Energy Research and Development Division
FINAL PROJECT REPORT

CASCADE CLEAN ENERGY SYSTEM FOR WATER AND WASTEWATER TREATMENT

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PREPARED BY:

Primary Author(s):
Dr. Charles C. Zhou
Sean Kilgrow
Jackie Ngo
Charles Hill

CASCADE Clean Energy, Inc.
10610 Morengo Dr
Cupertino, CA 95014
Phone: 408-203-8325 | Fax: 408-877-1730

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Prepared for:

California Energy Commission

Linda Schrupp
Contract Manager

Virginia Lew
Office Manager
Energy Efficiency Research Office

Laurie ten Hope
Deputy Director
ENERGY RESEARCH AND DEVELOPMENT DIVISION

Robert P. Oglesby
Executive Director

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PREFACE

The California Energy Commission Energy Research and Development Division supports public interest energy research and development that will help improve the quality of life in California by bringing environmentally safe, affordable, and reliable energy services and products to the marketplace.

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Cascade Clean Energy Waste Water Treatment System is the final report for the Clean Energy System for Water and Wastewater project PIR-10-011 conducted by CASCADE Clean Energy, Inc. The information from this project contributes to Energy Research and Development Division's Industrial/Agricultural/Water End-Use Energy Efficiency Program.

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ABSTRACT

Computer-Assisted Strain Construction and Development Engineering technology was used to select smart microbial consortia for the production of biogas. The microbes were cultured and added to anaerobic digesters to process wastewater sludge of more than 50,000 milligrams per liter chemical oxygen demand. The biogas produced from the anaerobic digesters was conditioned for use in direct fuel cells and electrical generators.

We designed and built a workable 1,000-gallon demonstration prototype of the Cascade Clean Energy System that is integrated into the existing wastewater treatment process at the Dublin San Ramon Service District. The system has been in operation for two years, during which time two sets of experiments have been conducted for the purposes of data collection. The Cascade Clean Energy System demonstrated that it has improved the existing waste-to-energy conversion efficiency at the Dublin San Ramon Service District by between 25.8 percent and 30 percent, depending on the quality of the sludge influents.

In addition, a skid-mounted 1,000-gallon anaerobic digester was constructed that is coupled to a precursor with the capacity to process or add microbe slurry of up to 100 gallons, sourcing the sludge from Dublin San Ramon Service District's anaerobic digesters, and piping methane to Dublin San Ramon Service District's existing fuel cell. This project improved both the chemical and biological oxygen demands of the treatment process, as well as the hydraulic retention characteristics of the wastewater stream, and has demonstrated that the Cascade Clean Energy System is scalable to a commercially acceptable level.

Keywords: Cascade, Microbes, Bioreactor, Biogas, Methane, Digester, Wastewater, Sludge

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EXECUTIVE SUMMARY

Introduction

Today's economy requires the rapid development of renewable energy alternatives to petroleum- based energy, as well as environmental bioremediation of industrial and municipal waste treatment and water purification. The Computer-Assisted Strain Construction and Development Engineering (CASCADE) Clean Energy solution is a sustainable wastewater treatment system that fulfills both these requirements. As an innovative solution in metabolic engineering, CASCADE takes advantage of nature's vast metabolic, genomic and genetic diversity to select "smart" microorganisms that clean wastewater more efficiently than existing processes, while simultaneously maximizing the recovery of renewable energy (in the form of methane, hydrogen or electricity) from wastewater and sludge.

Project Purpose

Wastewater treatment currently uses 5-7 percent of the United States' electrical output; however, only 1-2 percent of treatment facilities in the United States recover energy from wastewater. The state of California performs slightly better in that 10 of its 242 wastewater treatment plants (about 4 percent) generate electrical power as of 2008. These facilities use naturally occurring microbial strains in an anaerobic digestion process to recover methane from wastewater and sludge; the methane is then consumed by reciprocating engines, gas turbines, fuel cells and other systems to produce electricity.

Major efforts have not been undertaken to actively select microorganisms that can maximize energy recovery at those plants. As wastewater treatment plants consume a substantial amount of electricity, the possibility of amplifying energy recovery by selecting optimal microbial strains has enormous potential.

CASCADE technology uncovers the predictive relations between an organism's genomic fingerprint and its metabolic capabilities, and uses these relationships to find the organisms best suited for particular applications. Members of the project's team have achieved successful results in previous experiments using specially selected microbial strains in laboratory-produced wastewater and sludge to enhance energy recovery in the form of methane, hydrogen or electricity. This CASCADE Clean Energy System (CCES) pilot project is designed to demonstrate that this energy recovery process is more efficient than existing processes and is scalable to a commercial level.

The key measures for evaluating the success of the CCES are: 1) improvement in methane production, 2) reduction in Biological Oxygen Demand (BOD), 3) reduction in Chemical Oxygen Demand (COD) and 4) reduction in hydraulic retention time.

Project Results

A workable 1,000-gallon demonstration prototype of the CCES was built that is integrated into the existing wastewater treatment process at the Dublin San Ramon Service District (DSRSD). The system has been running for two years, during which time two sets of

experiments have been conducted, demonstrating that the CCES has improved DSRSD's existing waste-to-energy conversion by 26 percent, depending on the influents of the sludge.

To reduce construction time and costs, a skid-mounted 1,000-gallon anaerobic digester coupled to a precursor with the capacity to process or add 20 to 100 gallons of microbe slurry was built. This digester sources sludge from DSRSD and pipes methane gas to DSRSD's existing fuel cell. The project improved the COD, BOD, and hydraulic retention characteristics of the wastewater stream and has shown that the CCES is scalable to a commercially acceptable level.

CHAPTER 1: Administration

Planning of the project began on Jan. 2, 2011. On Feb. 17, 2011, Dr. Charles C. Zhou (Project Manager and Principal Investigator), Mr. Sean Kilgrow (Vice President), and Mr. Miguel J. Rea attended a kick-off meeting with the California Energy Commission (Commission) in Sacramento that included Kiel Pratt (Commission Agreement Manager), the Grants Officer, and a representative of the Accounting Office.

This meeting established the lines of communication and identified procedures for implementing this project's agreement. The project's administrative and technical aspects , the details of the terms and conditions of the Agreement and its execution, Critical Project Review (Task 1.2), match fund documentation and match fund plan from CCES team and DSRSD (Task 1.6 were discussed. DSRSD had already obtained all the necessary permits to begin the project at the District (Task 1.7) prior to the kick-off meeting. The work stream of the overall projects is organized into the following chapters.

CHAPTER 2:
Culture the Selected Bacteria

Task 2 involved culturing selected microbes, and was continued throughout the entire program. Cutting the costs of this task was a key technical and economic factor in preparing for large-scale commercialization.

We originally planned to culture one gallon each of five selected bacteria with pure media, per the instructions of bacteria vendors such as the American Type Culture Collection (ATCC). In actuality, each microbe was successfully cultured five times in order to improve our processes and attain the purest culture. Each microbe reached a population density of 100 million to 1 billion bacteria per milliliter. Details on making the microbe slurry are discussed below.

2.1 CASCADE Microbes Database

To build CASCADE's database of microorganisms and optimize the ecosystem inside an anaerobic digester, we used and reorganized information from the Kyoto Encyclopedia of Genes and Genomes (KEGG) database of Japan, the ATCC, the National Institute of Health (NIH), the United States Environmental Protection Agency (EPA) , and the Joint Genome Institute of the United States Department of Energy (DOE).

KEGG is a database resource that explains the high-level functions and utilities of biological systems (such as the cell, the organism, and the ecosystem) using molecular-level information, particularly large-scale molecular datasets generated by genome sequencing and other high-throughput experimental technologies. The specific databases/tables used within KEGG include:

KEGG PATHWAY	KEGG BRITEBRITE	KEGG MODULE
KEGG DISEASE	KEGG DRUG	KEGG ORTHOLOGY
KEGG GENOME	KEGG GENES	KEGG LIGAND
KEGG MEDIC	KEGG Organisms	KEGG Mapper: KEGG

PATHWAY/BRITE/MODULE mapping tools KEGG Atlas: Navigation tool to explore KEGG global maps, KAAS: KEGG automatic annotation server BLAST/FASTASequence similarity search SIMCOMPChemical structure similarity search PathPredBiodegradation/biosynthesis pathway prediction

Clostridium is one of the seven microbes we selected for our anaerobic bioreactors in this project. Figure 1 shows sample data from the KEGG database that tabulates a list of clostridium strains. Since clostridium strains were observed in all the sludge samples we collected, adding it to the anaerobic digester acted as an enrichment of the existing species, rather than the addition of a new species

Figure 1: Sample data from KEGG for one of the Clostridium strains used in this project

Clostridium	cbn	Clostridium botulinum BKT015925
	cbt	Clostridium botulinum E3
	cbf	Clostridium botulinum F Langeland
	cbe	Clostridium beijerinckii
	ckl	Clostridium kluyveri DSM 555
	ckr	Clostridium kluyveri NBRC 12016
	cpy	Clostridium phytofermentans
	cce	Clostridium cellulolyticum
	clj	Clostridium ljungdahlii
	csh	Clostridium saccharolyticum
	ccb	Clostridium cellulovorans
	cls	Clostridium sp. SY8519
	clb	Clostridium sp. BNL1100
	ccl	Clostridium clariflavum DSM 19732
	cdf	Clostridium difficile 630
	cdc	Clostridium difficile CD196
	cdl	Clostridium difficile R20291
	cst	Clostridium sticklandii
Filifactor	faa	Filifactor alocis
Alkaliphilus	amt	Alkaliphilus metalliredigens
	aoe	Alkaliphilus oremlandii
Arthromitus	asf	Candidatus Arthromitus sp. SFB-mouse-Japan
	asb	Candidatus Arthromitus sp. SFB-rat-Yit

ATCC is a global nonprofit bioresource center that acts as the main repository of cell lines in the United States, while providing biological products, technical services, and educational programs to private industry, government, and academic organizations around the world. The ATCC was a valuable resource for this project, by providing our source of microbes and the best method and source materials to culture them.

Figure 2 is taken from the ATCC webpage.

Figure 2: 57 bacteria strains were selected from the ATCC collections and microbes bank

NIH has a huge database called the National Center of Biotech Information (NCBI). Both KEGG and NCBI are organized in such a way that is conducive for information research, but have limitations for direct text mining or data mining to extract the information. In general, NCBI is a much more complete "Big Data" system, but KEGG is relatively easier to browse; it has information on more pathways, including those verified experimentally and simulated by computer ("in silico"). CASCADE analyzed these pathways and performed computer simulations to see which pathway had the most efficient energy costs and to find the pathway that would produce the most biogas. The CASCADE application involves no single strain, but rather a combination of multiple strains.

China Center for Type Culture Collection, Wuhan University (CCTCC) (http://www.wdcm.org/CCINFO/CCINFO.xml?611): The CCTCC claims to have more than 2300 bacteria in their collection, but we encountered problems accessing their data tables and did not use any of their information in our project.

The KEGG, NCBI, and ATCC database systems update their information daily; CASCADE updates its database on a weekly basis. Thus, some of the over 70 microbes we identified via the CASCADE system that have the potential to improve anaerobic digestion process can also be identified in the KEGG, ATCC and CCTCC databases.

2.1.1 CASCADE Data Mining

The aforementioned databases were used to update CACADE'S database of microorganisms. During the reporting period, the code of the CASCADE data collection module was modified to adapt to KEGG's new data formats and generated a summary, as seen in Table 1. As shown, KEGG dramatically increased the total number of organisms in its catalogue in recent years, which broadened our selection for this project. KEGG has systematically organized the genomic information and biochemical pathways for processes that are either proven by biological experiments or extrapolated from mathematical modeling; this information is quite valuable when we are trying to mine for enzymes related to a biochemical pathway. The subsequent codons and gene information can be further predicted or estimated.

Table 1: Comparison of KEGG data collected in 2006, 2007, 2008 and 2012

2006	366	139	1443
2007	503	138	1561
2008	718	186	1696
2012	1749	182	2271

During this period (2011 and 2012), the team researched the ATCC Bacteriology Collection database that represents the most diverse assemblage of prokaryotes in the world. Prokaryotes are a group of organisms whose cells lack a membrane-bound nucleus. This collection contains more than 18,000 strains in more than 750 genera, and represents every important physiological prokaryotic group. The collection holds more than 3,600 types of

cultures of validly described species, as well as nearly 500 bacteriophages, which are viruses that infect and replicate within bacteria. In the past three years, ATCC has benefited greatly from the work of genomic researchers worldwide.

Figure 3 shows the CASCADE software interface used for mining the microbe database. Researchers selected a total of 57 strains from KEGG and ATCC for this project. All 57 strains can be found in the ATCC, as shown in Figure 4. Eight of the methane-producing microorganisms or methanogen strains are very difficult to culture in the lab, and require extreme anaerobic conditions to produce. The production facility at the DSRSD wastewater treatment plant was used to enrich these methanogens.

With the help of Dr. Jeng Her and Sherry Wei, the team cultured the two methanogens directly inside the CMB. These methanogens can be enriched in the reactor itself before adding the additional five strains. This will eventually cut costs for culturing strains from $15,000 down to $1,500 per metric ton (2,200 pounds) of slurry.

Based on the results from the CASCADE engineering, the cultures were prepared in a biochemical lab located in Sunnyvale, California. Figure 6 shows part of the bench work in preparing the slurry of microbial strains.

Figure 3: Selecting microbes using our CASCADE method.

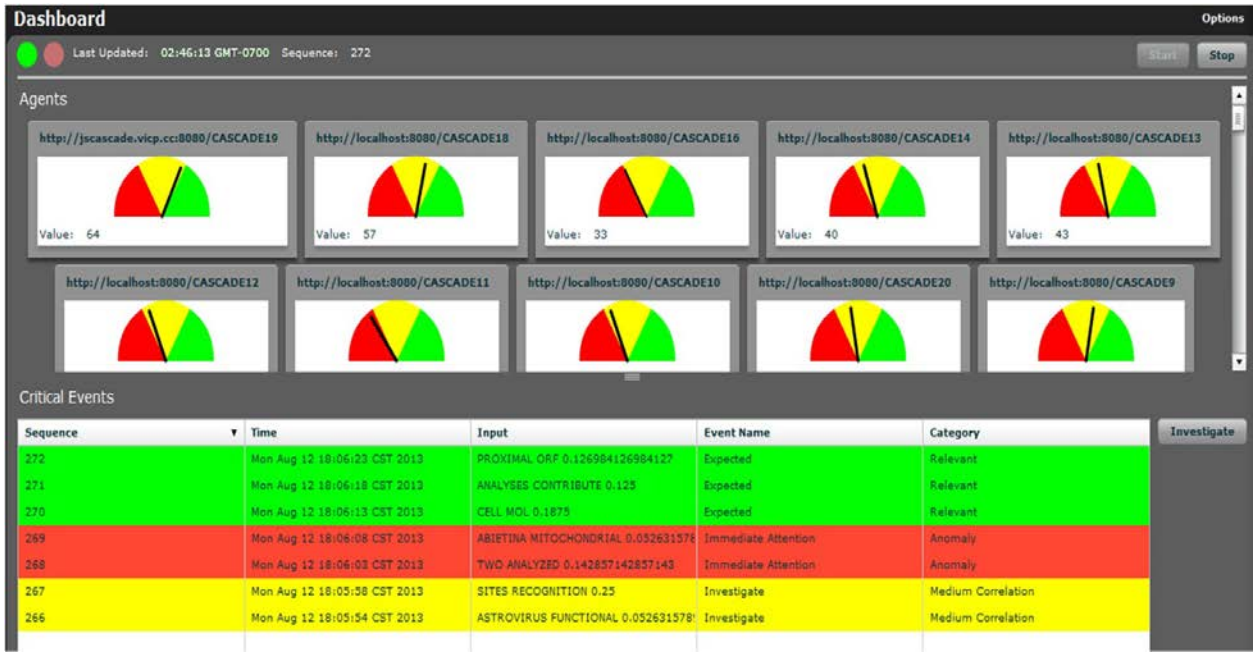


Figure 4: 57 bacteria strains were selected from ATCC's 18,000 known strains (from www.atcc.com)

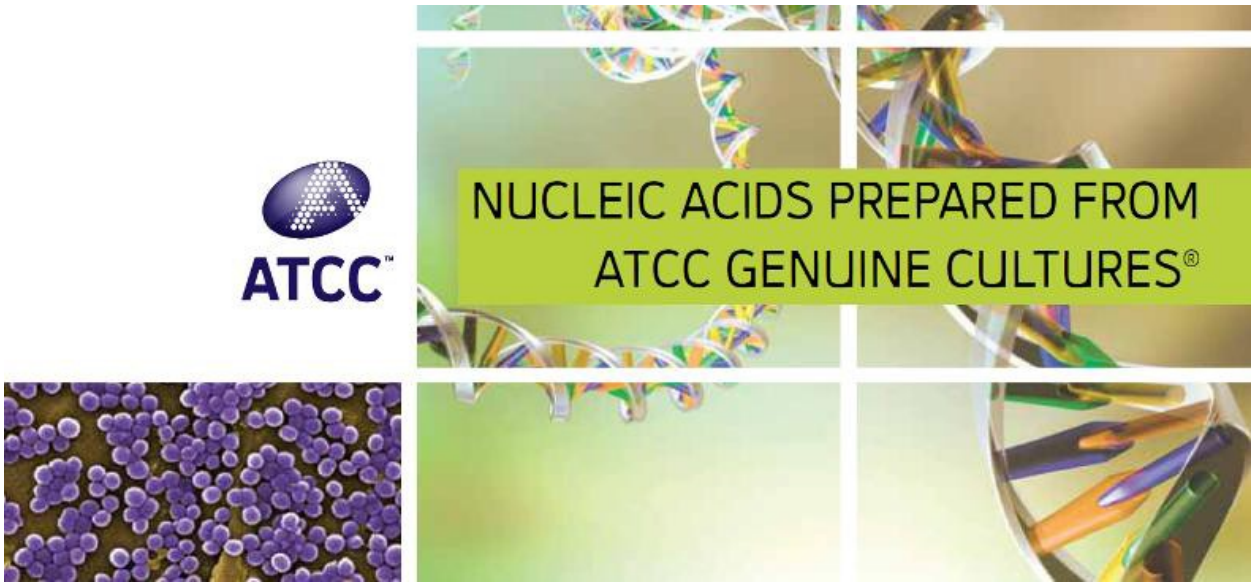


Figure 5: Biolab works in progress for selecting, culturing, and incubation.

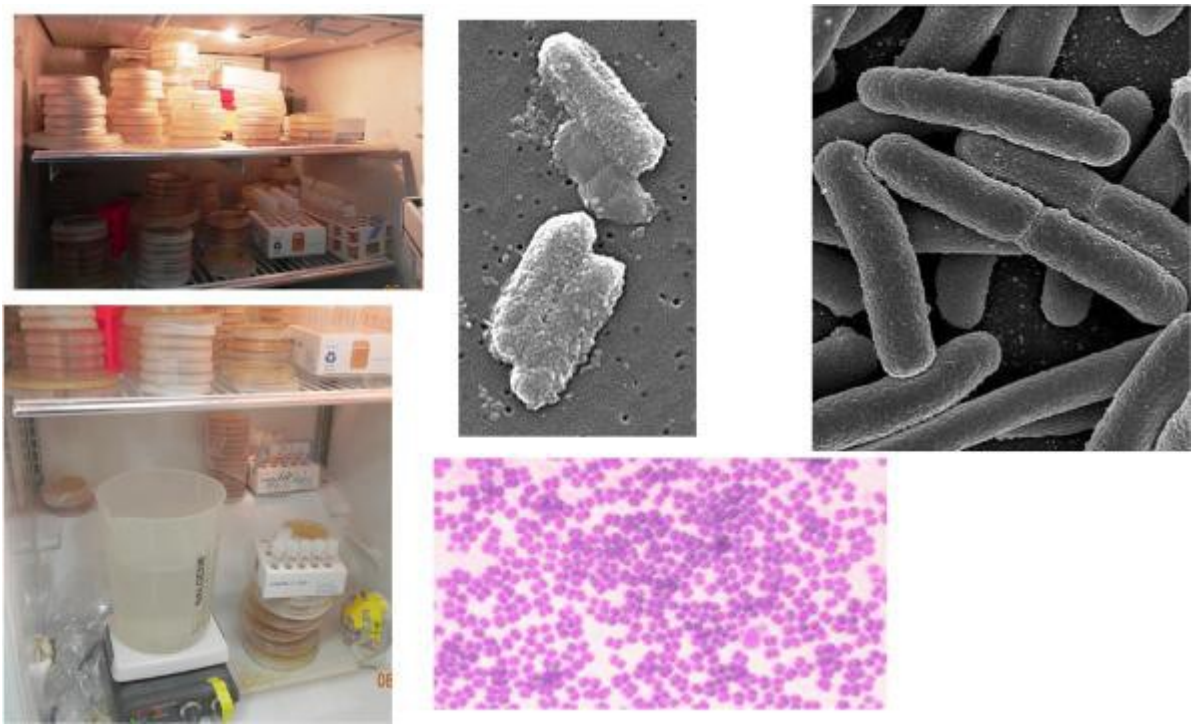
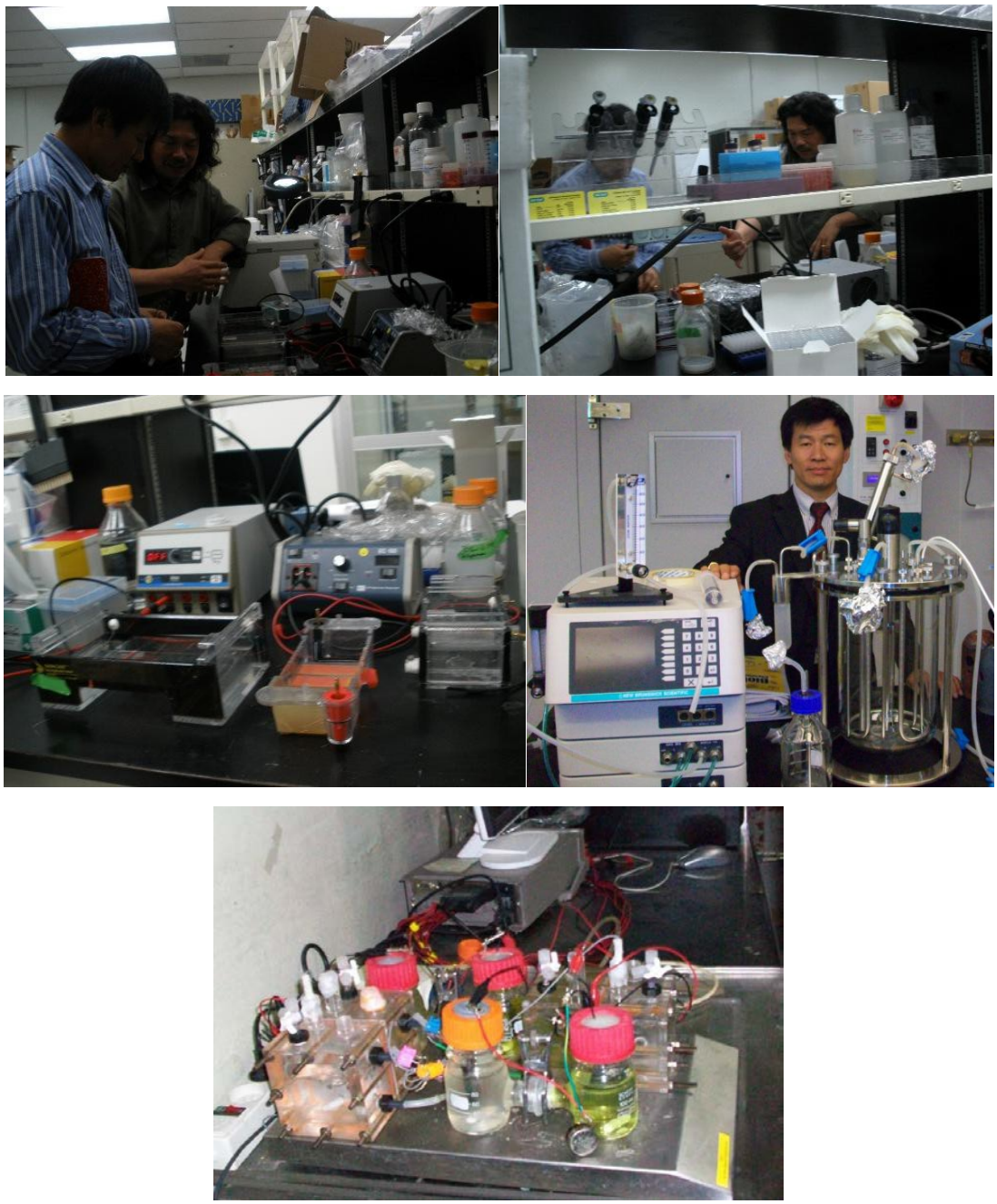


Table 2: Distribution of the Intake Properties of Domestic Wastewater Sludge Components among Organisms Involved in Anaerobic Digestion

																Sur-factants		Metal Major				Metal Cation			Major Anion	
Notes	AD (105)	Hydrolytic (47)	Acidogenic (56)	Acetogen (1)	Methanogenic (19)	Rank	Name	Alcohol	Primary alcohol	Aldehyde	Phenols	Methanethiol	(Ortho)phosphate	Sulfur	Hydrogen sulfide	Sorbitol 6-phosphate	Glycerine (Glycerol)	Potassium	Magnesium	Calcium	Sodium	Copper	Iron	Cobalt	Bicarbonate	Sulfate
								Alc	Pri	Alc	Ph	Me	Ort	Sul	Hy	Sor	Glyc	K	Mg	Ca	Na	Cu	Fe	Co	Bic	Sulfat
								C0	C0	C0	C0	C0	C0	C0	C0	C01	C00	C0	C0	C0	C0	C0	C1	C0	C0	C0005
Sulfate-Reducing						1	*Clostridium botulinum A ATCC 19397*							1		2	2									
Sulfate-Reducing						1	*Clostridium botulinum B1*	3						1		2	2									
Sulfate-Reducing						1	*Clostridium botulinum F*	3						1		2	2									
Sulfate-Reducing						1	*Clostridium botulinum A Hall*							1		2	2									
Sulfate-Reducing						1	*Clostridium botulinum A3 Loch Maree*							1		2	2									
Sulfate-Reducing						1	*Clostridium botulinum A*					4		1		2	2									
Sulfate-Reducing						1	*Clostridium difficile*					4		1		2										
Sulfate-Reducing						1	*Clostridium kluyveri*					4		1									3			3
strict anaerobic						1	*Fusobacterium nucleatum*							1												
aerobic, facultative anaerobic						2	*Escherichia coli UTI89 (UPEC)*						1	3		1	1		5				1			3
aerobic, facultative anaerobic						2	*Escherichia coli SECEC*						1	1		1	1						1			3
aerobic, facultative anaerobic						2	*Escherichia coli E24377A*						1	3		1	1						1			3
aerobic, facultative anaerobic						2	*Escherichia coli HS*						1	3		1	1						1			3
(e) acetoclastic methanogens						3	*Methanosarcina acetivorans*					1		1					1					1		
(e) acetoclastic methanogens						3	*Methanosarcina barkeri*					1		1					1					1		
						3	*Methanococcoides burtonii*					1		1					1					1		
						3	*Methanospirillum hungatei*					1							1					1		
(e) acetoclastic methanogens						3	*Methanosarcina mazei*							1				3	1					1		
						3	*Methanococcus maripaludis S2*							1												2
						3	*Methanobrevibacter smithii ATCC 35061*					1		1										1		
						3	*Methanosphaera stadtmanae*					1												1		
Sulfate-Reducing						4	*Clostridium acetobutylicum*					6		1												3
Sulfate-Reducing						4	*Clostridium novyi*							1			3									
Sulfate-Reducing						4	*Clostridium perfringens 13*							1			3	1								
Sulfate-Reducing						4	*Clostridium perfringens ATCC 13124*						4	1			3									
Sulfate-Reducing						4	*Clostridium perfringens SM101*							1			3									
Sulfate-Reducing						4	*Clostridium tetani E88*					6		1			3	1								
						5	*Lactococcus lactis subsp. lactis IL1403*					3														
						5	*Lactococcus lactis subsp. cremoris SK11*					3														
						5	*Lactococcus lactis subsp. cremoris MG1363*					3														
aerobic, facultative anaerobic						5	*Streptococcus agalactiae 2603 (serotype V)*						3	1			5									
aerobic, facultative anaerobic						5	*Streptococcus agalactiae A909 (serotype Ia)*						3	1			5									
aerobic, facultative anaerobic						5	*Streptococcus agalactiae NEM316 (serotype III)*						3	1			5									
						5	*Streptococcus gordonii*					3	3	1			5	2								
						5	*Streptococcus mutans*					3	3			5										
						5	*Streptococcus pneumoniae D39*					3					5	2								
						5	*Streptococcus pneumoniae TIGR4*										5	2								
						5	*Streptococcus pneumoniae R6*					3	3				5	2								
						5	*Streptococcus pneumoniae Hungary19A 6*										5									
						5	*Streptococcus sanguinis*					3	3				5	2								
						5	*Streptococcus suis 05ZYH33*										5									
						5	*Streptococcus suis 98HAH33*										5									
						5	*Streptococcus thermophilus CNRZ1066*					3														
						5	*Streptococcus thermophilus LMD-9*					3														
						5	*Streptococcus thermophilus LMG18311*					3														
						6	*Streptococcus pyogenes MGAS10394 (serotype M6)*										4						2			
						6	*Streptococcus pyogenes MGAS6180 (serotype M28)*										4						2			
						6	*Streptococcus pyogenes Manfredo (serotype M5)*										4									
						6	*Streptococcus pyogenes MGAS315 (serotype M3)*										4									
						6	*Streptococcus pyogenes MGAS10270 (serotype M3)*										4						2			
						6	*Streptococcus pyogenes MGAS10750 (serotype M3)*										4						2			
						6	*Streptococcus pyogenes MGAS2096 (serotype M3)*										4						2			
						6	*Streptococcus pyogenes MGAS9429 (serotype M3)*										4						2			
						6	*Streptococcus pyogenes MGAS8232 (serotype M18)*										4									
						6	*Streptococcus pyogenes SSI-1 (serotype M3)*										4									
						6	*Streptococcus pyogenes SF370 (serotype M1)*										4									
						6	*Streptococcus pyogenes MGAS5005 (serotype M1)*										4						2			

Figure 6: Dr. Charles Zhou and Dr. Jeng Her working in the microbial lab to culture and characterize the selected microbes



CHAPTER 3:
Build Strain Incubator

The goal of this task was to build a multiple compartment strain incubator to contain the selected bacteria cultures. Using additional match funding, various strain incubators with compartments were built that can contain one to 20 gallons of bacteria culture, as shown in Figure 7 and Figure 8. Each compartment has a reciprocating valve to control the volume and rate of the culture flowing to the bioreactor, as well as a 2-meter (6.5 feet) -long pump to measure the flow rate. More automation will be needed in the future for commercial applications.

Building a workable strain incubator presented more of a challenge than we had originally planned. Buying such a unit would cost $25,000 each, as shown in Figure 9, which was well beyond our projected budget. Instead, we looked for aid from our collaborators in the brewery and bioreactor industry, and we were able to build our own versions for this project within our budget. Our collaboration with customers and business partners, such as Mr. Charles Hill from Bluestone, Dr. Jeng Her from ProteoBio, and many collaborators from Anderson Valley Brewery, DSRSD wastewater treatment plant and DSRSD wet lab helped us avoid many technical and financial issues. In addition, Dr. Jeng Her from ProteoBio provided us with both the technology and the materials for our construction. Figure 7 shows simple strain incubators on a bench scale of 1 gallon size. Under lab conditions between 36 degrees Centigrade (°C) (96 degrees Fahrenheit [°F]) to 40°C (104°F), we were able to culture the microbes needed for the 1,000 gallon reactors. These were the precursors for our on-site experiments. Figure 8 shows the design that was developed with other partners for the bioreactors and incubators.

These testing methods helped us to scale up our reactors for future commercial production by controlling the temperature and acidity (pH values) of the slurry. The 1,000-gallon CASCADE methane bioreactor can be scaled for use as an incubator to culture microbial slurry for a one million gallon digester.

Figure 7: Bench-scale (1 gallon size) incubator tests in our lab for four of the five incubators.

Figure 8: These samples of incubators, or bioreactors, can be scaled up for commercial production.

Figure 9: A commercial bioreactor (incubator) shown in this photo would cost about $25,000

CHAPTER 4:
Build a CASCADE Methane Bioreactor to Mix the Bacteria from the Incubators with DSRSD Sludge

Within limitations imposed by the available project budget, a few modules for the 100-gallon CMB which can mix bacteria from the incubators with sludge influent from the DSRSD wastewater treatment facilities, were designed and tested. With support from the DSRSD facilities and a small brewery in Northern California, three different designs for a container with a capacity of 30 to 150 gallons were produced, while considering the factors of cost, functionality, and ease of operation. A small pump was installed to control the volume and rate of sludge to the bioreactor, with a flow meter and automatic switch valve. After testing the three modules in our lab, module #3 was selected for use in this project. It feeds the 1,000-gallon digester/bioreactor with concentrated sludge from DSRSD and microbe slurry in one easy operation module.

Figure 10: After testing three modules, we selected Module #3 for the CMB while considering cost, functionality, and ease of operation.

CHAPTER 5:
Build a 1000 Gallon Digester

One of the major engineering goals of this project was to design and manufacture a 1,000-gallon anaerobic digester to mimic the conditions of DSRSD's 600,000-gallon unit.

Currently, there are three commercial-scale anaerobic digesters within the DSRSD wastewater treatment facility. Digester #1 and Digester #2 have a capacity of 600,000 gallons (about 2,300 cubic meters), while Digester #3 has a capacity of 1,000,000 gallons (about 3,800 cubic meters). The additional input of microbial strain slurry should ideally be between 1/1000 and 1/100 of the commercial scale capacity. Therefore, the minimum capacity for the bioreactor needed to be 600 to 1,000 gallons in order to meet the requirements of all three digesters. Therefore, we designed a 1,000-gallon digester for use in this project. In addition, researchers needed to consider the flow patterns inside the system, which is a three- phase fluid flow process consisting of the gaseous phase (biogas), liquid phase (wastewater) and solid phase (BOD/COD particles in the sludge). A capacity of 1,000 gallons is thus simple enough to control the process, but large enough to simulate the fluid flow, biogas production, and temperature/acidity distribution in the commercial digesters. Completion of this system was a major milestone, and it has since been in operation for two years.

The temperature inside the 1,000-gallon digester is maintained at 96°F, with an automatic control system that circulates heated water provided by DSRSD into the inner wall surrounding the container. A mechanical pump was installed with a connection to Digester #2 to circulate the sludge at a slow and constant rate. A gas collection mechanism was created through a by-pass in the 1,000-gallon digester. An automatic switch valve with a flow meter for the gas and a separate meter for the measurement and verification (M&V) of gas production was installed. The biogas production data is presented in CHAPTER 7:

In order to measure gas flow rates from the digester, sludge-sampling valves were installed to provide an effluent connection to DSRSD headworks.

During this period, frequent meetings were held with DSRSD management staff who supported the project by providing management, electricity, plumbing, computer support, and other facilities and equipment. DSRSD also has all the permits for the CASCADE system to be installed and run on the DSRSD site.

Figure 11: Seismic reinforcement of the 1,000-gallon reactor against earthquakes.

Figure 12: Electronic Controller for the CASCADE bioreactors.

Figure 13: Shipping and installing the 1,000-gal digester.

Figure 14: Foundation and framework prior to installation of the 1,000-gal digester.

Figure 15: Laying down the 1,000-gal digester with the support of operators and engineers at DSRSD wastewater treatment facilities.

Figure 16: Foundation, framework, and installation of the 1,000-gal digester at DSRSD.

Figure 17: System installation: the sludge feed pump, 30 gallon pre-treatment tank), and electrical and electronic control system.

Figure 18: The sludge pump simultaneously 1) adds sludge if the pressure of the raw sludge is too low, 2) adds microbes, and 3) acts as a cleaning system in the event clogs occur.

Figure 19: Electrical system with the CASCADE CLEAN ENERGY logo.

Figure 20: Controlling system.

Figure 21: System is complete and ready for operation by June 30, 2012.

Figure 22: Researchers found that struvite crystals were formed during anaerobic digestion (Digester #2 shown)

These crystals are more effective than wastewater or sludge in absorbing heavy metal ions. This research result was totally unexpected and implies that sludge residues can be composted easily along with other agricultural wastes without the fear of heavy metals leaching into the groundwater table.This discovery can have scientific and technological significance for future research and development.

CHAPTER 6:
Connect Bioreactor's Gas System to DSRSD Line

Miguel Rea, Charles Zhou, Jeng Her and Charles Hill worked on both Task 6 (connecting the 1,000-gallon digester to the existing biogas line) and Task 7 (measuring the gas flow).

Special safety procedures have been taken into account due to the extremely flammable biogas generated. The biogas is 100% contained and flows via a gas line (with a diameter of 1 inch) as indicated in Figure 22. Engineers from the DSRSD plant inspected any potential leakage from the gas lines.

Figure 23: Left: The biogas line from the top of the 1,000 gallon bioreactor is connected to the commercial 600,000 gal anaerobic digester #2. Right: The liquid/sludge or flush piping lines between the bioreactor and the anaerobic digester #2.

Figure 24: Microbial slurry was added to the pre-processor, where the microbes are mixed with raw sludge and pumped into the 1,000 gal bioreactor.

CHAPTER 7:
Measure Gas Flow Rate from Digester

As shown in Figure 25, an EKM gas flowmeter was installed to measure the amount of biogas produced from the sludge, using standard measurements. A bypass was included to take samples of gas for analysis. These installations allowed researchers to compare results with those from existing processes that don't utilize microbes, in order to determine if enough biogas was produced to be harnessed for electricity.

Figure 26 presents comparison results between commercial anaerobic digester (AD) #2 and the 1,000-gallon CMB without adding any microbial strains. A total of 590,000 gallons of sludge was added to the AD #2 during a 31-day period. For each 1,000 gallons of sludge, AD #2 produced 2,471 standard cubic feet (scf) biogas with 68±4% methane, while the CMB produced 2,396 scf biogas with a similar methane content. In this set of controlled experiments, the commercial AD #2 was 5% more efficient than the CMB.

Figure 27 presents the comparative results between the AD #2 and the 1000-gallon CMB with 5 gallons of microbial slurry added. A total of 590,000 gallons of sludge was added to AD #2 during the study period. For each 1,000 gallons of sludge in this study, the AD #2 produced 2,857 scf biogas with 68±4% methane, while the CMB produced 3,597 scf biogas with a similar methane content. In this set of experiments, the CMB produced 26% more biogas.

The results indicated that the CASCADE system had successfully reached the goal of improving methane production for the DSRSD. Based on the 1,000 gallon digester (CMB), it should increase their existing waste-to-energy conversion by 26% in this project.

The total BOD in the sludge can vary between 20,000 milligrams per liter (mg/L) to 52,000 mg/L before digestion. The BOD was reduced by about 60%. For DSRSD sludge prepared from a Dissolved Air Flotation Thickener (DAFT) process, each 34 grams of BOD will generate about 1 scf methane (28.3 liters).

The DAFT system was initially not operational but has since been repaired and data collection was not impacted. A six month time extension granted by the Commission for the project was important in allowing collection of this valuable data to occur.

Figure 25: Measuring biogas with a flowmeter

Figure 26: Biogas data collection for Digester #2 and the 1,000-gallon CMB for a period of 31 days. Both data are calibrated to biogas production per 1,000 gallons of raw sludge.

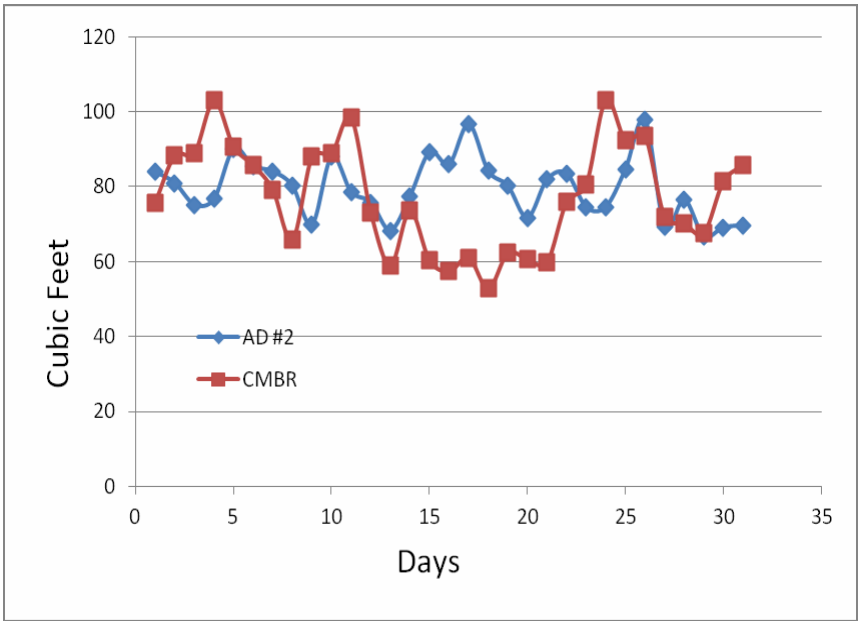
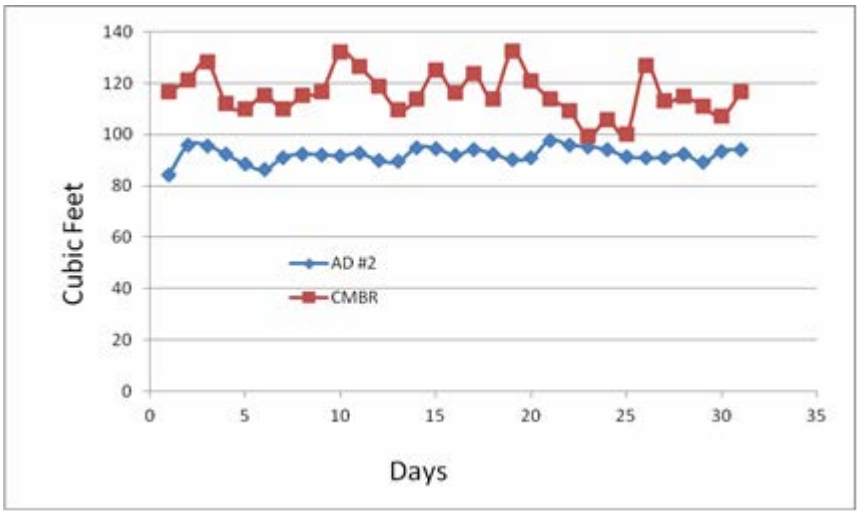


Figure 27: Biogas data collection for Anaerobic Digester #2 and the 1,000-gallon CMB (with 5 gallons of microbial slurry added) for a period of 31 days. Both curves are represented in biogas production per 1,000 gallons of raw sludge.



CHAPTER 8:
Tune the Process to Optimize Methane Production

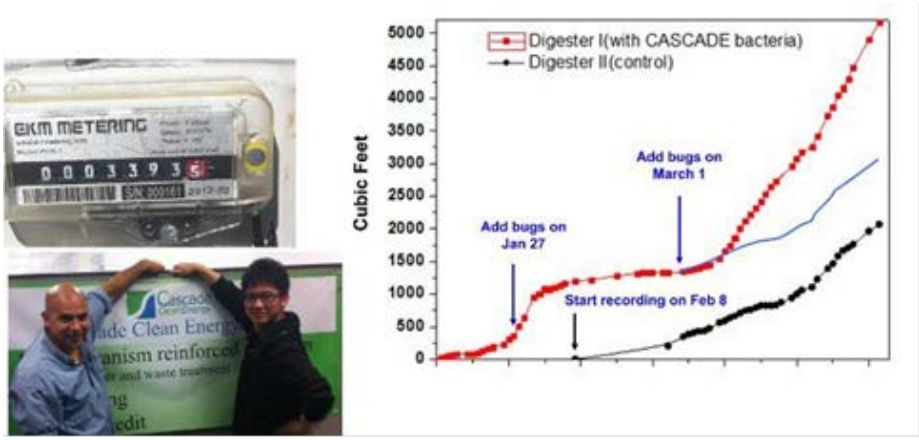
The anaerobic digestion process is one of the most complicated processes in terms of reaction mechanisms, and deals more with empirical knowledge than a scientific understanding. Its influents are never the same, due to the various sources of the wastewater. Data comparisons do not mean much if they are not from the same batch of raw sludge for the same set of experiments.

For all three commercial digesters in the DSRSD wastewater treatment plant, the inside temperatures were controlled at 1020F, although the Recipient attempted to set the temperature at 100oF with an uncertainty of 20F for the CMB. The pH values are completely controlled by the DAFT commercial process, which is operated by the field engineers at the treatment plant.

The key difference between the digestion in our current study and other anaerobic digestion is that the enrichment of microbial strains added to the digesters improved biogas production. A microbial slurry level of between 0.5 percent and 1.0 percent of the sludge capacity optimizes methane production. Due to reasons of economics, this project used the lower 0.5 percent concentration level. Tuning the digester with enriched microbial slurry, temperature, and pH values on a bench scale is relatively easier than for full-scale commercial anaerobic digesters. For this task, we tuned our process to optimize methane production within the safety parameters prescribed by DSRSD. The amount of microbial slurry for the 1,000-gallon CMB is controlled below 10 gallons but 5 gallons were used for all the tests in this project due to economics.

In the following Figure 28, we showed an accumulative curve of the biogas production for the 1,000- gallon CMB over 60 days. We added microbial slurry twice during this run. After 10 days of culturing digestion of the sludge at BOD/COD values of 52,000 mg/L, we began to add the microbial slurry on the tenth day, and stopped adding any new sludge to the CMB. Biogas production slowed down significantly, as shown by the flat part in the red line in Figure 28. By the thirty-fifth day, we began to add the second batch of microbial slurry as well as 33 gallons of sludge to the system each day at 8 a.m.

Figure 28: Tuning biogas production and measurements. The red line is the accumulative biogas in scf added together for the biogas produced each day.



CHAPTER 9:
Initiate Technology Transfer Activities

Since the project began, we have attended the California Clean Tech Open (CCTO, now called the Clean Tech Open or CTO) in San Francisco and San Jose, as well as the Advanced Research Projects Agency-Energy (ARPA-e) in Washington, D.C., in order to initiate technology transfer activities and explore the possibilities of using CASCADE technology in commercial wastewater treatment and livestock farming, and recover biogas from animal manure and farming waste.

We also looked at the potential applications of CASCADE technology to the dairy farm industry. According to the January 19, 2014, issue of the journal "Progressive Dairyman", there are 9.3 million dairy cows in United States, and California alone has 1.78 million dairy cows. By using the CASCADE process, it is estimated that 25 billion scf of biogas can be generated and 1.4 billion kWh of electricity can be produced annually from the manure wastes of the cows in California alone.

The oil industry is also interested in using the CASCADE technology for water, oil and methane recovery from oil field sludge.

Figure 29: Presenting at an investors' presentation for the Clean Tech Open

CHAPTER 10:
Create Production Readiness Plan

The next step of the project was to create a plan to expand the commercial and manufacturing viability of CASCADE technology, as well as to improve its stability and efficiency. To support Tasks 9 and 10,to the research team attended the World Ag Expo in Tulare, California, where more than 200 potential customers and business partners were gathered. We also attended the Armed Forces Communication and Electronic Association West 2011, 2012 and 2013 conferences in San Diego to promote awareness of the technology. At these conferences, it was shown that CASCADE microbes can be made into powder form for use on United States Navy ships, indicating that this technology will have much wider applications than was originally expected.

Various businesses from the United States and other countries have approached us in the last two years expressing an interest in using CASCADE technology for various clean energy production applications. In addition to producing biogas from wastewater sludge, we also explored bringing our solutions to other markets as part of this project. These include:

1) Producing biogas and organic fertilizers from dairy wastes, sourced from dairy farms in California, Wisconsin, Mongolia, and China (see Figure 30 below for specific example)

2) Cleaning black wastewater more effectively than the conventional methods currently in use on large ships, such as aircraft carriers

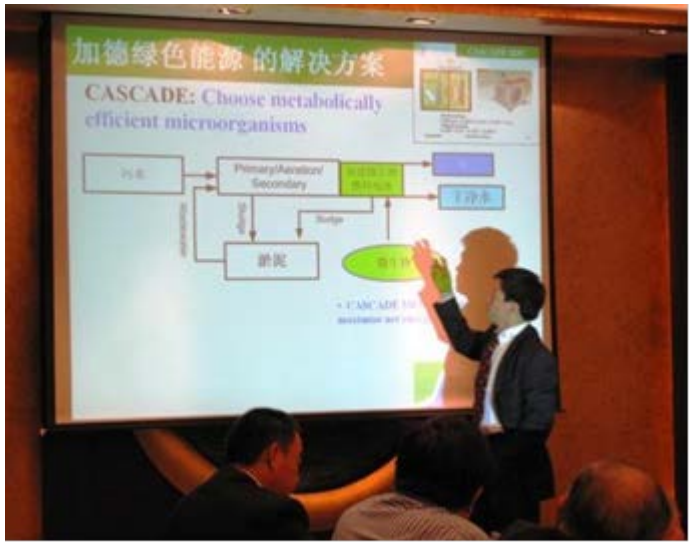
3) Acting as second-generation technology of shale gas extraction by using the shale bed underground as a natural anaerobic digester and replacing hydraulic fracking.

4) Facilitating coal bed methane extraction by using the coal bed seam as a natural digester and converting traditional coal to biogenic coal bed methane as clean energy.

5) Cleaning oil spills, from which biogas can be generated.

Figure 30: The research team worked with a brewery and used their wastewater as one of the media for making microbes which reduced the cost of making microbe slurry from $15,000 down to $1,500 per ton for future commercialization purposes.

Figure 31: Presenting to international investors.



CHAPTER 11: Conclusions

In this project, a bio-database containing microbe strain data from 2001 and 2009 was utilized to develop a patented technology called CASCADE. The CASCADE Bio-database thus created was used in a special application for the production of biogas from sludge of a commercial municipal wastewater treatment plant, and eventually the biogas is converted to electricity used in the same wastewater treatment plant. The CASCADE machine learning method was used to select appropriate smart microbial consortia to facilitate the CASCADE process. The microbes were cultured and added to anaerobic digesters to process wastewater sludge of more than 50,000 mgL COD. The biogas produced from the anaerobic digesters was conditioned for use in direct fuel cells and electrical generators.

A 1,000-gallon prototype of the CCES that is integrated into the existing wastewater treatment process at the DSRSD was designed and constructed. The system has been successfully operating for two years, during which time two sets of experiments have been conducted to collect data on system performance. It was clearly demonstrated that the CCES has improved DSRSD's existing waste-to-energy conversion rate between 25.8 percent and 30 percent, depending on the quality of the sludge influents.

In addition to the wastewater treatment industry, there are strong indications that this technology is fully scalable and can be used in the dairy farm industry to convert farming wastes to clean energy. Subsequent work is also continuing in many areas, such as converting sludge produced in the coal and petrochemical industries, and wastewater produced in the food industry to useful, clean energy. There are even applications for sewage treatment aboard large ocean-going vessels such as United States Navy aircraft carriers.

GLOSSARY

Term	Definition
AD	Anaerobic digester
ATCC	American Type Culture Collection
BOD	Biological oxygen demand
°C	Degrees Centigrade
CAM	Commission Agreement Manager
CASCADE	Computer Assisted Strain Construction and Development Engineering
CCES	Cascade Clean Energy System
CCTO	California Clean Tech Open
CCTC	China Center for Type Culture Collection
CMB	CASCADE Methane Bioreactor
COD	Chemical oxygen demand
Commission	California Energy Commission
DAFT	Dissolved Air Flotation Thickeners
DSRSD	Dublin-San Ramon Service District
°F	Degrees Fahrenheit
EPA	United States Environmental Protection Agency
KASS	KEGG automatic annotation server
KEGG	Kyoto Encyclopedia of Genes and Genomes
kWh	Kilo-watt hours
M&V	Measurement and verification
mg/L	Milligrams per liter
MITCNC	Massachusetts Institute of Technology Club of Northern California
NCBI	National Center of Biotech Information
NIH	National Institutes of Health
RD&D	Research development and demonstration

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APPENDIX A:
Information of CASCADE Bioreactor and Designs

USER'S MANUAL of CMBR

CASCADE CLEAN ENERGY

http://www.ccleanenergy.com

CMBR Manual

What's CMBR?

CMBR（CASCADE methane bioreactor）is a continuous flow anaerobic digester that consists of a feeding tank, anaerobic fermentation tank, circulating pump, heater and an electric control box. The circulating pump can also be used for sludge feeding. This piece of equipment was mainly used for anaerobic digestion in a pilot scale experiment.

Specification

Bioreactor			
Total Volume	1000 gallons	**Effective Volume**	800 gallons
Feeding tank	26 gallons	**Effective Volume**	21 gallons
Plane Size	6.56×9.84ft	**Height**	11.7ft
Temperature	<131°F	**Heating Power**	4.0kw
Feeding Tank	26.5 gallons	**Pump Capacity**	35gallons/min
Gas Meter	EKM METERING		
Control Box			
Timer Swith	H5S OMRON		
Temperature	E5EZ OMRON		
Size	1.6ft×2.3ft		
Power Suppy	480V,60Hz for pump 240V,60Hz for heating elements		

Installation Guide:

1. Equipment Base

The whole bioreactor adopts a skid-mounted installation. The equipment base should be placed smoothly on a flat concrete platform that is at least 7.8 inches thick, then fixed with anchor bolts.

2. *Equipment Connection: In accordance with the equipment connection diagram, put all the*

equipment (the reactor body, feeding tank, electric control box, and water pump) in place, and fix with bolts. Connect all pipes and gas pipes; all connections must use leak-proof measures. Pay attention to the import and export of the pump and flow direction by pump installation.

3. *Electrics Installation:*

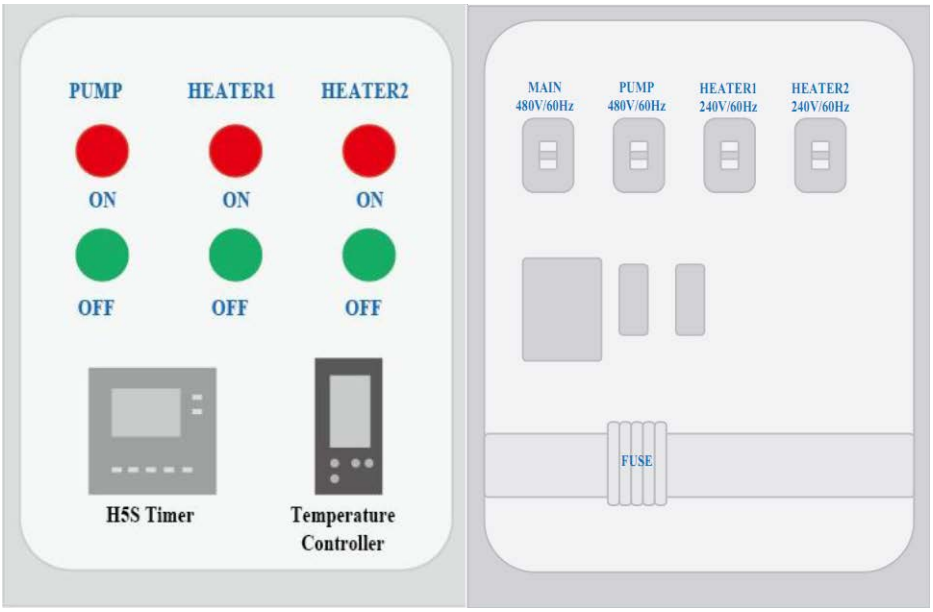
This section includes a control box and the heating element installation.

The heating element consists of two 150ft sections of self-regulated, explosion-proof tracing band, with a total length of 300ft. The band passes through the hole on the reactor wall and wraps around the wall, so that the final connection line is located in the central power connection box. Each section can be wrapped about 12-13 times.

The black rubber is used as the insulation layer, with the nylon rope used as reinforcement. The insulation layer is covered by the plastic material to prevent aging.

Parameter Setting

1. Control Box



E5EZ Temperature Controller Setting

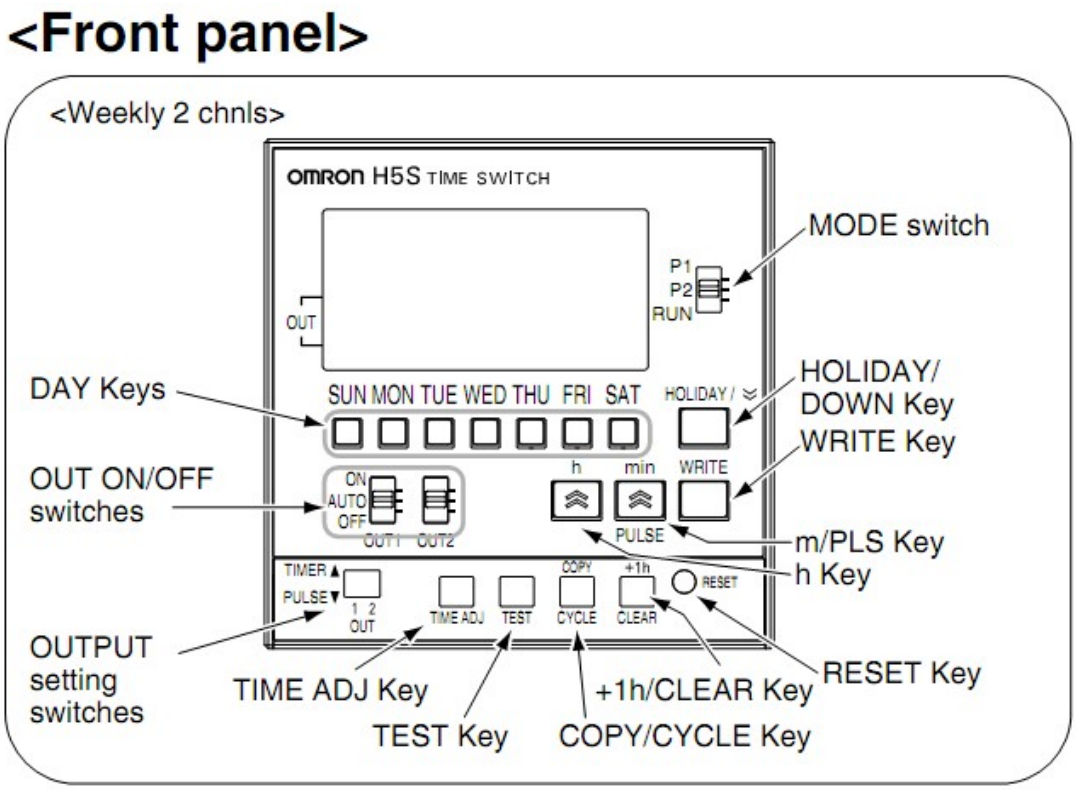
Up and down keys:

Use the keys to change the values displayed on the NO.2 display.

Each press of the key increments (advances) the values displayed on the NO.2 display.

Each press of the key decrements (returns) the values displayed on the NO.2 display.

H5S Timer Switch Setting



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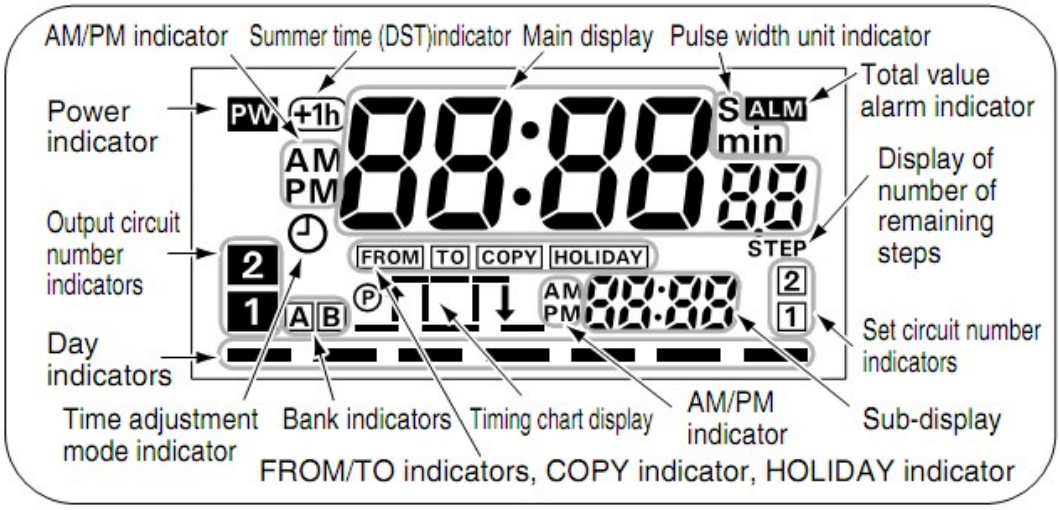
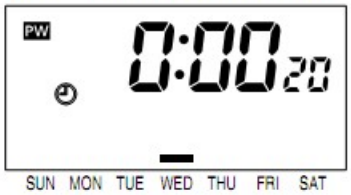


【Example】The current time (day/hour/minute) is set to Saturday 17:28.

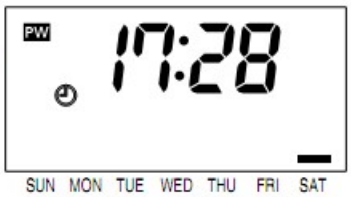
① Set the Mode Switch to RUN.

The color indicates flashing.

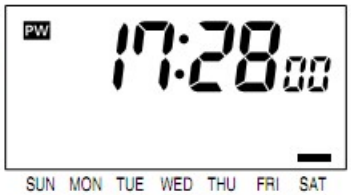
② Press TIME ADJ for 2 s or more.
The symbol flashes.



③ Press SAT.
(The bar (▬) mark at the Saturday position will turn ON.)
Set the time with h and m . ※1

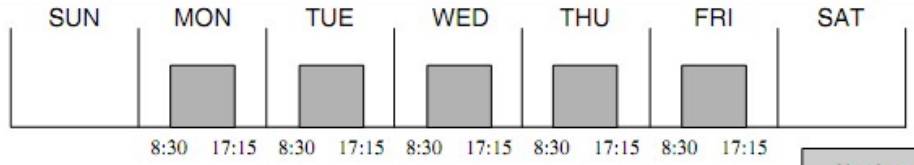


④ Press WRITE to confirm the setting, and the Time Switch will start timing from 0 second.

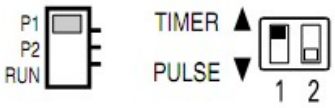


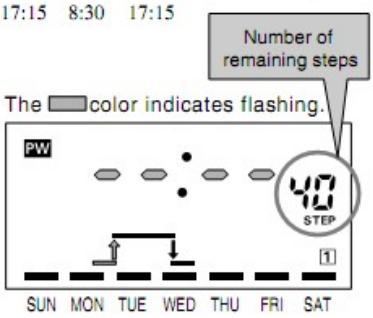
※1 Holding down these Keys rapidly advances the value.
Pressing ⌄ decrements the value of the Key that was last pressed.

【Example】The Time Switch turns ON circuit 1 or circuit 2 at 8:30 and turns it OFF at 17:15 from Monday through Friday.

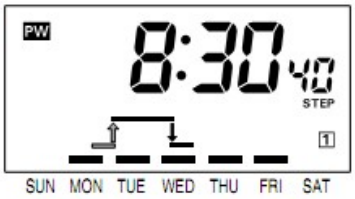


① Set the Mode Switch to P1 or P2. ※1
→See Section 4-5 for 4 channel type.



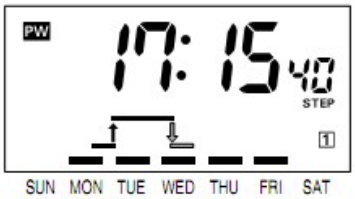


② Press the DAY Keys to turn on the bars (—) at the positions of Monday through Friday. Set the ON time with h and m. ※2



③ Press WRITE.
Set the OFF time with h and m. ※2

④ Press WRITE to confirm the settings.



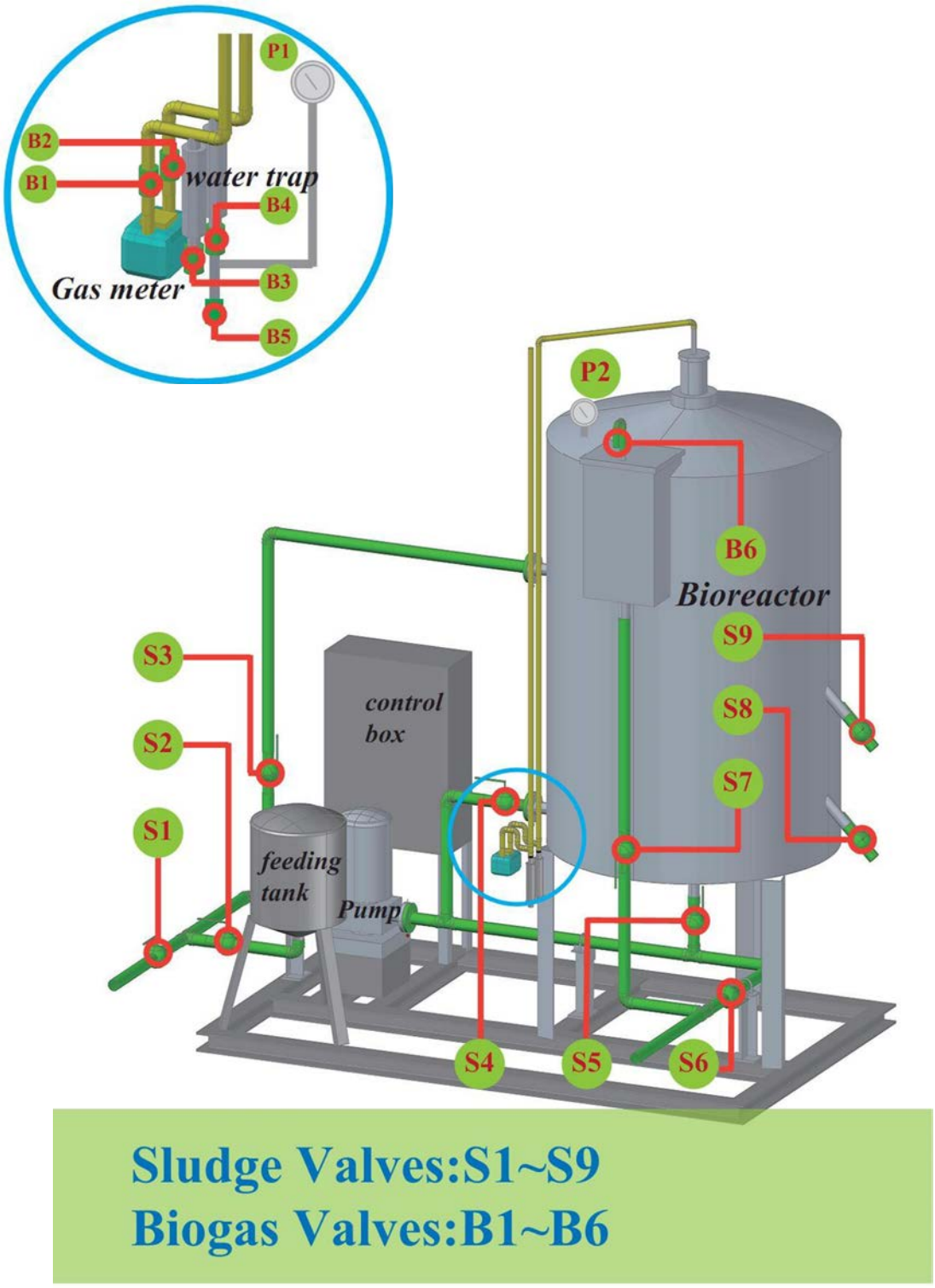
※1 If one or more programs have already been set, the display starts showing the set programs. To add another program, press WRITE repeatedly until "- - : - -" is displayed.

※2 Holding down these Keys rapidly advances the value. Pressing ⌄ decrements the value of the Key that was last pressed.

≪Note≫

- If multiple settings are required, repeat the steps ② through ④.
- Both the ON and OFF times must be set.
- All the set weekly programs can be checked by pressing WRITE in program setting mode.
- When the Mode Switch is set to P1 or P2 (to PRGM for 4 channel type), the Time Switch stop automatic operation. To forcibly turn ON or OFF the output, use the OUT ON/OFF switches.
- The set data will be cleared if the OUTPUT setting switch is moved between the TIMER and PULSE positions after the data has been set.

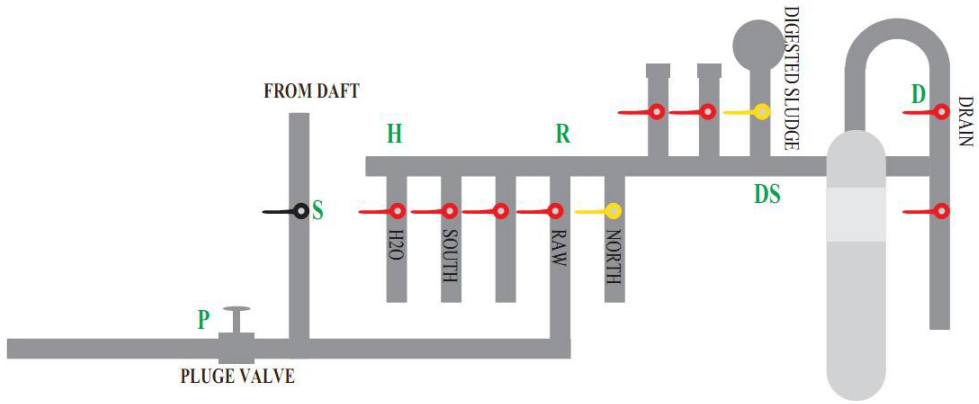
2. Valves of CMBR



Water test

Equipment and control point number

1. Definition of the shared valves:



H_2O valve: H valve

Raw sludge valve: R valve

Drainage valve: D valve

Sludge shifting valve: S

valve Plug valve: P valve

The biogas valve on top of Digester Two: M valve

Yellow sample valve of the Digester Two: DS valve

2. Water Test Procedure:

The water test is used to check all the connections and functions of all parts of the entire system.

It is absolutely necessary to conduct this test before the sludge experiments and after any reconnections of the system.

First check that:

(1) All the sludge valves and biogas valves are closed, and that the vent valve B6 is always open. The main power is off, so water leakage won't damage the electrical part of the system.

(2) The H/R/D/S/P/M valves are a shared part with DSRSD WWTP. Return them to their original positions after use.

(3) The M valve is closed before every test is conducted, and the system is already filled with sludge.

(4) The OUT1 on/off switch of the H5S timer is set to off.

3. *Operation:*

Steps 1-7 are used for a water-proof test of sludge pipe connections.

(1) Open valve H. Make sure the water supply is available.

(2) Close valves D and S. Open valves R and P.

(3) Open valves B1, B2, B4. Close valves B3 and B5.

(4) Open valves S1, S5, S7 to fill the CMBR with water.

(5) Close valve S1 while the water overflows from the overflow box.

(6) Check all the connections in 24 hours. There should be no leakage in the system.

(7) Leakage in any location must be fixed.
If no leakage occurs, continue with the following steps:

When the bioreactor is fully filled with water, the air in the bioreactor will be compressed, and

the pressure may not exceed 4.5 kPa.

Steps 8-10 are used for an air-proof test of gas pipe connections.

(8) Spray the bubbling agent onto every connection of the biogas pipe arrangement.

(9) Record the pressure of the pressure gauge.

(10) No bubbles or pressure drop in 24 hours means that the gas connections do not leak.

Steps 11-14 are used for a normal operation test with water.

(11) Turn on the pump power switch, the heater power switches, and the two main power switches.

(12) Set the bioreactor temperature to 98°F. Record the initial temperature and final temperature of the bioreactor. It may take two days to heat the bioreactor from 50°F to 98°F.

(13) Open sludge valves S3, S4.

(14) Set the OUT1 on/off switch to on. Check the noise and motor temperature of the pump.

(15) Open sludge valve S6 to drain the water after the water test.

Note: Never run the pump (either manually or automatically) when the sludge valves S3 and S4 are closed and the system is empty.

Normal Digestion Operation

1. Biogas pressure less than 17.7 inch water column.

Once the water test is complete, the system is run normally as preset, and the sludge experiments can be started. The sludge operation procedure is listed below:

First check that:

(1) All the sludge valves and biogas valves B3, B5 are closed, and the gas valves B1, B2, B4 and B6 are open.

(2) The H/R/D/S/P/M valves are closed.

(3) The OUT1 on/off switch of the H5S timer is set to off.

Feed the bioreactor with sludge.

(1) Open the sludge valves DS, R, P. The digested sludge flows from Digester Two to the CMBR.

(2) Open the sludge valves S1, S5, S7 to fill the CMBR with sludge.

(3) Close valve S1 while the water overflows from the overflow box.

(4) Turn on the pump power switch, the heater power switches, and the two main power switches.

(5) Set the bioreactor temperature to 98°F. Program the H5S timer as required for the experiments. For example, the pump runs 10 minutes every 8 hours.

(6) Record all the parameters in the blank form required for the experiments.

(7) Close sludge valve P/R/DS valve. Open valve D.

Replenishment of sludge for daily operations:

(1) Open sludge valve S/P while Digester Two is being fed with sludge from DAFT (Dissolved Air Flotation Tank).

(2) Open sludge valves S1/S5. Let the sludge flow into the bioreactor for seconds.

(3) Close sludge valve S1.

(4) Close sludge valves P/S.

2. *Biogas pressure more than 17.7 inch water column.* *First check:*

(1) All the sludge valves and biogas valves B3, B5 and B6 are closed, and the gas valves B1, B2

and B4 are always open.

(2) The H/R/D/S/P/M valves are closed.

(3) The OUT1 on/off switch of the H5S timer is set to off.

Feed the bioreactor with sludge.

(1) Open sludge valves DS/R/P. The digested sludge flows from Digester Two to the CMBR.

(2) Open sludge valves S1, S5, S7 to fill the CMBR with sludge.

(3) Close sludge valves S1/S7 while the water overflows from the overflow box.

(4) Turn on the pump power switch, the heater power switches, and the two main power switches.

(5) Set the bioreactor temperature to 98°F. Program the H5S timer as required for the experiments. For example, the pump runs 10 minutes every 8 hours.

(6) Record all the parameters in the blank form required for the experiments.

(7) Close sludge valve P/R/DS. Open valve D.

Replenishment of sludge for daily operation:

(1) Open sludge valve S/P while Digester Two is being fed with sludge from DAFT (Dissolved Air Flotation Tank).

(2) Open sludge valves S1/S5. Let the sludge flow into the bioreactor for seconds.

(3) Close sludge valve S1.

(4) Open sludge valve S7 for seconds to discharge the sludge. Then close S7.

(5) Close sludge valves P/S.

Calibration of sludge level

In order to keep the sludge level at a certain position, please complete calibration of the sludge level every week.

(1) Open biogas valve B3. Release the pressure inside the bioreactor to the 400mm H_2O column.

(2) Open biogas valve B6. Keep the pressure in the overflow box equal to 1 atm.

(3) Open sludge valve S7 until the sludge doesn't flow out.

(4) Close sludge valve S7.

Cautions:

1. Never close the sludge valves S3/S4 when the pump is running or automatically controlled by the H5S Timer switch.

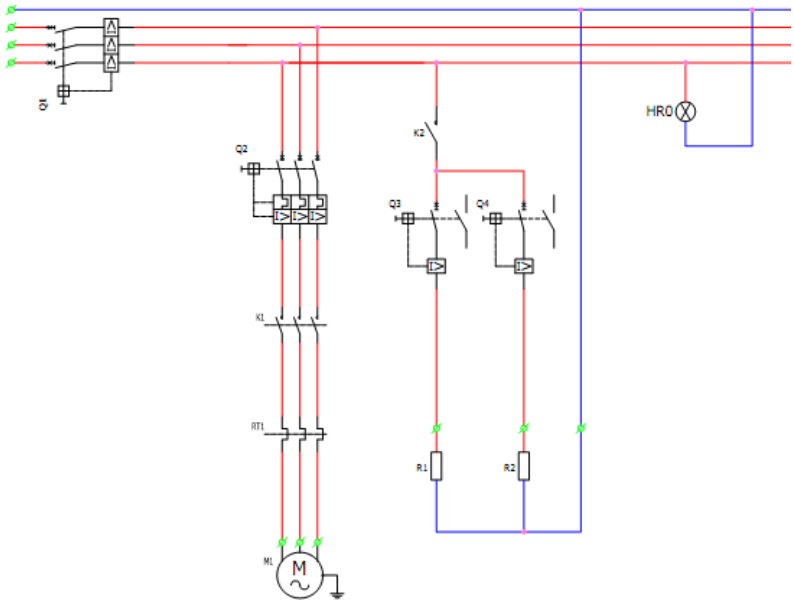
2. If an accident occurs, the system needs to be shut down by shutting off the main power, closing biogas valves S1/S2, and opening valves B6/S5/S6 to drain the sludge.

3. Sludge valves S3/S4 are closed when sludge is feeding into the bioreactor.

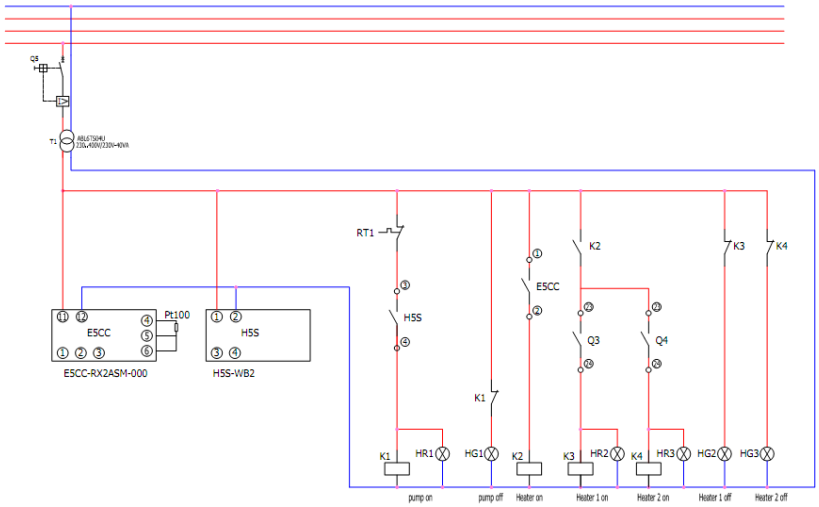
4. Gas valves B1, B2 are only for gas meter maintenance. Close valves B1, B2 before taking off the gas meter.

5. Gas valves B3, B5 are used for condensing water discharge and the biogas sample.

Scheme of Primary Circuit



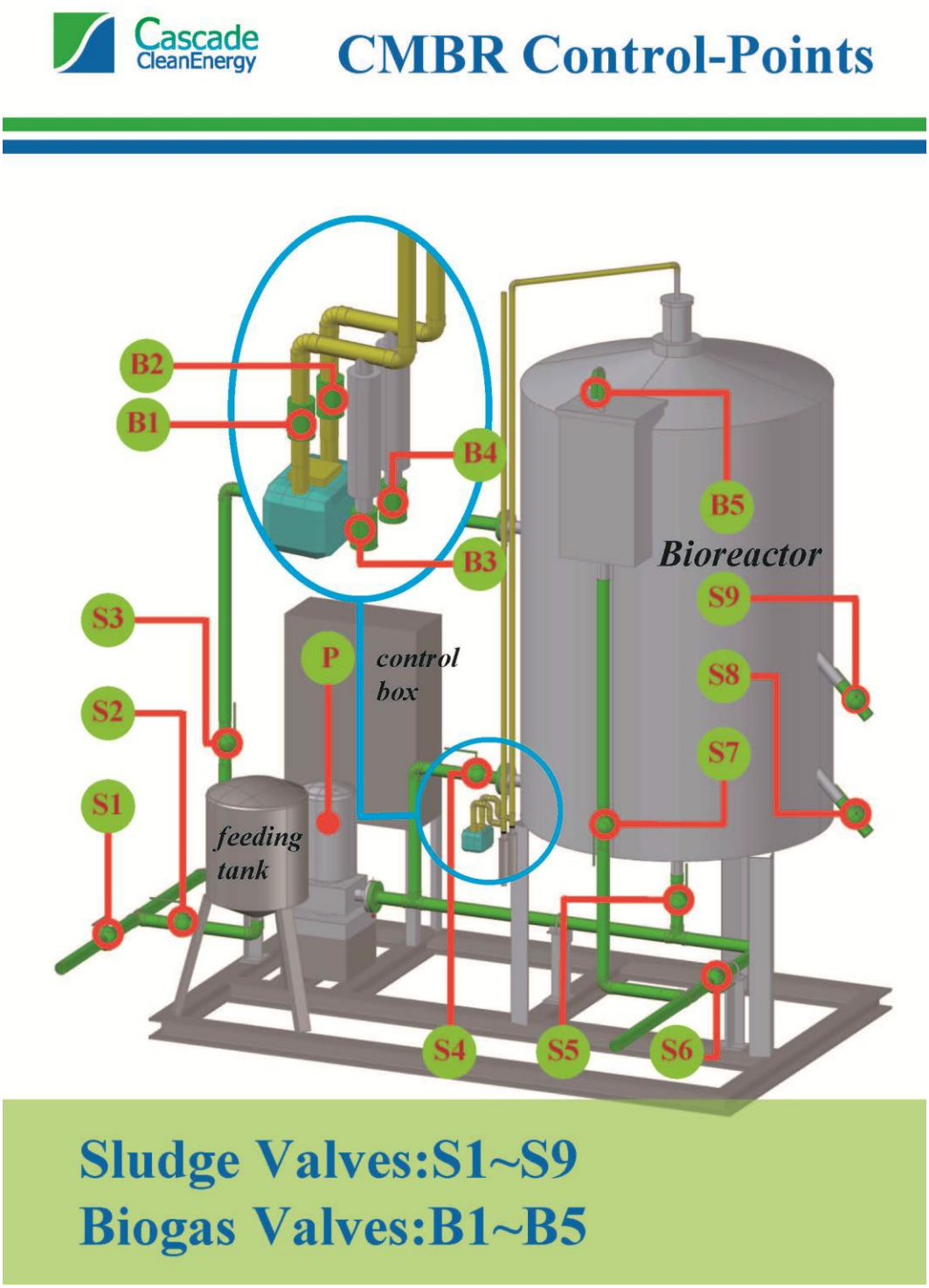
Scheme of Secondary Circuit



2. Worksheet

Date	Feed volume	COD	pH	T	Gas Volume	CH_4 content	Recorder

APPENDIX B:
Operation Procedure of CASCADE Bioreactor



CMBR Water-proof Test

Check First!

1. Close all the Sludge valves before Water-proof test.
2. Power supply is ON.
3. B5 is always open. Do not close it!

Normal Operation:

1. Manipulate the valves in sequence.

Water Supply → S1 S3 S4 S5 S7

→ overflow → S1 → S7

Check for water overflowing

2. Make sure that no leakage happens in 12hours.
3. Feeding and circulation.

Water Supply → Feed tank → S7 → S3 → S2

Feed water on the top

Pump On → Feed tank → Pump Off →

Check for low water level

S2 → S3 → Pump Automode

4. Drainage

S6 → Drain off the water

OPEN CLOSE CHECK

★ Any leakage is not allowed in the test!

Cascade CleanEnergy CMBR Start-up

Check First!

1. Close all the valves before Start-up.
2. Open the valve BS.Do not close it!
3. Temperature is right between 90-lOSF.

Normal Operation:

1. Manipulate the valves in sequence.

Check for sludge overflowing

2. Sludge replenishment and circulation

Feeding raw sludge to high sludge level

Check for low sludge level

3. Biogas pipe

When the pressure is higher than O.Spsi. Open all the biogas valves.

OPEN CLOSE

when pressure is lower than 0.5psi, open valves lead to leakage.

CMBR Shut-off

Check First!

1. All the system is running correctly.

2. Open the valve B5. Do not close it!

Normal Operation:

1. The main power supply is shut down.
2. Close all the biogas valves but biogas valve B5.
3. Open valve S5 to drain the sludge. B5
4. Run water-proof procedure 3 and 4 to clean the reactor.
5. Turn off the power.

OPEN CLOSE CHECK

★Rapid Sludge Drainage may cause damages to the reactor.