Archaea: Arkea® Substrate

What, When, How and Why?

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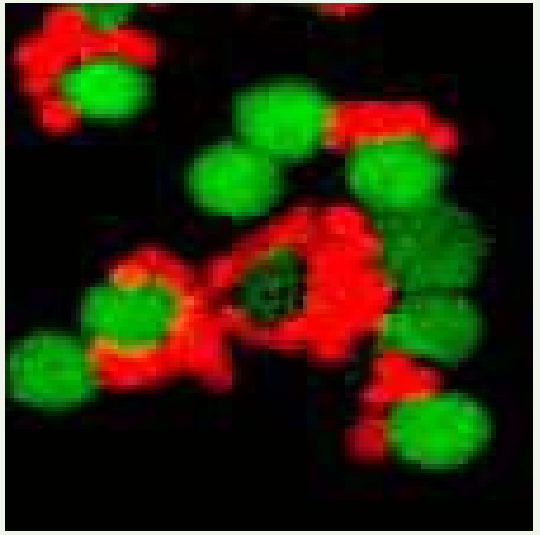
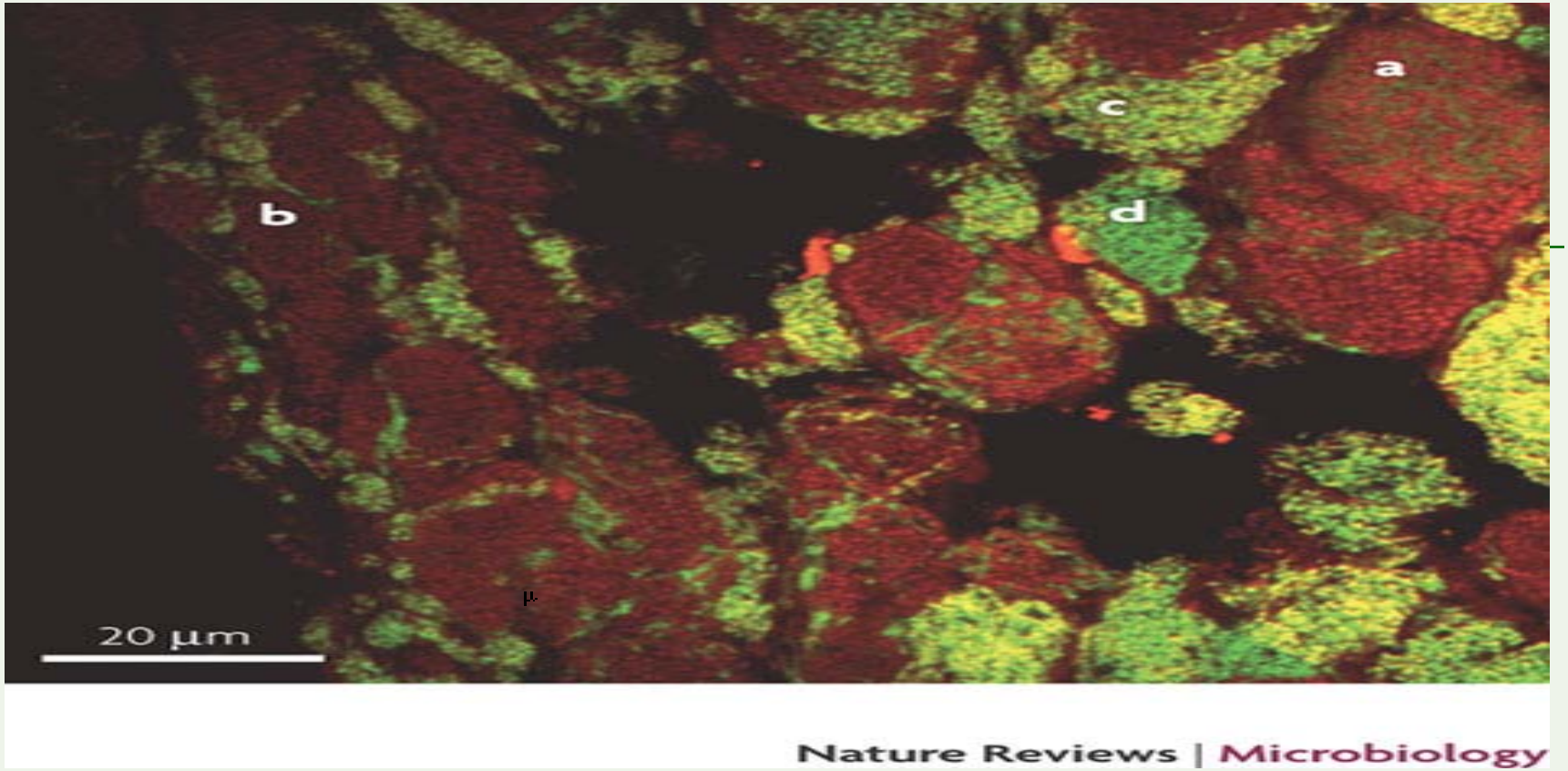
How Archaea Are Unique

- Generally Archaea live co-operatively with other “microbes”, not alone.
- Archaea control their genes like we do, not like bacteria.
- Thus, they can tolerate harsh conditions and environmental changes.

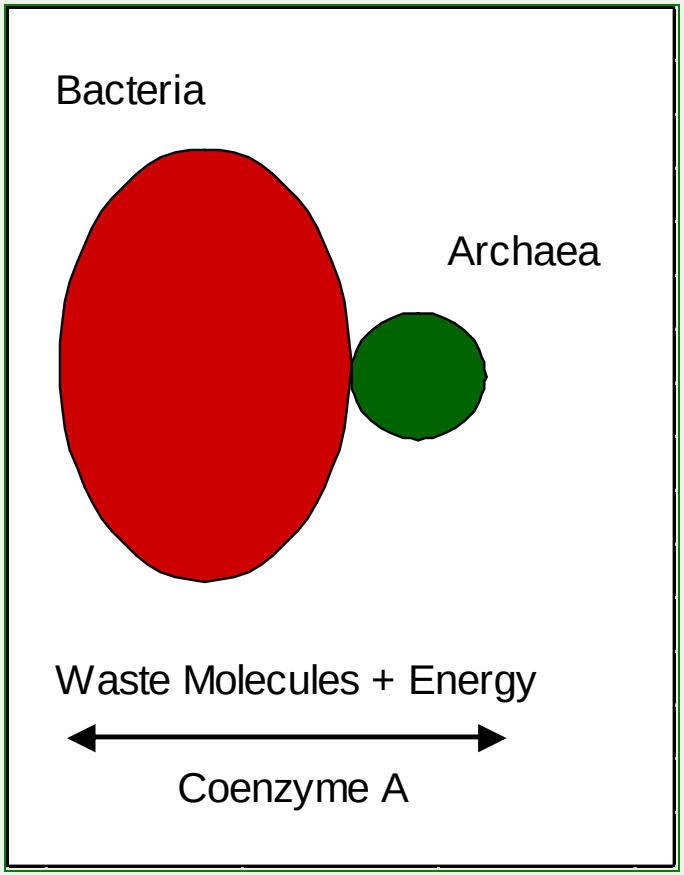




Sections of syntrophic communities in a sludge granule were stained to reveal the archaea (green), bacteria (red) and ***Methanosaeta*** sp. (red); the double (red and green) labelling of ***Methanosaeta*** sp. results in yellow fluorescence and distinguishes it from the bacteria. The microcolony labelled **a** is a syntrophic colony with bacteria intertwined with filamentous archaea. The part labelled **b** indicates a microcolony that probably consists of fermenting bacteria, **c** indicates a ***Methanosaeta*** microcolony and **d** indicates an unspecified archaeal microcolony. The left side of the section is the outside of the granule and the right side is towards the middle. This confocal laser scanning microscopy micrograph includes signals from a single focal plane that is 0.7 m thick, obtained with the two specific filter sets for the fluorescent labels. Photograph courtesy of H. J. M. Harmsen, University of Groningen, The Netherlands

Syntrophy

- *Bacteria and Archaea share a syntrophic relationship.*
- As bacteria accumulate waste, waste and the energy to break it down are sent to Archaea.
- Archaea break waste down.
- Archaea send back Coenzyme A which provides more reaction sites in bacteria.
- As a result, waste breakdown is accelerated.



Nature Reviews Microbiology 7, 568-577 (August 2009) | doi:10.1038/nrmicro2166

Electron transfer in syntrophic communities of anaerobic bacteria and archaea

Alfons J. M. Stams & Caroline M. Plugge

Abstract

Interspecies electron transfer is a key process in methanogenic and sulphate-reducing environments. Bacteria and archaea that live in syntrophic communities take advantage of the metabolic abilities of their syntrophic partner to overcome energy barriers and break down compounds that they cannot digest by themselves. Here, we review the transfer of hydrogen and formate between bacteria and archaea that helps to sustain growth in syntrophic methanogenic communities. We also describe the process of reverse electron transfer, which is a key requirement in obligately syntrophic interactions. Anaerobic methane oxidation coupled to sulphate reduction is also carried out by syntrophic communities of bacteria and archaea but, as we discuss, the exact mechanism of this syntrophic interaction is not yet understood.

Impact of Syntrophy

- Improved microbial performance
- Enhanced microbial robustness
- Establishment of “micro” ecology zones
- “Improved microbial communication”

Archaea In Nature

- Ubiquitous
  - Waterways, soil, plants, animals, man
  - Up to 40% of biomass on Earth
- Key role in carbon cycle
- Key role in nitrogen cycle
- Key role in sulfur cycle

Carbon Cycle

- Breakdown of complex carbohydrates
  - Archaea organisms alone utilize hydrogen freed from complex carbohydrates
  - Archaea organisms break down difficult to metabolize molecules
- Fixing CO_2
  - Carbon dioxide fixation into organic compounds in the oceans is driven by Archaea organisms

Nitrogen Cycle

- Archaea organisms can perform essentially all steps in nitrogen cycle.
- Archaea organisms have on order of magnitude more ammonia oxidation capability than other microbes in soil.
- ANAMMOX

Sulfur Cycle

- Across Archaea domain, strong sulfide oxidase enzymes to break down hydrogen sulfide or its salts.
- *Sulfolobus* group of Archaea
  - Convert oxidized/reduced sulfur compounds into sulfuric acid

Why Archaea

Why Archaea

- Archaea are the world's shock absorber.
- Archaea are known as "Extremophiles".
- Archaea grow and divide in extreme environments where nothing else can grow.
- Archaea have been found in geysers at Yellowstone National Park and around hydrothermal vents on the ocean floor.

Arkea® Substrate

- ArchaeaSolutions, Inc. produces Arkea® substrate containing a proprietary blend of Archaea organisms and other selected microbes.
- Archaea is key driver
- Other supporting microbes
  - Bacteria
  - Fungi
  - Nutrients/Micronutrients
  - Job specific microbes

Archaea Identification

- Several species of Archaeal methanogens have been identified in Arkea® through gene sequencing analysis.

Archaea vs. Bacteria

TABLE 6.1. Bacteria and archaea share many features to the exclusion of eukaryotes

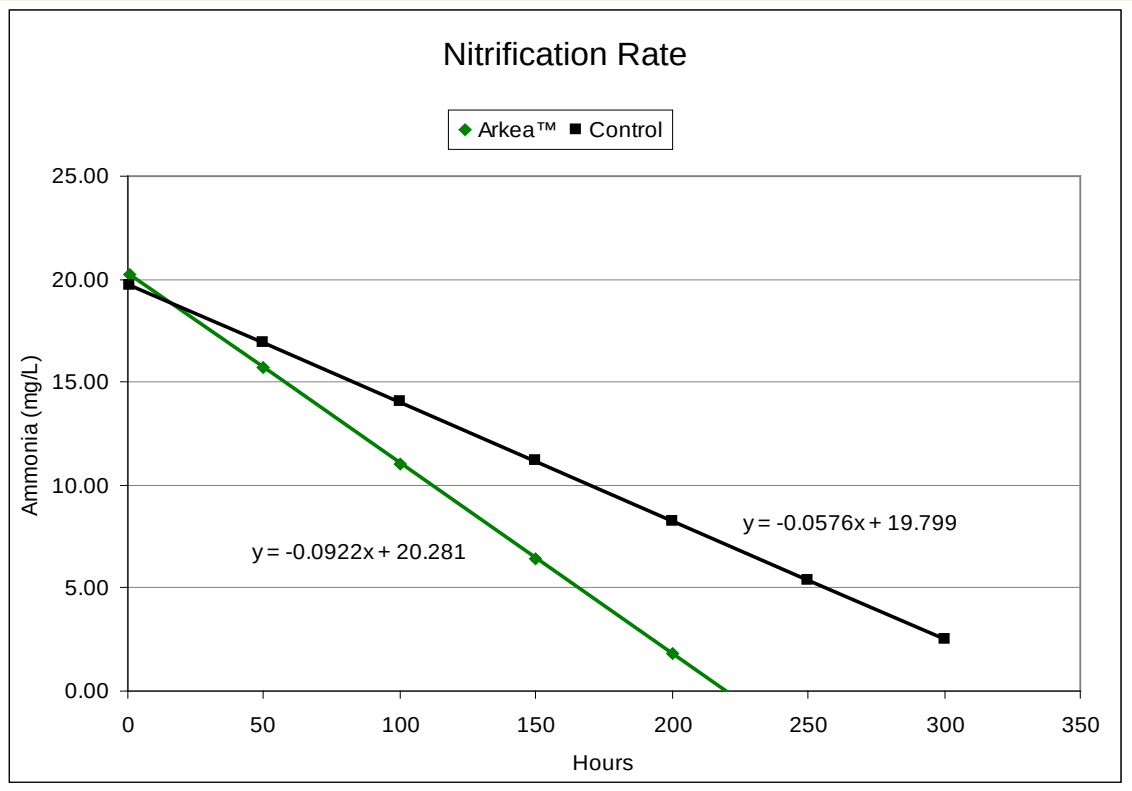
Feature	Archaea and Bacteria	Eukaryotes
Nuclear membrane	Absent	Present
Genomes	Usually one circular chromosome; frequently one or more small DNA circles (plasmids)	Multiple linear chromosomes
Mitosis	Absent	Present
Introns	Rare	Common
Cytoskeleton composed of microtubules	Absent	Present
Internal membranes	Simple and rare	Complex and common
Primary mode of genetic recombination	Lateral gene transfer	Sexual reproduction
Size	Small, 1–5 µm	Larger, 20–100 µm
Organelles	No	Yes
Ploidy	Haploid	Diploid or more

Based on Madigan M.T., Martinko J., and Parker J. 1997. *Brock biology of microorganisms*, 8th ed. Prentice Hall, New York, Table 3.5.
What we list here are features of most of the members of these groups.

Nitrogen Cycle

Forms syntrophic relationship with both nitrifiers and denitrifiers, enhancing robustness. Also occurs at low carbon levels, indicating direct reaction of ammonia and nitrate to yield nitrogen gas plus water.

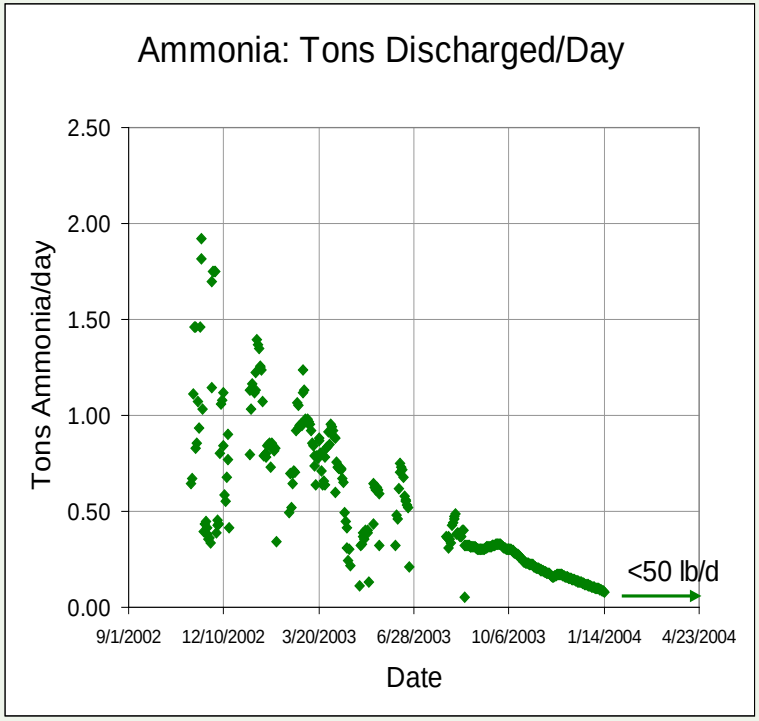
Ammonia Oxidation



- Ammonia breaks down faster.

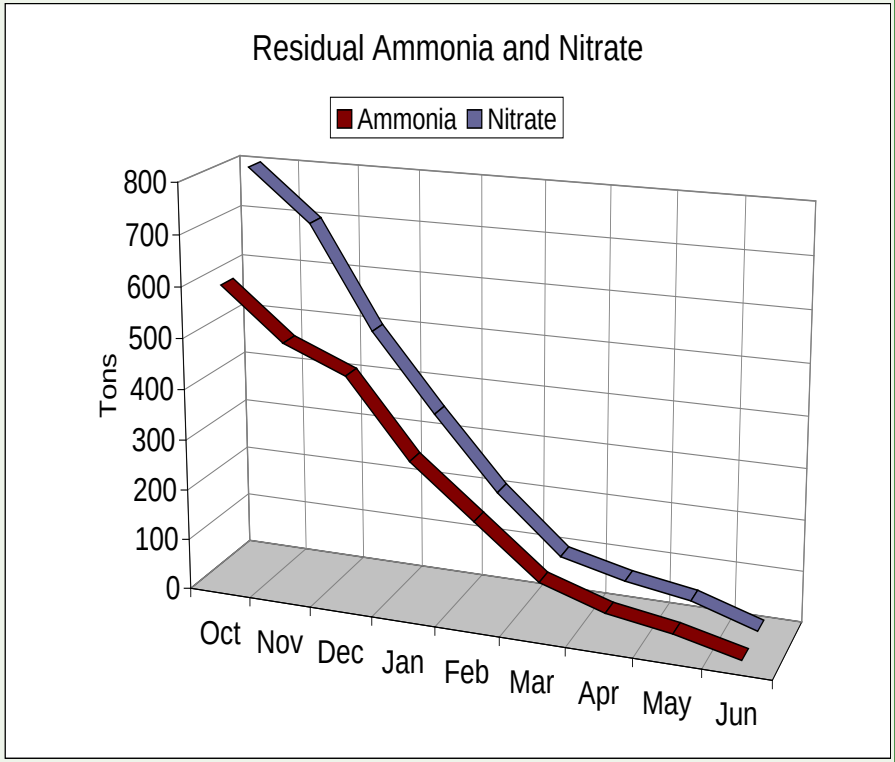
Explosives Manufacturer

- 150 million gallon lagoon with COD 8-15 mg/L.
- Discharged 2 Tons of ammonia per day prior to Arkea® treatment.
- Over a period of over one year, ammonia discharge reached levels less than 50 lbs/day.
- Have maintained discharge of significantly less than 50 lbs/day for years.
- Nitrate pollution prevention followed same path.
- Firm went from “shut down” threat to winning state EPA award (“Black Diamond”) for effective wastewater processing.

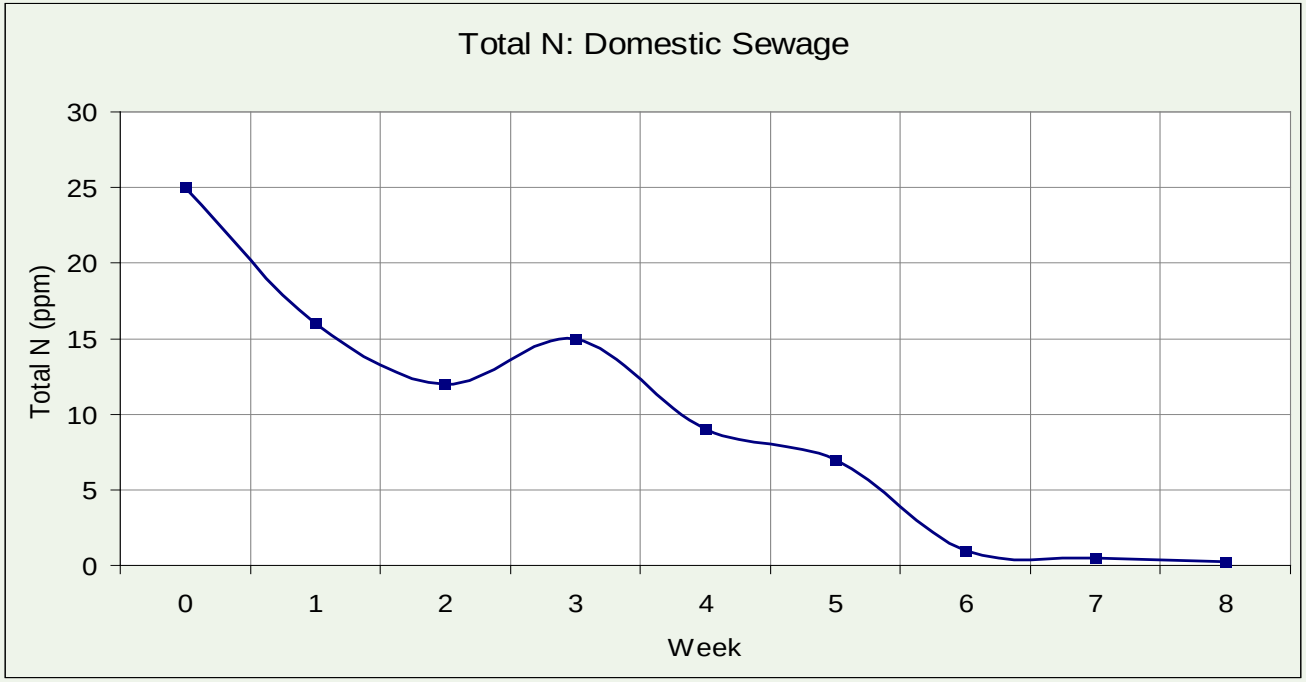


Practical Application: Nitrogen

- Plant is producing sulfuric acid, nitric acid, and ammonia for explosives.
- Plant has 150 million gallon “lake” to dilute it.
- At limits - so they must eliminate ammonia, nitrate, and sulfate.
- Very little carbon - so they must go ANAMMOX.



Domestic Sewage



- As per norm, about 6 weeks needed to reduce Total N
- Remained at <1 ppm

Municipal TKN

- In less than 6 months, TKN dropped to +/- 1 mg/L and has remained there for almost five years.
- Plant is in the state of New York where winters are quite cold.
- Science now knows Archaea organisms have 3,000-fold more ammonia oxidase genes than other soil microbes.

Time Period	mg/L
6/2004 - 5/2005	>7.00
7/2005 - 9/2005	<2.00
9/2005 - Present	+/- 1.00

Phosphorus Reduction

Field Results

- Phosphorous management has not been a historical goal of clients.
- Our most complete example of work is on dairy farms in the central CA valley in 2001 through 2003.
- Normally the dairy lagoons would be filled with bio-solids and often have a bio-solid crust on top.

- During a typical 90-day clean-up period, there was a reduction in environmentally sensitive components of the lagoon.
- Anecdotal information indicated phosphorous removed from the liquid phase was in the insoluble material in the bottom of the lagoon.

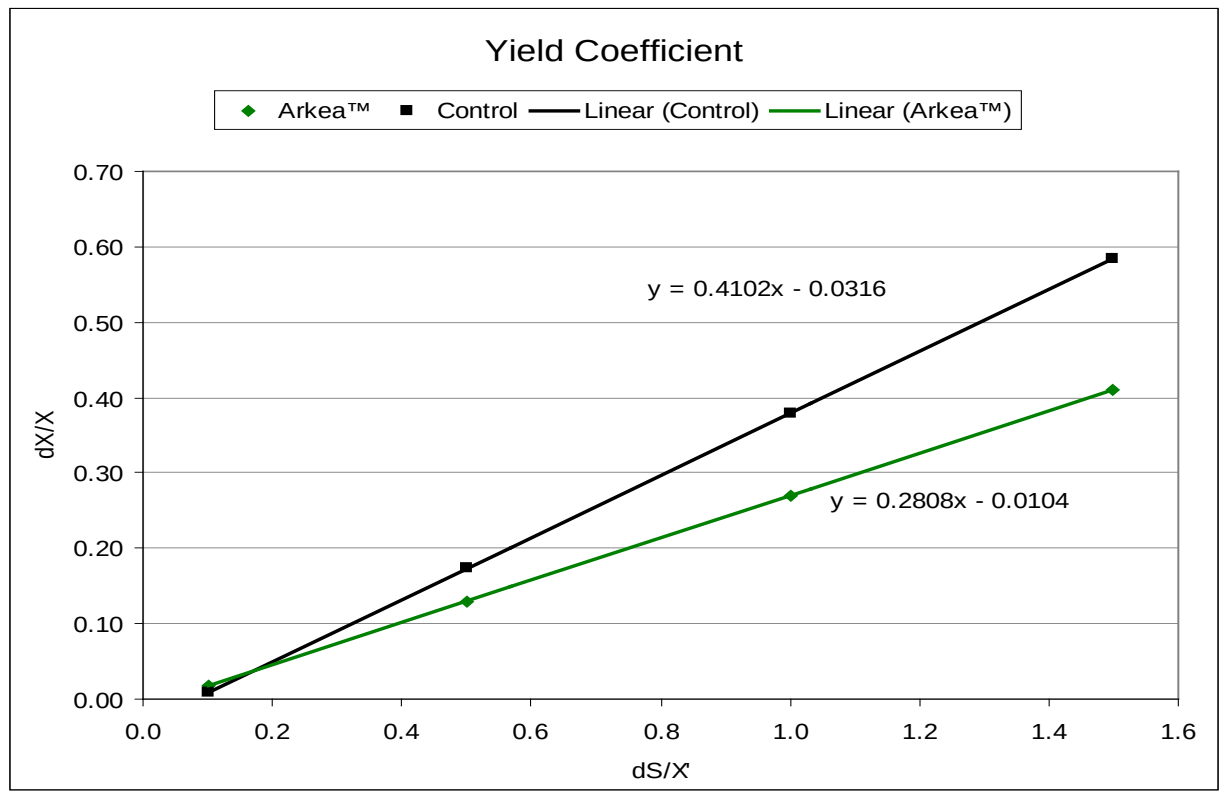
Sludge Management

Increased endogenous respiration drives reduced yield efficiency. Main effects of particulate organic carbon breakdown to fuel “one-carbon” Archaea metabolism are on horizon.

How Arkea® Substrate Cause This?

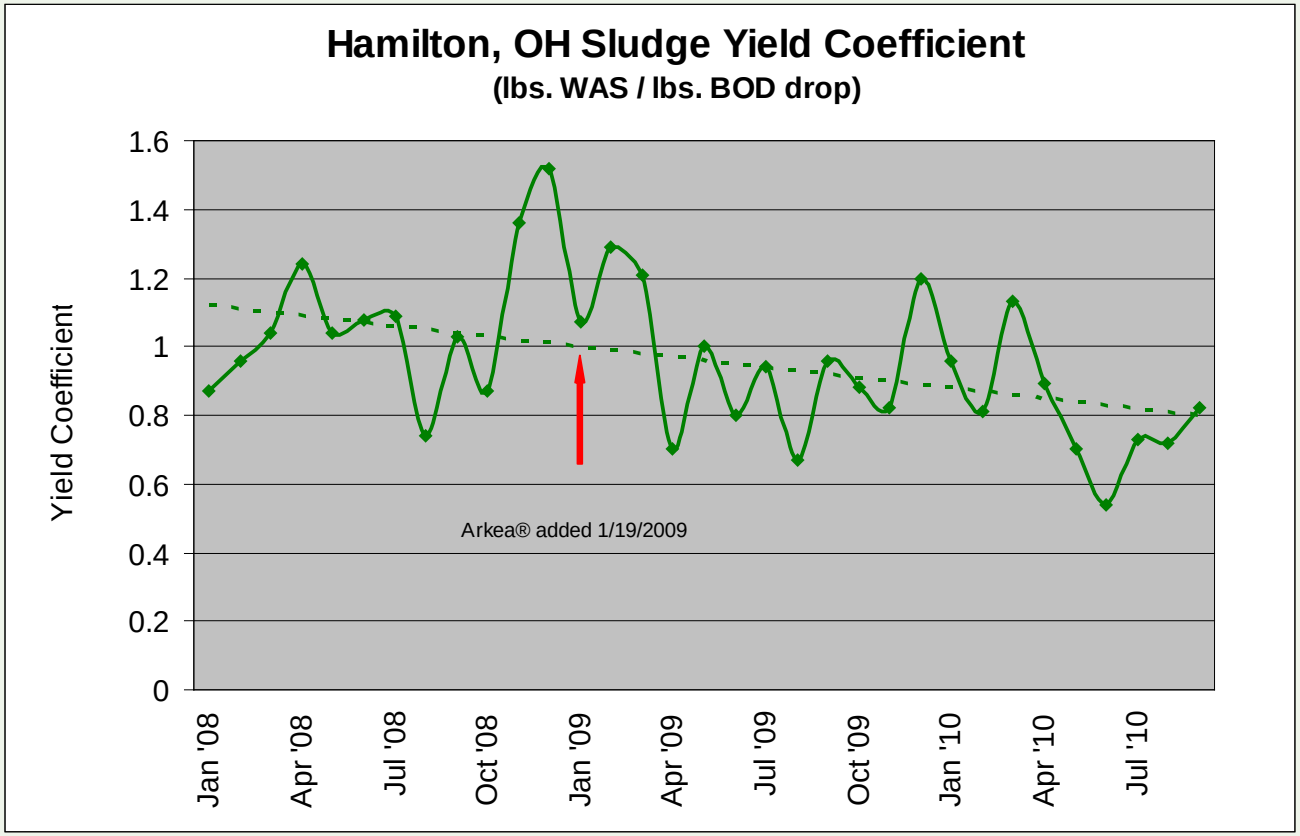
- Increase oxygen utilization efficiency
- Accelerate metabolism
- This reduces sludge formation

Sludge Yield



- Less sludge is produced with Arkea® substrate.

Sludge Yield – Hamilton, OH

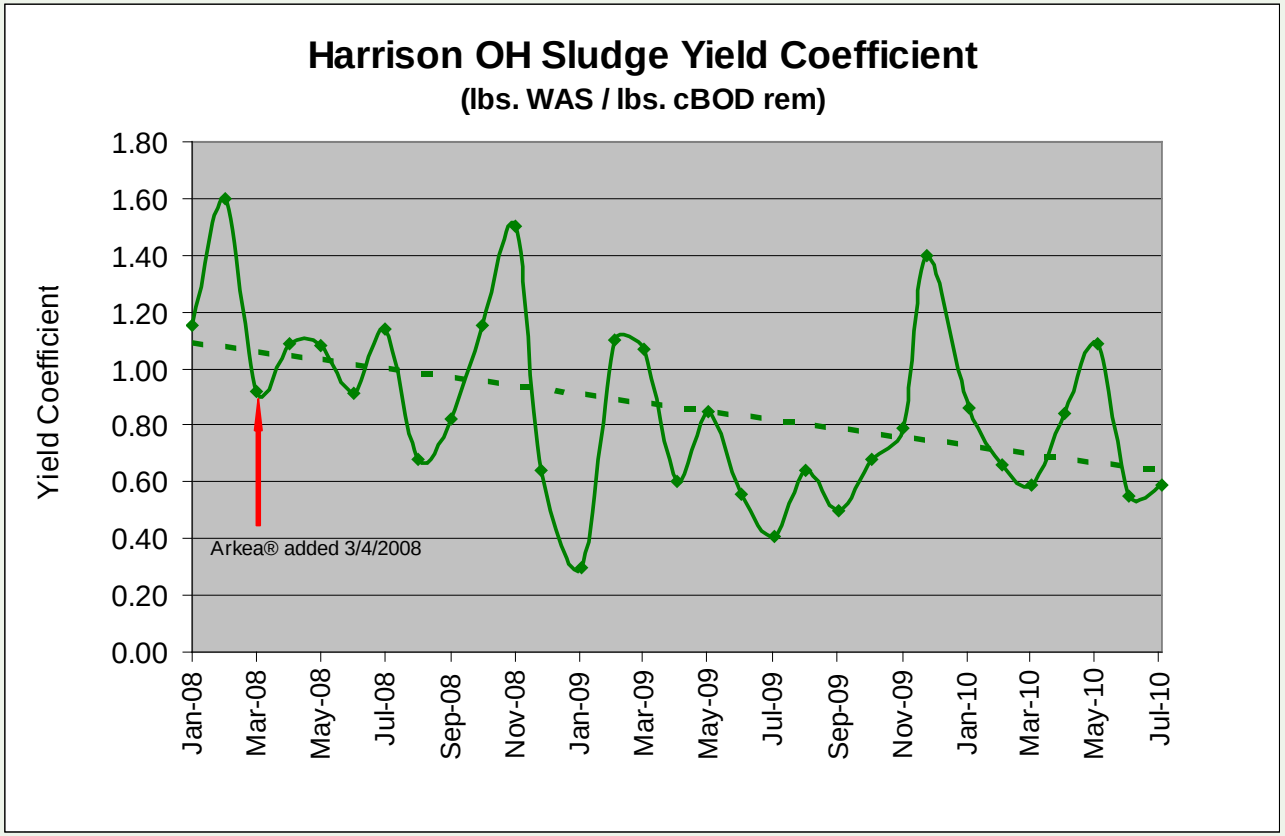


WAS & Sludge Yield – Hamilton, OH

- Since the addition of Arkea® on 1/19/2009, lbs. of WAS continue to decline.
- Sludge yield coefficient also decreased.

Year	WAS (lbs.)	Sludge Yield (lbs. WAS / lbs. BOD drop)
2008	15,806	1.07
2009	11,581	.95
2010	11,090	.81

Sludge Yield – Harrison, OH

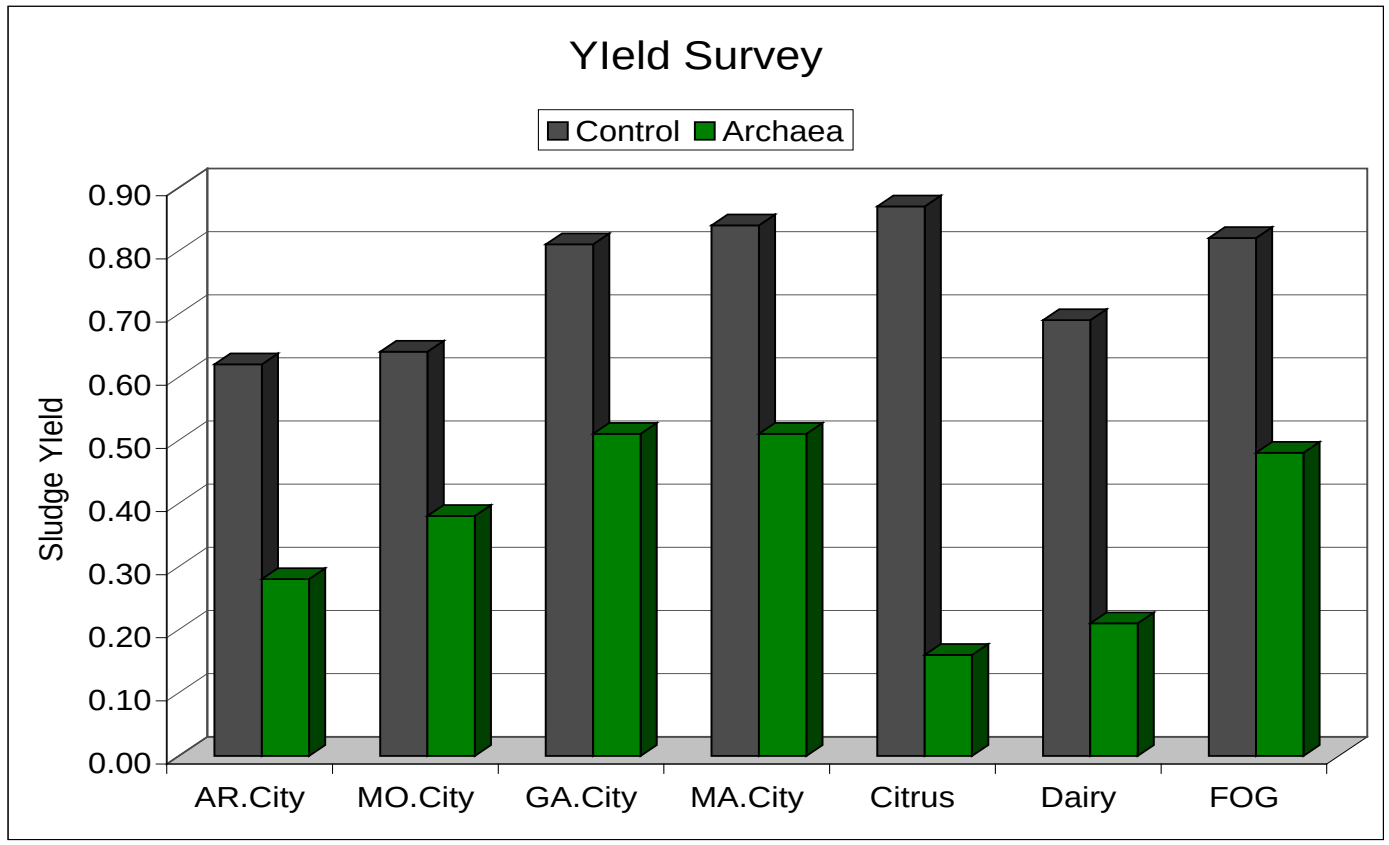


Sludge Yield – Harrison, OH

- Data show average sludge yield calculations
  - 2007: 1.28
  - 2008: 1.06
  - 2009: .74
  - 2010: .74
- Represents a **42%** reduction in sludge yield

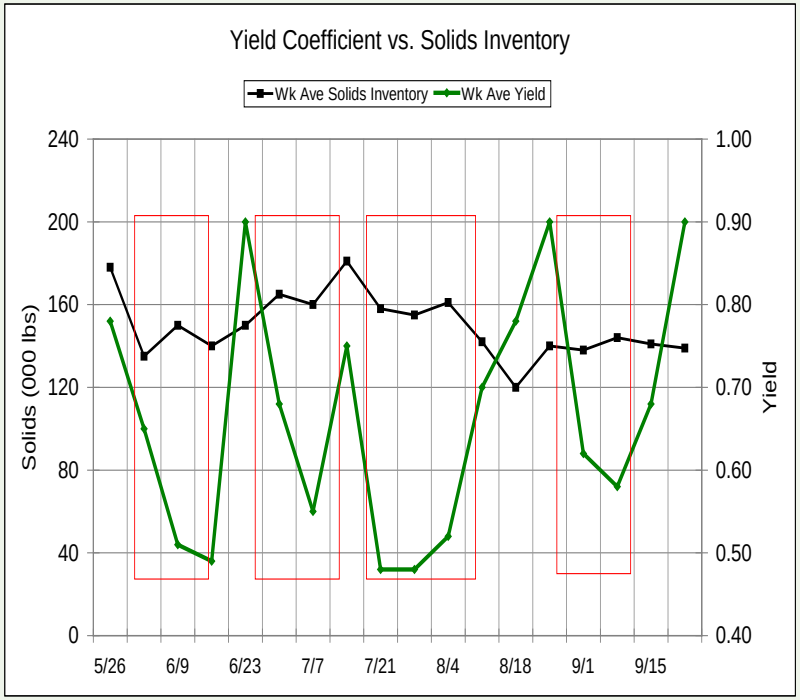
Year	Sludge Yield (lbs. WAS / lbs. cBOD rem)
2007	1.28
2008	1.06
2009	.74
2010	.74

Sludge Yield



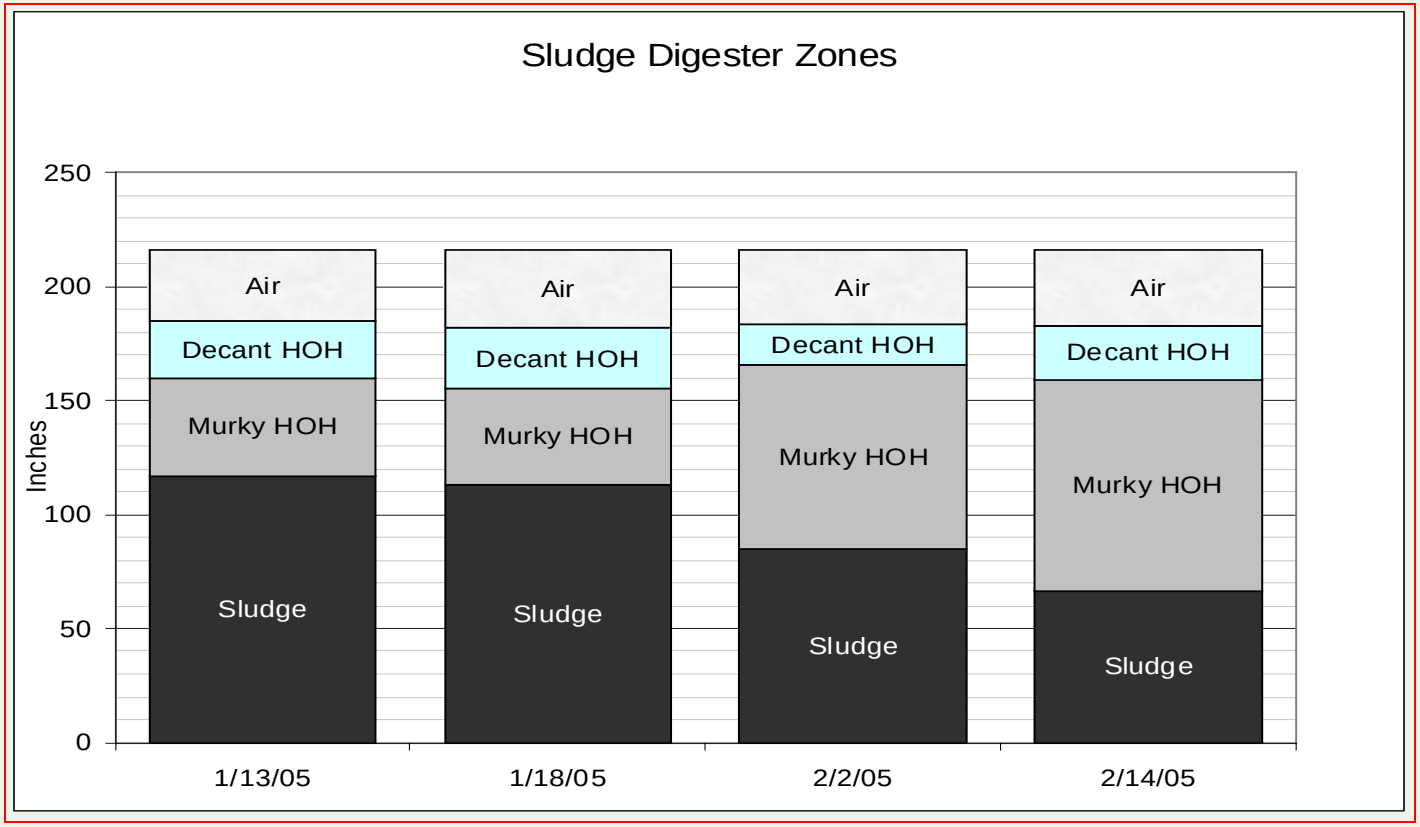
“Off-On-Off” Yield Work

- 19 MGD plant
- Management wanted to alternately turn on (inside red boxes) Arkea® substrate treatment and then turn it off for several cycles.
- Followed solids inventory
- When Arkea® substrate were “on”, yield dropped. When “off”, the yield climbed.

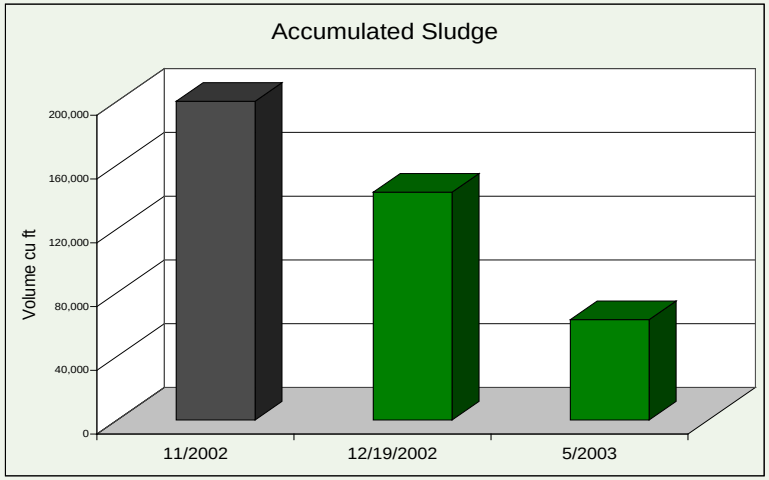


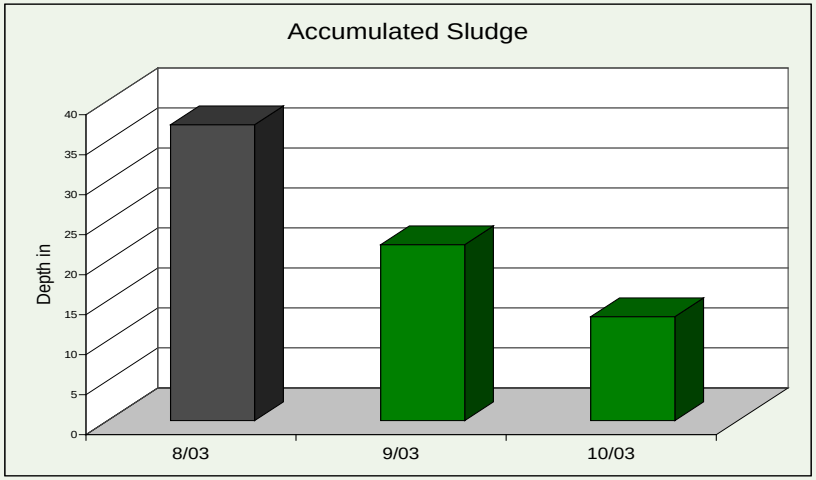
Biological Dredging

Desludging Digester



Examples: Biological Dredging





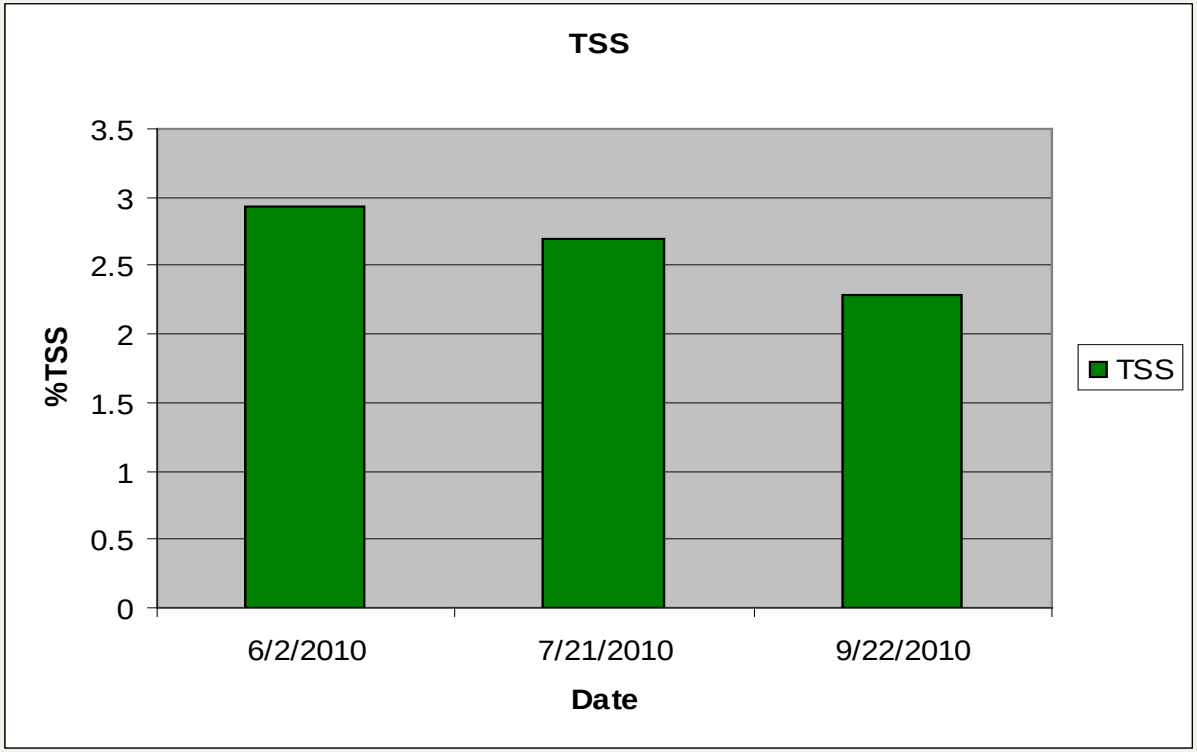
- Two mid western cities with excess accumulated sludge in lagoons
- In both cases, biological dredging with Arkea® substrate reclaimed significant hydraulic volume at about 20-25% the cost of mechanical dredging and without shutting down.

Lagoon Bio-Dredging Results

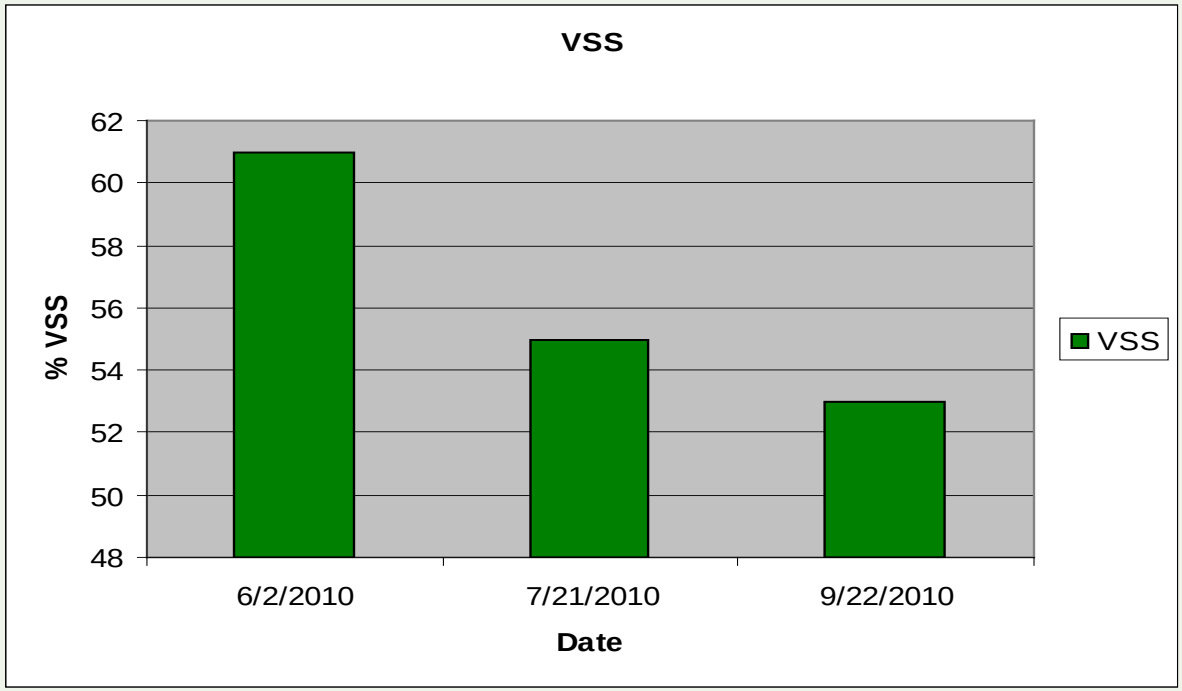
Before: note dark gray color

After: note green color and improved appearance

TSS Reduction – Wooster, OH

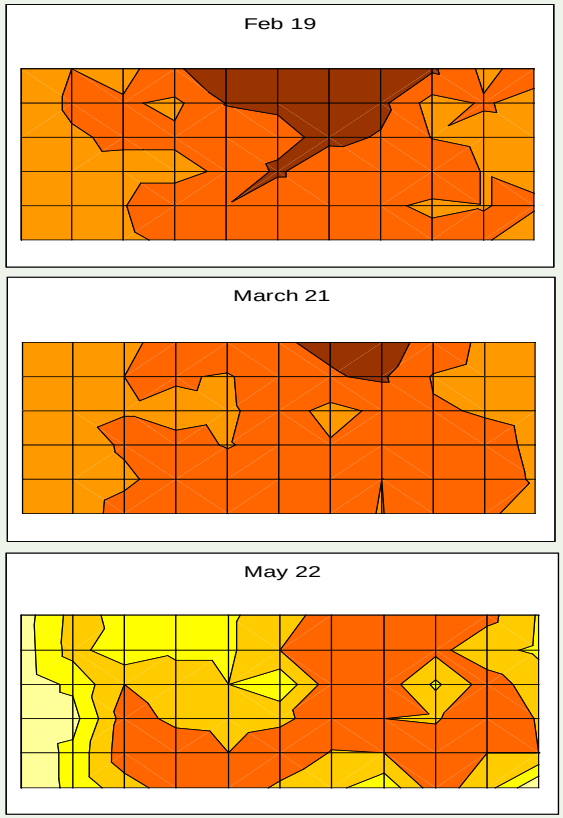


VSS Reduction – Wooster, OH



Beef: BioDredging Accumulated Sludge

- A municipality with a slaughterhouse killing 480 animals/hr on a 24/7 basis could not cost effectively manage their bio-solids using mechanical dredging. An Arkea® product was developed to directly consume their accumulated sludge.
- The two-dimensional topographic charts to the right show Bio-dredging effectively reducing sludge depth. The dark brown area is >100” in depth. Orange is 80 – 100”. Gold is 60-80”. Dark yellow is 40-60”. Yellow is 20-40”. Vanilla is <20”.
- Accomplished at a fraction of the cost of mechanical dredging.



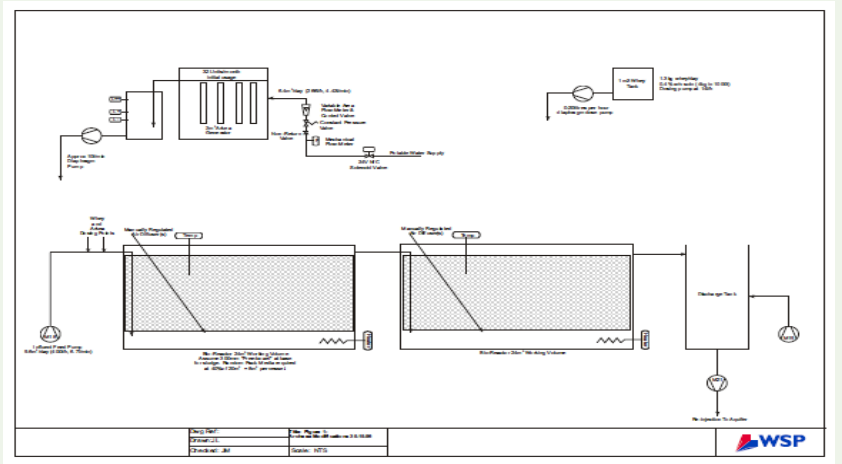
More Case Studies

Sweden Refinery

Ohio Installations

Indiana Installations

London 2012 Olympic Stadium- Water Treatment



Arkea® Substrate Generation On Site

Economics

Cost Effective Nutrient Reduction

- Arkea® is a cost effective means for primary or secondary treatment in the management of nutrient reduction.

Cost Savings

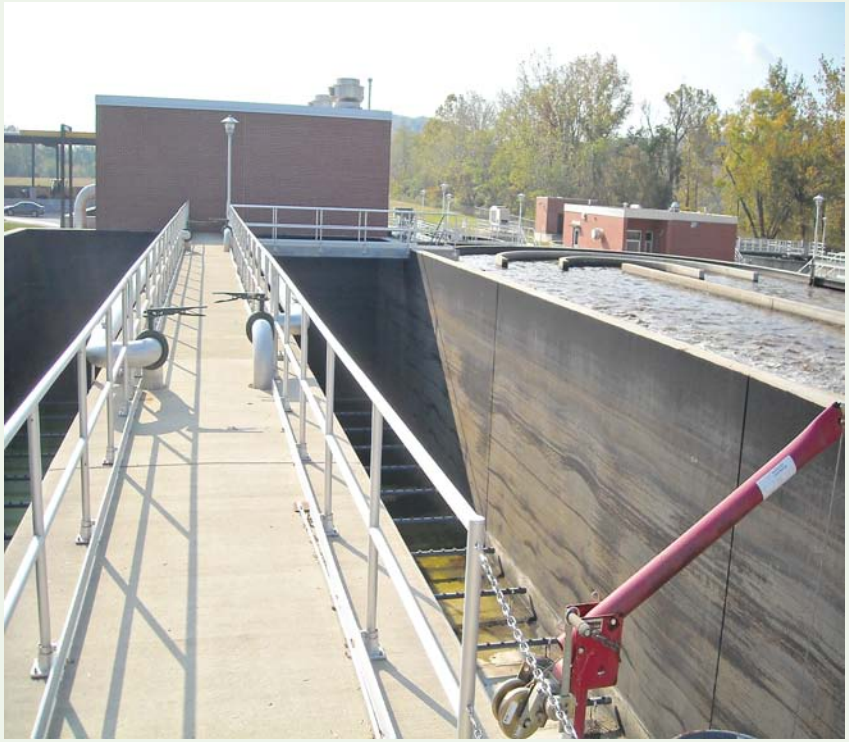
- Arkea® substrate improves operating profit.
- The operational cost savings will come from the following areas:
  - Decreased energy costs
  - Reduced sludge handling costs
  - Decreased chemical usage
  - Improved overall plant operation

Hamilton, OH Practical Measurement

- Sludge generated at the Hamilton WWTF
  - Spread on fields
  - Disposed of in a landfill
  - Composted
- With Arkea® augmentation
  - Number of truckloads of sludge going to fields or landfill significantly declined
  - Consistently >= **67% fewer truckloads** of sludge leaving the WWTF

Harrison, OH Operational Impact

- When project began, plant operated all four available sludge digesters.
- Due to impact of Arkea® only two sludge digesters currently in use.
- Convincing confirmation of reduced sludge yield



Arkea® Safety

Archaea Are Safe

- Checked by official USEPA independent testing protocols for acute, chronic, reproductive, and developmental safety.
- No known pathogens.
- Archaea are biologically very distant from pathogens.

Phoenix, AZ WWTP

- Severe pathogen problem in WRF because of climate
- In year long project, dosing with Arkea® in headworks consistently caused a 2-log reduction in all coliforms prior to disinfection
- Published results in peer-reviewed publication

Archaea Augmentation and Coliform Reduction at an Arizona Water Reclamation Facility

Source: *Proceedings of the Water Environment Federation, WEFTEC 2008: Session 101 through Session 115, pp. 7718-7729(12)* ***Publisher:*** *Water Environment Federation*

Lake Edith YMCA Camp

- Swimming and canoeing had been closed because of pathogenic microbes.
- After Arkea® treatment, pathogen counts were consistently 3 ppm.
- Dam at sewage lagoon next to camp broke in third year of treatment. No increase in pathogen counts 48 hours post event.

Summary

- Archaea are required for waste breakdown.
- Archaea are nature's oldest nitrogen removal group of organisms.
- Oxygen toxicity and slow cell division prohibited commercial use.
- ArchaeaSolutions, Inc. developed technology to overcome these limitations.
- Since Archaea divide so slowly and are overgrown by bacteria, ArchaeaSolutions, Inc. adds Arkea® substrate.

Summary (continued)

- Arkea® substrate's presence accelerates oxygen utilization.
- This results in increased endogenous metabolism and reduced sludge yield.
- If sludge has accumulated, Arkea® substrate cause its breakdown.
- Arkea® substrate do all this in a cost effective manner.
- Arkea® are proven safe in the environment.

Jobs Arkea® Substrate Can Do

- Reduce sludge production
- Reduce built up sludge
- Protect against slugs/shocks
- Reduce accumulated nutrients
- Increase digestion in anaerobic cells
- Reduce ammonia levels
- Improve disinfection
- Reduce levels of E.coli, coliforms, etc.

ArchaeaSolutions, Inc.

- Science-focused company
- Discovered how to use Archaea organisms to resolve environmental problems
- Ongoing work in North America, Europe, and Africa
- Usual approach is to evaluate problem and situation, perform lab analysis, complete pilot program, and undertake full scale work.

Questions?

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