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Acclimated cultures of freshwater archaea and bacteria degrade synthetic sewage in a salt-water environment.

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Abstract

Cultures of archaea and bacteria were acclimated from a freshwater environment to a salt-water environment where they degraded synthetic wastewater. Arkea®, a blend of freshwater archaeal and bacterial cultures was slowly acclimated over 3 months to degrade synthetic sewage in Cromaglass sequencing batch reactors (SBRs). The results to date demonstrate the potential ability of acclimated archaea and bacteria to degrade wastewater in salt-water. Because freshwater or potable water may not be available, limited in quantity, or expensive to use, the potential use of salt-water acclimated cultures may provide acceptable treatment of wastewaters including bilge at marinas and "closed waste systems" from planes and airports. Wastewater treatment with Arkea® acclimated cultures represents reduced use of potable water and monetary savings.

INTRODUCTION

Biological, wastewater treatment processes contain numerous groups of freshwater aerobic, facultative anaerobic, and anaerobic archaea and bacteria that degrade carbonaceous and nitrogenous compounds in polluted freshwater (wastewater). However, in some geographic locations, freshwater may not be available, limited in quantity, and expensive to use or produce through desalination technology. This may occur in some locations where treatment of bilge from sailing vessels at marinas and aircraft at airports is required. In order to reduce the use of freshwater/wastewater treatment of bilge, this study was conducted to determine the following: 1) if archaea and bacteria could be

acclimated to a salt water environment, 2) if acclimated cultures could degrade carbonaceous compounds (cBOD), and 3) if acclimated cultures could degrade carbonaceous compounds (cBOD) in the presence of a commercial deodorizer and disinfectant.

Although most treatment plant operators are familiar with bacteria and their ability to degrade cBOD, they may not be familiar with archaea. Archaea were known as “ancient” bacteria. However, they are now recognized as a separate domain of life with significant structural and metabolic differences to bacteria. Perhaps, the most significant difference between archaea and bacteria with respect to their ability to degrade cBOD is the extremophilic ability of archaea. As extremophiles, archaea can tolerate and grow under extreme ranges in pH, temperature, and salinity. The ability to survive and grow under extreme or harsh environmental or operational conditions is due to: 1) the presence of ether bonds in the lipids that make-up the cell wall and cell membrane in archaea as opposed to ester bonds that make-up the cell wall and cell membrane in bacteria and 2) the more tightly coiled protein molecules that are used by archaea for enzyme synthesis. Ether bonds are stronger than ester bonds, and tightly coiled protein molecules are more tolerant of harsh conditions that denature proteins.

The ability of archaea to grow under extremes in salinity and the ability to augment wastewater treatment processes with cultures of archaea and bacteria offers an opportunity to degrade wastewater cBOD in salt-water. Augmentation or more appropriately, bioaugmentation may be used to establish a colony of archaea and bacteria to 1) treat cBOD and 2) tolerate and degrade the in the presence of a deodorizing and disinfecting compound use to treat bilge from sailing vessels and aircraft. Additionally, fixed film media may be used to develop a biofilm of archaea and bacteria to improve treatment efficiency obtained by a suspended growth of archaea and bacteria as monitored through changes in mixed liquor volatile suspended solids (MLVSS). However, approximately 30 percent of the MLVSS consists of bacteria, and approximately 3% of the MLVSS consists of archaea.

To enhance microbial growth and consequently, degradation of cBOD, humates may be added to a treatment process. Humates or humic substances are organic compounds including long chain acids that are used as a fertilizer supplement. The acids act as chelating agents that improve the uptake and utilization of vital nutrients for indigenous bacteria and augmented bacteria.

MATERIALS

Archaea and bacteria used for the acclimation study consisted of a proprietary blend of cultures in Arkea® that was provided by ArchaeaSolutions, Inc. (Tyrone, Georgia). Acclimation testing was performed with two fiberglass, 250-gallon sequencing batch reactors (SBRs) provided by Cromaglass® Corporation

(Williamsport, Pennsylvania). Reef Crystal, Synthetic Sea Salt was obtained from Aquarium Systems, Incorporated (Mentor, Ohio). Mesa Verde Resources (Placitas, New Mexico) provided the humate that was used in the study. Process Tech, LLC (New Harmony, Indiana) performed adenosine triphosphate (ATP) testing on LuminUltra Junior LB 9509, LuminUltra Photon Master, and LumiCalc software (LuminUltra, Fredericton, New Brunswick).

Except for Total Kjeldahl Nitrogen (TKN) testing and ATP testing, the Clean Water Institute, Biology Department, Lycoming College (Williamsport, Pennsylvania), made all field and laboratory tests. Seewald Testing Laboratories (Williamsport, Pennsylvania) performed TKN tests, and Process Technology, LLC (New Harmony, IN) performed ATP tests.

Synthetic sewage was used as the substrate for archaea and bacteria (Table 1), and Sani-Pak® Toilet Deodorant Liquid, SP-97000M Series was used as the chemical deodorizer and disinfectant (Cleleste Industries Corporation, Easton, Maryland). Sani-Pak® does not contain quaternary ammonia and is used to control odors in marine, mass transit, portable toilets, rail, and recreational vehicles.

Table 1

Formula for Synthetic Sewage*

Compound, g/L of Deionized Water	
Compound	Quantity, g
Ammonium chloride (NH_4Cl)	0.0761
Glucose ($C_6H_{12}O_6$)	0.3000
Magnesium sulfate ($MgSO_4 \bullet 7H_2O$)	0.0176
Sodium bicarbonate ($NaHCO_3$)	0.2433
Sodium carbonate (Na_2CO_3)	0.1622
Sodium phosphate ($Na_2PO_4 \bullet 12H_2O$)	0.0462
Calcium chloride ($CaCl_2 \bullet H_2O$)	0.0047

*Acetate (CH_3COOH) was added as needed to increase the cBOD value, and sodium hydroxide (NaOH) was added to adjust pH.

EXPERIMENTAL PROCEDURE

In order to evaluate the ability of archaea and bacteria to acclimate to a salt-water environment, two SBRs, control reactor and test reactor, were filled with 150 gallons of chlorinated potable water each. After chlorine stripping, the SBRs were operated on 6 cycles per day with two phases per cycle. Phases consisted of 2.5 hours of aeration and 1.5 hours of settling. There was no discharge or decant phase. At scheduled times, 1.5 or 3 L of supernatant was removed during the settling phase for laboratory testing from each reactor, and then 1.5 or 3 L of synthetic sewage was added to each reactor. Supernatant removal and synthetic sewage addition were performed approximately twice per week.

Arkea® addition was approximately 0.001 ppm or 0.002 ppm. Although salinity increased throughout the study period, Arkea® was added in an effort to off set the loss of archaea and bacteria when supernatant was removed. In addition to Arkea® addition, fixed film media was submerged in each reactor to maintain a large and active biomass. The insertion of fixed film media converted the SBRs from a suspended growth system to a suspended growth (floc particles) and fixed film growth (biofilm) or integrated fixed film activated sludge (IFFAS) process.
Field tests included: chlorine (total), conductivity, dissolved oxygen, hardness, pH, salinity, total dissolved solids (TDS), and temperature. Laboratory tests include: ammonia, BOD, cBOD, COD, nitrate, nitrite, orthophosphate, total phosphorus, TKN, and TSS.

RESULTS AND DISCUSSION

The study was performed from March 21, 2013 to July 19, 2013. The control reactor and test reactor were operated in the same aeration and settling mode and were sampled and monitored at the same time. Salt and Arkea® additions to each reactor were performed on the same date and time, and the quantity of salt and Arkea® added to each reactors also was the same. Basic monitoring parameters for 1) dissolved oxygen, 2) pH, 3) temperature, and 4) total dissolved solids were very similar in range of values (Table 2).

Table 2

Range of Values for Dissolved Oxygen, pH, Temperature, and
Total Dissolved Solids over the Study Period for Control and Test Reactors

Operational Parameter	Reactor	
	Control	Test
Dissolved oxygen, mg/L	4.3 to 8.1	4.9 to 8.3
pH, standard units	7.8 to 8.4	7.7 to 8.5
Temperature, ^{o}C	17 to 24	17 to 24
Total dissolved solids, mg/L	130 to 39,000	180 to 38,100

Salt addition to increase the total dissolved solids (salinity) of freshwater (< 500 mg/L) in each reactor to the salinity of salt water (30,000 to 35,000 mg/L) was performed slowly over time to permit acclimation of archaea and bacteria to the salinity of salt water (Table 3). Arkea® addition throughout the study period (Table 4) and insertion of fixed film media to promote the growth of biofilm were performed in order to compensate for the loss of archaea and bacteria through the removal of supernatant. Approximately four square feet of fixed film media were submerged in each reactor on April 11, 2013. A light, chocolate-brown biofilm was observed on the fixed film media on June 3, 2013. The total dissolved

solids were approximately 21,000 mg/L on June 3, 2013. Inoculation of typtic soy agar (TSA) with biofilm removed from the media resulted in the growth of numerous colonies of microbes.

Table 3

Total Pounds of Salt Added to Each Reactor and Resulting Total Dissolved Solids Value

Date	Total Pounds Added	Total Dissolved Solids, mg/L	
		Control Reactor	Test Reactor
March 21	0	130	134
April 16	4	386	328
April 25	12	6,870	6,650
April 30	16	7,830	8,600
May 7	20	10,700	10,800
May 9	24	11,000	11,500
May 14	28	13,600	14,300
May 16	32	14,600	14,000
May 21	36	15,800	16,000
May 23	40	19,000	19,000
June 4	44	20,400	20,900
June 5	49	21,300	21,600
June 18	50	22,200	21,500
June 21	52	23,800	23,900
June 24	54	28,300	28,800
June 25	55	29,000	29,400
June 26	56	28,900	28,900
June 27	60	28,400	28,000
July 1	64	31,000	30,500
July 2	66	38,900	33,600
July 10	71	31,200	31,800
July 19	73	39,000	38,100

Table 4

Total Pounds of Arkea® Added to Each Reactor

Date	Total Pounds of Arkea® Added, grams	
	Control Reactor	Test Reactor
March 21	4	4
March 26	8	8
March 28	12	12
April 2	16	16
April 4	28	28
April 9	40	40
April 15	52	52
April 16	64	64
April 19	76	76
April 22	88	88
April 29	100	100
May 2	112	112

Because adequate quantities of micronutrients are critical for the growth of archaea and bacteria, approximately 8 g of Miracle-Gro® were added to each reactor. Miracle-Gro® contains boron, copper, iron, manganese, molybdenum nitrogen, phosphorus, potassium, and zinc. 60 mL of Sani-Pak® was added to the test reactor only on July 22, 2013.

ATP testing was performed on four supernatant samples from each reactor. Samples were taken on May 28, June 7, July 15, and July 22, 2013 (Table 5). All samples were shipped cold from Williamsport, Pennsylvania to New Harmony, Indiana. All samples, except for the July 7, 2013 arrived in New Harmony, Indiana with two days of the sampling date. The samples from July 7 arrived in New Harmony five days after sampling.

Table 5

ATP Values Obtained with Increasing Salinity

Date	Reactor			
	Control		Test	
	ATP, ng/mL	TDS, mg/L	ATP, ng/mL	TDS, mg/L
May 5	ATP_t , 90	10,700	ATP_t, 136	10,800
	ATP_d, 77		ATP_d, 40	
	ATP_c, 14		ATP_c, 95	
June 7	ATP_t, 191	21,300	ATP_t, 100	21,600
	ATP_d, 229		ATP_d, 382	
	ATP_c, -38		ATP_c, -285	
July 11	ATP_t, 88	31,200	ATP_t, 60	31,800
	ATP_d, 75		ATP_d, 42	
	ATP_c, 13		ATP_c, 18	
July 22	ATP_t, 192	39,000	ATP_t, 54	38,100
	ATP_d, 206		ATP_d, 61	
	ATP_c, < 5		ATP_c,< 5	

ATP is formed in the cell when energy is produced from the degradation of substrate. There are three forms of ATP that are determined during ATP testing, total ATP (ATP_t), cellular ATP (ATP_c), and dissolved ATP (ATP_d). ATP_t consists of ATP_c and ATP_d. The production of ATP_c is an indicator of cellular activity and the degradation of substrate. The increase in ATP_d is an indicator of a harsh environmental condition that results in the death and autolysis of cells. As a result of autolysis, ATP_d increases in the bulk solution.

Also, a portion of the supernatant from each reactor obtained on July 22, 2013 was streaked on TSA. Numerous colonies of microbes were observed growing on the agar plates within 48 hours. The total dissolved solids were approximately 38,000 mg/L on July 22, 2013. *Bacillus* was the dominant colony.

CONCLUISION

Results from culture plating and ATP testing demonstrated that archaea and bacteria were able to acclimate from freshwater salinity to salt-water salinity and were able to degrade cBOD. Archaea and bacteria were acclimated to > 38,000 mg/L of total dissolved solids. However, floc formation, which is inhibited at salinity values > 5,000 mg/L of total dissolved solids, was observed in the study. To compensate for the lack of floc particle maturation, fixed film media was used to provide for the growth of biofilm.

Addition research is recommended to determine the following: 1) if archaea and bacteria can be acclimated more quickly, 2) if archaea and bacteria can degrade

wastewater rather than synthetic sewage, and 3) if nitrification can occur with acclimated cultures of nitrifying organisms. It is also recommended that a pilot study using acclimated cultures to degrade bilge be performed.

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