Microbial Ecology of Dairy Wastewater Lagoons

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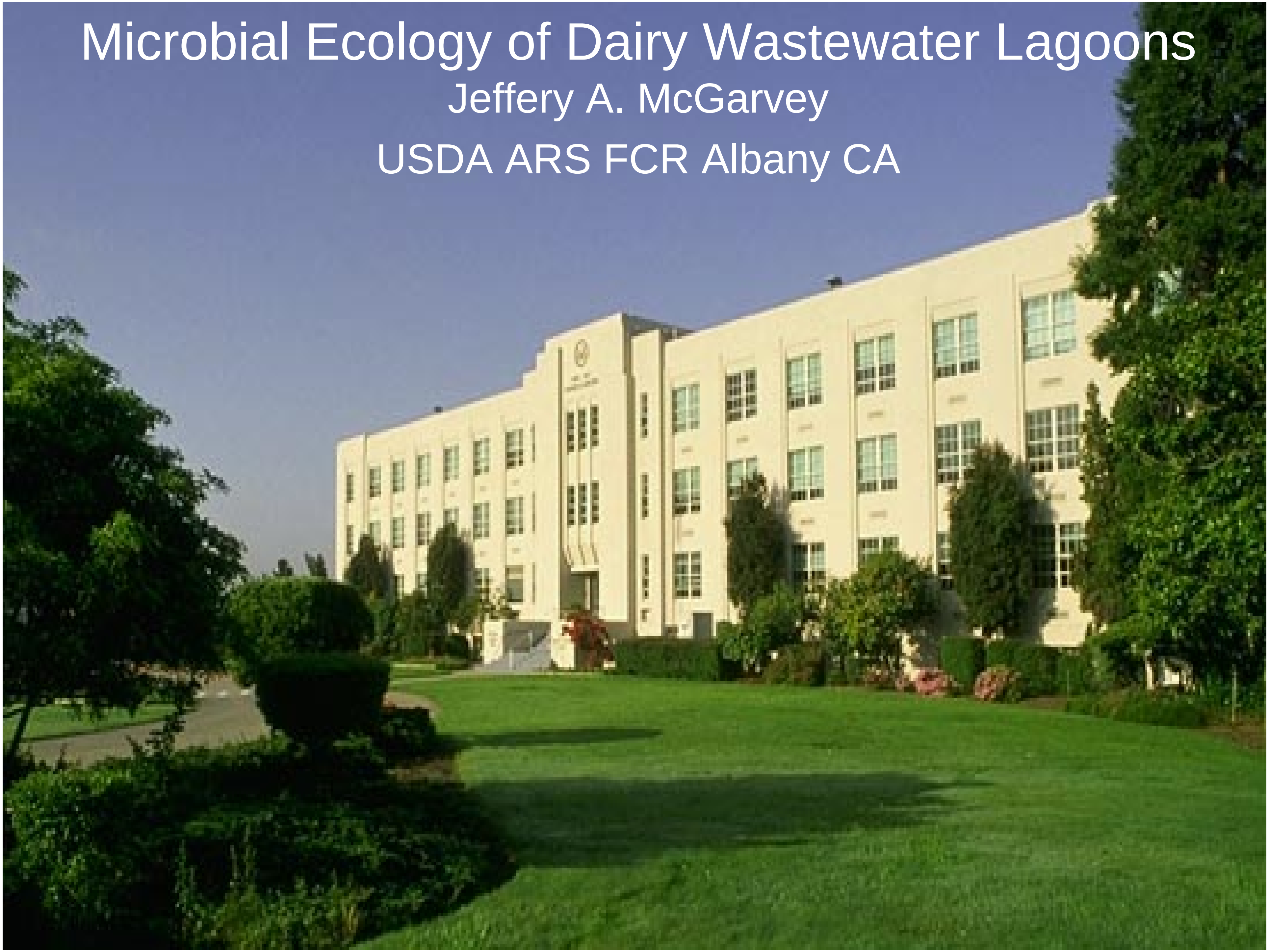


Bacterial Nutrition

Bacteria like all living creatures need:

1. Energy to perform tasks.
   ATP (Adenosine Tri-Phoshpate)

2. Carbon for growth.

Energy

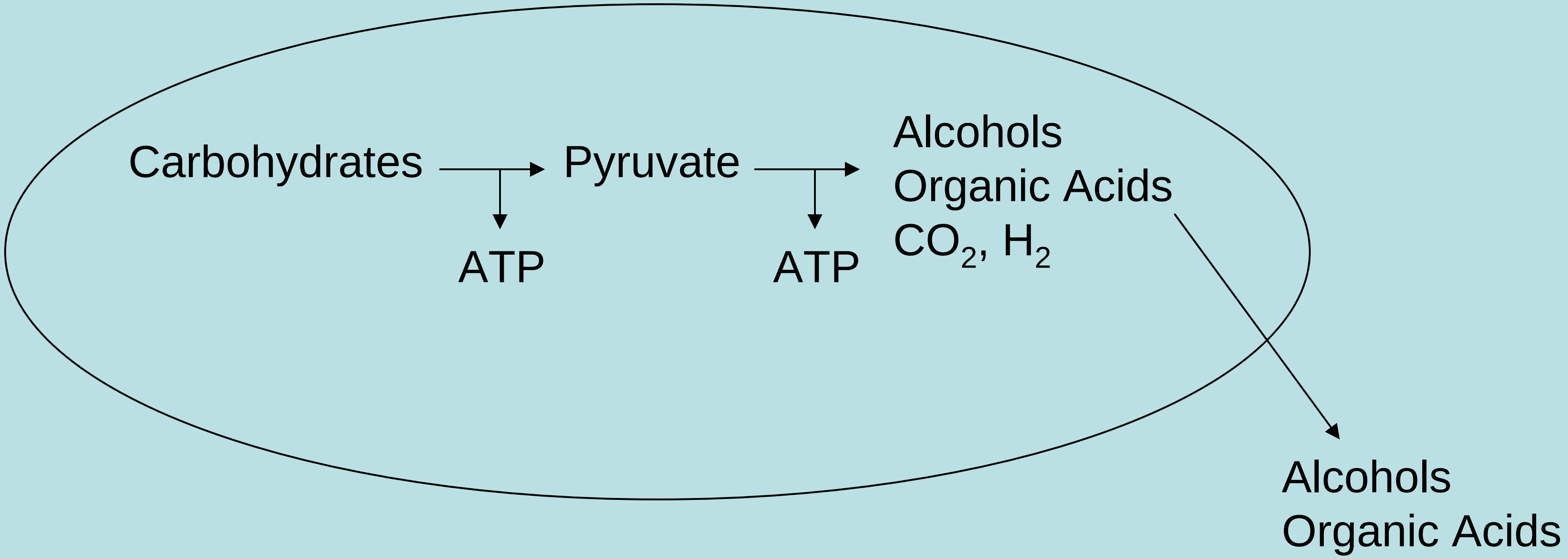
There are 2 ways bacteria can obtain energy

- The breakdown of high energy chemicals, bacteria that do this are called chemotrophs
- The capture of light energy, bacteria that do this are called phototrophs

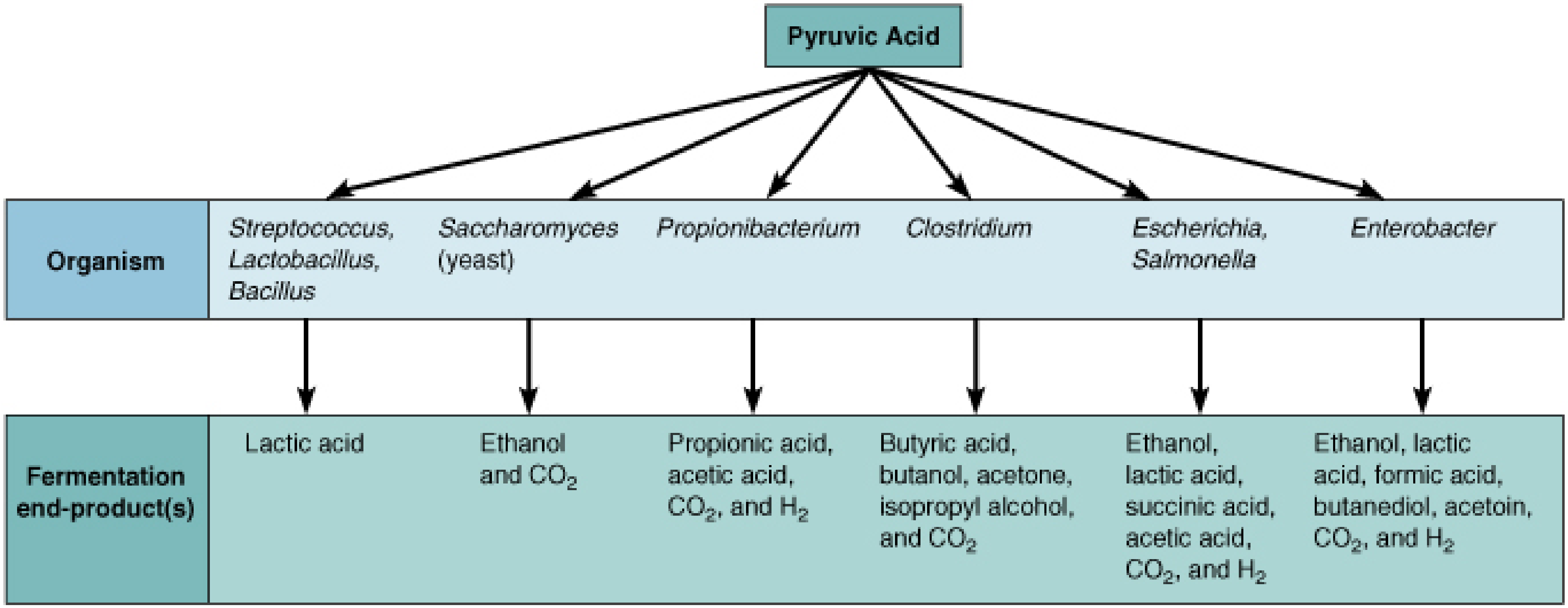
Chemotrophic Metabolism

1. Fermentation

A series of chemical reactions in which carbohydrates are converted to pyruvate then to alcohols, organic acids, CO_2 and H_2. A key point to this process is that all of the reactions take place within the cell and the end-products are then excreted.



Fermentative Bacteria and their End-Products

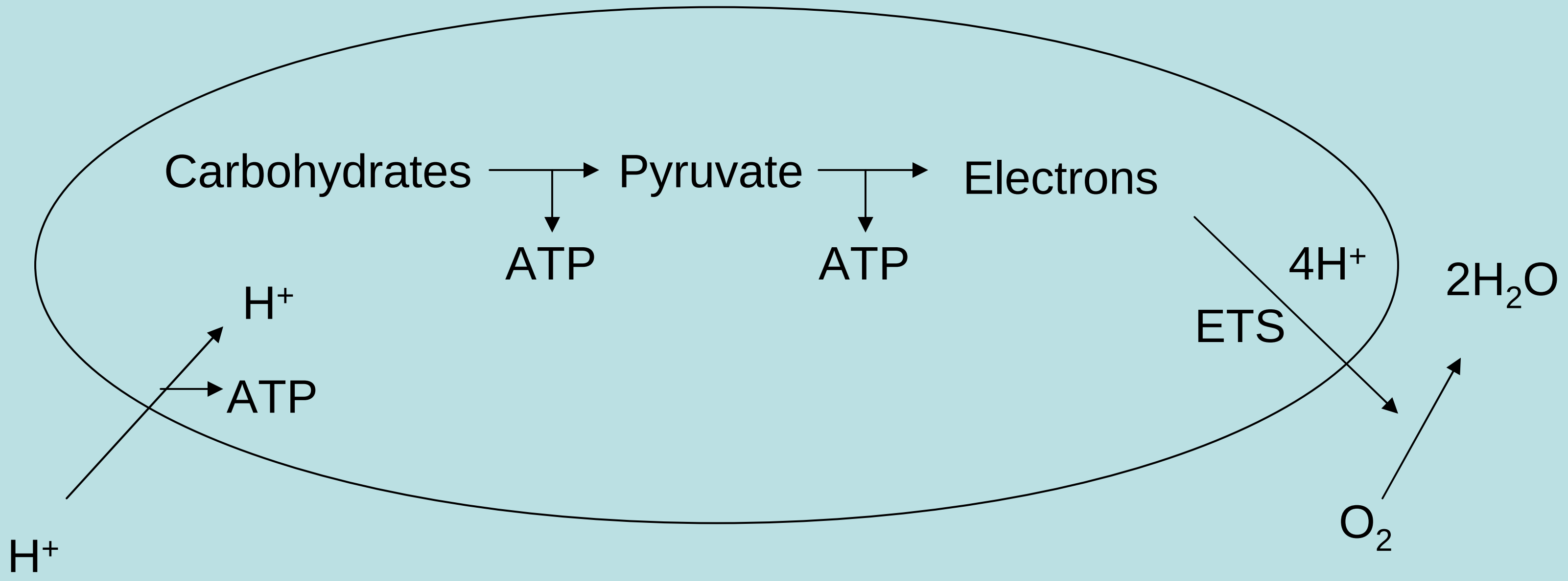


(b)

Chemotrophic Metabolism

2. Aerobic Respiration

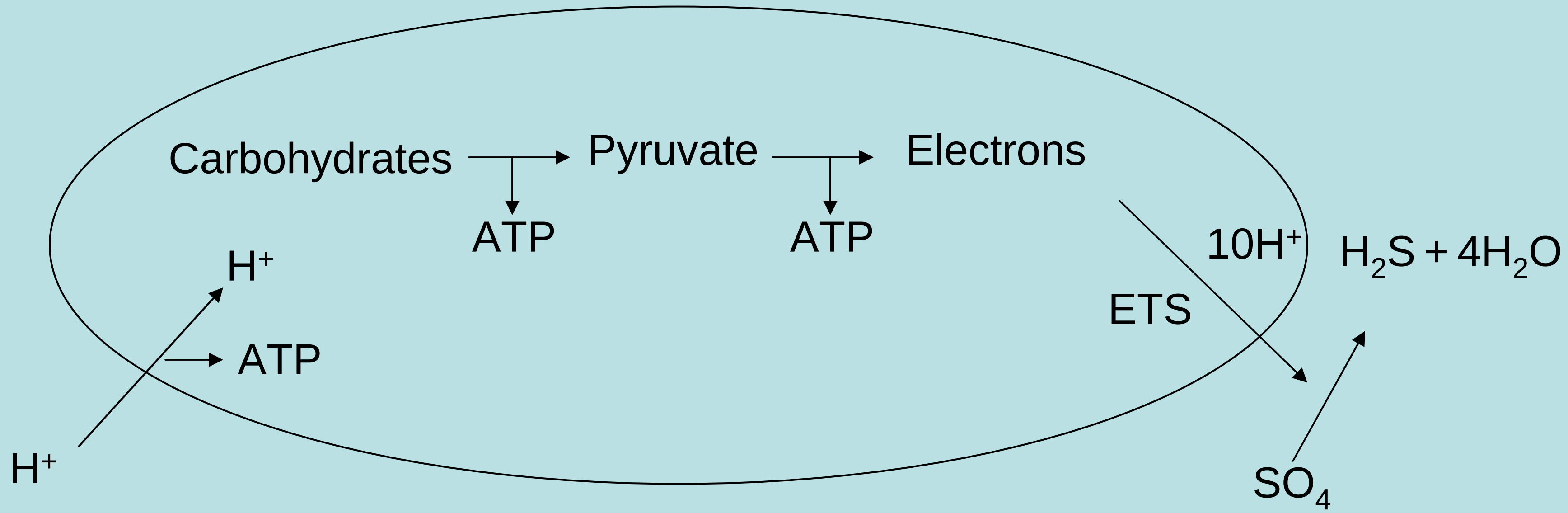
Similar to fermentation in that carbohydrates are usually converted to pyruvate but the end-products are CO_2 and H_2O. A key to point in this process is that electrons are shuttled outside the cell and thus the end-products are made outside the cell.



Chemotrophic Metabolism

3. Anaerobic Respiration

Similar to aerobic respiration but the terminal electron acceptor is not oxygen but some other compound such as SO_4, NO_3, etc.

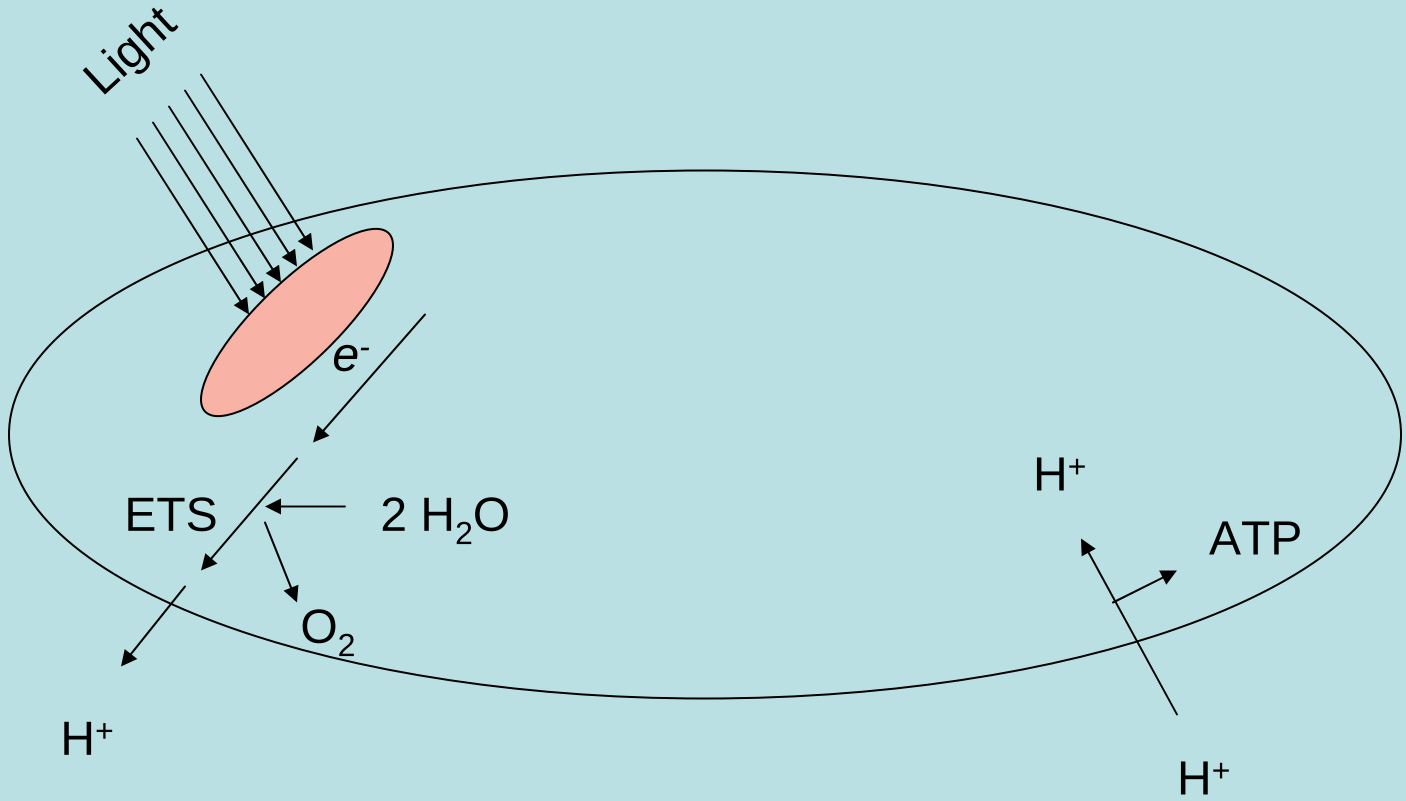


Phototrophic Metabolism

- Two types of phototrophic metabolism
  - Oxygenic Photosynthesis
    - Using H_2O as an electron donor and generates oxygen in the process.
    - Plants and some bacteria do this.
  - Anoxygenic Photosynthesis
    - Uses compounds such as H_2S or organic acids as an electron donor.
    - Only bacteria can do this.

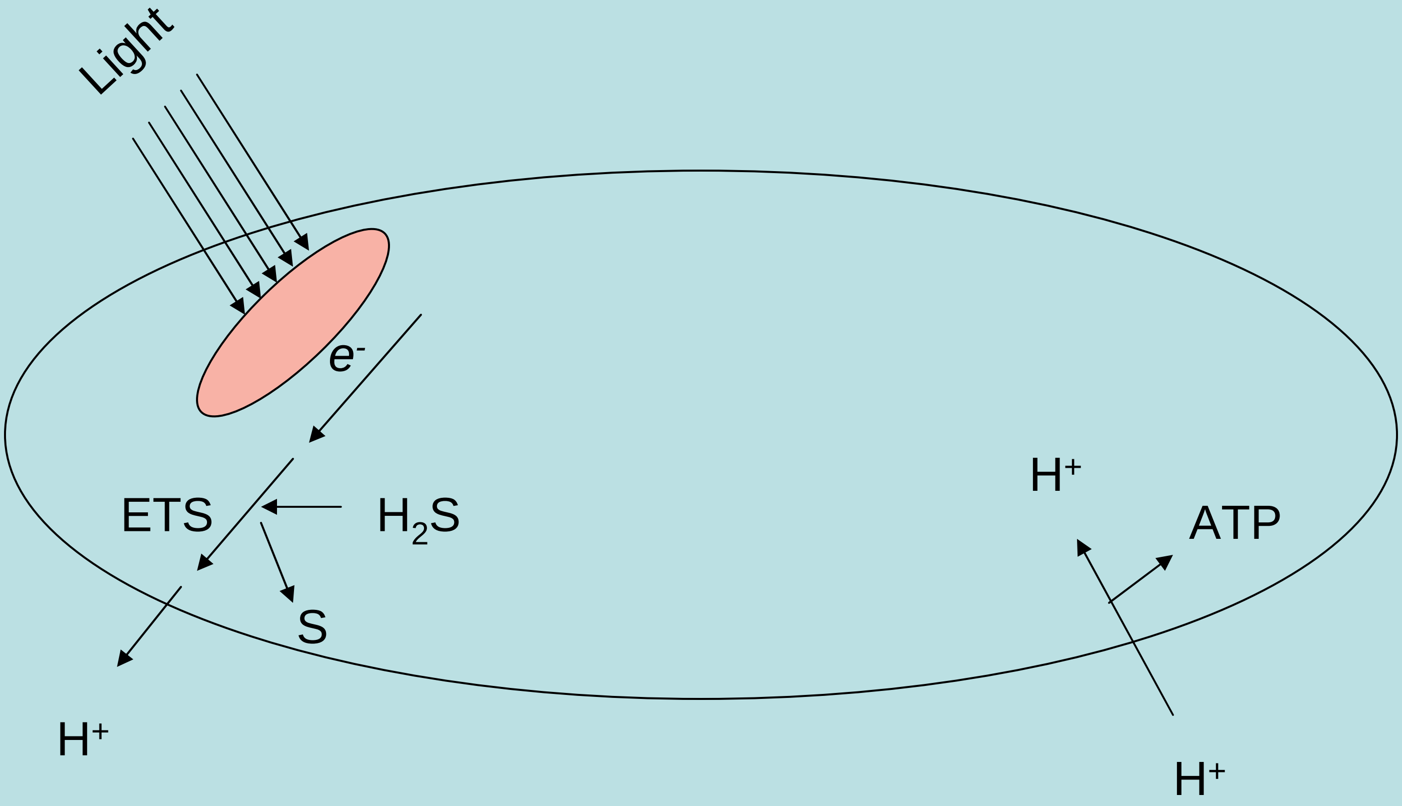
Phototrophic Metabolism

Oxygenic photosynthesis occurs in the bacterial membrane and uses light to strip electrons off of H_2O, to create molecular oxygen and a hydrogen ion gradient.



Phototrophic Metabolism

Anoxygenic photosynthesis is similar to oxygenic photosynthesis but uses light to strip electrons off of donor chemicals such as H_2S, organic acids, or H_2 to create a hydrogen ion gradient.



Carbon for Growth

Two ways to get it:

1. Assimilate organic carbon sources such as sugars, organic acids and alcohols.

   Bacteria that do this are called heterotrophs.

2. Fix CO_2 into simple sugars and convert these into all the different things you need.

   Bacteria that do this are called autotrophs.

Energy Production

Chemotrophs

Fermentation

(Carbohydrates $\longrightarrow$ acids and alcohols)

Aerobic Respiration

(Carbohydrates $\longrightarrow$ $CO_2 + H_2O$)

Anaerobic Respiration

(Carbohydrates $\longrightarrow$ $CO_2 + H_2S$)

Phototrophs

Oxygenic ($H_2O \longrightarrow O_2 + H^+$)

Anoxygenic ($H_2S \longrightarrow S + H^+$)

Carbon Assimilation

Heterotrophs

Obtain carbon form complex molicules (sugars, alcohols, proteins, etc.) and convert them as needed.

Autotrophs

Fix carbon from CO_2 and make all compounds needed for growth.

Combinations

Chemo-Autotroph

Energy from chemicals Carbon from CO_2

Chemo-Heterotroph

Energy from chemicals Carbon from Complex chemicals

Photo-Autotroph

Energy from sun Carbon from CO_2

Photo-Heterotroph

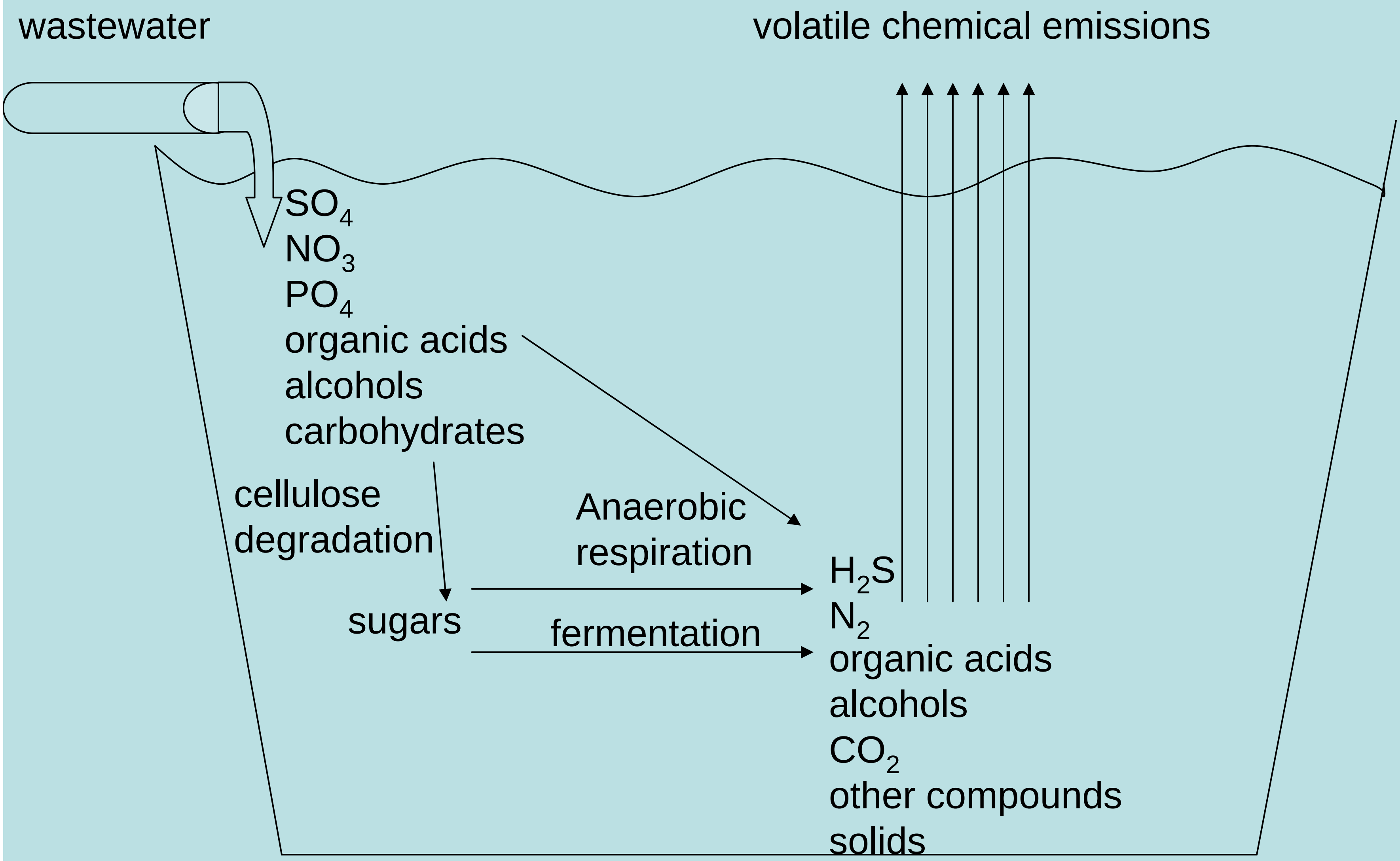
Energy from sun Carbon from Complex Chemicals

Note: Many bacteria can do more than one combination depending on the environment!!!!!!

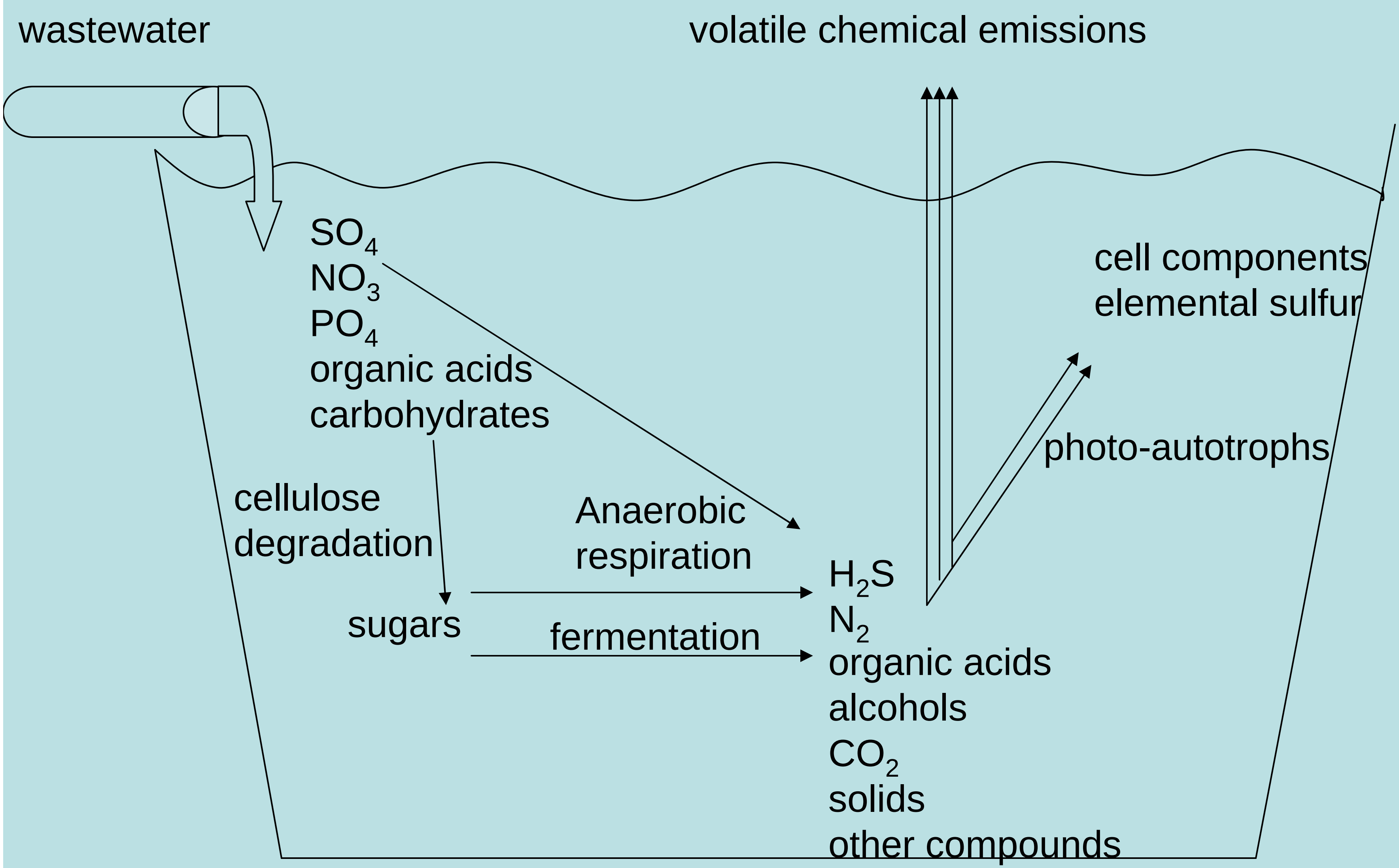
So what does any of this have to do with wastewater lagoons?

- Wastewater lagoons contain chemotrophs, phototrophs, autotrophs, and heterotrophs
- Complex interactions occur between these bacteria
- Changing the levels of these types of bacteria can alter the biochemistry of the lagoons dramatically

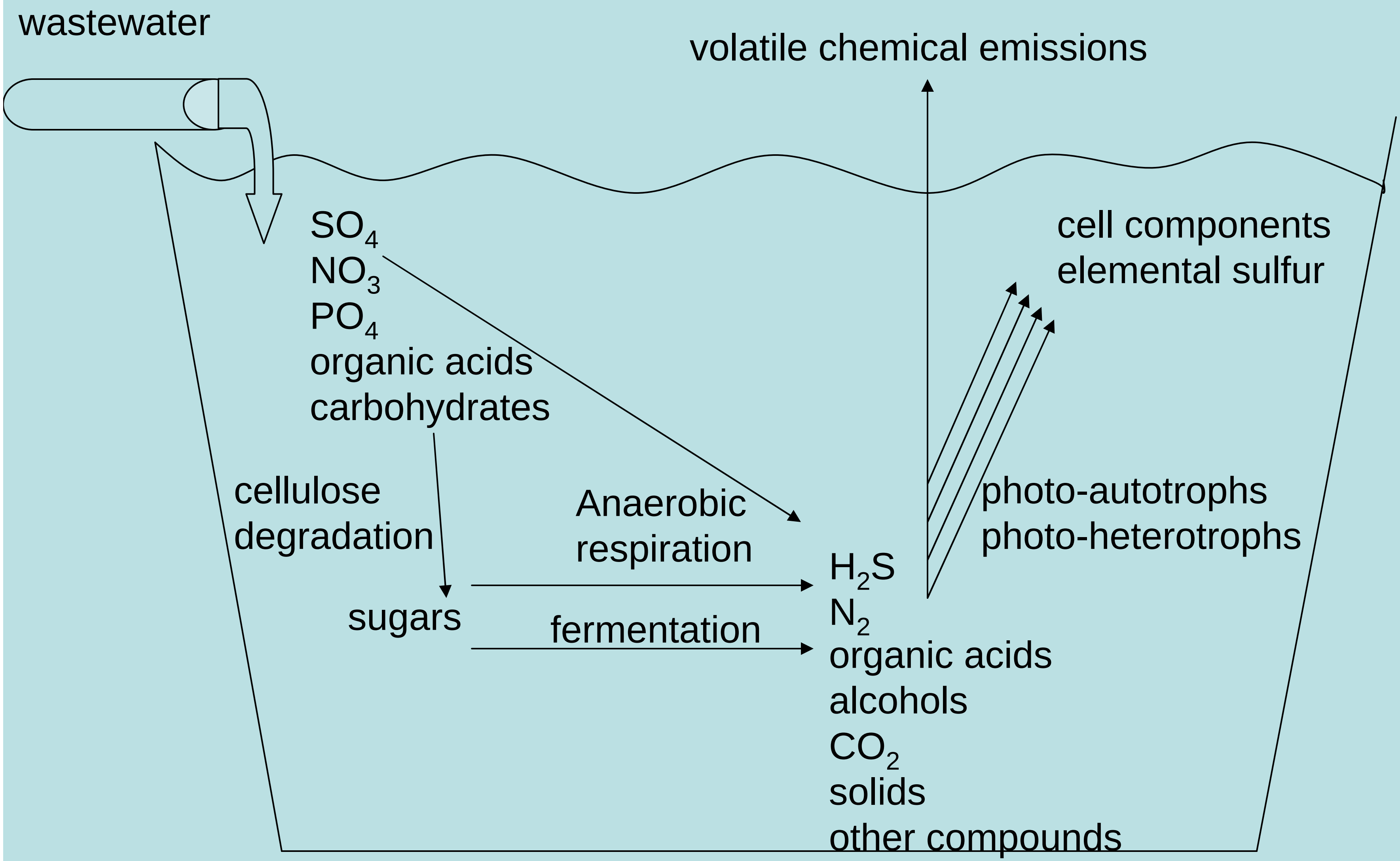
Biology of wastewater lagoons with only chemo-heterotrophs



Biology of Wastewater Lagoons with chemo-heterotrophs and photo-autotrophs



Biology of wastewater lagoons with chemo-heterotrophs, photo-autotrophs and photo-heterotrophs



Two Farms With Different Waste Water Treatments

Circulated Lagoon

Stagnant Lagoon

Farms chosen because of similarities in the following

- Number of Cows
- Type of Cows
- Lagoon Volume
- Animal Diet
- Geographic Location

Gross Microbiological and Chemical Analysis

	Stagnant	Circulated
APC	$5.9X10^5$	$1.3X10^6$
AnPC	$2.6X10^5$	$2.3X10^5$
CPC	$7.9X10^2$	$6.8X10^2$
Chem. Sp.	Conc.	Conc.
TKN	303 mg/L	255 mg/L
NH_3	229 mg/L	195 mg/L
NO_3	< 0.5 mg/L	< 0.5 mg/L
NO_2	< 0.5 mg/L	< 0.5 mg/L
SO_4	71.7 ppm	56.7 ppm
P	47.7 ppm	39.4 ppm
BOD	314 mg/L	589 mg/L
COD	2040 mg/L	2413 mg/L
Na	6.4 mM	6.2 mM
pH	7.4	7.5
Dis. Solids	3646 ppm	2735 ppm

Phyla of Bacteria

Phylum	% of Total Clones Sequenced	
	Stagnant	Circulated
Unknown	33	32
Firmicutes	26	27
Proteobacteria	15	31
Actinobacteria	3	2
Bacteroidetes	7	3
Spirochetes	2	0.3
Chloroflexi	7	0.2
Verrucomicrobia	2	1.5
Other	5	3

Proteobacteria

Class	% of Total Clones Sequenced	
	Stagnant	Circulated
α	0.8	0.5
β	3.1	2.9
γ	5.1	23.2
δ	6.0	3.8
ε	0.3	0.8
Unknown	0.1	0.2

Major differences in bacterial types

Chromatiaceae (γ *Proteobacteria* “purple sulfur bacteria”) were 4.6-fold more numerous in the circulated lagoon

Chloroflexi (green nonsulfur bacteria) were 5.4-fold more numerous in the stagnant lagoon

Twenty Most Prevalent OTUs

Stagnant

OTU	Most Similar In GenBank	# Clones
S1	AY218558	32
S2	AY438769	32
S3	AJ009469	26
S4	*Clostridium lituseburense*	23
S5	AY438715	20
S6	*Achromatium sp.* HK15	19
S7	AF050542	17
S8	AF050627	17
S9	U81676	17
S10	*Cytophaga sp.*	14
S11	AF050534	14
S12	AF050570	13
S13	AJ278162	13
S14	U81706	12
S15	AY438835	11
S16	AF050628	10
S17	AJ278163	10
S18	Lachnospiraceae 19gly4	9
S19	AF001698	9
S20	AF050534	9

Circulated

OTU	Most Similar In GenBank	# Clones
C1	*Achromatium sp.* HK15	109
C2	*Clostridium lituseburense*	58
C3	*Pseudomonas anguilliseptica*	56
C4	UBZ93999	53
C5	AY438769	32
C6	*Thiolamprovum pedioforme*	29
C7	AY438880	28
C8	U81676	28
C9	*Eubacterium tenue*	20
C10	AY438948	20
C11	U81706	21
C12	*Rhabdochromatium marinum*	18
C13	*Desulfobulbus sp.* RPf35L17	12
C14	AY438835	12
C15	AF050627	10
C16	AF050628	10
C17	U81676	10
C18	AY438730	9
C19	*Aquaspirillum metamorphum*	7
C20	*Arcobacter cryaerophilus*	7

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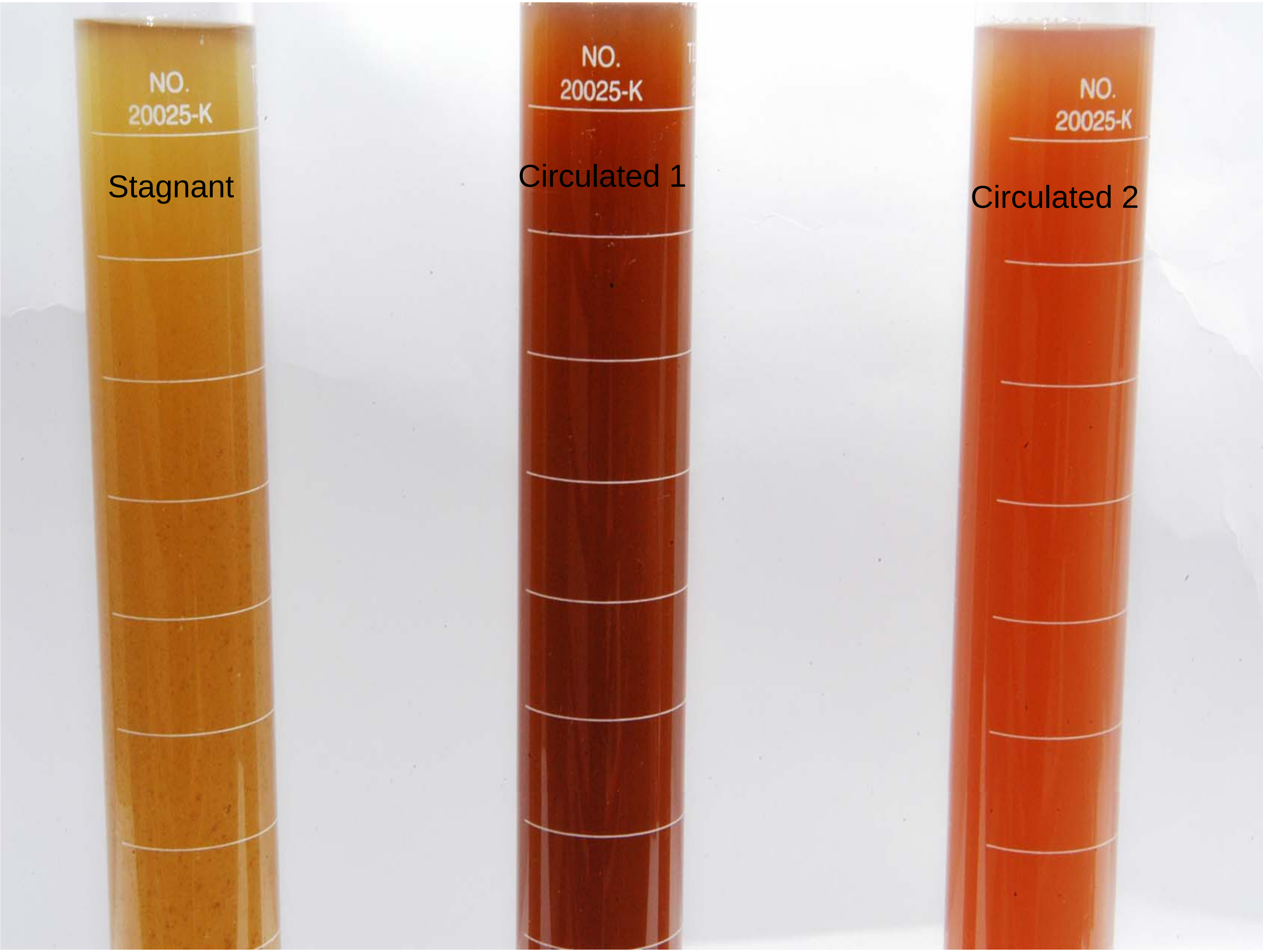
Phototrophautrotrophs Photoheterotrophs Chemoautotrophs Chemoheterotrophs Unknown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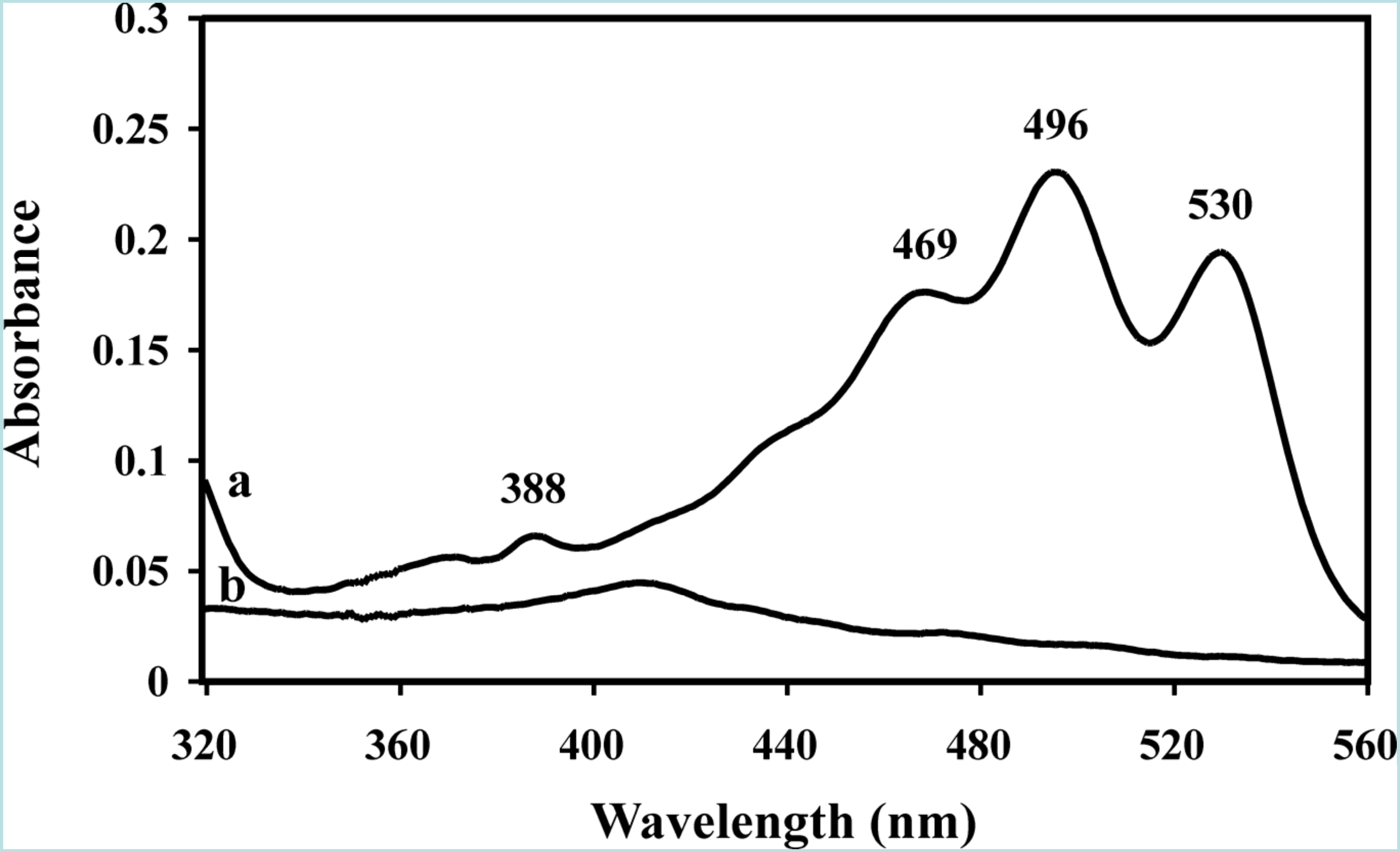
Stagnant
NO.
20025-K
Circulated 1
NO.
20025-K
Circulated 2



0.3
0.25
0.2
0.15
0.1
0.05
0
Absorbance
320
360
400
440
480
520
560
Wavelength (nm)
a
b
388
469
496
530

Conclusions

- Gross chemical and biological parameters are similar in both systems.
- Bacterial populations are similar in both systems but circulated lagoons select for photosynthetic *Proteobacteria* (purple sulfur bacteria) while stagnant lagoons favor *Chloroflexi* (green non-sulfur bacteria)
- Stagnant lagoons have greater bacterial diversity than circulated lagoons. Indicative of less treatment?

Future and Ongoing Studies

- How does aerobic and anaerobic pretreatment of waste effect the bacterial composition and VOC emissions from wastewater lagoons?

- How does surface aeration of wastewater lagoons effect the microbiology and VOC emissions from wastewater lagoons?

- How does circulation or stagnation effect VOC emissions from dairy wastewater lagoons?

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