MANAGEMENT OF NITROGEN CYCLING AND MICROBIAL POPULATIONS IN BIOFLOC-BASED AQUACULTURE SYSTEMS

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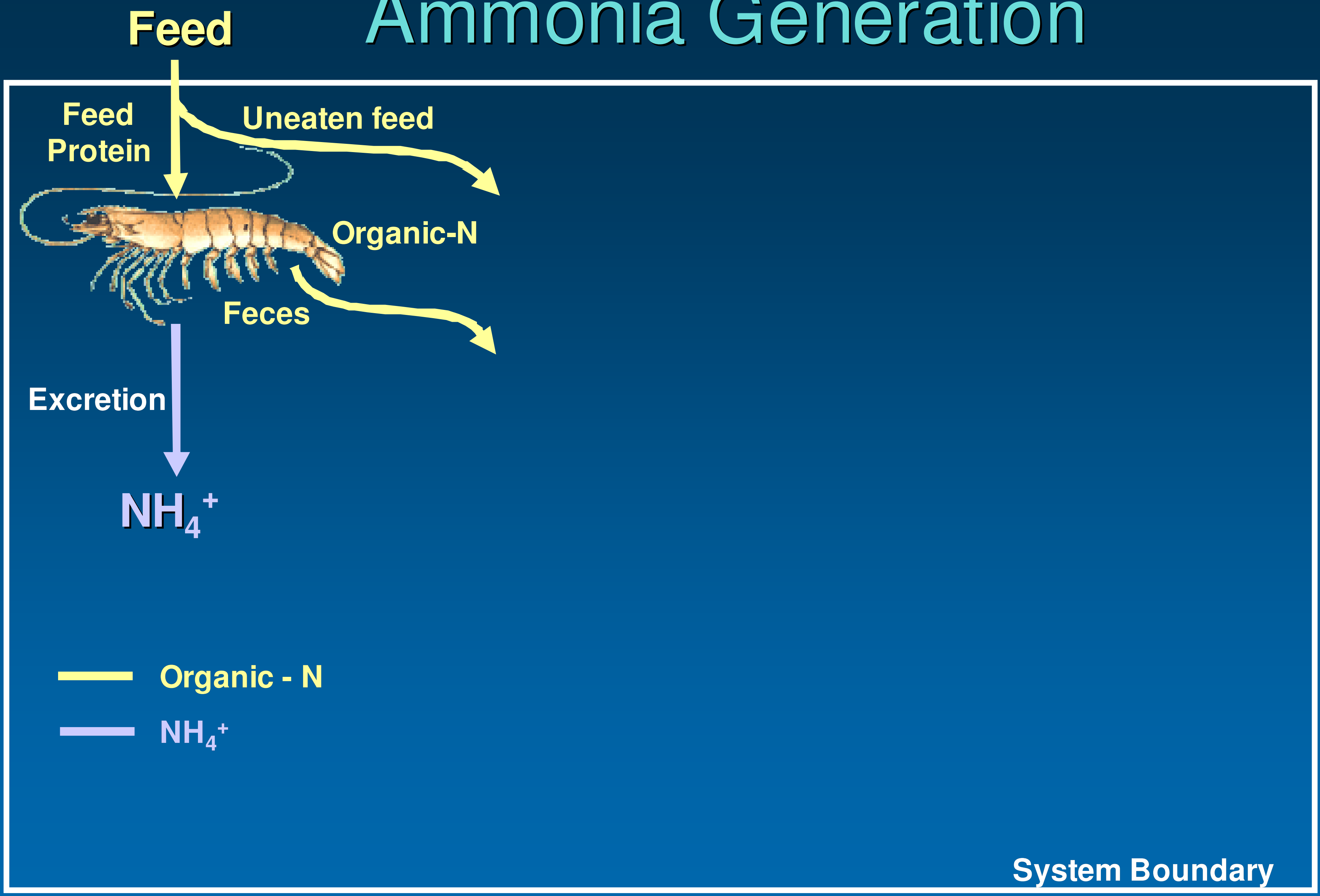
The Nitrogen Syndrome

- Feed protein is inefficiently utilized by aquatic organisms.
  - Shrimp protein utilization efficiency ≈ 20%
  - Fish protein utilization efficiency ≈ 25%
- 70-80% of the nitrogen in feed is converted into ammonia either by direct excretion or mineralization by bacteria

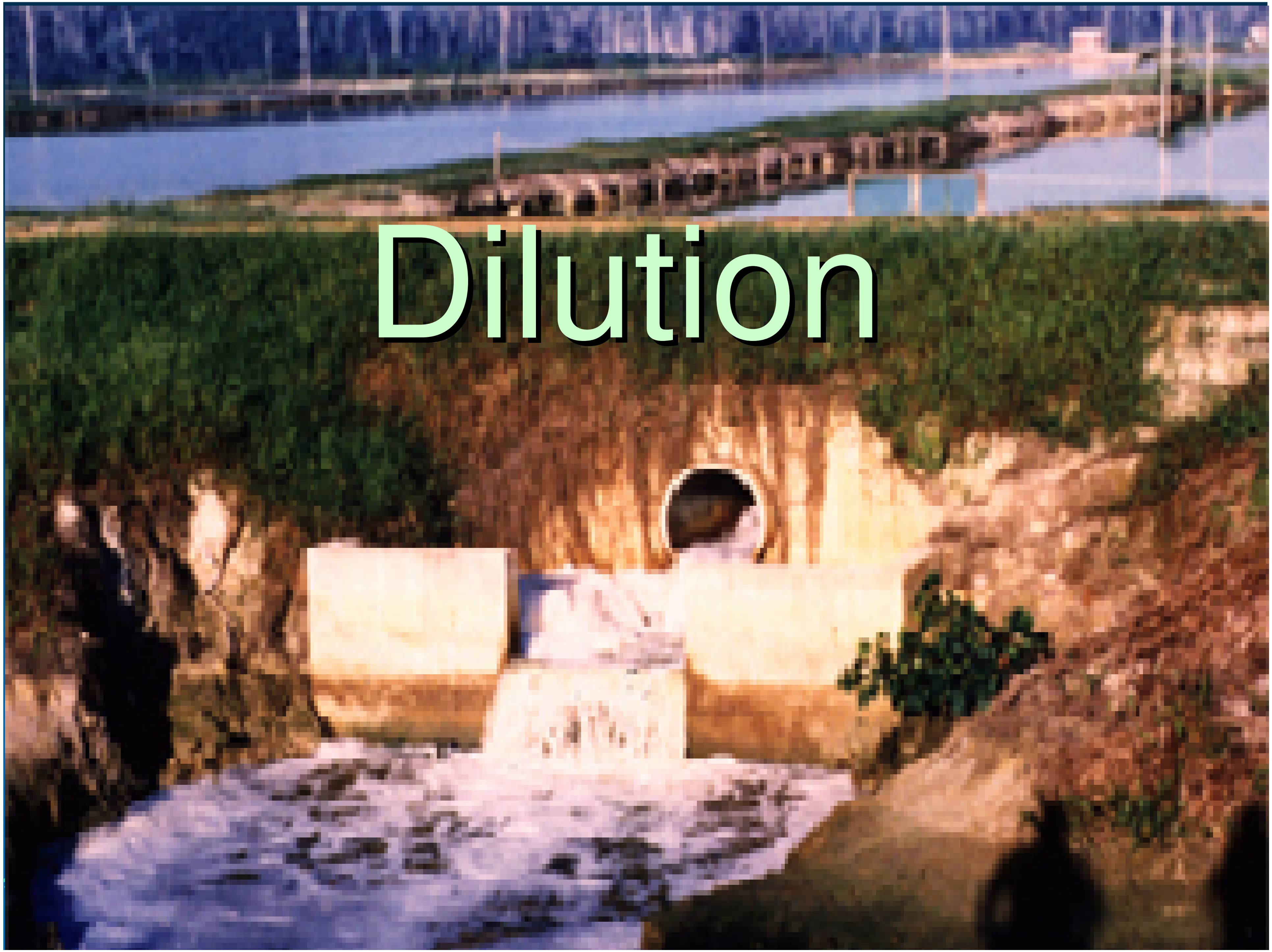
Ammonia Generation

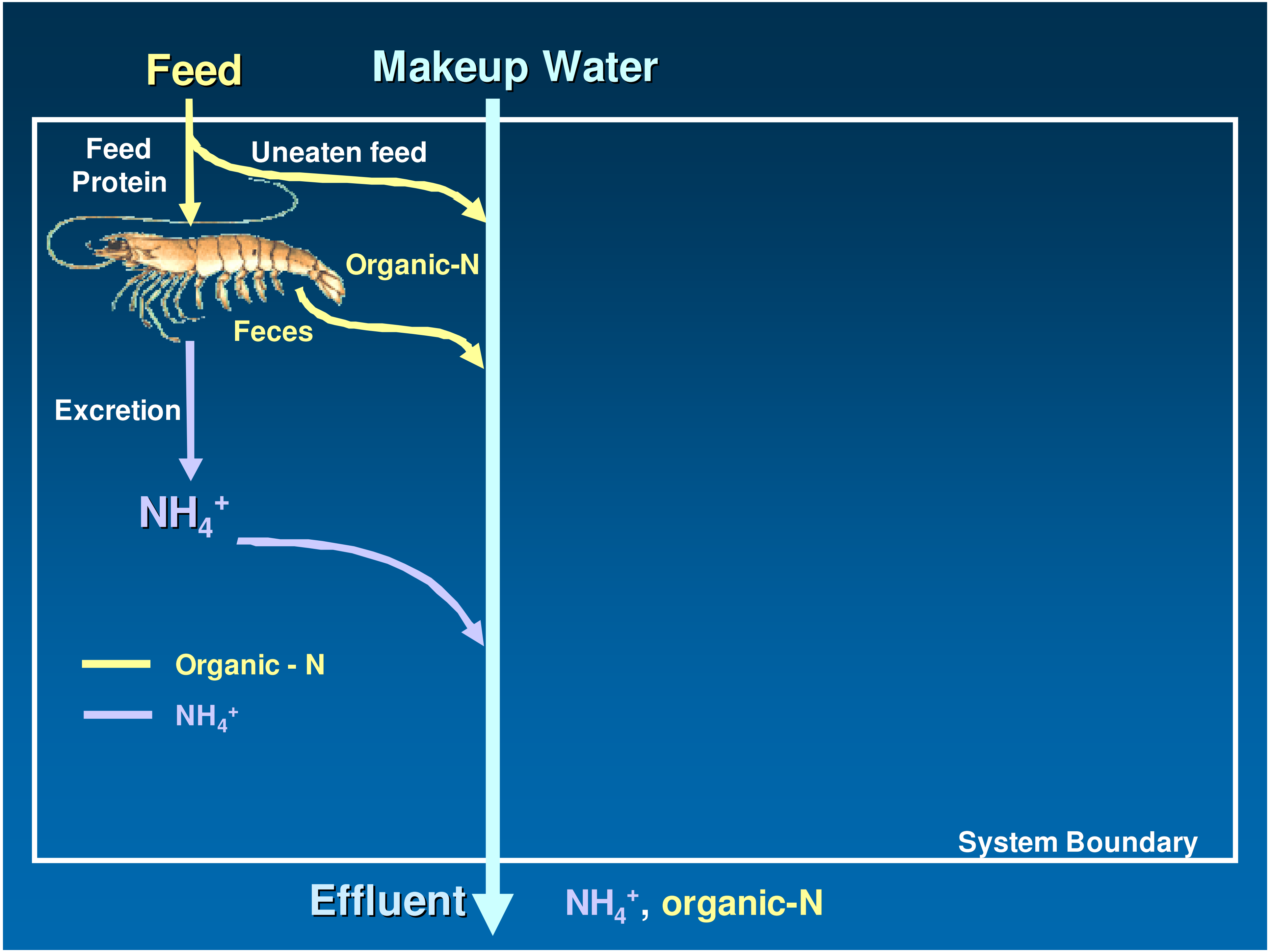


Mechanisms for Removal of Nitrogenous Wastes

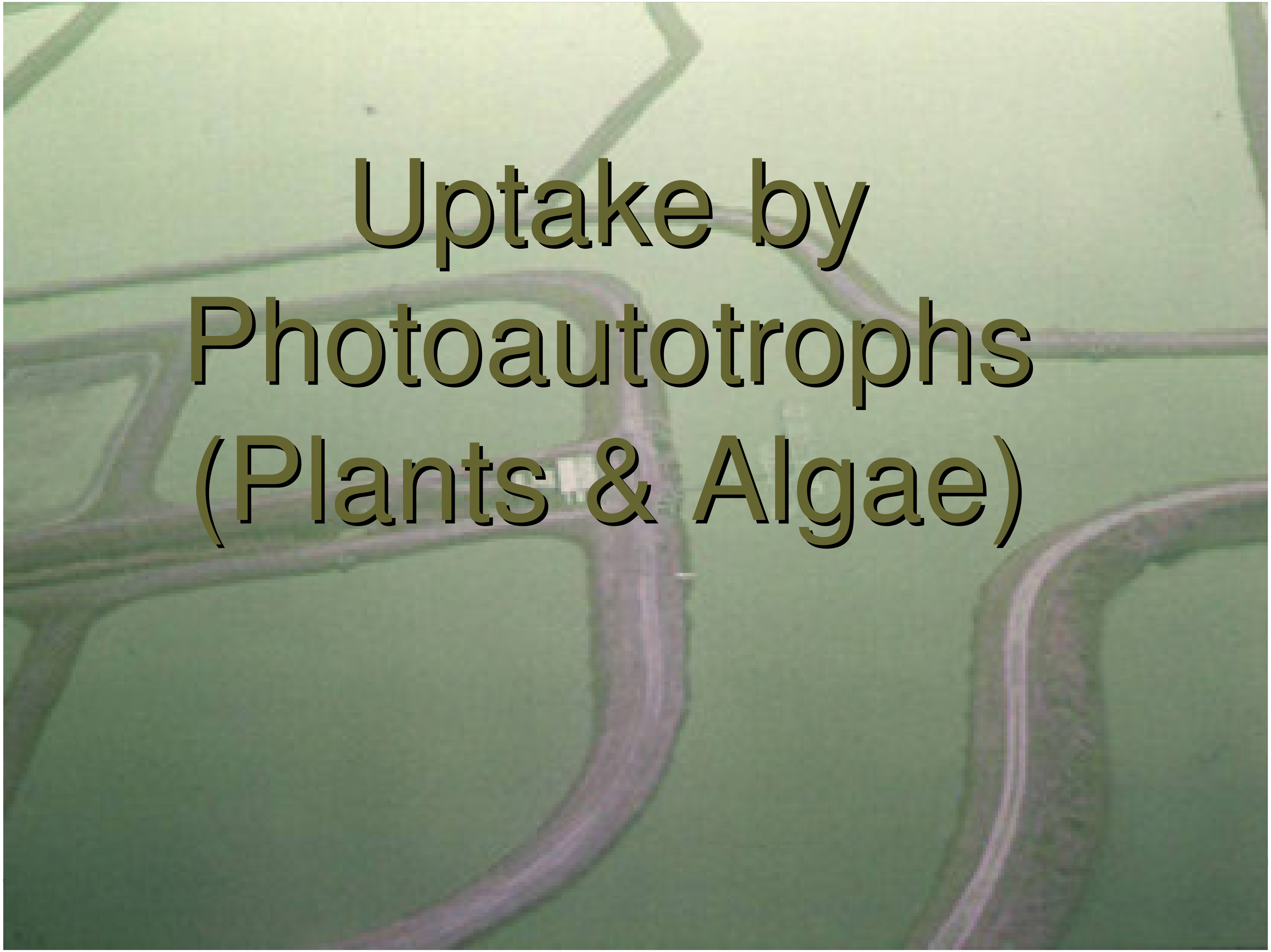
- Dilution
- Plant and algal uptake
- Nitrification by autotrophic bacteria
- Assimilation by heterotrophic bacteria

Dilution

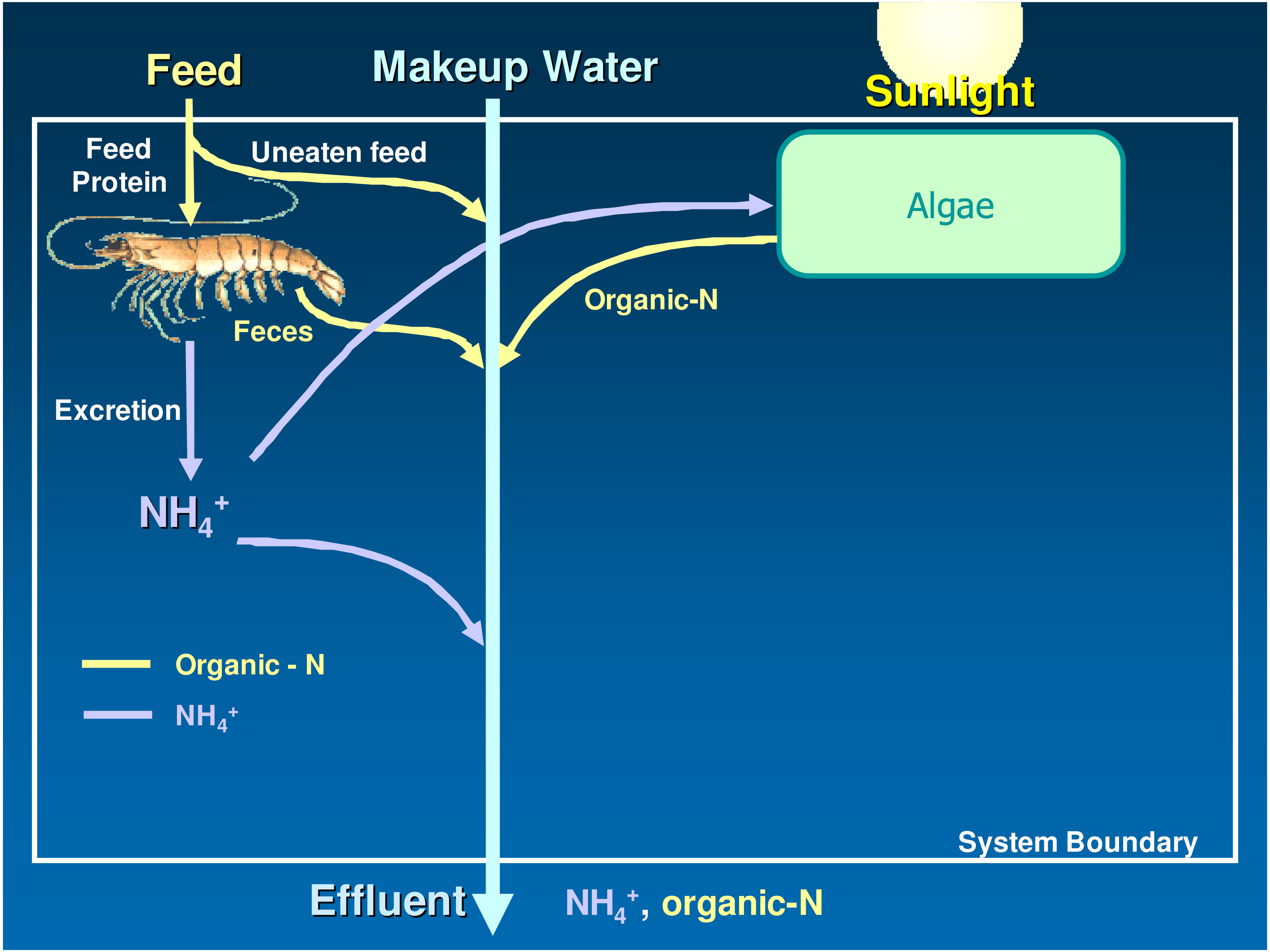




Feed
Makeup Water
Feed Protein
Uneaten feed
Organic-N
Feces
Excretion
NH_4^+
Organic - N
NH_4^+
System Boundary
Effluent
NH_4^+, organic-N

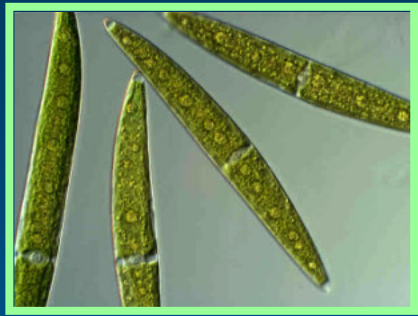


Uptake by Photoautotrophs (Plants & Algae)


Feed
Makeup Water
Sunlight
Feed
Protein
Uneaten feed
Algae
Organic-N
Feces
Excretion
NH_4^+
Organic - N
NH_4^+
System Boundary
Effluent
NH_4^+, organic-N

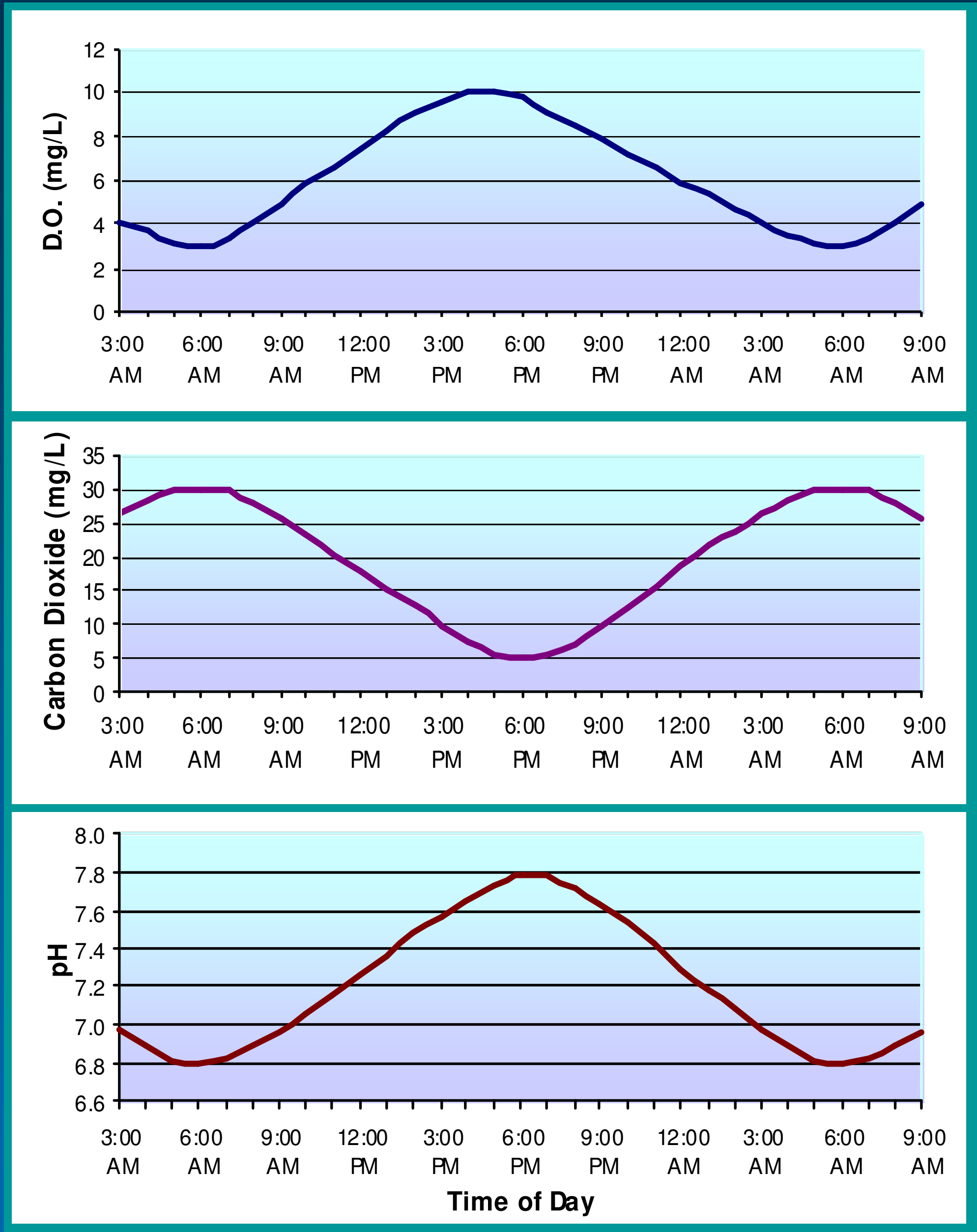
Nutritional Value of Algae

- Principle advantage of an algae-based production system is the nutritional value of phytoplankton
  - HUFAs
  - Carotenoids & other pigments

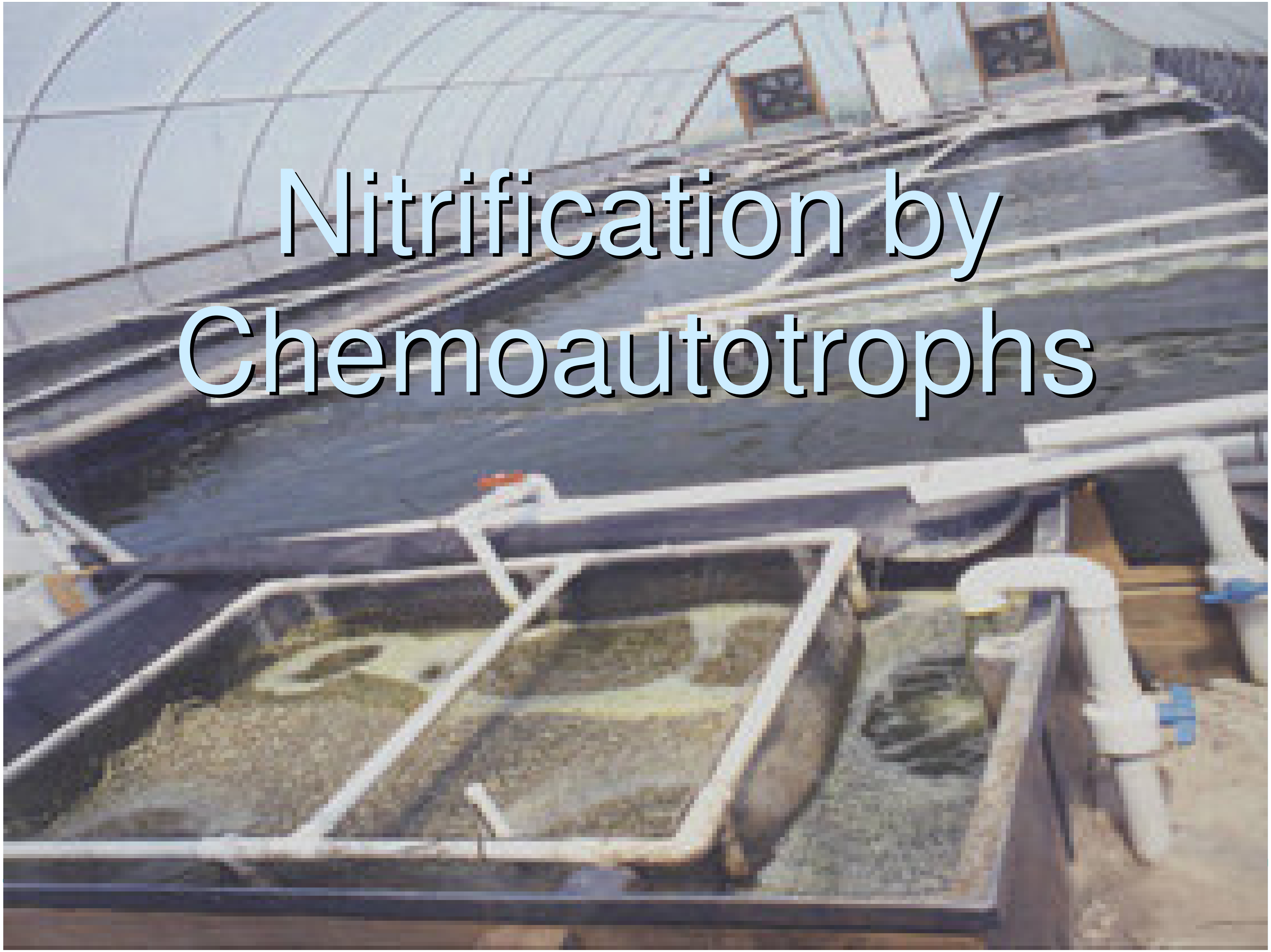


Diurnal Swings in Water Quality

- Algal-dominated systems are characterized by diurnal swings in water quality
  - Dissolved Oxygen
  - Carbon Dioxide
  - pH
  - Unionized ammonia
- Stressful for fish & shrimp
- Can affect growth & survival



Nitrification by Chemoautotrophs



Nitrification

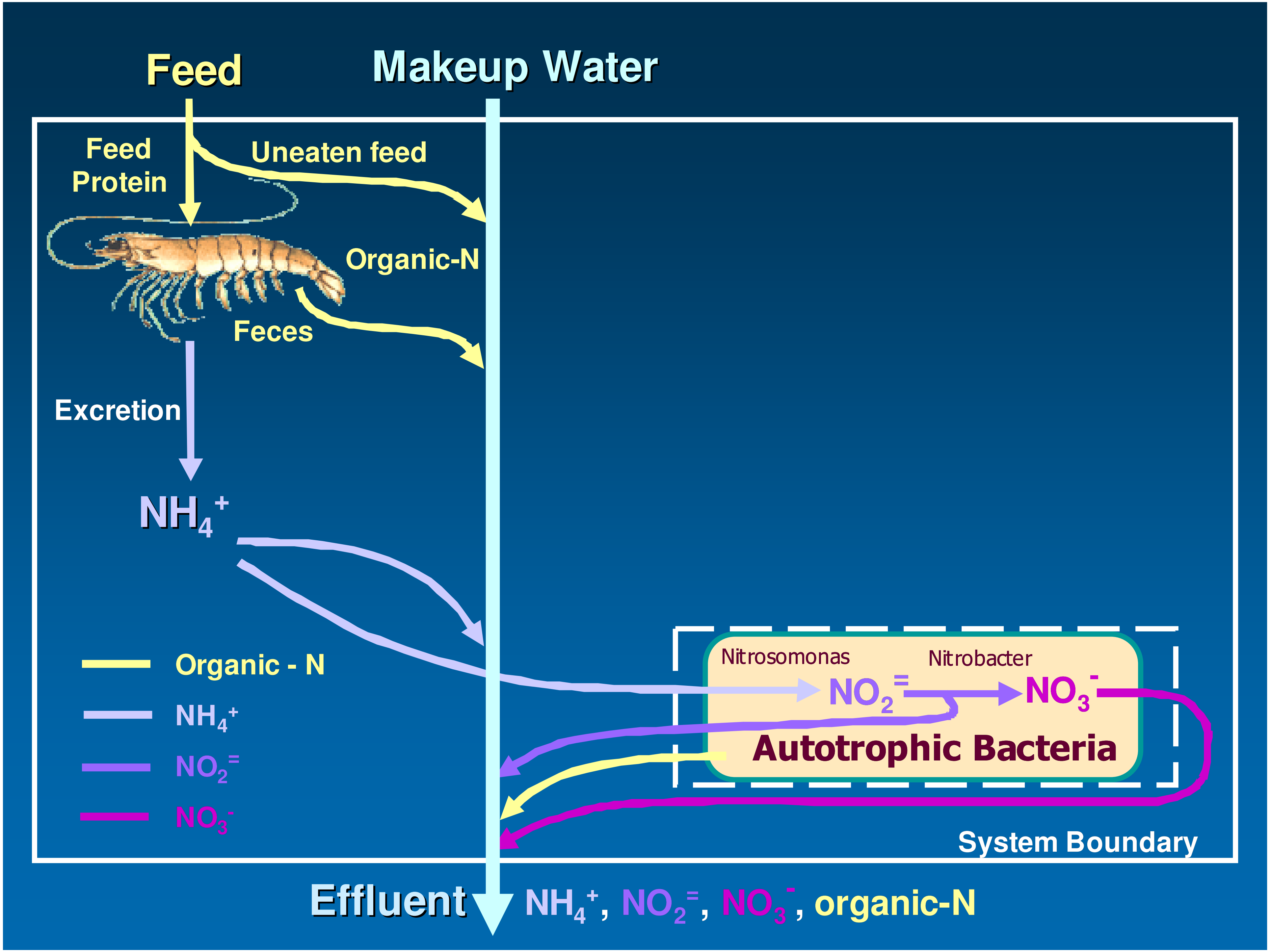
Nitrification is the two step process in which ammonia is oxidized first to nitrite and then to nitrate by two different microbial populations.

$$NH_4^+ + 1.5O_2 \xrightarrow{\textit{Nitrosomonas}} 2H^+ + H_2O + NO_2^-$$

ammonium ion → nitrite

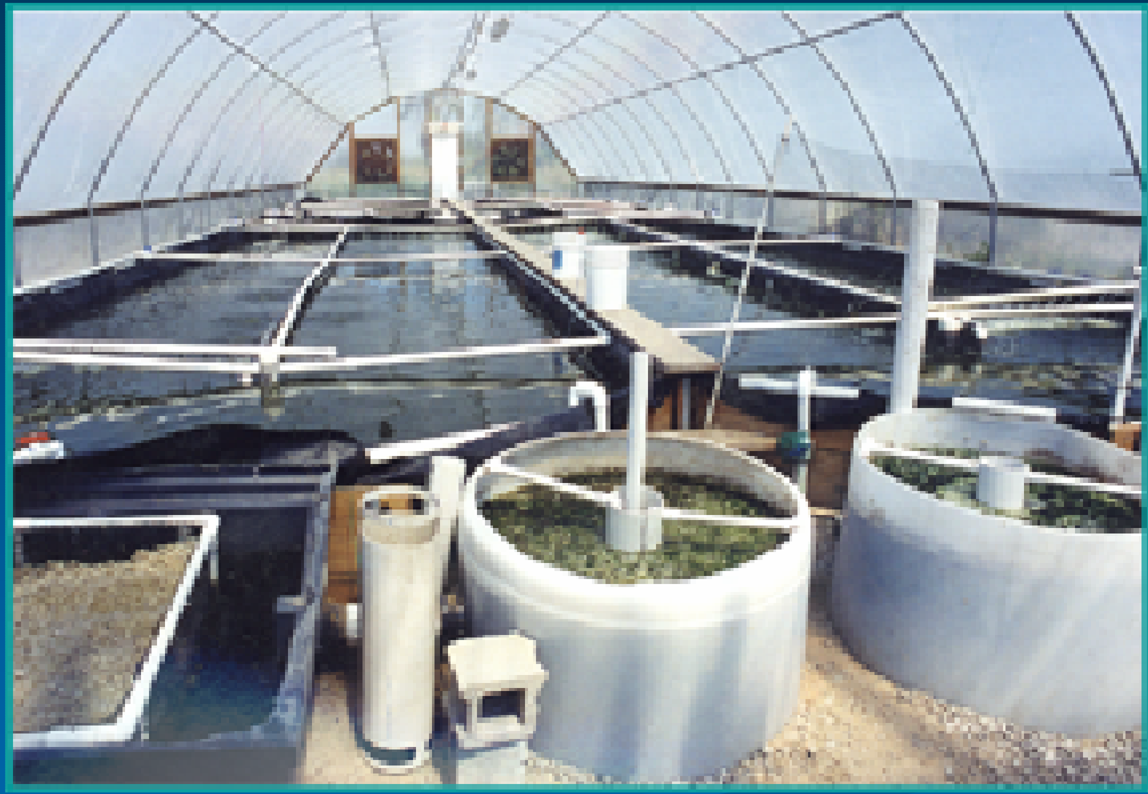
$$NO_2^- + 0.5O_2 \xrightarrow{\textit{Nitrobacter}} NO_3^=$$

nitrite → nitrate

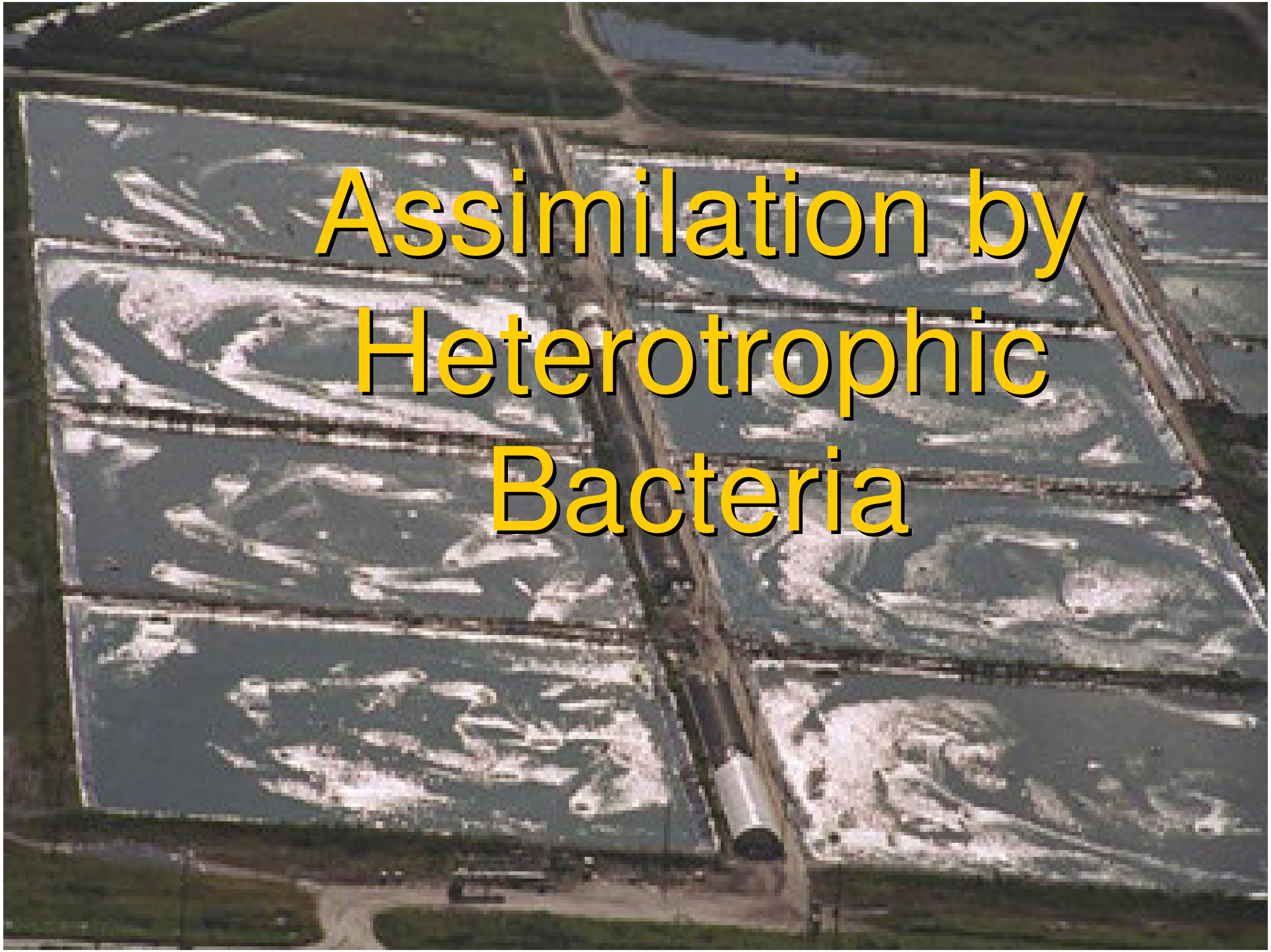

Feed
Makeup Water
Feed
Protein
Uneaten feed
Organic-N
Feces
Excretion
NH4+
Organic - N
NH4+
NO2=
NO3-
Nitrosomonas
Nitrobacter
NO2=
NO3-
Autotrophic Bacteria
System Boundary
Effluent
NH4+, NO2=, NO3-, organic-N

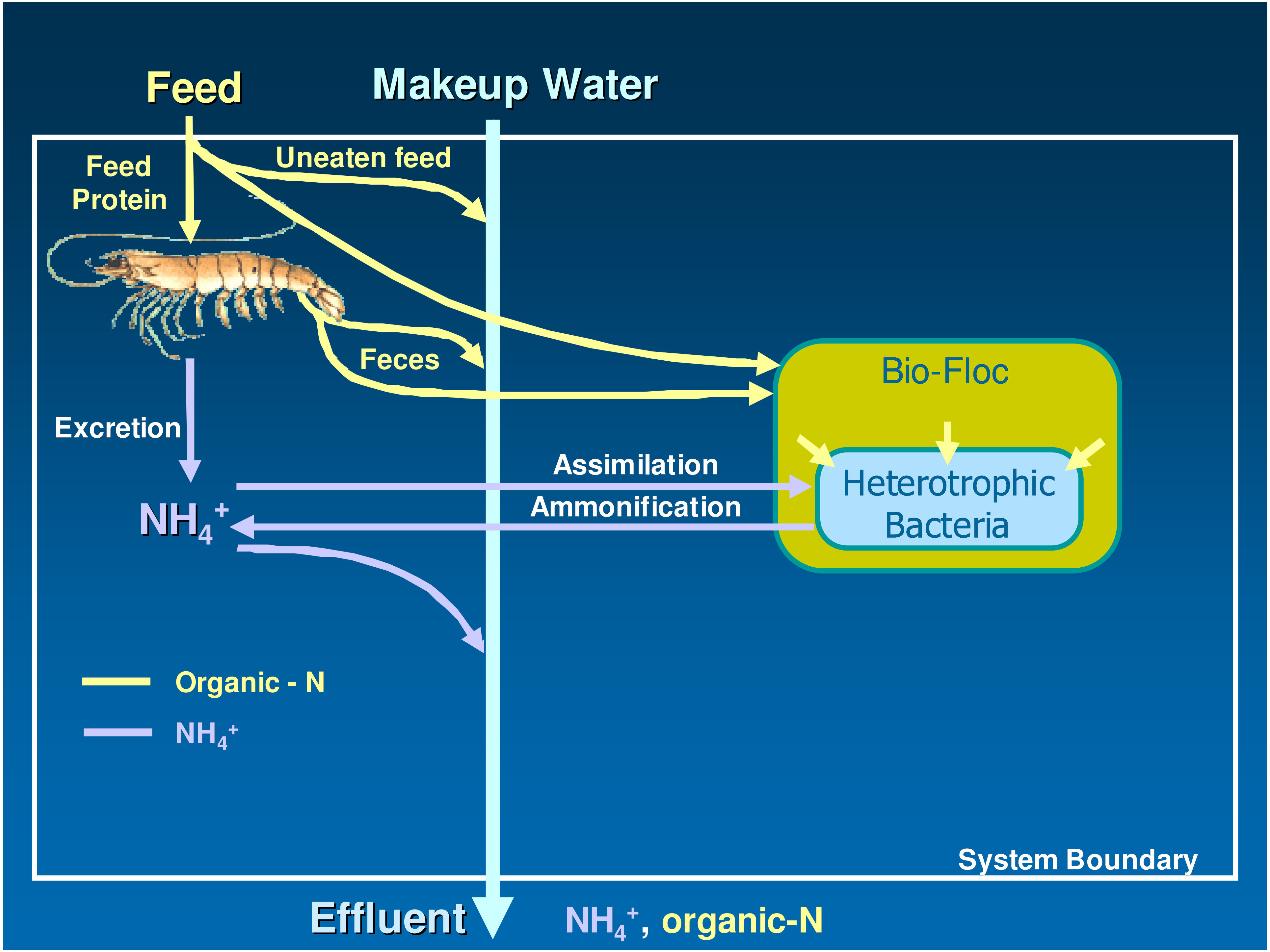
Autotrophic System Requirements

- Provision of large amount of surface area for nitrifying bacteria
- Rapid & efficient removal of suspended solids
- Chemical supplementation with bicarbonate



Assimilation by Heterotrophic Bacteria





Feed
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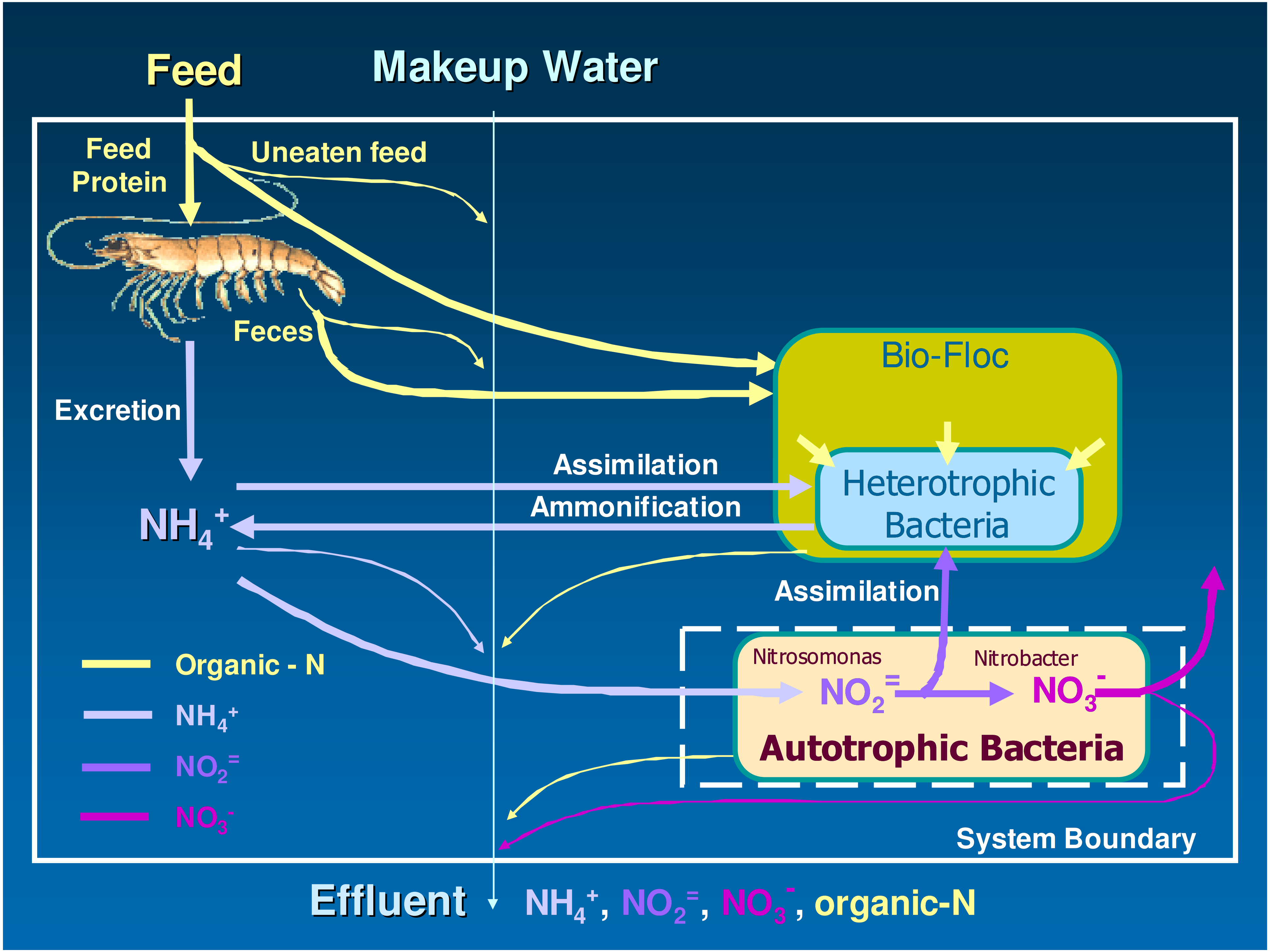
Feces
Excretion
Bio-Floc
Assimilation
Ammonification
Heterotrophic Bacteria
NH_4^+
Organic - N
NH_4^+
System Boundary
Effluent
NH_4^+, organic-N

Effect of C/N Ratio on Nitrogen Utilization by Heterotrophic Bacteria

- C/N Ratio < 10
  - Organic nitrogen sources used preferentially
  - Ammonification of nitrogen leads to increase in ammonia
- C/N Ratio >10
  - Both organic and inorganic sources utilized
  - Net consumption of ammonia

Hybrid Production Systems

- In reality, no system is purely autotrophic, heterotrophic, or photoautotrophic
- Composition of the microbial community determined by a variety of factors, including:
  - light intensity
  - C/N ratio of feed inputs
  - rate of solids removal
  - surface area available for colonization by nitrifiers
  - bicarbonate alkalinity
- By controlling these factors one can control which of these groups is dominant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Nitrosomonas
Nitrobacter
$NO_2^=$
NO_3^-
Autotrophic Bacteria
Organic - N
NH_4^+
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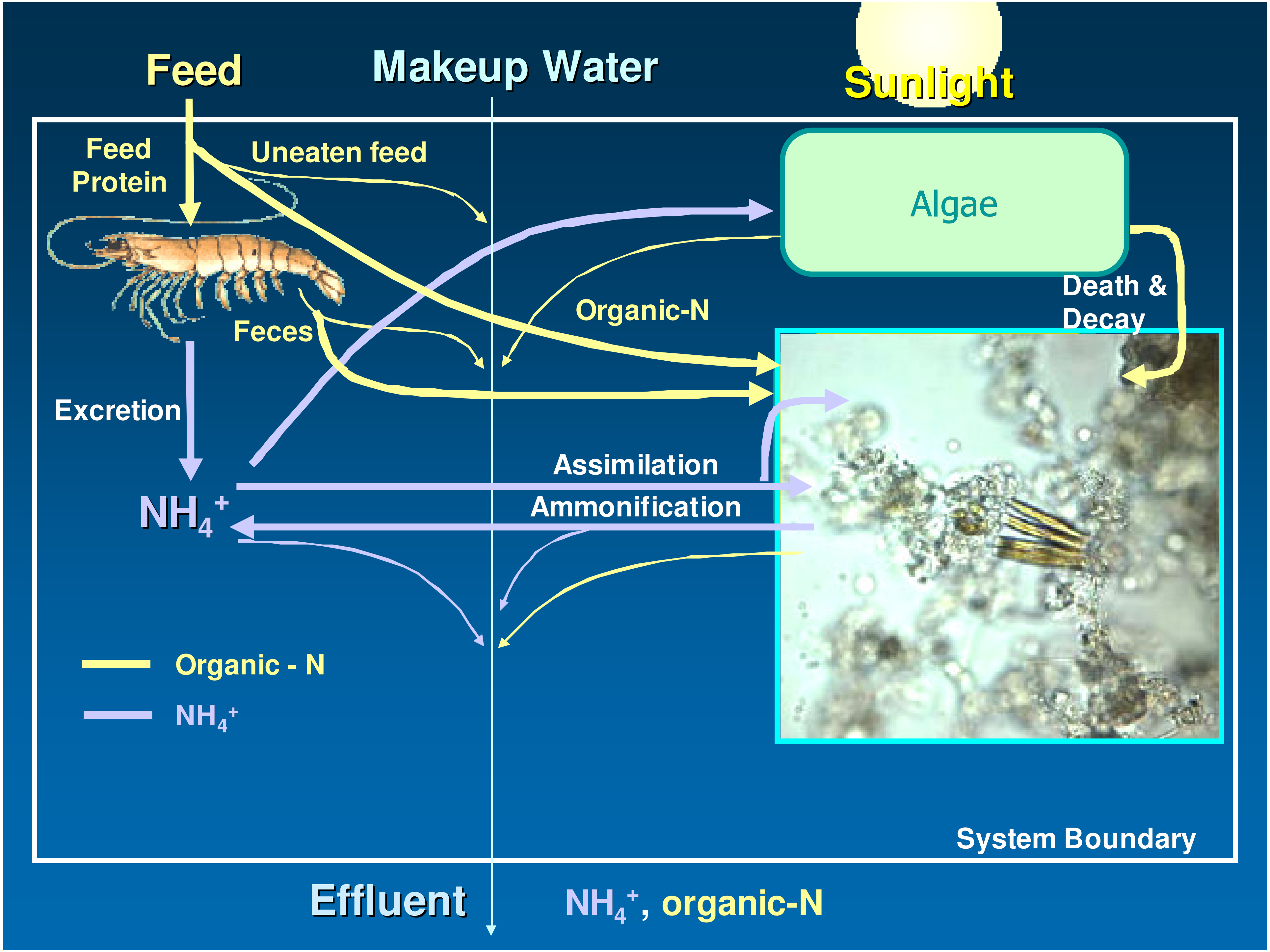
System Boundary
Effluent
NH_4^+, $NO_2^=$, NO_3^-, organic-N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Death & Decay
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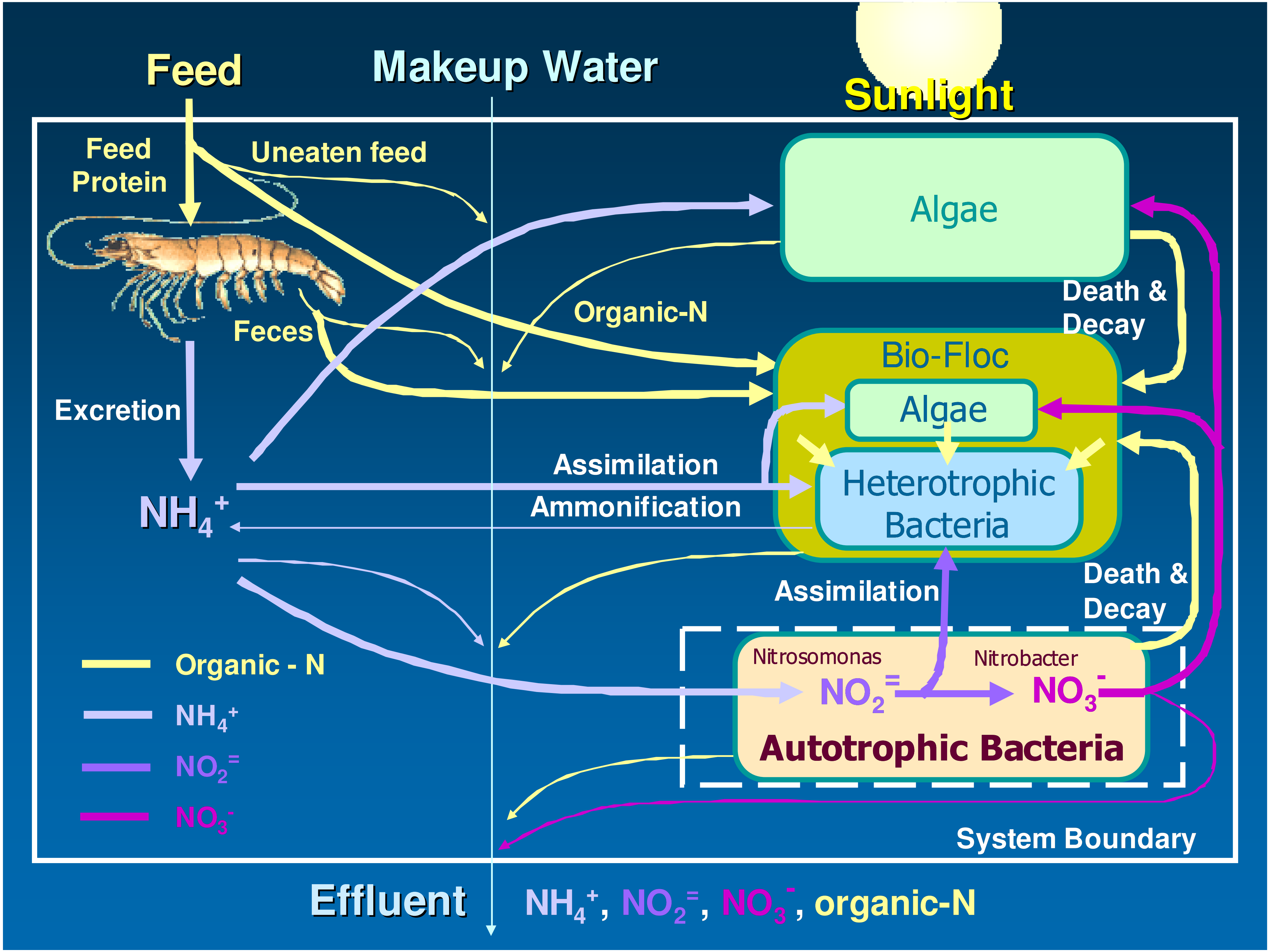
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NH_4^+, $NO_2^=$, NO_3^-, organic-N

How to Promote Biofloc Development

- Reduce water exchange to near zero
- Aerate heavily
- Increase C/N ratio of feed inputs

Strategies for Increasing C/N Ratios

- **Reduce protein & increase carbohydrate content of feed**

Feed Protein Content	C/N Ratio
35%	8.9
30%	10.4
25%	12.5

- **Supplementing the regular feed with carbohydrate source**
  - Sugar or molasses
  - Starchy grains (e.g. corn meal, cassava meal, wheat flour)

Low Protein-High Carbohydrate Feeds

- Feed protein levels in biofloc systems may be 25-40% lower than in conventional systems
  - Lower protein and higher carbohydrate content promotes development of biofloc
  - Protein utilization efficiencies of tilapia and Pacific white shrimp are nearly doubled in biofloc systems
  - FCRs are often in the range of 1.0 – 1.25
- Low protein biofloc diets may need to be fortified with higher levels of vitamins and minerals

Carbohydrate Required for 100% Removal of NH_4-N

- 20 kg of carbohydrate are required for heterotrophic bacteria to utilize 1kg of the ammonia-nitrogen
- The total amount of carbohydrate supplementation required to remove the ammonia-nitrogen generated from a given amount of feed can be calculated using the following relationship:

$$\text{kg CHO} = \text{kg feed} \times \text{kg N/kg feed} \times \text{kg } NH_4\text{-N/kg N} \times 20 \text{ kg CHO/kg } NH_4\text{-N}$$

kg CHO = kg feed x kg N/kg feed x kg NH_4-N/kg N x 20 kg CHO/kg NH_4-N

Example

- **1 kg 35% protein feed contains 0.056 kg N**

  Feed N = 1 kg x 0.35 kg protein/kg feed x 0.16 kg N/kg protein

  = 0.056 kg N/kg feed

- **Assume 50% of N in feed is excreted by shrimp**

  N excretion = 0.056 kg N/kg feed x .5 kg NH_4-N excreted/kg N

  = 0.028 kg NH_4-N excreted/kg feed

- **Assume 20 kg CHO required per kg of NH_4-N.**

  kg CHO = .028 kg NH_4-N excreted/kg feed x 20 kg CHO/kg NH_4-N

  = 0.56 kg CHO required/kg feed

Carbohydrate Supplementation Requirements

Shrimp Feed		100% NH_4-N Assimilation by Heterotrophs	
% protein	Feed C:N Ratio	kg CHO/kg feed	C:N Ratio
35%	8.9	0.56	13.9
30%	10.4	0.48	15.4
25%	12.5	0.40	17.5

Carbohydrate Supplementation Requirements

Shrimp Feed		50% NH_4-N Assimilation by Heterotrophs	
% protein	Feed C:N Ratio	kg CHO/kg feed	Net C:N Ratio
35%	8.9	0.28	11.4
30%	10.4	0.24	12.9
25%	12.5	0.20	15.0

Net Protein Content of Feed + Carbohydrate Supplement

The net protein content of the feed and carbohydrate supplement is calculated as follows:

$$\text{Net PC} = PC_{feed} / (1 + F_{CHO})$$

where,

Net PC = protein content of the feed (fractional basis)

F_{CHO} = kg CHO supplement / kg Feed

Example:

30% protein feed, F_{CHO} = 0.48

Net PC = 0.30 / 1.48 = 0.2027

Calculation of Net C/N Ratios

The net C:N Ratio resulting from carbohydrate supplementation can be calculated:

$$\text{Net C/N} = 0.5 / (\text{Net PC} \times 0.16)$$

<u>Example:</u>

30% Protein Feed, $F_{CHO} = 0.48$

Net PC = 0.30 /1.48 = 0.2027

Net C:N = 0.5 / (0.2027 * 0.16)

= 15.42

Which Carbohydrate Supplement is Best?

<u>Considerations</u>:

- How quickly is the carbohydrate utilized?
  - Simple carbohydrates (e.g. sugar, starch, molasses) are taken up almost immediately.
    - Advantage: Rapid response
    - Disadvantage: Must be added continuously to prevent boom & bust cycles
  - Complex carbohydrates (e.g. cellulose, cassava meal, starchy grains) take time for the bacteria to break down and use
    - Advantage: Stable carbohydrate levels
    - Disadvantage: Slower response times

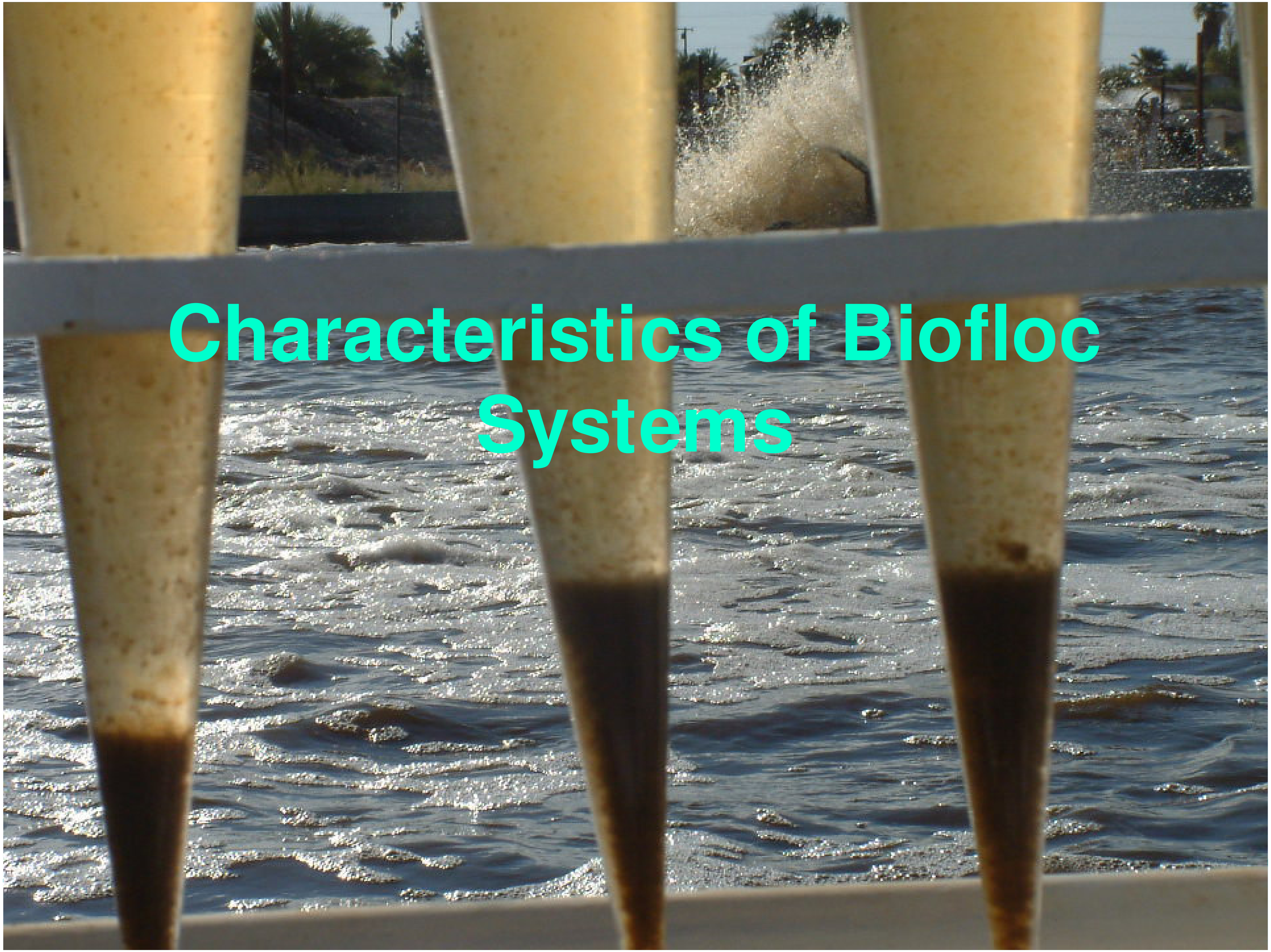
Which Carbohydrate Supplement is Best?

Considerations:

- Protein content:
  - Complex carbohydrates often contain protein, which must be taken into account when calculating carbohydrate requirement
- Digestibility:
  - Slowly digested matter may accumulate in culture vessel
- Cost per unit of carbohydrate

Sources of carbohydrate

- Sugar
- Starch
- Cellulose
- Cassava Meal (1-2 % protein)
- Molasses (6% protein)
- Corn Meal (8% protein)
- Wheat Meal (10% protein)
- Sorghum Meal (11% protein)


Characteristics of Biofloc
Systems

Oxygen Requirements

- Systems with high rates of solids removal
  - Significant BOD exported from the culture system
  - ∴ Lower oxygen requirements within the system

- Biofloc systems
  - Most of BOD retained within the system
  - ∴ Higher oxygen requirements

High Production Rate of Bacterial Biomass

Ammonification of organic nitrogen and assimilation of inorganic nitrogen result in very high rates of production of bacterial biomass (8.1 g VSS/g NH4-N)

- Oxygen requirements very high
- Carbon dioxide production very high
  - High [CO_2] → Low pH
- Volatility
  - Doubling time of heterotrophic bacteria very short (20 minutes - 2 hrs)
  - Water quality parameters can change very quickly in response to changes in nutrient availability

Aeration & Oxygenation

- A key design element for a biofloc-based system is a robust aeration system
- Aeration fulfills 3 functions:
  - Supplies oxygen to water
  - Circulates water to keep bio-floc in suspension
  - De-gases CO_2
- 1.0-1.2 kg O_2/kg feed
- Oxygen supplementation may be necessary when loading rates exceed 4.0 kg/m^2

Excessive TSS leads to instability

- Excessive TSS → O_2 consumption, CO_2 production, & low pH
- High sludge age → Proliferation of protozoans
  - Protozoans feed on heterotrophic bacteria, reducing their numbers → Reduced uptake of ammonia and nitrite
  - Protozoans consume oxygen, and generate ammonia and CO_2
- Increased potential for sludge accumulation

Management of TSS levels

- Characterization of heterotrophic production systems as “zero-exchange” systems is unfortunate, because limited solids removal is necessary for making these systems stable.
- Solids removal & control of sludge age is one most important management tools for managing biofloc-based systems
- More research is necessary to identify ideal TSS concentrations and sludge age

Conclusions

- Nitrogen can be managed by multiple pathways
  - Photoautotrophic
  - Chemoautotrophic
  - Heterotrophic
- Heterotrophic systems promoted by:
  - Managing C/N Ratio of feed inputs (>10:1)
  - Reducing water exchange, solids removal
  - Increasing aeration, water circulation

Design and Management Critical

Design keys

- Adequate aeration or oxygenation
- Good water circulation (eliminate dead spots in the tank)
- Good degassing capability
- Solids removal capability

Management keys

- Manage C/N ratios at minimum need to maintain low TAN and nitrite concentration
- Filter solids as needed to control TSS