kinetic tests, the decay region often represents only about one-fourth of the total database. The decay rate estimated from these tests often is higher than those observed in continuous cultures tests, probably due to accumulation of storage products during the log growth phase.

Note that μ_m or q_m values do not effect $S_{e,min}$ but they do affect substrate concentrations at real SRT values, in particular at short SRTs. The K_S and b_a values used in Figure 5.7 produce S_{e-min} values of 0.008, 0.040, 0.081, and 0.202 mg COD/L, respectively, for K_S values of 1, 5, 10, and 25 mg COD/L. This $S_{e,min}$ relationship applies only for CMAS reactors and chemostats. For plug flow and multiple tanks-in-series systems it is possible to achieve effluent concentrations below the $S_{e,min}$ calculated using equation 5-11.

5.4 Relationship Between Kinetic Parameters and Oxygen Uptake

The impact of individual kinetic parameters on oxygen uptake reactions often can be evaluated by visual observation of the O_u vs time plots. Figure 5.9 illustrates the effect of μ_m (and q_m) and K_S on the oxygen uptake versus time for initial conditions used in Figure 5.6: S_o = 100 mg COD/L, X_o = 5 mg COD/L and the Monod kinetic parameters: μ_m = 0.25/hr (or as noted on the graph), K_S = 5 mg COD/L, Y_g = 0.35 mg VSS/mg COD_r, and b_a = 0.008/hr. This graph shows that the oxygen uptake rate is completed more rapidly as μ_m or q_m increase. K_S primarily affects the shape of the oxygen uptake curve and has a relatively small effect on the time for completing the reaction.

Figure 5.10 shows the same data as that in Figure 5.9, but plotted as OUR versus time. This transformation shows that the μ_m (or q_m) primarily affects the time required to complete the biodegradation reaction and the maximum OUR achieved. In this case, S_o = 100 mg COD/L, X_o = 5 mg COD/L and the Monod kinetic parameters: μ_m = 0.25/hr (except as noted on the graphs), K_S = 5 mg COD/L, Y_g = 0.35 mg VSS/mg COD_r, and b_a = 0.008/hr. K_S primarily affects the sharpness of the peak of the OUR vs time curve and has a substantial effect on the maximum OUR achieved, but as was seen in the O_u versus time plot, it has a relatively small impact on the time required to complete the reaction.

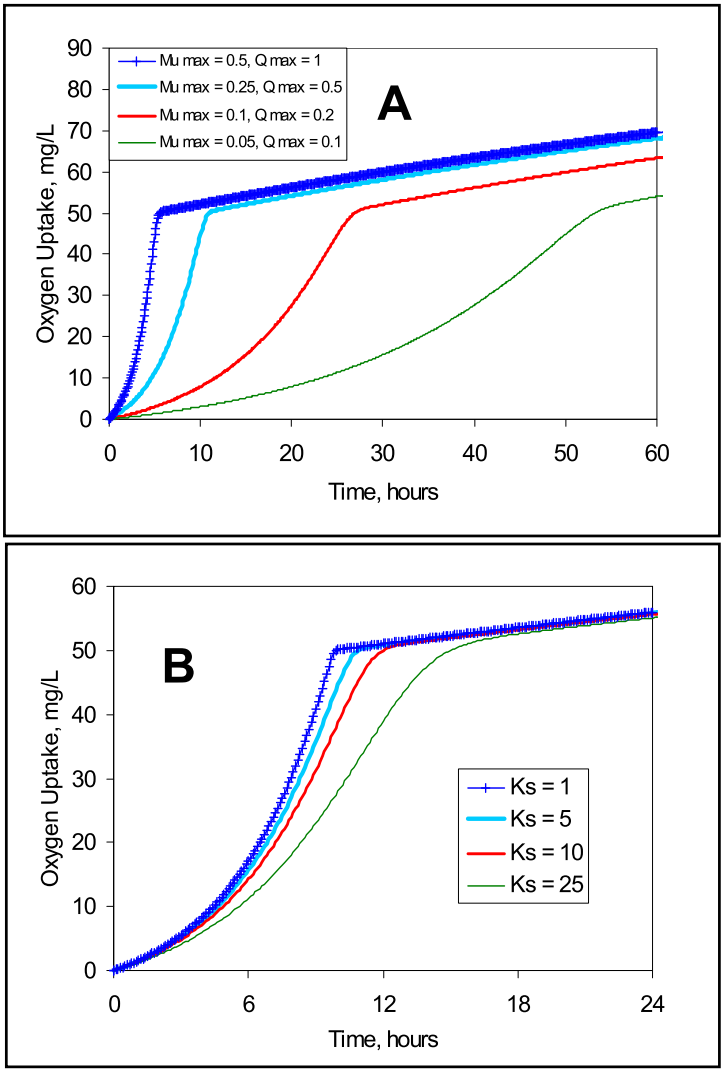


Figure 5.9

Effect of μ_m and q_m (A) and K_S (B) on oxygen uptake versus time (S_o = 100 mg COD/L, X_o = 5 mg COD/L or 3.5 mg VSS/L)

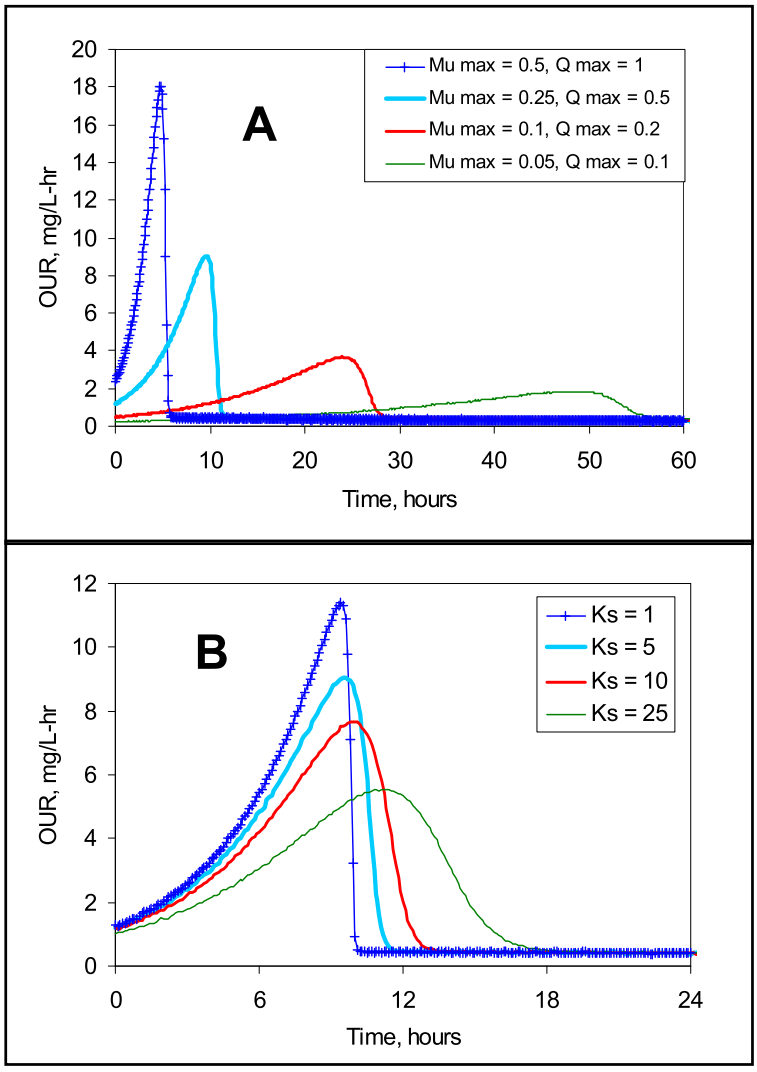


Figure 5.10

Effect of μ_m and q_m (A) and K_S (B) on oxygen uptake rate versus time.

Another, and perhaps more useful, method of transforming data is to plot OUR versus cumulative oxygen uptake, O_u, as shown in Figure 5.11. This approach helps evaluate the data quality for its use in obtaining kinetic parameter estimates, and helps to indicate which kinetic model is most appropriate (the non-inhibitory substrate Monod model or the inhibitory substrate Haldane or Andrews model). For pure Monod kinetics, the curve should increase somewhat linearly upward from an initial OUR to a maximum. The substrate concentration at the maximum OUR is approximately equal to $\mathbf{K_S}$. Beyond this peak, the OUR decreases rapidly to the rate associated with endogenous respiration. The sharpness of the peak – in the absence of mass transfer limits – reflects the value of $\mathbf{K_S}$. Sharp peaks reflect low $\mathbf{K_S}$ values; rounded peaks represent high $\mathbf{K_S}$ values. Excessively rounded peaks can represent either the occurrence of mass transfer limits, low but stoichiometrically adequate nutrient concentrations (e.g. nitrogen), or an insufficient supply of oxygen to the culture (Smets *et al.*, 1996). Chiang *et al.* (2004) proposed using this analytical approach to estimate short-term BOD of wastewater samples.

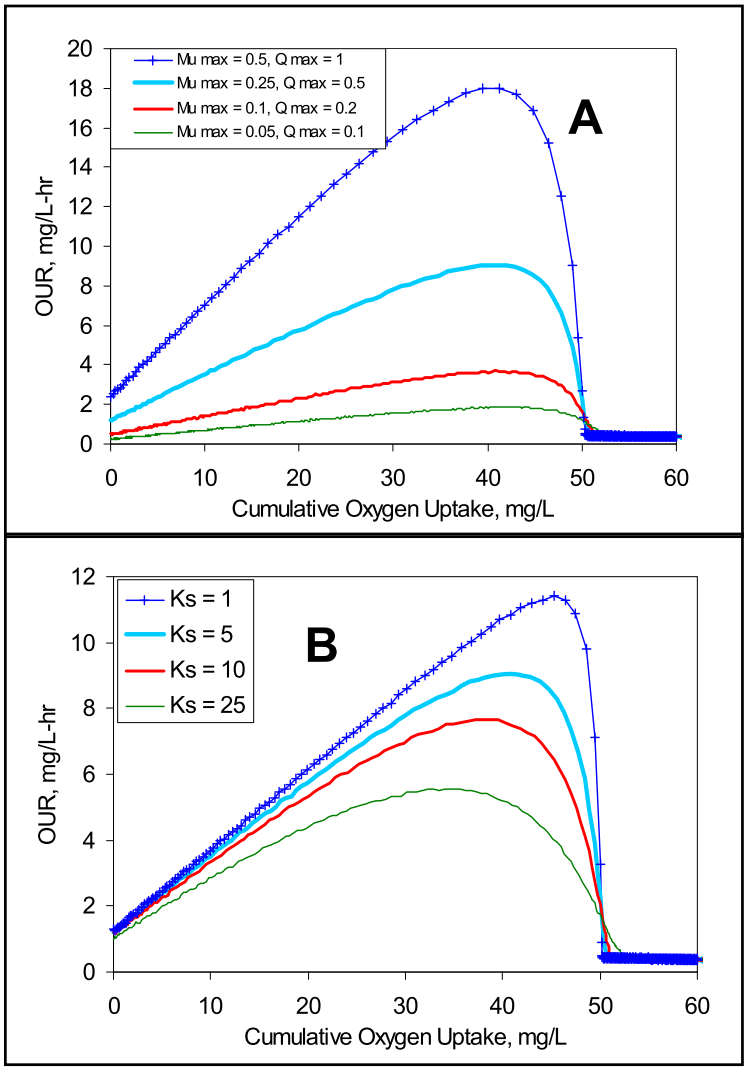


Figure 5.11

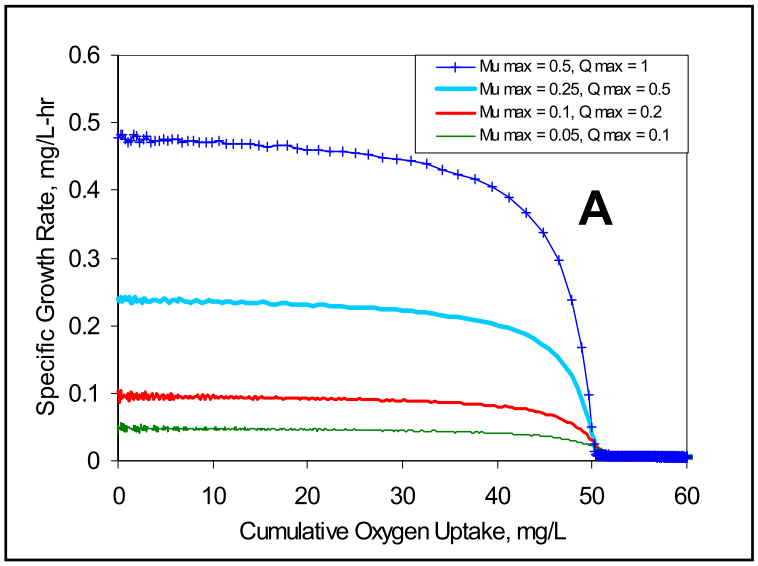
Effect of μ_m and q_m (A) and K_S (B) on OUR versus cumulative oxygen uptake.

A final useful transformation is to plot the calculated specific growth rate (μ_m), versus oxygen uptake (O_u) (or q_m vs O_u). Figure 5.12 shows this type of plot for a non-inhibitory substrate concentration of 100 mg COD/L and indicates that μ or q versus O_u produces an initial near horizontal, or slowly decreasing, slope intercepting the specific growth rate axis at values approaching μ_m. ($S >> K_S$ is needed for the specific growth rate to closely approach the asymptotic value of μ_m).

Significant deviations from this pattern indicate problems with initial culture acclimation or erroneous estimates of initial biomass concentration. For pure Monod kinetics, the slope of the μ_m (or q_m) versus O_u curve remains relatively flat as long as the reaction remains kinetically saturated ($S >> K_S$). The slope should decrease gradually until the reaction approaches complete degradation of the substrate. Or more accurately, as S approaches K_S the value of μ starts to drop and the slope of the μ vs O_u line becomes increasingly negative. Note

that increasing values of K_S cause the slope to decrease at lower O_u values, have a less sharp, and a more poorly defined intercept at the point of substrate exhaustion (Figure 5.12). This figure also indicates that the model is more sensitive at low cumulative oxygen uptake values, i.e. that the data plotted this way will look noisy at low O_u values even when there is little error in the data. The intercept at the cumulative oxygen uptake axis ($O_{u,p}$ = approximately 50 mg/L for this data) represents the point of complete substrate conversion and gives a convenient first estimate of the yield coefficient. That is,

$$Y_g \cong (1 - O_{u,p}/S_o - Y_p)/\beta \qquad (5\text{-}12)$$



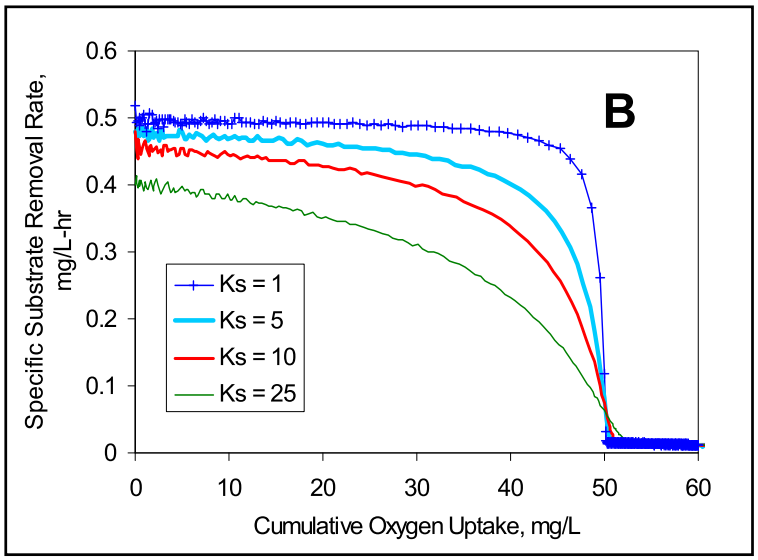


Figure 5.12

Effect of μ_m and q_m (A) and K_S (B) on fit of μ vs O_u curves.

5.5 Intrinsic Kinetic Tests with Chemicals Exhibiting Substrate Inhibition

As described in Chapter 2, some chemicals exhibit inhibition to their own biodegradation when present in sufficiently high concentrations. Examples include phenolic compounds and chlorinated hydrocarbons. In fact, almost any chemical can exhibit substrate inhibition if tested at sufficiently high concentrations. As described by Eqs. 2-31 and 2-32, substrate inhibition is a special case of competitive inhibition so that as the concentration of substrate decreases, inhibition subsides and the substrate conversion reaction approaches Monod kinetics (Eq. 5-5).

The most common model used for representing the effect of inhibitory substrates on specific growth rates and specific substrate removal rates is that of Andrews (1968). This model is a modification of the Monod equation and has the same mathematical function as the enzyme inhibition kinetics equation of Haldane (1930). Mathematically this relationship typically is given as:

$$\mu = \frac{\mu_m S}{K_S + S + S^2/K_I} = \frac{\mu_m S}{K_S [1 + S^2/K_I K_S] + S} \qquad \textbf{(5-13)}$$

where K_I = Haldane (or Andrews) inhibition coefficient, mg^2/L^2, and all other terms are as referred to for Eqs. 5-1 and 5-2. The second form of Eq. 5-13 shows that substrate inhibition is a special case of competitive inhibition (see Eq. 2-25).

An example of such modeling of phenol biodegradation is shown in Figure 5.13. The factors controlling estimation of kinetic parameters for chemicals exhibiting substrate inhibition are essentially the same as for non-inhibited cases.

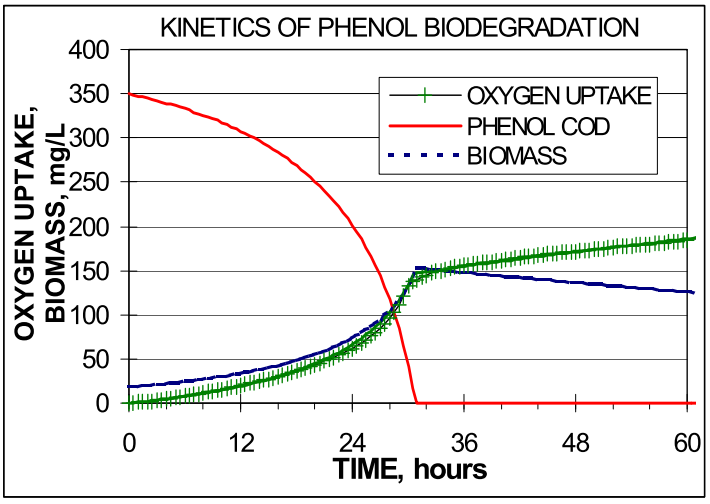


Figure 5.13

Kinetics of phenol biodegradation. Symbols represent measured data; lines represent modeled values.

The impact of the Haldane (Andrews) relationship on specific growth rate versus substrate concentration is illustrated graphically in Figure 5.14. At low values of $\mathbf{K_I}$, the highest specific growth rate achieved is far below the maximum for a non-inhibitory substrate. The kinetic parameters used here were μ_m = 0.25/hr, K_S = 5, and K_I values varied between 5 and 20,000 mg^2/L^2. When K_I = 20,000 mg^2/L^2, the μ vs substrate concentration curve is indistinguishable from a Monod model so the substrate is effectively non-inhibitory. Clearly, lower values of K_I represent more inhibitory substrates.

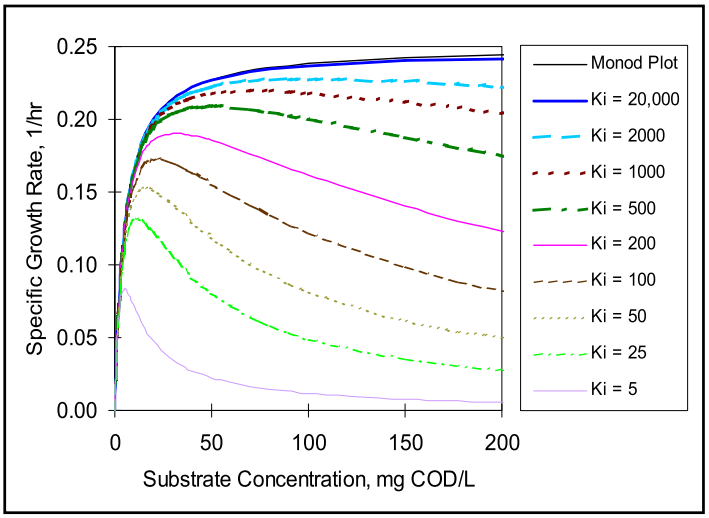


Figure 5.14

Specific growth rate vs. substrate concentration when μ_m = 0.25 1/hr, K_S = 5, and for K_I values between 5 and 20,000 mg^2/L^2.

The concentration at which the specific growth rate (and specific substrate removal rate) is at the maximum attainable value, **S*** can be estimated as follows and illustrated in Figure 5.15:

$$S^* = (K_S K_I)^{0.5} \qquad \textbf{(5-14)}$$

The corresponding specific growth rate is:

$$\mu^* = \frac{\mu_m}{2\,(K_s/K_I)^{0.5} + 1} \qquad \textbf{(5-15)}$$

Substrate concentrations above this point cause a decrease in substrate removal rate that leads to a further increase in concentration.

Eq. 5-15 illustrates that the ratio of K_S/K_I, and not K_I alone represents the degree of inhibition (Grady *et al.*, 1999). The values for **S*** and **μ*** for the curves shown in Figure 5.14 are shown in Table 5-1 for the indicated K_I values.

Figure 5.15

The Andrews (or Haldane) equation plot for μ_m = 0.25/hr, K_S = 5.0 mg COD/L, K_I = 25 mg^2/L^2.

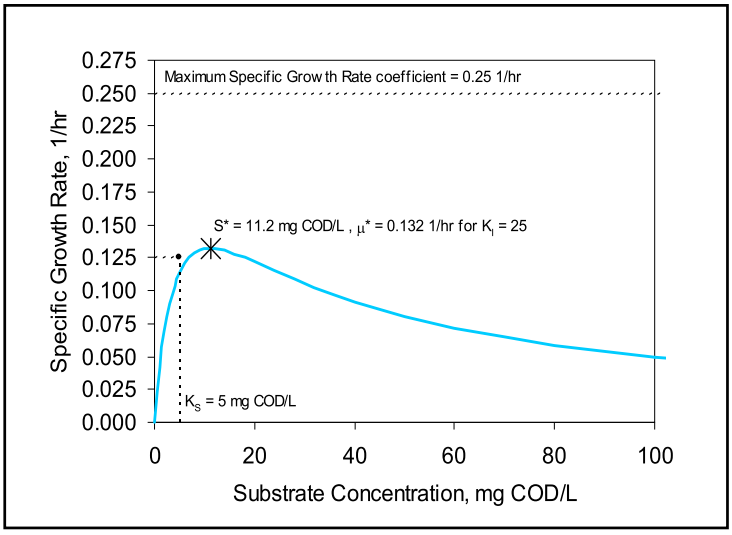


Table 5-1. Effect of K_I value on S* and μ^*.

K_I	20,000	10,000	5,000	2,000	1,000	500	200	100	50	25	5
S*	316	224	158	100	71	50	32	22	16	11	5
μ^*	0.242	0.239	0.235	0.227	0.219	0.208	0.190	0.173	0.153	0.132	0.083

The kinetic parameters used here were μ_m = 0.25 1/hr, K_S = 5, and the K_I values shown.

A series of oxygen uptake versus time curves for the different values of K_I used in Figure 5.15 is shown in Figure 5.16A when using S_o = 100 mg/L, μ_m = 0.25/hr, K_S = 5.0 mg/L, X_o = 5 mg/L, and b_a = 0.008/hr. The oxygen uptake reaction takes significantly longer time to complete when K_I values are low because the substrate/culture pair having these characteristics is more greatly inhibited at the initial substrate concentration. The initial substrate concentration has a major impact on the initial oxygen uptake rate and the time required to complete the biodegradation reaction as shown in Figure 5.16B when using a K_I value of 25 mg^2/L^2.

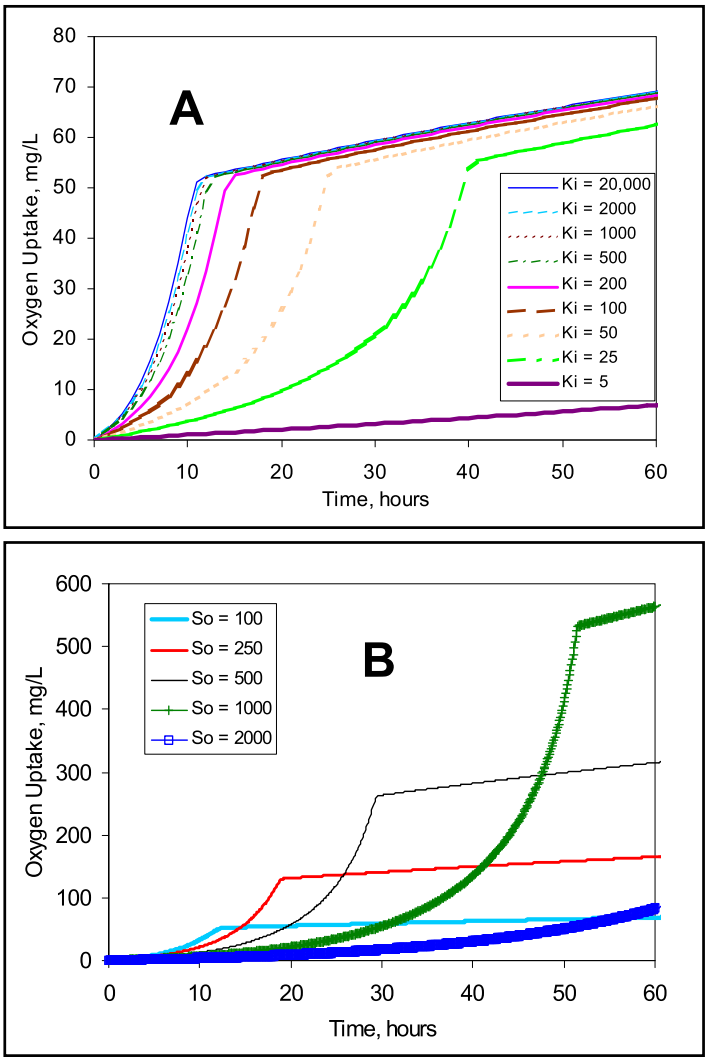


Figure 5.16

Effect of K_I and S_0 on the shape of the oxygen uptake vs. time curve for inhibitory substrates following Andrews (Haldane) kinetics. (Compare to Figure 4.10)

The transformed data respirograms shown earlier for Monod kinetics become even more interesting and important for cases of substrate inhibition. The OUR versus time respirograms simulated at the same $\mathbf{S_o}$ shows greater inhibition at low $\mathbf{K_I}$ values at the initial substrate concentration (at time zero) as a lower initial oxygen uptake rate, but the maximum observed oxygen uptake rate is only suppressed slightly (Figure 5.17A). When different initial substrate concentrations are used for the same $\mathbf{K_I}$ of 25 mg^2/L^2 (Figure 5.17B), the higher initial concentrations cause greater inhibition and thus greater suppression of the initial OUR, but the maximum OUR increases with increasing S_o. These effects are observed because, by the time the maximum OUR occurs, the substrate concentration has decreased to S* (see Eq. 5-14) and the biomass concentration has increased to approximately $(\mathbf{S_o} - \mathbf{S^*})\mathbf{Y_g}$. The gradually increasing slope at low $\mathbf{K_I}$ values occurs because toxic impacts are reduced as biodegradation occurs. The increasing apparent lag time with oxygen uptake rates increasing to a maximum is a fingerprint for substrate inhibition reactions.

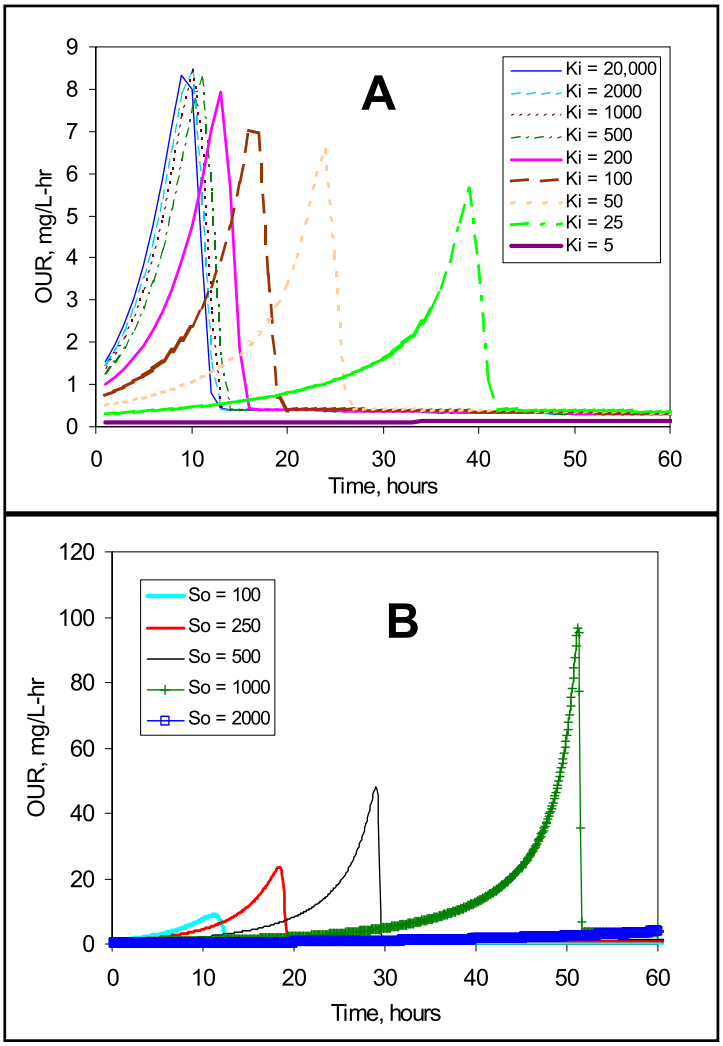


Figure 5.17

Effect of K_I and S_o on the shape of the OUR vs. time curve for inhibitory substrates following Andrews (Haldane) kinetics. (Compare to Figure 2.9)

OUR versus $\mathbf{O_u}$ transformations for the substrate inhibition kinetics simulations are given in Figure 5.18. This transformation is particularly helpful in identifying substrate inhibition because it changes the shape of the OUR versus $\mathbf{O_u}$ curve from that which is characteristic of non-inhibitory substrates (see Figure 5.11). As seen in Figure 5.18A, the leading side of the OUR versus $\mathbf{O_u}$ curves become concave when $\mathbf{S_o}$ is greater than or equal to $\mathbf{K_I}$.

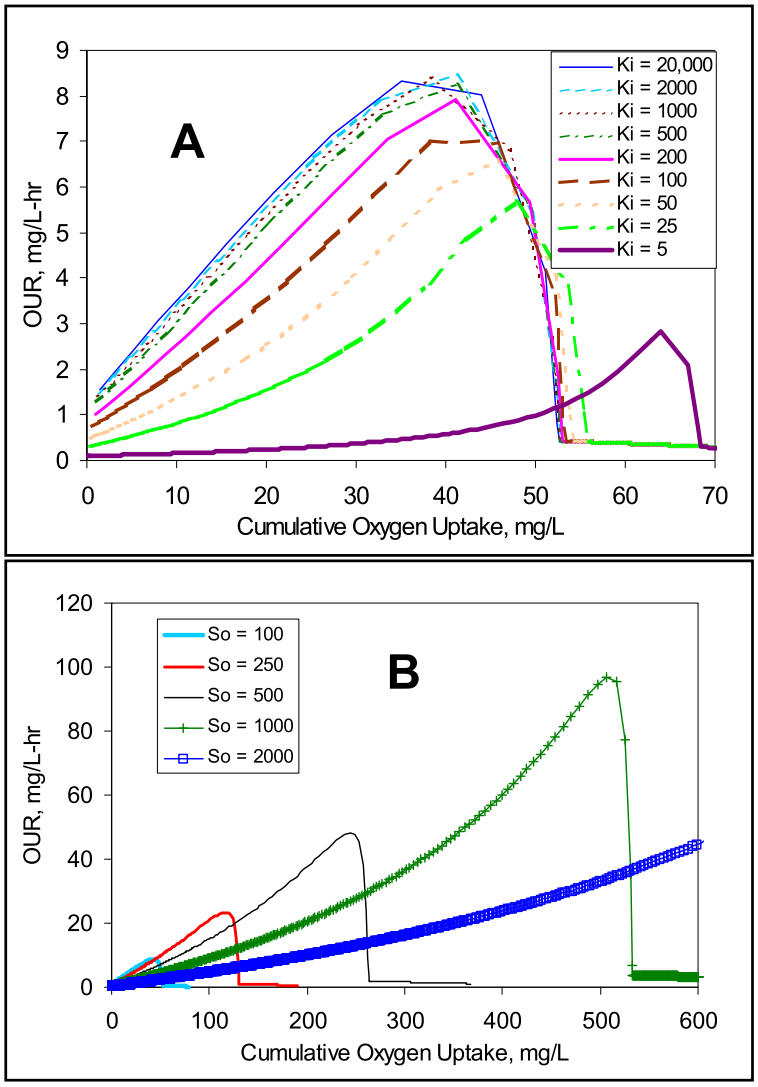


Figure 5.18

Effect of K_I and S_0 on the shape of the OUR vs O_u curve for inhibitory substrates following Haldane (Andrews) kinetics.

The specific growth rate versus $\mathbf{O_u}$ transformation for the same data shows decreasing μ intercepts with decreasing values of $\mathbf{K_I}$ (Figure 5.19B). Looking back at Figure 5.14 it is clear why the lower initial observed μ intercept occurs at lower $\mathbf{K_I}$ values at an $\mathbf{S_o}$ of 100 mg COD/L (Figure 5.18A) or higher $\mathbf{S_o}$ values at a $\mathbf{K_I}$ = 25 mg^2/L^2 (Figure 5.19B). The specific growth rate for the cultures initially under inhibited conditions increases the most with increasing levels of oxygen uptake. This increase in μ occurs because the substrate concentration is decreasing. $\mathbf{S_t}$ would equal $\mathbf{S^*}$ at the point where the maximum value of μ is observed. Changes in initial substrate concentration show more pronounced increases in slope at the higher concentrations (Figure 5.19B).

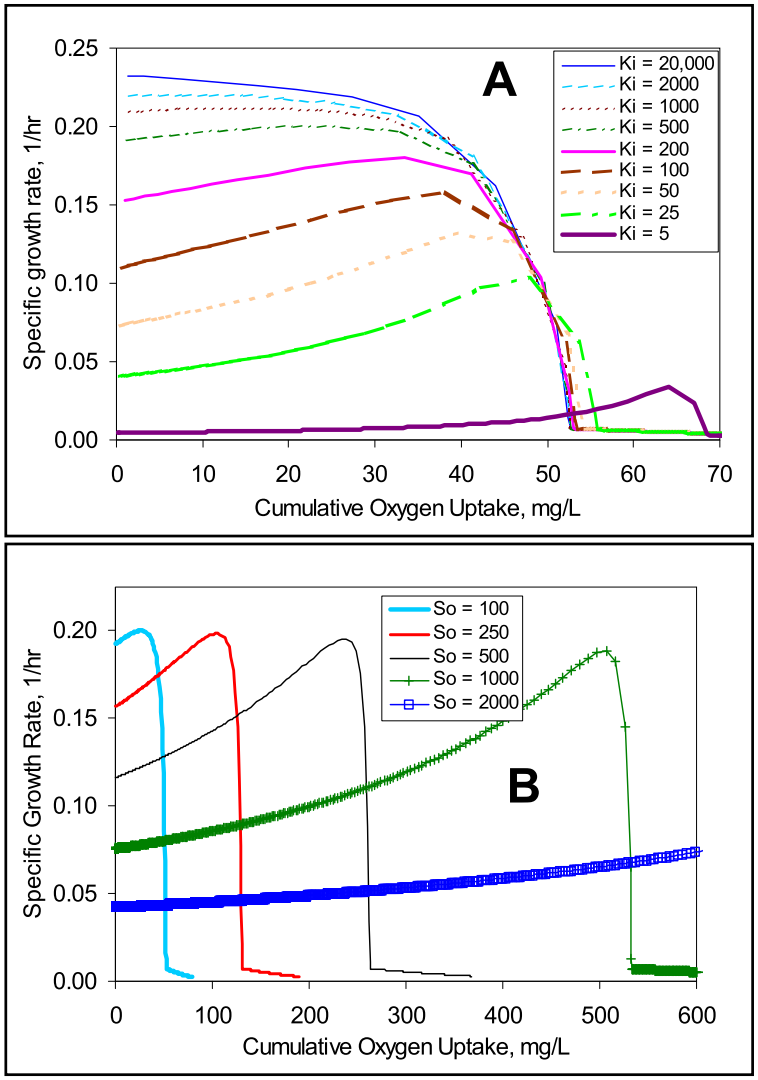


Figure 5.19

Effect of K_I (A) and S_o (B) on the shape of the μ vs O_u curves for inhibitory substrates following Andrews (Haldane) kinetics.

The above simulations show clearly that it would be difficult to distinguish between inhibitory and non-inhibitory conditions for K_I values that are substantially higher than the initial substrate concentration of the test. Therefore, it is important to test higher initial substrate concentrations when substrate inhibition is suspected.

5.6 Effect of Non-Substrate Toxicity on Effluent Concentrations

Non-substrate toxicity can be expressed as an effective reduction in the maximum specific growth rate or the maximum specific substrate utilization rate (non-competitive inhibition), an increase in the half-saturation coefficient (competitive inhibition), or both (mixed inhibition). As presented in Chapter 2, these effects are expressed by the impact of the toxicant on the effective values of μ_m and q_m, as follows:

$$R_S = \frac{q_m [q^*] S X_a}{K_{so}[K_S^*] + S} = \frac{q_{m,e} S X_a}{K_{s,e} + S} \quad \textbf{(5-16)}$$

where $q_{m,e} = q_{mo}\, q^*$ = the effective inhibited maximum specific substrate removal

$K_{S,e} = K_{so}\, K_S^*$ = the effective Half saturation coefficient

As discussed in Chapter 2, generally accepted terms for describing q^* and K_S^* as a function of toxicant concentration were developed by Han and Levenspiel (1988). Specifically:

$$q^* = [1 - I/I^*]^n \quad \textbf{(5-17)}$$

$$K_S^* = [1 - I/I^*]^{-m} \quad \textbf{(5-18)}$$

These terms have three specific characteristics of interest. First, I^* represents the toxicant concentration at which substrate conversion ceases, that is, complete inhibition has occurred. Second, when I is low or I^* is high, the kinetic coefficients are reduced to the values for non-toxic environments, or q_{mo} and K_{so}.

Applying Eq. 5-16 to the calculation of residual effluent substrate concentration from a CMAS reactor receiving a non-substrate inhibitor, we find the equation becomes:

$$S_e = \frac{K_{Se}(1 + b_a\, SRT)}{SRT\,(Y_g\, q_{m,e} - b_a) - 1} \quad \textbf{(5-19)}$$

Eq. 5-16 indicates that competitive inhibition ($K_S^* > 1.0$) increases the effective K_S and causes S_e to increase with increasing SRTs (Figure 5.20A). Relatively small impacts are seen at SRTs greater than 10 days. Figure 5.20B illustrates that non-competitive inhibition ($q^* > 1.0$) produces greater deterioration of S_e with increases in SRT. Mixed inhibition compounds the deterioration of effluent S_e versus SRT (graph not shown).

It is clear from this analysis that the closer the inhibitor concentration is to the critical inhibitor concentration, I^*, the greater the effect on the effluent substrate concentration at any given SRT, and higher SRTs are required to obtain a given effluent substrate concentration when an inhibitor is present.

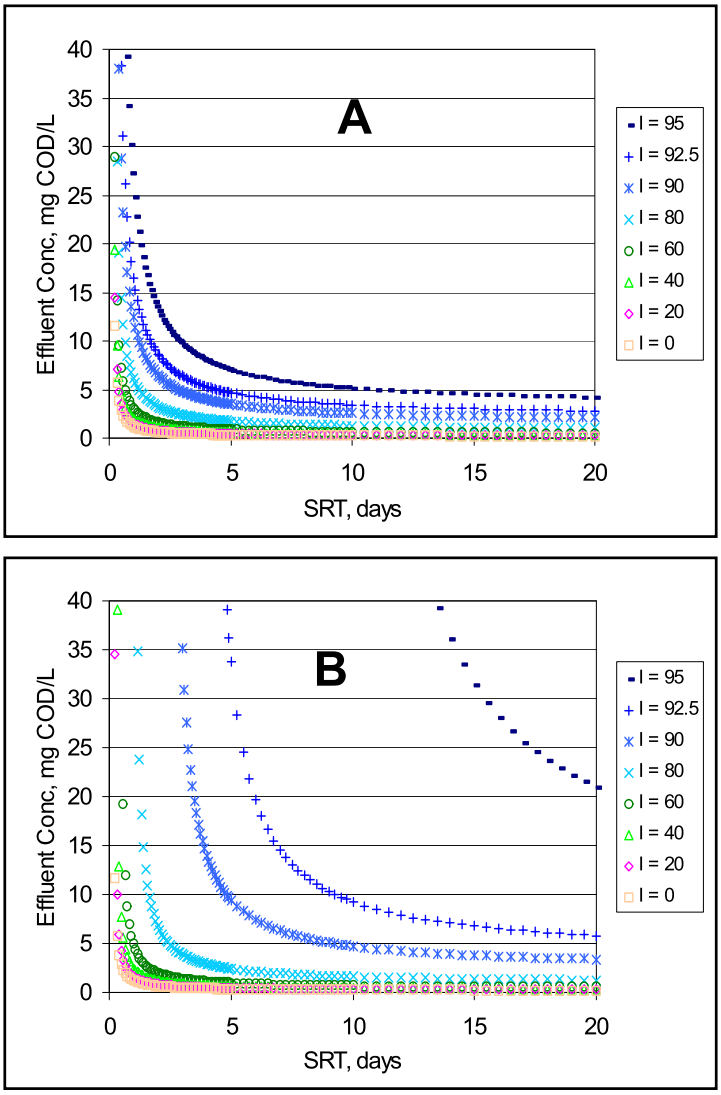


Figure 5.20

Impact of competitive Inhibition (A) and non-competitive (B) inhibition on S_e vs SRT relationship.

Figure 5.21 illustrates the impact of mixed inhibition on COD removal efficiency vs inhibitor (toxicant) concentration at SRTs of 1, 2, 5, 10 and 20 days. The influent substrate concentration used for these calculations was 100 mg COD/L and **I*** = 100 mg/L. As indicated, a toxicant would show little impact on efficiency until a threshold concentration is reached. Beyond this concentration, rapid decreases in effluent quality would be expected.

The best method to collect batch respirometric data when one is interested in investigating non-substrate toxicity is to add the toxicant at various concentrations to a series of respirometer vessels having the same $\mathbf{S_O}$ and $\mathbf{X_O}$ concentrations. Eqs. 5-4 and 5-5 would then be fit to the oxygen uptake data to obtain the best estimates of $\boldsymbol{\mu_m}$ and $\mathbf{K_S}$ for the given inhibitor concentration. The values of the parameters collected in this way are then tabulated and plotted to provide information concerning the observed parameter values as a function of the inhibitor concentration as described in Section 2.7.1. Eqs. 5-17 and 5-18 can then be fit to this data to produce values for **I*, m**, and **n**.

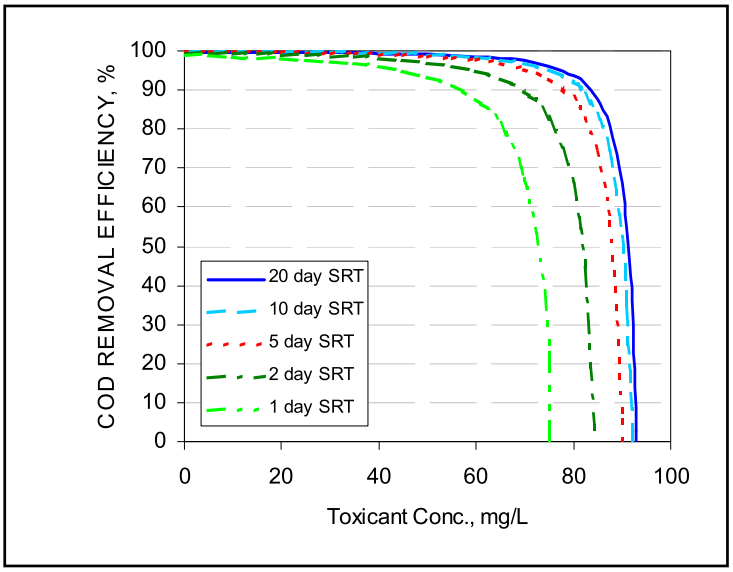


Figure 5.21

Impact of mixed inhibition on COD removal efficiency. when S_o and K_I = 100 mg/L.

5.7 Extant Kinetic Tests – Pure Compounds

Extant kinetic procedures fall into two categories: single compound tests and multiple-compound tests. The principal use of extant kinetics to date has been to estimate the biodegradation characteristics of specific chemicals in activated sludge environments. In some cases, extant kinetic tests have been conducted using high-sensitivity dissolved oxygen probes in specially designed vessels, as illustrated in Figure 5.22 and described by Ellis *et al.* (1996). Measurement of extant kinetic parameters for specific chemicals in this manner requires the use of a respirometer that can accurately detect small differences in oxygen uptake between the endogenous rate of activated sludge liquors and the endogenous rate plus the oxygen uptake due to biodegradation.

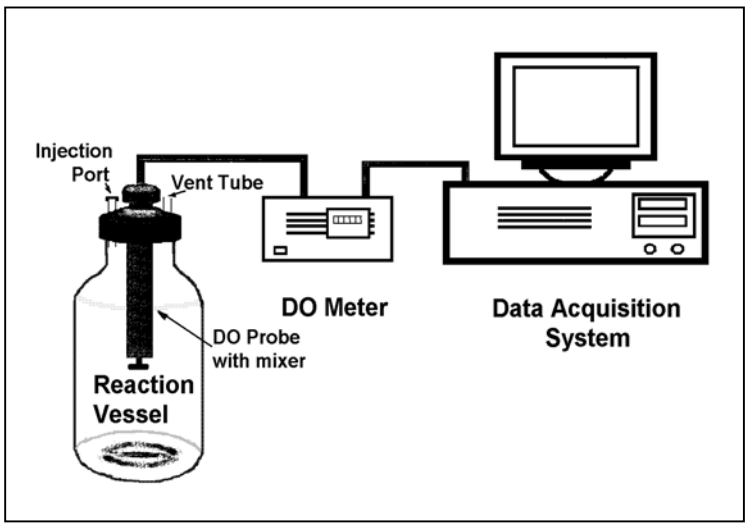


Figure 5.22

Schematic diagram of a respirometer system used to collect data for estimating extant kinetic parameters for specific compounds. (based on method described by Ellis *et al.*, 1996).

The test procedure involves 1) aerating a mixed liquor sample until it is in an endogenous state, 2) placing the mixed liquor in the respirometer vessel, 3) adding pure oxygen to bring the dissolved oxygen concentration to between 10 and 20 mg/L, and 4) measuring the dissolved oxygen depletion over time, as illustrated in Figure 5.23A. The test is repeated with injection of 1 to 5 mg/L of test compound immediately after oxygenating the sample. In some cases, measures of endogenous oxygen uptake and endogenous plus substrate oxygen uptake are conducted simultaneously using dual oxygen probes. Subtracting the endogenous oxygen uptake from that for the sample receiving substrate gives a net oxygen depletion associated with biodegradation of the organic substrate (Figure 5.23B).

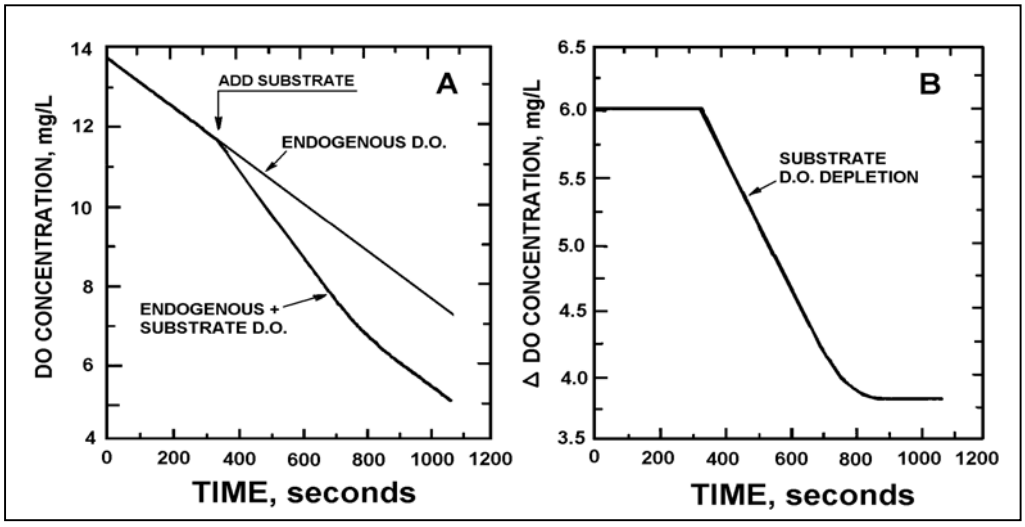


Figure 5.23. Data from extant kinetics test using phenol as the test substrate (Data from Ellis *et al.*, 1996)

A basic characteristic of Ellis-type extant kinetic tests is that the endogenous behavior of the test culture must be the same in the presence and absence of the added substrate so that the difference between the two dissolved oxygen curves represents the oxygen uptake due only to biodegradation of the test compound. Figure 5.24 shows the kinetic analysis using the test data shown in Figure 5.23. Symbols represent measured data; lines represent model output. A characteristic result of this type of extant kinetic test is that estimated values of $\mathbf{K_S}$ usually are lower than those experienced with low-rate intrinsic kinetic tests. One feature of this type of extant kinetic test is that it applies to only single and known substrates.

Ellis-type extant kinetic tests with specific compounds require that the ratio of substrate to biomass be small enough to prevent significant change in the physiological state of the competent biomass during the test duration and to prevent complete DO depletion during the tests. Eliosov *et al.* (2001) conducted extensive single-substrate extant tests with chemical doses ranging from about 3 to 5 mg/L to mixed liquors having total volatile solids concentrations around 2,500 mg/L. These dose levels represented substrate to competent biomass ratios $\mathbf{(S_0/X_0)}$ as high as 0.1:1 if competent biomass represents only 2% of the total

volatile solids in the test culture. Detailed procedures for single-substrate extant kinetic tests are presented by Eliosov *et al.* (2001). Typical extant kinetic parameters are listed in Table 5.2 for a selected number of chemicals. Magbanua *et al.* (2003) compared the predictions of calculated effluent substrate concentrations using both intrinsic and extant kinetic measurements to values measured in operating activated sludge processes. Their conclusion was that predictions using extant values more closely matched the actual in-plant values. However, the manner in which the kinetic parameter values and competent biomass estimates were made for the extant kinetics tests helped to better calibrate these results to the observed effluent data. This is necessary for obtaining the kinetic parameter value estimates using the Ellis-type extant kinetics methods but is not necessary for intrinsic kinetic parameter estimates or high rate – multiple substrate "extant" kinetics tests.

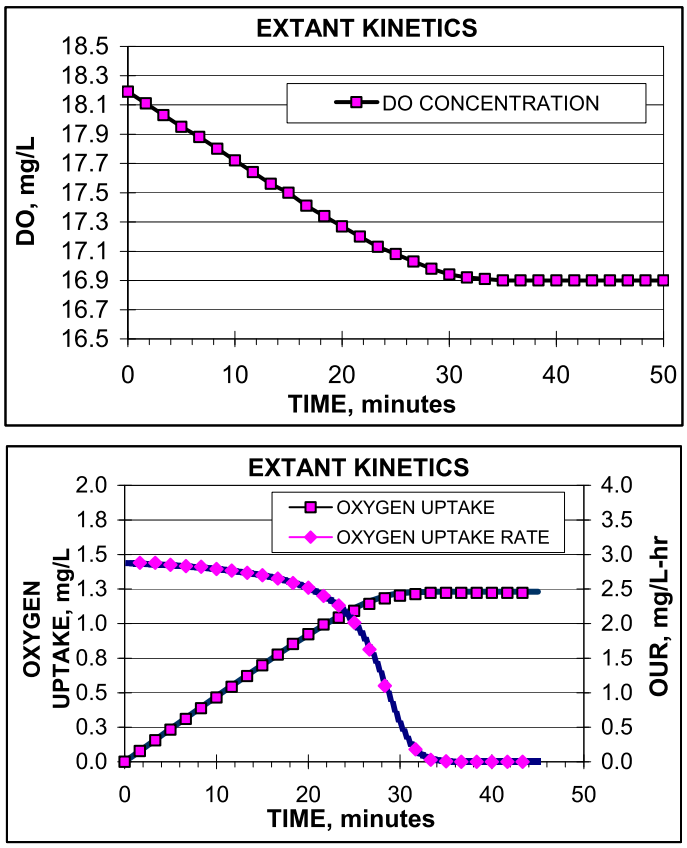


Figure 5.24

Net dissolved oxygen depletion (above) and modeling using Eq. 5-5 (below) for the phenol data shown in Figure 5.23.

Table 5.2. Summary of extant kinetic parameters for specific organic substrates (data from Eliosov *et al.*, 2001; Magbanua *et al.*, 2003).

Substrate	Y_g	q_m	μ_m	K_s
Acetone	0.493	0.361	0.178	0.540
Ethylene glycol	0.373	0.423	0.158	0.368
Phenol	0.465	0.155	0.072	0.35

Notes: Y_g = mg VSS/mg COD_r , q_m = mg COD/mg VSS/hr, K_S= mg COD/L

Extant kinetic tests for single chemicals are complicated by two phenomena. First is the difficulty of measuring, or even estimating accurately, the competent biomass responsible for degrading the specific compound. This difficulty arises because a number of species can degrade a given compound and the total competent biomass is a function of the total COD and not only the COD contributed by the specific compound. Eliosov *et al.* (2001) reported competent biomass fractions for activated sludge mixed liquors ranged from 2 to 30% depending on the chemical being tested and the state of acclimation of the culture used for testing.

A second difficulty is that the biodegradation of a specific compound in biological tests usually is a sequential reaction involving more than one metabolic intermediate and more than one species of microorganisms. The consequence of this phenomenon is that estimates of $\mathbf{K_S}$ usually are much lower than values estimated using low-rate intrinsic test conditions. Estimates of yield coefficients also will be erroneously low if the substrate is only partially degraded during the short-duration test. Fortunately, the solids retention time in most treatment systems is sufficiently long that these situations are infrequent.

Estimating the appropriate competent biomass concentration for modeling of extant kinetics data is the final hurdle that must be overcome in order to allow the widespread application of extant kinetics techniques. The volatile suspended solids present in a sample of mixed liquor from an activated sludge reactor include four types of particulate matter: 1) biomass that is capable of degrading a given substrate that has been selected for study, 2) biomass that is alive and capable of degrading other substrates but not the selected substrate, 3) dead biomass and organic, but non-biodegradable, solids that were present in the influent, and 4) slowly degrading volatile solids in the influent wastewater.

Figure 5.25 shows $\mathbf{S_e}$ concentrations that would be expected in an Ellis-type extant kinetics test for biomass having μ_m = 0.25/hr, $\mathbf{K_S}$ = 5 mg COD/L, $\mathbf{Y_g}$ = 0.35 mg VSS/mg COD_r , $\mathbf{b_a}$ = 0.008/hr, $\mathbf{S_o}$ = 2.0 mg COD/L and where 100%, 50%, 25%, 10%, 5%, or 1% of the 375 mg/L active biomass is competent. This simulation indicates clearly the importance of being able measure or estimate accurately the concentration of competent biomass (see Section 6.4).

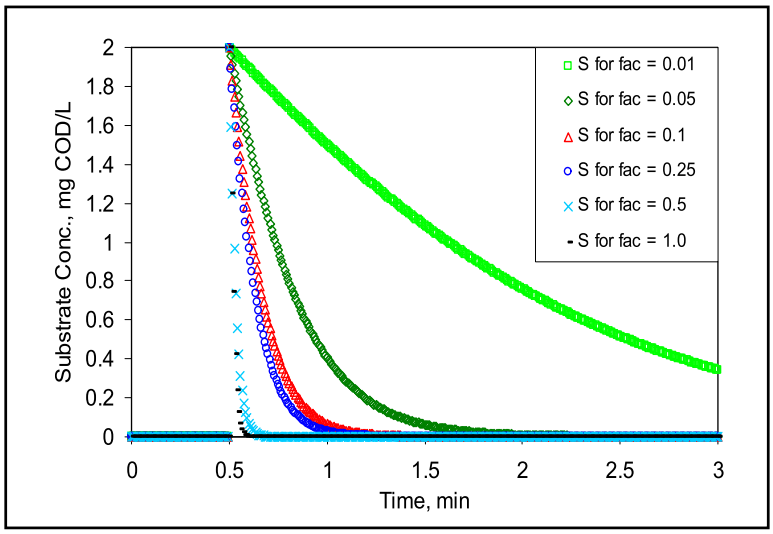


Figure 5.25

Effect of the fraction of active competent (fac) biomass on effluent substrate concentrations in an extant kinetics test.

5.8 Extant Kinetic Tests – Mixed Constituents

Activated sludge mixed liquors contain a variety of organic compounds and multiple species of organisms that are capable of degrading these compounds. The residual concentration of each organic compound in the effluent from a biological treatment process typically is less than 1 mg/L. However, the mixed liquors in plug-flow, step-feed, and SBR type processes would see higher compound concentrations at the inlet of the reactor so that the total oxygen uptake rate for these processes can exceed 200 mg/L-hr. Measuring oxygen uptake for these high-rate extant reactions requires use of respirometers that can accurately measure such high rates without sample dilution or oxygen transfer limits. Respirometers that have been used for this application include those that provide sequential measurement of dissolved oxygen depletion (Brouwer *et al.*, 1998; Kong et al, 1996), ΔP (Arthur and Arthur, 1994), or direct input respirometers that measure oxygen uptake continuously (Young, 1999). In a typical application, raw wastewater is mixed with endogenous mixed liquor solids or return activated sludge in ratios that simulate the loading conditions to the reactor. In this case, the total COD to total VSS ratio can range from 0.5:1 or higher for the inlet to SBRs and plug-flow activated sludge processes to 0.05:1 for completely mixed reactors and effluent zones of plug-flow reactors (Young, 1999; Insel *et al.*, 2003).

An example of the results of a high-rate, multiple-component, extant test is shown in Figure 5.26. The symbols represent measured oxygen uptake rate; the lines represent the results of modeling the three major constituents using Eqs. 5-4 and 5-5. In this case, the three constituents included: 1) a highly biodegradable organic fraction, 2) a slowly degraded organic fraction, and 3) a third substrate thought to represent nitrification. Corresponding kinetic parameters are shown in Table 5.3. Kappeler and Gujer (1992) and Brouwer *et al.* (1998) used similar modeling to determine kinetic parameters for mixed industrial wastewaters. Insel *et al.* (2003) used a similar modeling approach and identified a number of factors affecting the precision of fitting biological growth and substrate conversion reactions to oxygen uptake data from multi-constituent textile and domestic wastewaters and the resulting accuracy of the estimated kinetic parameters. This type of analysis and its application to the control of activated sludge processes are described in greater detail in Chapter 6.

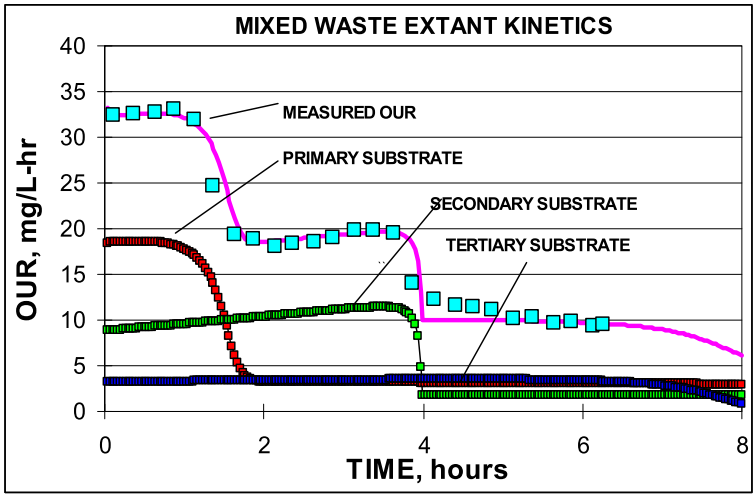


Figure 5.26

Results of multi-substrate extant kinetic tests using a high-rate respirometric method (Young, 1999).

Table 5.3. Kinetic and biological growth parameters for the three substrates shown in Figure 5.26.

Parameter	Wastewater # 1		
	S_1	S_2	S_3
S_O, mg COD/L	77	85	8
M_a, mg VSS/L	235	110	7
Y_g, mg VSS/mg COD_r	0.50	0.50	0.50
q_m, mg COD/mg VSS-hr	0.25	0.25	0.21
K_s, mg COD/L	4	1	0.3
b_a , /day	0.30	0.30	0.20

5.9 Kinetics of Anaerobic Reactions

Kinetics of anaerobic biodegradation reactions also can be modeled using Eqs. 5-4 and 5-5. However, in this case, it is more common to model the sequential reactions that typically occur in anaerobic (methanogenic) environments rather than to model only the single rate-controlling reaction. An example of the estimation of kinetic parameters for ethanol biodegradation is shown in Figure 5.27. This case involved simultaneous modeling of ethanol conversion, acetate production and subsequent acetoclastic methanogenesis as well as hydrogen production and subsequent hydrogenotrophic methanogenesis. The kinetic parameters for acetoclastic and hydrogenotrophic methanogenesis were determined from single-substrate tests (Kim *et al.*, 1996). A summary of kinetic parameters for these reactions is shown in Table 5.4.

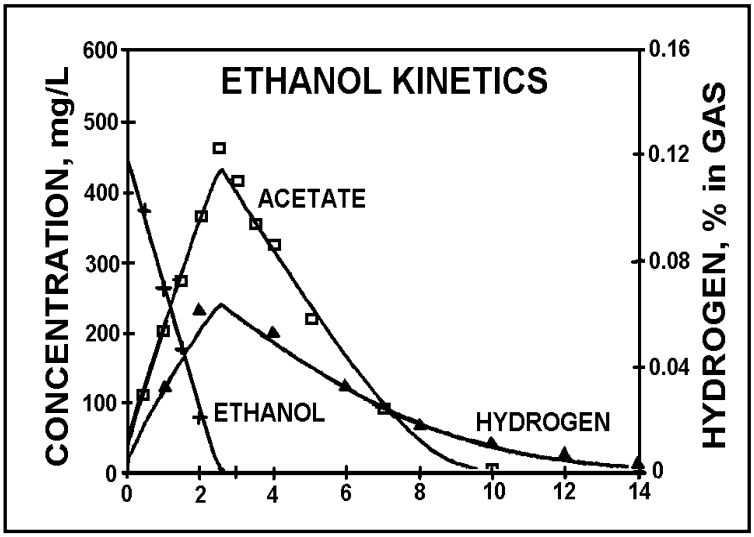


Figure 5.27

Results of kinetic tests for anaerobic reactions using substrate concentration as a modeling parameter (Kim, 1991).

Table 5.4. Kinetic parameters for methanogenic reactions during anaerobic biodegradation of ethanol (From Kim *et al.*, 1991).

Substrate	Y_g	q_m	μ_m	K_{Ss}	b_a
Ethanol	0.022	1.34	0.0295	8.5	0.001
Acetic acid	0.038	0.261	0.001	62	0.001
Hydrogen	0.046	1.0	0.046	1.67	0.001

Units: Y_g = mg VSS/mg COD converted, K_S = mg COD/L, q_m = mg COD/mg VSS/hr, b = /d

Kinetic parameters also can be estimated from anaerobic respirometer tests that use methane production as a monitoring parameter. In this case, the rate of methane production represents a measure of the rate-limiting reaction. In most cases, the rate limiting reaction is acetoclastic methanogenesis, but in other cases, the rates of hydrolysis or hydrogenotrophic methanogenesis may control the reaction rate. An example of the estimation of kinetic parameters using methane production is shown in Figure 5.28. In this case, acetoclastic methanogenesis was considered to be the rate-limiting step so that estimates of methane production for hydrogen conversion could be made. The rates were modeled using Eqs. 5-4 and 5-5 and assuming that decay rates were negligible. This latter assumption is reasonable when conducting short-term anaerobic tests because of the very low decay rate for acetogens and methanogens.

Assessment of kinetic parameters for more complex hydrolysis and fermentation reactions requires the identification of the specific rate-controlling reaction and often involves measurement of intermediates to confirm that methane production actually reflects the progress of the biodegradation reaction (Young and Tabak, 1993).

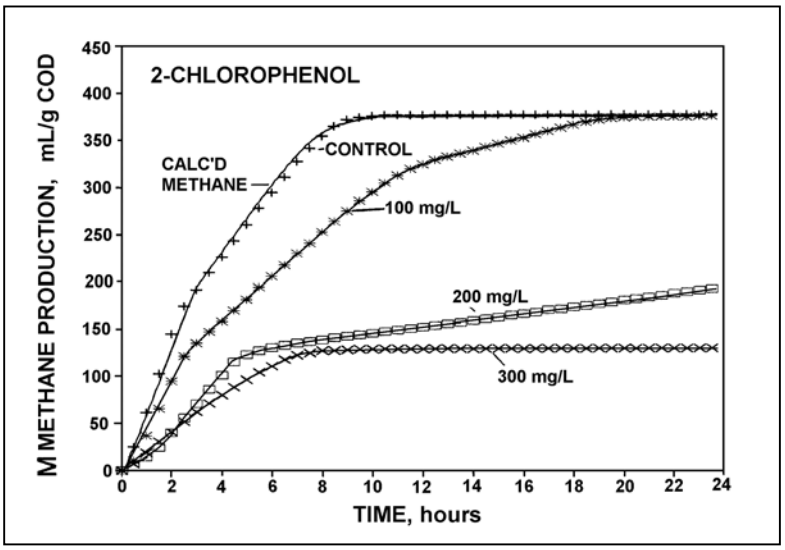


Figure 5.28

Analysis of the kinetics of inhibited anaerobic reactions using methane production as a modeling parameter (Data from Kim *et al.*, 1996; used with permission of Elsevier Science, Ltd).

5.10 Kinetics of Volatile Organic Compound Biodegradation

When measuring oxygen uptake of volatile organic compounds using headspace gas respirometers, the volatile substrate, benzene for example, must be allowed to achieve equilibrium between liquid sample and headspace gas by Henry's law before beginning data collection. Otherwise, pressure changes early in the test will cause errors in apparent oxygen uptake measurements. Corrections then must be made to account for changes in the amount of volatile compound in the headspace gas during the test.

Figure 5.29 shows a simulation of the biodegradation of the highly volatile compound, ethylene. The parameter values used as the basis for this simulation were taken from Tambwekar (2002). The curves shown in Figure 5.29 represent 1) the total substrate present in the respirometer vessel as if it were all dissolved, 2) the actual dissolved phase substrate concentration, 3) the observed oxygen uptake (Total $\mathbf{O_u}$), 4) the amount of that oxygen uptake that was used for biological reactions (Actual $\mathbf{O_u}$), and 5) the amount of apparent oxygen uptake (Apparent $\mathbf{O_u}$), i.e. that delivered to the respirometer vessel to replace the ethylene that had been in the gaseous phase and the extra oxygen that dissolves into the liquid as the oxygen concentration in the headspace increases. Because ethylene is so volatile most of it is in the headspace at the start of the reaction.

Generally, volatile substances having a Henry's coefficient less than 100 atm (0.075 m^3/m^3) present few if any problems with most commercial respirometers. The reader is referred to articles by Tambwekar (2002), Goudar and Strevett (1998), and Naziruddin *et al.* (1998) for more detail on procedures for estimating kinetic parameters for volatile compounds.

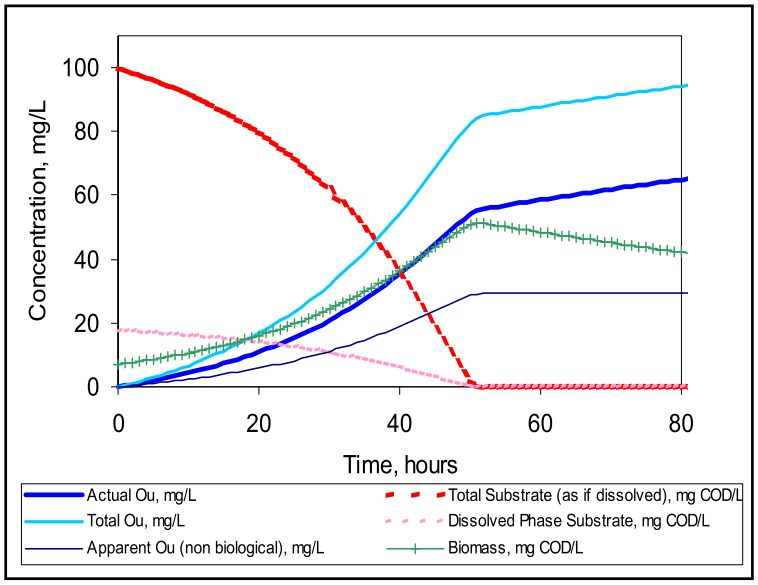


Figure 5.29

Simulated progress of ethylene biodegradation experiment using respirometry (Tambwekar, 2002).

5.11 Factors Affecting Kinetic Measurements

While kinetic methods have been applied to a number of chemicals and aerobic microbial environments (Grady and Magbanua, 1998, 2003; Magbanua *et al*., 2003) a basic assumption is that oxygen uptake is a direct measure of substrate mineralization. However, many substrates are degraded sequentially through a number of intermediates that in turn may be degraded at different rates by different species of microorganisms. Therefore, kinetic parameters often represent composite reactions between these intermediates and a mixed microbial community. Consequently, it is not surprising that intrinsic kinetic parameters vary widely among tests conducted by different investigators who used cultures from different sources that are grown under widely varying conditions.

Even under closely controlled conditions, intrinsic and extant kinetic coefficients have ranged widely in value even when measured under apparently similar test conditions (Smets *et al.*, 1996; Grady and Magbanua, 1998; Eliosov *et al.*, 2001). A number of factors that contribute to this variation include, but are not necessarily limited to, the composition of the microbial culture, solid/liquid interactions such as adsorption and volatilization, presence of other toxic substances, culture history, nutrient availability, and physical arrangement of the microbial solids matrix, as discussed in greater detail in the following sections. For a more comprehensive analysis and discussion of the measurement of intrinsic kinetic parameters, the reader is referred to publications by Brown et al.(1990), Grady and Magbanua (1998) and Smets *et al.* (1996).

5.11.1 Different Strains of Microorganisms

Yang and Okos (1987) observed 20-fold differences in substrate uptake and biomass growth rates by three strains of methane-formers when cell growth conditions were held constant, acetate was the only substrate, the temperature was 35^{o}C, and the only variable was the dilution rate. In other tests, when operating at 35^{o}C and when using acetate as the organic substrate, K_s values ranged from 27 to 300 mg/L. Speece (1988) reported that in acetate enrichment cultures, *Methanothrix* predominated at low organic loading rates (< 1 g COD/L-day) while *Methanosarcina* predominated at high organic loading rates (>10 g COD/L-day). This shifting culture predominance seemed to be related to the balance of substrate and nutrient compounds and to the different maximum uptake rates of the cultures. Other factors no doubt were involved.

The species-specific nature of the kinetic parameters indicates that supposedly intrinsic kinetic coefficients determined for one environment may not be universally applicable to other seemingly similar environments. The apparent dependence of kinetic parameters on the microbial species means that respirometer test protocols must be designed to maintain a constant culture composition or provide some means of monitoring and compensating for shifts in culture predominance.

5.11.2 Environmental Stresses

Some bacteria are more sensitive to environmental stresses and chemical and physical factors and have slower growth rates than other bacterial groups present in anaerobic or aerobic processes. Therefore, outside interferences – physical, such as temperature, or pH; or chemical, such as produced by toxic substances – will affect the balance of reaction pathways

and the population dynamics of the microbial culture. Consequently, environmental conditions in test cultures must be maintained as constant as possible to minimize test variability, and the environmental conditions must be as close as possible to those in the treatment or natural environment for which kinetic parameters are being determined. Respirometers that are used for assessing extant kinetic parameters then must be able to operate under the natural conditions of the test environment.

5.11.3 Physiological State of the Culture

A number of investigators have demonstrated that the physiological state of a culture affects the measurement of kinetic parameters (Daigger and Grady, 1982a,b; Sokol, 1987; Chuboda *et al.*, 1992). The causes for these impacts includes culture history – the growth state of the culture prior to the test, changes in species predominance during the test, and the inability to determine the concentration of biomass responsible for the biodegradation reaction, ie, the competent biomass. As indicated by Eq. 2-17, the ratio of active to total biomass changes in response to solids retention time of the process from which the culture was taken. Therefore, using volatile solids as a measure of active biomass concentration can lead to erroneous estimates of kinetic parameters. The computation method should allow estimation of initial biomass as one of the kinetic parameters. Also, as solids retention time changes, the species predominance shifts, thereby causing changes in specific substrate uptake rate.

5.11 References

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APPENDIX B

JUSTIFICATION FOR RESPIROMETER PURCHASE

JUSTIFICATION FOR RESPIROMETER PURCHASE

Screening experiments could be performed in shake flasks with hand sampling and significant analysis of these samples, but frequent hand sampling is required in order to obtain sufficient data to allow kinetics and stoichiometry of growth to be accurately determined. The need for frequent hand sampling could significantly limit the number of experiments that could be performed or greatly increase labor and analytical costs and produce data with greater relative error (and thus greater uncertainty) with respect to the kinetics and stoichiometry of algae growth. It would be much more difficult to use the hand-sampled data to provide information on multiple substrate growth behavior, nutrient limitations, and the evaluation of inhibition effects (all of these effects are expected to be seen in this research). It may be possible to investigate all of these effects with hand-sampled microbial growth experiments, but doing so can require significant investment in time, analytical chemistry work on samples, and replication of experiments if samples are not collected at the right time. With the continuous oxygen uptake data collection provided through respirometry, it is impossible to miss the timing of critical events. Additionally, for experiments where samples need to be collected at critical times in the growth cycle, the oxygen uptake data being collected during a respirometer run can used to identify when the samples should be collected. This greatly decreases analytical costs by eliminating the need to analyze extra samples to make sure samples were collected at the critical time. Without a respirometer, the project will cost more in the long run and lead to less knowledge being gained from the screening experiments.

A cost comparison estimate between hand sampling and respirometry was performed. The assumption was developed assuming an algae growth experiment having duration of 15 hours with hand sampling of the reactors every hour so that 16 data points are obtained (time 0 through time 15 hr). 15 hours was selected because 15 data points is about the minimum required to obtain data that are good for use in regression of the kinetics and stoichiometry of microbial growth. Slower or faster growth would require more or less frequent sampling. If eight reactors are run simultaneously, the experimentalist will be taking a sample every 7.5 minutes, which is quite challenging. In operating the hand-sampled experiment this way, we will obtain 16 samples that need to be analyzed for substrate and biomass concentrations. The least expensive way to do this is the use of chemical oxygen demand (COD) and total suspended solids (TSS) analysis which will cost approximately $25/sample. This means the hand-sampled experiment will cost at least 15 hours of labor (not including setup and cleanup time) plus eight reactors * 16 samples * $25/sample = $3200 in analytical cost. The labor for the same experiment in the respirometer (not including setup and cleanup) is estimated to be 1 hour, with the analytical cost for two samples per reactor (initial and final) being $400. This means the hand-sampled experiment will cost 14 hours more in labor (~$700) and $2800 more in analytical cost than the respirometer experiment every time a set of reactors is run. Even without the labor cost savings, the analytical cost savings (including only the most basic analytical measurements) will take less than nine respirometer runs to offset the full cost of the respirometer. It is expected that there will be more than nine respirometer runs for this Phase 1 research.

APPENDIX C

PERTINENT INFORMATION RELATED TO THE PROPOSAL

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Value of Algae in Livestock Feed

Kelp (a macroalgae) used as a feed additive has been studied extensively. The retail cost of kelp meal is generally ~$1/lb, with wholesale prices slightly lower. This same price range can be expected for microalgae sold as animal feed. Kelp and microalgae fed to livestock results in the following:

- Increased milk production:
  - 4 lb/d or more with 4 oz/d of kelp meal[1]
  - 20% more with algae concentrates[2]
- Reduced care bills[2,3]
- Increased meat quality/omega-3 content in meat[4–6]
- Increased egg production and omega-3 content[7–9]

A practical example of kelp fed to beef cattle is the premium Kobe beef from Japan. Genetics and diet, which includes seaweed (kelp), contribute to the highly marbled meat quality that Kobe beef is known for around the world.

Many of the studies cited in this proposal show omega-3 enrichment where algae are fed to poultry, swine, and other livestock. Microalgae, as compared to kelp, have a higher fat content (~20% vs. ~3%) and will grow very well in fermenters on carbon sources from North Dakota ethanol and biodiesel by-products.[10–12] Certain algae are selected to cause the fats to contain a high percentage of essential omega-3s, which greatly improves the fat quality of the dairy, eggs, and meat.

Importance of Omega-3 Fatty Acids and Market Potential

Omega-3 and omega-6 fatty acids are distinct families of polyunsaturated fatty acids that are essential for human growth and development and for many aspects of health. These fatty acids have very different biochemical roles in the body and cannot be exchanged with each other. The primary source of omega-6 fatty acids is vegetable oil, whereas the primary sources of omega-3s are fish, seafood, and some seed oils such as flax. Our diets evolved from near-equal proportions of omega-3 and omega-6. However, omega-6s now account for the majority of the polyunsaturated fatty acids (PUFAs) in our food. This change has accelerated in the past century, so the U.S. population is presently consuming 10–50 times more omega-6 than omega-3 fatty acids. Importantly, the lack of dietary omega-3 fatty acids has been associated with many diseases and disorders, including cardiovascular disease, the dysregulation of lipid levels, immune function, diabetes, osteoporosis, chronic inflammation, respiratory diseases, visual problems, cancer, neuronal development, and numerous brain disorders including depression and violence.

There are three main omega-3 fatty acids in foods. Alpha-linolenic acid (ALA) (18:3n-3) is the only omega-3 available in terrestrial plants. Eicosapentaenoic acid (EPA) (20:5n-3) and docosahexaenoic acid (DHA) (22:6n-3) are found almost exclusively in fish and seafood, but

ultimately they are bioaccumulated from algae. Research indicates EPA and DHA are the forms responsible for the majority of the omega-3 beneficial effects. Although EPA and DHA can be made from ALA, this process is very inefficient in humans. Therefore, to meet the body's needs to optimize health, DHA and EPA must be obtained from the diet.

The importance of omega-3 fatty acids has resulted in the demand for their food sources and supplements to increase significantly in the past several years. Omega-3-enriched foods now make up the strongest sector of the functional foods market. The U.S. market value increased from approximately $100 million to more than $2 billion in 4 years. A new report predicts that foods with added omega-3 will reach $7 billion in sales by 2011, and DHA and EPA are predicted to dominate over ALA within the omega-3 category by 2011.[13] Importantly, these forms can only be obtained from our diets through the consumption of ocean fish and seafood or through supplements made from fish sources.

In the marine system, EPA and DHA originate in macroalgae and microalgae and accumulate up the food chain to fish. Some freshwater fish and diadromous species such as Atlantic salmon and trout have enzymes to produce EPA and DHA from ALA, but marine fish do not. Therefore, to maximize growth and nutrient content, aquaculture farms use large quantities of fishmeal and fish oil made from less valuable wild-caught species. Aquaculture's share of global fishmeal and fish oil consumption more than doubled over the past decade to 68% and 88%, respectively. However, according to the Food and Agricultural Organization of the United Nations, 74% of the world's commercially fished species are depleted, overfished, or fully fished. They further predict that fish oil demand by 2015 will be 145% of global production capacity.

Therefore, to meet this increasing demand for omega-3-rich fish, freshwater and marine aquaculture must rapidly expand, even though global aquaculture is the fastest-growing sector in agriculture. Distressingly, fishmeal availability for aquaculture is declining because of overfishing and environmental disasters. Consequently, alternative plant food sources for aquaculture are in high demand and are being explored. Since algae are the primary manufacturers of omega-3 PUFAs in the marine food chain and the only plant source available for EPA and DHA, they are an ideal source for omega-3 additives in feedstocks, not only in aquaculture but for other animal feedstocks as well. However, the importance of DHA and EPA incorporation into fish and animal feeds is currently being overlooked.

References

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APPENDIX D

LETTERS OF COMMITMENT

EERC®
Energy & Environmental Research Center

UNIVERSITY OF NORTH DAKOTA
15 North 23rd Street — Stop 9018 / Grand Forks, ND 58202-9018 / Phone: (701) 777-5000 Fax: 777-5181
Web Site: www.undeerc.org

April 29, 2011

Ms. Karlene Fine
Executive Director
Attn: Renewable Energy Program
North Dakota Industrial Commission
State Capitol – Fourteenth Floor
600 East Boulevard Avenue
Bismarck, ND 58505

Dear Ms. Fine:

Subject: Center for Biomass Utilization® Commitment to EERC Proposal No. 2011-0227 Entitled "Improving the Profitability of North Dakota Ethanol and Biodiesel Plants with Algae"

This letter is in regard to the cost share to be provided by the Energy & Environmental Research Center (EERC) for the "Improving the Profitability of North Dakota Ethanol and Biodiesel Plants with Algae" project submitted to the the North Dakota Industrial Commission Renewable Energy Program (NDIC REP). The EERC, with the U.S. Department of Energy (DOE) approval, will provide $200,000 toward the total project cost of $421,750 under the 2010 DOE-sponsored Center for Biomass Utilization (CBU®) Program. The CBU is an ongoing program within the EERC that has been in existence for over 7 years. The funds that are committed toward this project are available now.

The CBU has a history of funding research projects that involve the development of technologies and tools to advance electricity, heat, and fuel production from renewable resources. The approach and concepts outlined in the proposed project should provide the data and information needed to address critical questions that remain unanswered and allow biomass to play a larger role as greenhouse gas issues are addressed.

I am hopeful that the NDIC REP will view this proposal favorably and look forward to supporting and participating in this project.

If you have any questions, please feel free to contact me by phone at (701) 777-5243, by fax at (701) 777-5181, or by e-mail at bfolkedahl@undeerc.org.

Sincerely,

Bruce C. Folkedahl
Senior Research Manager

BCF/cs

Mr. Peter A. Letvin
Research Engineer
Energy & Environmental Research Center
15 North 23rd Street, stop 9018
Grand Forks, ND 58202-9018
Phone: 701-777-5040
Fax: 701-777-5181
E-mail: pletvin@undeerc.org

April 20, 2011

RE: NDIC-Renewable Energy Council solicitation for May 1, 2011; EERC proposal #2011-0277;
"Improving the Profitability of North Dakota Ethanol and Biodiesel Plants with Algae"

Dear Mr. Letvin:

Blue Flint Ethanol, LLC is a joint venture between Great River Energy (GRE) and Headwaters Incorporated. Utilizing primarily waste heat from the adjacent Coal Creek Station Blue Flint is the first co-located, directly integrated ethanol plant in the world. Production began at the facility in February of 2007. Since that time, Blue Flint has continued to pursue innovative projects to drive down production costs, diversify our revenue, achieve greater value for our co-products and reduce our carbon footprint.

The efforts at the EERC to research algae growth on ethanol co-product streams may prove valuable to our industry. If this can be achieved economically, this research will likely play a role in increasing the product value of Blue Flint Ethanol distiller grains products. There is potential for significant impact on our industry.

We are aware of the capabilities existing at the Energy & Environmental Research Center to complete this research and fully support the approaches you have proposed for this effort. Blue Flint Ethanol

would also like to commit 200 hours of our labor at a rate of $75/hour for a total of $15,000 towards assisting in the research and economic analysis of utilizing algae in North Dakota ethanol plants.

We look forward to working with you to develop this technology that will be valuable to our industry and North Dakota.

Sincerely,

Jeff Zueger

General Manager and Chief Operating Officer
Blue Flint Ethanol
2841 3rd St SW
Underwood, ND 58576
(701) 442-7500

NONCASH COST-SHARE FORM

Instructions **Date: 04/19/2011**

This form provides information and instructions for an organization/individual, hereinafter referred to as sponsor, that are providing a noncash (in-kind) cost-share contribution to the EERC project listed below. Please review the entire document, **complete Section A, complete Section C and attach the information requested, and sign Section D. Return by April 26, 2011 to:**

via Mail: Peter Letvin, EERC, 15 N 23rd St, Stop 9018, Grand Forks, ND 58202-9018.
via Email (as a PDF document): pletvin@undeerc.org
via Fax: (701) 777-5181

If you have any questions, please contact Becky Faulhaber at (701) 777-5111, bfaulhaber@undeerc.org

Section A: Identifying Information

EERC
Prime Sponsor Name (source of funds): US Dept. of Energy – Center for Biomass Utilization
EERC Proposal No: 2011-0227
Project Title: Improving the Profitability of North Dakota Ethanol and Biodiesel Plants with Algae
Project Period of Performance: 09/01/2011 – 08/31/2012

SPONSOR

Organization/Individual Name: Red Trail Energy

Contact Name: Eli Sotillo

Organization Type: X Small Business☐ Large Business☐ Nonprofit☐ Other_____

Total Noncash Cost Share Committed: $4800.00

Section B: Federal Guidelines – Cost Share Criteria

Noncash cost share is the value of noncash contributions provided by nonFederal third parties. Contributions may be in the form of real property, equipment, supplies and other expendable property, and the value of goods and services directly benefiting and specifically identifiable to the project or program.

Contributions must meet all of the following criteria:

1. They are verifiable from the recipient's records.
2. They are not included as contributions for any other federally assisted project or program.

3. They are necessary and reasonable for proper and efficient accomplishment of project or program objectives.

4. They are allowable under the federal cost principles applicable to the EERC for the project. Please consult with the Project Manager if there is a question regarding the allowability of the cost-share contribution.

5. They are not paid by the federal government under another award unless authorized by federal statute to be used for cost sharing or matching.

6. They are provided for in the approved budget.

7. The expense is incurred within the period of performance of the project.

Section C: Required Documents

Please complete the following and attach the documents to this form.

1. A commitment letter which describes the proposed cost-share contribution to the project, signed by an authorized official of your organization.

2. Documentation of the valuation of the proposed cost-share. The valuation method and documentation examples are provided below for the various types of cost-share.

Description of Proposed Cost-Share :

Contribution Value: $4800.00

Method/Documentation of Contribution Value (catalog price, invoice copies, billing rate, appraisal etc. See below for guidelines on determining the value of the contribution):

Commercial Goods
Commercial goods contributed by an organization are valued at the fair market value of the goods. Please consider educational discounts for which UND EERC may be eligible in your valuation. Documentation of fair market value may include an advertised catalog or website price. If the item you are contributing is not generally available in the marketplace, please include copies of invoices for the same item sent by your organization to other organizations, or describe how you determined the fair market value.

Commercial Services/Employee Services
Commercial services contributed by an organization are valued at the best customer rate of the service, typically an hourly or daily billing rate. If your organization does not provide a billing rate, the employee services are valued at the employee's regular rate of pay, plus fringe benefits and overhead, provided the donated services are of the same skill for which the employee is normally paid.

Equipment
Equipment can be donated or loaned as noncash cost share. Loaned equipment is valued at a fair rental value. Donated new equipment is valued based on the use value (accounting for depreciation and project life). Donated used equipment is valued at the use value (accounting for depreciation and project life), taking into consideration the age and condition of the equipment at the time of donation. Documentation of the value of new equipment donated or loaned includes catalog or web advertised prices (if available on the open market). If the item donated is used or is not available on the open market, please describe how the value of the donation was determined.

Supplies
Donated supplies may include such items as office supplies, laboratory supplies, workshop supplies or minor equipment (<$5000). Donated supplies are valued at the fair market value of the property at the time of the donation. Documentation of donated supplies could include a copy of the purchase price of the donated items.

Land/Buildings
Land and buildings may be donated or loaned as noncash cost share. Donated land and buildings are valued at the fair market value at the time of donation by an independent appraiser. Rental charges for land or buildings are valued at fair rental value.

Other Costs: Describe any other costs and the method of valuation.

Indirect/Overhead Costs: Indirect or overhead costs incurred as a result of contributing employee services or other costs may be included. Please provide the basis for the overhead rate (i.e., percentage of salaries, percentage of direct costs).

Profit/Fee: Foregone profit or fee cannot be reported as noncash cost share.

Section D: Cost-Share Certification

Please review the certification and have an authorized official of the sponsoring organization sign.

CERTIFICATION

As a sponsor of the federally funded project, I understand that the organization is subject to all applicable federal guidelines, including the guidelines identified in Section B.

The noncash cost share contributed to this project will not be charged to the respective project and will otherwise be paid from qualifying and nonfederal sources. The expenditures are necessary and reasonable for the accomplishment of the project/program objectives.

Commercial Goods (if included): The value of commercial goods does not exceed the fair market value.

Commercial Services (if included): The value of commercial services does not exceed our best customer rate or the best market rate for the service.

Salaries and Benefits (if included): The value of effort represents the actual amount that will be paid to the respective individuals at their regular rate of pay. The value for fringe benefits does not include an overhead component and reflect reasonable, allowable, and allocable amounts.

Equipment (if included): The value of equipment represents the fair market value of the item.

Supplies (if included): The value of supplies and equipment represents the cost of those items and does not exceed the fair market value of the items.

Other Costs (if included): The value of other costs are true and correct and does not exceed the actual cost or fair market value.

Indirect (Overhead, F&A, G&A if included): The indirect, F&A, or G&A rate is true and correct and does not include any costs that are unallowable per the applicable federal guidelines or that can be claimed as a direct cost.

Documentation of actual expenditures and/or market values is available if necessary.

I HEREBY CERTIFY THAT THE ABOVE INFORMATION IS TRUE AND ACCURATE.

Authorized Official Name (print): _____

Title: Ops. Manager

_____ _____

Authorized Official Signature Date

RED TRAIL ENERGY, LLC

"Our Farms, Our Fuel, Our Future"

PO Box 11 Richardton, ND 58652 (701)-974-3308 FAX (701)-974-3309

Mr. Peter A. Letvin
Research Engineer
Energy & Environmental Research Center
15 North 23rd Street, stop 9018
Grand Forks, ND 58202-9018
Phone: 701-777-5040
Fax: 701-777-5181
E-mail: pletvin@undeerc.org

RE: NDIC-Renewable Energy Council solicitation for May 1, 2011; EERC proposal #2011-0277;
"Improving the Profitability of North Dakota Ethanol and Biodiesel Plants with Algae"

Dear Mr. Letvin:

Red Trail Energy, LLC (RTE) is a North Dakota-based investor group formed to finance, construct and operate a corn-based ethanol production facility located near Richardton, North Dakota. This vision became a reality when the $99 million, state-of-the-art plant began producing ethanol, in January of 2007. As one of the first coal-fired ethanol plants in the nation, RTE produces 50 million gallons of ethanol, using 18-20 million bushels of corn and ~100,000 tons of coal, annually. RTE now employs 41 personnel with an annual payroll of $1.8 million.

The efforts at the EERC to research algae growth on ethanol product streams may prove valuable to our industry. If this can be achieved economically, this research will likely play a role in increasing the product value of Red Trail Energy *livestock feed products or DDGs*. This may have a significant impact on our industry.

We are aware of the capabilities existing at the Energy & Environmental Research Center to complete this research and fully support the approaches you have proposed for this effort. Red Trail Energy would also like to commit 40 hours of our labor at a rate of $120.00/hour for a total of $4800.00 towards assisting in the research and economic analysis of utilizing algae in North Dakota ethanol and biodiesel plants.

We look forward to working with you to develop this technology that will be valuable to our industry and North Dakota.

Eli Sotillo
Operations Manager
Red Trail Energy

Chem E Inc.

4810 Meadow Creek Drive
Fargo, ND 58104
Ph: (701) 793-0277
Fx: (509) 267-6245

Mr. Peter A. Letvin
Research Engineer
Energy & Environmental Research Center
15 North 23rd Street, stop 9018
Grand Forks, ND 58202-9018
Phone: 701-777-5040
Fax: 701-777-5181
E-mail: pletvin@undeerc.org

RE: NDIC-Renewable Energy Council solicitation for May 1, 2011; EERC proposal #2011-0277;
"Improving the Profitability of North Dakota Ethanol and Biodiesel Plants with Algae"

Date: 27 April, 2011

Dear Mr. Letvin:

Chem E Inc is a process engineering company operating out of Fargo, ND for the past 10 years. We provide processing engineering consultancy services to manufacturing facilities around the globe, but have a special interest in our local North Dakota industries. We specialize in the sugar, food, and bio energy industries such as biodiesel and ethanol production. We are very supportive of innovative energy efforts and we hope to support North Dakota as it continue to expand the renewable energy efforts begun by some of our local providers.

The efforts at the EERC to research algae growth on ethanol and biodiesel product streams may prove valuable to these industries in North Dakota. If this can be achieved economically, this research will likely play a role in increasing the profitability of ethanol and biodiesel plants in ND. This will have a significant impact on the industry.

I am aware of the capabilities existing at the Energy & Environmental Research Center to complete this research and fully support the approaches proposed for this effort. Chem E Inc. would also like to commit 50 hours of our labor at a rate of $135/hour for a total of $6,750 towards assisting in the research and economic analysis of utilizing algae in North Dakota ethanol and biodiesel plants.

We look forward to working with you to develop this technology that will be valuable to our industry and North Dakota.

Paul Fry
President
Chem E Inc

EERC

BP-1d

NONCASH COST-SHARE FORM

Instructions

Date: 04/27/2011

This form provides information and instructions for an organization/individual, hereinafter referred to as sponsor, that are providing a noncash (in-kind) cost-share contribution to the EERC project listed below. Please review the entire document, **complete Section A, complete Section C and attach the information requested, and sign Section D. Return by April 26, 2011 to:**

via Mail: Peter Letvin, EERC, 15 N 23rd St, Stop 9018, Grand Forks, ND 58202-9018.
via Email (as a PDF document): pletvin@undeerc.org
via Fax: (701) 777-5181

If you have any questions, please contact Becky Faulhaber at (701) 777-5111, bfaulhaber@undeerc.org

Section A: Identifying Information

EERC
Prime Sponsor Name (source of funds): US Dept. of Energy – Center for Biomass Utilization
EERC Proposal No: 2011-0227
Project Title: Improving the Profitability of North Dakota Ethanol and Biodiesel Plants with Algae
Project Period of Performance: 09/01/2011 – 08/31/2012

SPONSOR

Organization/Individual Name: Chem E Inc.

Contact Name: Paul Fry

Organization Type: X Small Business ☐ Large Business ☐ Nonprofit ☐ Other_____

Total Noncash Cost Share Committed: $6,750

Section B: Federal Guidelines – Cost Share Criteria

Noncash cost share is the value of noncash contributions provided by nonFederal third parties. Contributions may be in the form of real property, equipment, supplies and other expendable property, and the value of goods and services directly benefiting and specifically identifiable to the project or program.

Contributions must meet all of the following criteria:

1. They are verifiable from the recipient's records.
2. They are not included as contributions for any other federally assisted project or program.

3. They are necessary and reasonable for proper and efficient accomplishment of project or program objectives.

4. They are allowable under the federal cost principles applicable to the EERC for the project. Please consult with the Project Manager if there is a question regarding the allowability of the cost-share contribution.

5. They are not paid by the federal government under another award unless authorized by federal statute to be used for cost sharing or matching.

6. They are provided for in the approved budget.

7. The expense is incurred within the period of performance of the project.

Section C: Required Documents

Please complete the following and attach the documents to this form.

1. A commitment letter which describes the proposed cost-share contribution to the project, signed by an authorized official of your organization.

2. Documentation of the valuation of the proposed cost-share. The valuation method and documentation examples are provided below for the various types of cost-share.

Description of Proposed Cost-Share : Provide Chemical Engineer V technical consulting to project.

Contribution Value: $6,750

Method/Documentation of Contribution : 50 hours at the standard Chemical Engineer V billing rate of $135/hr .

Commercial Goods
Commercial goods contributed by an organization are valued at the fair market value of the goods. Please consider educational discounts for which UND EERC may be eligible in your valuation. Documentation of fair market value may include an advertised catalog or website price. If the item you are contributing is not generally available in the marketplace, please include copies of invoices for the same item sent by your organization to other organizations, or describe how you determined the fair market value.

Commercial Services/Employee Services
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Equipment can be donated or loaned as noncash cost share. Loaned equipment is valued at a fair rental value. Donated new equipment is valued based on the use value (accounting for depreciation and project life). Donated used equipment is valued at the use value (accounting for depreciation and project life), taking into consideration the age and condition of the equipment at the time of donation. Documentation of the value of new equipment donated or loaned includes catalog or web advertised prices (if available on the open market). If the item donated is used or is not available on the open market, please describe how the value of the donation was determined.

Supplies
Donated supplies may include such items as office supplies, laboratory supplies, workshop supplies or minor equipment (<$5000). Donated supplies are valued at the fair market value of the property at the time of the donation. Documentation of donated supplies could include a copy of the purchase price of the donated items.

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Land and buildings may be donated or loaned as noncash cost share. Donated land and buildings are valued at the fair market value at the time of donation by an independent appraiser. Rental charges for land or buildings are valued at fair rental value.

Other Costs: Describe any other costs and the method of valuation.

Indirect/Overhead Costs: Indirect or overhead costs incurred as a result of contributing employee services or other costs may be included. Please provide the basis for the overhead rate (i.e., percentage of salaries, percentage of direct costs).

Profit/Fee: Foregone profit or fee cannot be reported as noncash cost share.

Section D: Cost-Share Certification

Please review the certification and have an authorized official of the sponsoring organization sign.

CERTIFICATION

As a sponsor of the federally funded project, I understand that the organization is subject to all applicable federal guidelines, including the guidelines identified in Section B.

The noncash cost share contributed to this project will not be charged to the respective project and will otherwise be paid from qualifying and nonfederal sources. The expenditures are necessary and reasonable for the accomplishment of the project/program objectives.

Commercial Goods (if included): The value of commercial goods does not exceed the fair market value.

Commercial Services (if included): The value of commercial services does not exceed our best customer rate or the best market rate for the service.

Salaries and Benefits (if included): The value of effort represents the actual amount that will be paid to the respective individuals at their regular rate of pay. The value for fringe benefits does not include an overhead component and reflect reasonable, allowable, and allocable amounts.

Equipment (if included): The value of equipment represents the fair market value of the item.

Supplies (if included): The value of supplies and equipment represents the cost of those items and does not exceed the fair market value of the items.

Other Costs (if included): The value of other costs are true and correct and does not exceed the actual cost or fair market value.

Indirect (Overhead, F&A, G&A if included): The indirect, F&A, or G&A rate is true and correct and does not include any costs that are unallowable per the applicable federal guidelines or that can be claimed as a direct cost.

Documentation of actual expenditures and/or market values is available if necessary.

I HEREBY CERTIFY THAT THE ABOVE INFORMATION IS TRUE AND ACCURATE.

Authorized Official Name (print): Paul Fry

Title: President

PF

Authorized Official Signature

27 April 2011

Date

GREAT RIVER ENERGY®

Bismarck Office • 1611 East Century Avenue • Suite 200 • Bismarck, North Dakota 58503 • 701-250-2165 • Fax 701-255-5405

Mr. Peter A. Letvin
Research Engineer
Energy & Environmental Research Center
15 North 23rd Street, Stop 9018
Grand Forks, ND 58502-9018
Phone: 701-777-5040
Fax: 701-777-5181
E-mail: pletvin@undeerc.org

April 29, 2011

RE: NDIC-Renewable Energy Council solicitation for May 1, 2011: EERC proposal #2011-0277;

"Improving the Profitability of North Dakota Ethanol and Biodiesel Plants with Algae"

Dear Mr. Letvin:

Blue Flint Ethanol. LLC (BFE) is a joint venture between Great River Energy (GRE) and Headwaters Incorporated. We Utilize primarily waste heat form the adjacent Coal Creek Station. BFE is the first co-located, directly integrated ethanol plant in the world. Production began at the facility in February of 2007. Since that time, BFE has continued to purse innovative projects to drive down production costs, diversify our revenue, achieve greater value for our co-products and reduce our carbon footprint.

The efforts at the Energy & Environmental Research Council (EERC) to research algae growth on ethanol co-products streams may prove valuable to our industry. If this can be achieved economically, this research will likely play a role in increasing the product value of BFE distiller grains products. There is potential for significant impact on the ethanol industry.

We are aware of the capabilities existing at the EERC to complete this research and fully support the approaches you have proposed for this effort.

GRE looks forward to working with you to develop this technology that will be valuable to the ethanol industry in North Dakota.

Sincerely,

Al Christianson
Manager, North Dakota Business Development & Governmental Affairs

NDEPA
NORTH DAKOTA ETHANOL PRODUCERS ASSOCIATION

May 10, 2011

Mr. Peter A. Letvin
Energy & Environmental Research Center
15 North 23rd Street, stop 9018
Grand Forks, ND 58202-9018

RE: NDIC-Renewable Energy Council solicitation for May 1, 2011; EERC proposal #2011-0277;
"Improving the Profitability of North Dakota Ethanol and Biodiesel Plants with Algae"

Dear Mr. Letvin:

On behalf of the North Dakota Ethanol Producer's Association (NDEPA), I am submitting this letter in support of the Energy & Environmental Research Center's (EERC) research efforts surrounding algae growth on ethanol production streams. The NDEPA was formed in 2006 to provide strong leadership and a clear, unified voice for the state's ethanol industry. The goals of the NDEPA are to further develop the ethanol production industry, to promote and increase ethanol use, and to increase public understanding about the benefits of ethanol.

The efforts at the EERC to research algae growth on ethanol product streams may prove valuable to our industry. If this can be achieved economically, this research will likely play a role in increasing the product value of livestock feed products and DDGs produced by ND ethanol producers. This will have a significant economic impact on our industry.

We are aware of the capabilities existing at the EERC to complete this research and fully support the approaches you have proposed for this effort. We look forward to working with you to develop this technology that will be valuable to our industry and North Dakota.

Sincerely,

Randy Schneider
President

1605 E. Capitol Avenue
PO Box 1091 · Bismarck, ND 58502
701.355.4458 · 701.223.4645 (fax) · www.ndethanol.org

APPENDIX E

RESUMES OF KEY PERSONNEL

PETER A. LETVIN
Research Engineer
Energy & Environmental Research Center (EERC), University of North Dakota (UND)
15 North 23rd Street, Stop 9018, Grand Forks, North Dakota 58202-9018 USA
Phone: (701) 777-5040, Fax: (701) 777-5181, E-Mail: pletvin@undeerc.org

Principal Areas of Expertise

Mr. Letvin's principal areas of interest and expertise include algae growth and culturing, large-scale algae cultivation, low-cost algae photobioreactors, and low-cost algae-processing equipment.

Qualifications

M.S., Mechanical Engineering, Colorado State University, 2008.
B.S., Mechanical Engineering, University of North Dakota, 2004
Proficient in the use of ProEngineer, MS Excel, MS Project, and MS Visio

Professional Experience

August 2010–Present: Research Engineer, EERC, UND. Mr. Letvin's responsibilities include process and equipment design, bench- and pilot-scale testing of new cutting-edge technologies, and analysis of design and test data using a wide range of design and analysis tools. His work focuses on technologies involving algae energy, wastewater cleanup, hydrogen production, and emission control.

2006–2010: Director of Operations, Solix Biofuels, Fort Collins, Colorado. Mr. Letvin's responsibilities included management of indoor and outdoor research facilities, design and execution of experiments, and management of a team of operators and students. He was heavily involved in commissioning of the pilot-scale algae production facility south of Durango, Colorado, and the introduction of new technology and retrofits to that facility.

2005–2007: Graduate Research Assistant, Engines and Energy Conversion Laboratory (EECL), Colorado State University, Fort Collins, Colorado. Mr. Letvin's responsibilities included CAD modeling for various projects and proposals, complete installation of and operator training on a large waterjet, installation of engines, emission measurement devices, test apparatus setup, and contract work for companies doing research at EECL. Mr. Letvin was also an instructor for a mechanical engineering course, teaching the use of ProEngineer.

2005: Temporary Maintenance Engineer, Poly America, Grand Prairie, Texas. Mr. Letvin's responsibilities included maintenance of machines, troubleshooting with operators and mechanics, data analysis of past downtime, and the start of a planned maintenance system using those data. Mr. Letvin trained the production planners to analyze the data in the absence of a proper system in place.

2004: Engineering Internship, Southwest Airlines, Dallas, Texas. Mr. Letvin's responsibilities included analysis of the over-the-wing dual-boarding bridge project installed and tested by Southwest Airlines to decrease ground time of aircraft.

2003: Engineering Internship, Bobcat, Bismarck, North Dakota. Mr. Letvin's responsibilities included CAD modeling and design for the new turbo engine to be installed in the Toolcat 5600. The design included high-flow auxiliary hydraulics and a larger turbo-charged engine. Mr. Letvin assisted in the manufacture of prototype parts and installation of those parts in the factory. The 5600T has now completely replaced the 5600.

2001–2004: Teaching Assistant, UND. Mr. Letvin's responsibilities included classroom time with undergraduate students, computer lab assistance, and final design project planning. While in this position, Mr. Letvin also performed several consulting type services to local industries in need of design, CAD, and FEA analysis assistance.

Publications and Presentations

Letvin, P.A. Alternative Launcher for Target Drones. Presented to the 2004 National Defense Industrial Association, sponsored by the Office of the Secretary of Defense, Operational Test and Evaluation, Charleston, SC.

Patents

Willson, B.; Babbitt, G.; Turner, C.; Letvin, P.; Weyer-Geigel, K.; Ettinger, A.; Boczon, A.; Rancis, N.; Murphy, J. Diffuse Light Extended Surface Area Water-Supported Photobioreactor. U.S. Patent No. 11871728, Oct 12, 2007.

Willson, B.D.; Turner, C.W.; Babbitt, G.R.; Letvin, P.A.; Wickrmasinghe, S.R. Permeable Membranes in Film Photobioreactors. U.S. Patent No. 12481418, June 9, 2009.

Hentges, P.F.; Barlow, T.C.; Gorham, D.S.; Quinn, J.C.; Letvin, P.A.; Turner, C.W.; Babbitt, G.R.; Echter, N.P.; Howland, J.W. Systems and Methods for Harvesting Algae from Photobioreactors. U.S. Patent No. 0120070, May 26, 2011

Babbitt, G.R.; Turner, C.W.; Letvin, P.A. Low Shear Pumps for Use with Bioreactors. U.S. Patent No. 0199904, Aug 13, 2009.

Turner, C.W.; McCarty, B.R.; Letvin, P.A.; Willson, B.D.; Herboldsheimer, D.R. Systems and Methods for Positioning Flexible Floating Bioreactors. U.S. Patent App. No. 13/046,559 March 11, 2011.

DR. ROBERT M. COWAN
Research Engineer
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Principal Areas of Expertise

Dr. Cowan's principal areas of interest and expertise include separations science, industrial wastewater treatment, air pollution control, carbon dioxide capture, and bioremediation.

Qualifications

Ph.D., Civil (Environmental) Engineering, State University of New York at Buffalo, 1994
Dissertation: Effect of Interspecies Interactions on Population Dynamics in a Chemostat as Related to Bacterial Supplementation Processes

M.S. (departmental honors), Chemical (Biochemical) Engineering, State University of New York at Buffalo, 1987. Thesis: Separating Lactic Acid from Fermentation Media Using Liquid Membrane Emulsions

B.S. (graduated with distinction), Chemical Engineering, State University of New York at Buffalo, 1984

Professional Experience

2010–Present: Dr. Cowan's work focuses on addressing the issue of global climate change through carbon capture and storage (CCS), including developing novel capture technologies, designing equipment and conducting experiments, and assisting with the development of programs to minimize and treat water used or produced during CCS activities. Current projects include Carbon Dioxide Capture Technology Review, Anaerobic Digestion of Feedlot Waste, and Water Use and Water Quality Impacts of Carbon Dioxide Capture.

2006–2009: Laboratory Director and Senior Scientist, Carbozyme, Inc., Monmouth Junction, New Jersey. Dr. Cowan's responsibilities included managing all aspects of CO_2 capture technology research and development including writing monthly progress reports and task milestone reports on a $7,500,000 U.S. Department of Energy (DOE)-funded project; managing a scientific and technical team of four Ph.D. engineers, one M.S. professional engineer, and two technicians; developing enzyme immobilization methods for carbonic anhydrase; developing methods for testing immobilized enzyme performance; developing data analysis procedures for quantifying component and system performance, which included analysis of flux, permeance, stoichiometry, and kinetics, as well as electrical power requirements for electrodialysis; designing experimental apparatus and experiments for study of all aspects of CO_2 capture; directing the progress of modeling efforts; designing, constructing, and operating prototype CO_2 capture devices; developing and writing research grant proposals; and performing technical liaison functions between company, subcontractors, and vendors.

2008: Lecturer, Environmental Engineering, Civil and Environmental Engineering, Princeton University. Dr. Cowan taught an upper-level engineering class, CEE 303 – Introduction to Environmental Engineering.

2005–2006: Process Specialist, EnviTreat, LLC., Springdale, Alaska. Dr. Cowan's responsibilities included consulting with industries, municipalities, and engineering firms on industrial wastewater treatability; assessing the stoichiometry and kinetics of biodegradation of industrial wastewater; evaluating wastewater treatability and toxicity; providing recommendations of process improvements based on results of laboratory testing; developing methods for and performing regression analysis of respirometric data; developing proposals and quotes for laboratory and process analysis services; and designing respirometry experiments and data analysis methods; and providing training on the use of respirometers.

2001–2006: Independent Consultant, RMC Environmental, Dayton, New Jersey. Dr. Cowan's responsibilities included consulting with industry and testing laboratories on wastewater treatment and respirometry. Clients included RespirTek, Inc., Biloxi, MS; ELAN Chemicals, Newark, NJ; Mitsubishi Chemical Company, Japan; OLI Systems, Inc., Morris Plains, NJ; and Challenge Environmental Laboratories, Fayetteville, AR.

2001–2003: Senior Research Scientist and Laboratory Director, Sapient's Institute, Monmouth Junction, New Jersey. Dr. Cowan's responsibilities included developing technology for the capture and sequestration of carbon dioxide from air, flue gas, and natural gas; researching water treatment and water recycling technology for NASA; managing research program to develop capture and sequestration technology using enzyme-based contained liquid membrane reactor for use in advanced life support and in CO_2 capture from air; and developing and writing successful grant proposals for the National Aeronautics and Space Administration (NASA) and DOE.

1994–2001: Assistant Professor of Environmental Engineering, Department of Environmental Sciences, Rutgers University. Dr. Cowan's responsibilities included managing a large research group with over $1,500,000 in sponsored research; research and training in the areas of bioremediation, industrial wastewater treatment, solid waste management, and air pollution control; conducting biological treatment and toxicity studies of refinery wastewaters experiencing shock loads of MEA, biodegradation studies on MTBE and other fuel oxygenates as well as gasoline components, and degradation of ethylene and ammonia in air treatment biofilters and composting; implementing major changes in both the Environmental Engineering and the Environmental Sciences curricula; designing, developing, and teaching two new laboratory courses and three new lecture courses; leading multidisciplinary Waste Processing and Resource Recovery (WP&RR) research team of the New Jersey–NASA Specialized Center of Research and Training for Bioregenerative Life Support (NJ-NSCORT), a 5-year multi-investigator project funded by NASA; and collaborating with faculty from departments of Chemical and Biochemical Engineering, Biochemistry and Microbiology on a multidisciplinary DARPA project.

Professional Registration
Engineer in Training (EIT) certification, South Carolina

Professional Memberships
American Chemical Society
Water Environment Federation
American Institute of Chemical Engineers

Publications and Presentations
Has coauthored several technical publications.

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Principal Areas of Expertise

Dr. Raymond is a Research Manager and oversees the Natural Materials Analytical Research Laboratory (NMARL) the Analytical Research Laboratory (ARL), and the Cell and Tissue Culture Laboratory at the EERC. As the Research Manager of the Health and Analytical Research Group, Dr. Raymond's work involves evaluating potential human health risks resulting from environmental exposures to toxins as well as strategies for prevention, protection, and remediation. Dr. Raymond's principal area of interest and expertise involves evaluating the protective effects of selenium against mercury toxicity and the effects of mercury exposure on selenium-dependent physiology. This involves a series of investigations to determine the mutual influences of Hg and Se on each others' uptake, retention, distribution, and neurobehavior and neurofunctional effects. Studies are performed at the molecular, cellular, tissue, and population levels. To find ways to decrease Hg exposure, Hg bioaccumulation and remediation are also being addressed. In addition to mercury exposure, other areas of research interest include investigations of exposure to particulate matter (PM), carcinogenic zeolites, and other trace metals and their interactions in environmental systems.

Qualifications

Ph.D., Biochemistry and Molecular Biology, University of North Dakota, 2002.
B.S., Microbiology, University of Arizona, 1993.

Professional Experience

2004–Present: Research Manager, EERC, UND. Dr. Raymond's research examines biochemical and analytical approaches involved in evaluating potential human health effects and risks resulting from environmental toxins. Dr. Raymond is also the Research Manager of the Health and Analytical Research Group and oversees the Natural Materials Analytical Research Laboratory, the Analytical Research Laboratory, and the Cell and Tissue Culture Laboratory at the EERC.

2002–2004: Postdoctoral Research Associate, EERC, UND. Dr. Raymond's research examined biochemical and analytical approaches involved in evaluating potential human health effects and risks resulting from environmental exposure to air, water, and food toxins, in particular, the role and mechanisms of mercury exposure and mercury–selenium interactions.

1996–2002: Predoctoral Research Fellow, Biochemistry and Molecular Biology Research, Human Nutrition Research Center, Agricultural Research Service, U.S. Department of Agriculture, Grand Forks, North Dakota. Research focused on molecular cell-signaling

mechanisms of oxidative damage involving cellular redox status in free radical signaling and antioxidant mechanisms.

1994–1995: Graduate studies in nutritional metabolism, North Dakota State University, Fargo, North Dakota.

1981–1992: Medic, United States Air Force (USAF) and USAF Reserve.

Publications and Presentations
Has authored and coauthored several publications.

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Principal Areas of Expertise

Dr. Schlasner's principal areas of interest and expertise include hydrogen, CO_2 capture, petroleum-refining and microbial bioprocess technologies, and advanced process control.

Education and Training

Ph.D., Chemical Engineering, Ohio State University, 1987
M.S., Chemical Engineering, Ohio State University, 1983
M.B.A., University of South Dakota, 1977
B.S., Chemical Engineering, South Dakota School of Mines & Technology, 1980
B.A., Chemistry and Mathematics, St. Olaf College, 1974
Diploma, Air War College, 1997
Diploma, Air Command and Staff College, 1993
Air University by correspondence
Professional Engineer, Ohio and Oklahoma
U.S. Department of Defense Acquisition Professional in Systems Planning, Research, Development, and Engineering, certified Level II (1995), trained Level III (2000)
Master of Process Technology, certified (1997); one of the first 18 engineers certified by Phillips
Top Secret/SBI Security Clearance, updated 2004

Professional Experience

2010–Present: Research Engineer, EERC, UND, Grand Forks, North Dakota. Dr. Schlasner works on projects related to hydrogen production technology, petroleum refinery emission control, and hot-gas filtration.

1987–2009: ConocoPhillips Company (formerly Phillips Petroleum Company), Bartlesville Technology Center, Oklahoma.

2001–2009: R&D Team Lead and Chief Engineer, CO_2 Capture/H_2 Production Team, R&D Senior Engineer, Long-Range Technology.

1992–2001: Refinery Senior Engineer, Advanced Process Control, Sweeny Petrochemical Complex, Texas.

1991–1992: Refinery Engineer, Process/Operations, Bartlesville Corporate Engineering, Oklahoma.

1987–1991: Process Engineer, Plastics, Bartlesville Research Center, Oklahoma.

Process Automation Engineer, Advanced Composites
R&D Engineer, Advanced Composites
R&D Engineer, Biotechnology Division

1980–2004: Colonel, Directorate Senior Reservist, U.S. Air Force Reserve, Air Force Research Laboratory (AFRL), Ohio.
Lieutenant Colonel, Division Senior Reservist
First Lieutenant – Major:

- Biotechnology Project Engineer
- Nonmetallic Materials Project Engineer
- Chemical Research Officer

1974–1978: Second Lieutenant – First Lieutenant, U.S. Air Force Active Duty, 44th Strategic Missile Wing (SAC), South Dakota.

- Wing Operations Staff Officer
- Missile Combat Crew Commander
- Deputy Missile Combat Crew Commander

Professional Memberships
National Hydrogen Association, Director (2006–2007)
American Chemical Society
American Society for Microbiology
Tau Beta Pi
Beta Gamma Sigma