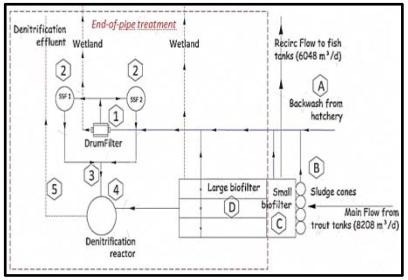
Transforming waste into new resources: optimizing sludge hydrolysis to improve nitrogen removal in aquaculture through denitrification

Carlos Octavio Letelier Gordo

February 2017



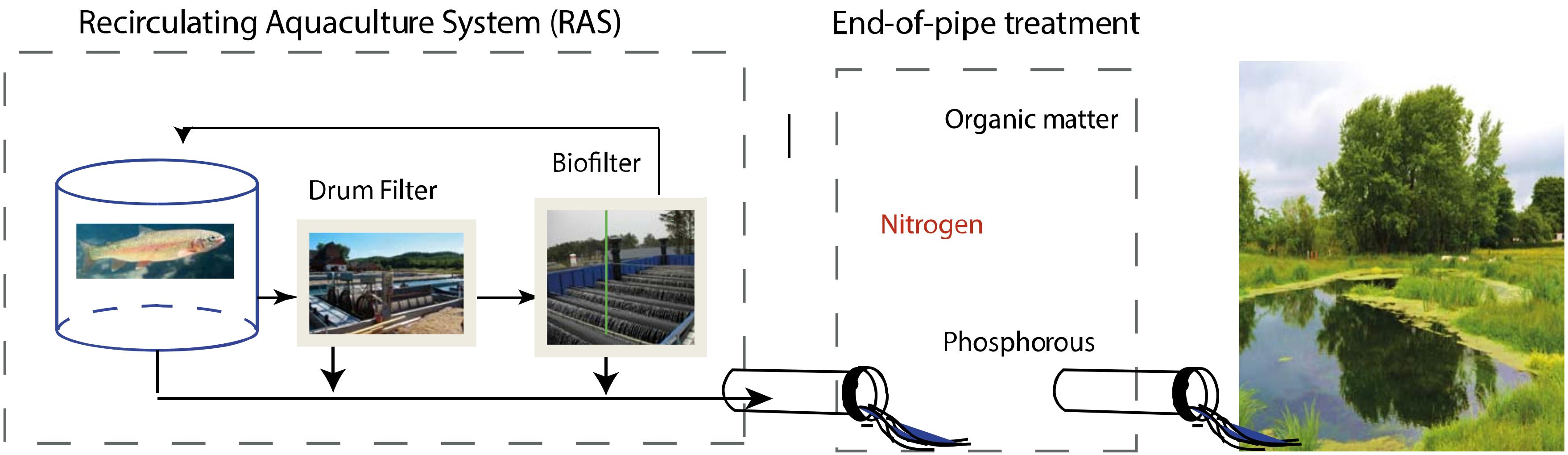
DTU Aqua
National Institute of Aquatic Resources

OUTLINE

1. **INTRODUCTION** TO THE PROBLEM
2. **PART I**: CHARACTERIZING AND DESCRIBING THE HYDROLYSIS AND FERMENTATION PROCESSES OF SOLID WASTE DERIVING FROM TWO DIETARY PROTEIN SOURCES, FISH MEAL AND SOYBEAN MEAL
3. **PART II**: OPTIMIZATION OF THE HYDROLYSIS AND FERMENTATION PROCESSES
4. **PART III**: APPLICABILITY OF INTERNAL CARBON SOURCES FOR DENITRIFICATION ON A DANISH BROOD STOCK FARM (A MASS BALANCE APPROACH).
5. **PART IV**: CONCLUSIONS AND FUTURE PERSPECTIVES

The problem

Environmental regulations has forced the improvement of water treatment technologies (End-of Pipe treatment)



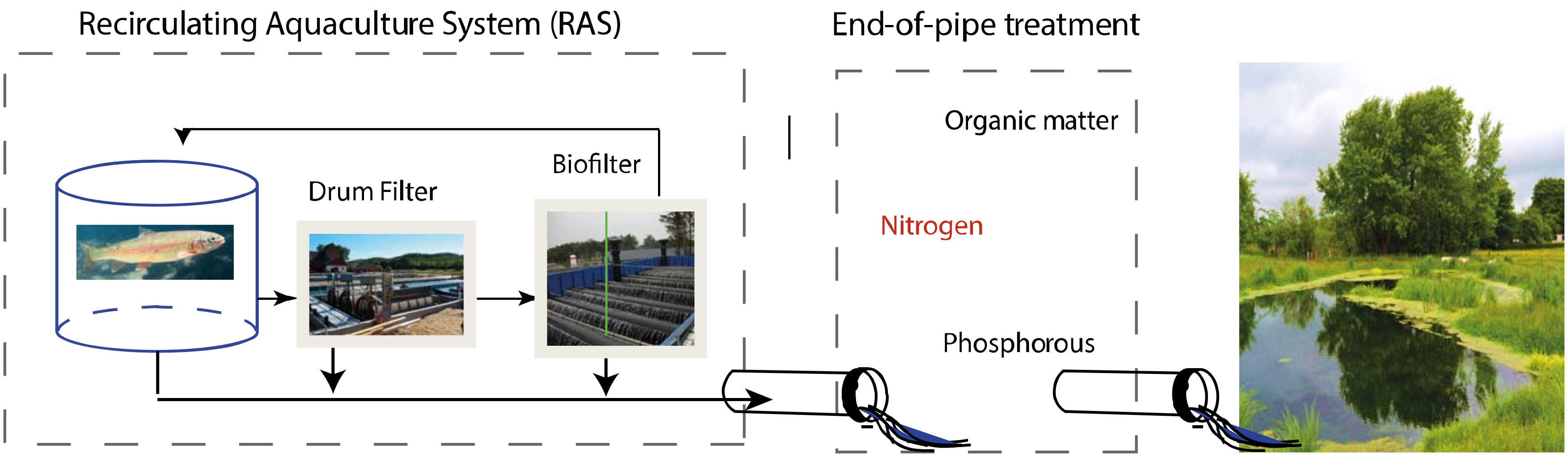
The problem

Environmental regulations has forced the improvement of water treatment technologies (End-of Pipe treatment)

Major challenge is removal of N
(15-50% best available technology)

System	TN	TP	BOD	Feed allowance (ton)	Cultivable Ton Fish
Traditional	7%	20%	20%	100	143
Type 3	15%	65%	80%	109	156
Type 3 (Wetland)	50%	76%	93%	186	266

(Jokumsen and Svendsen, 2010)



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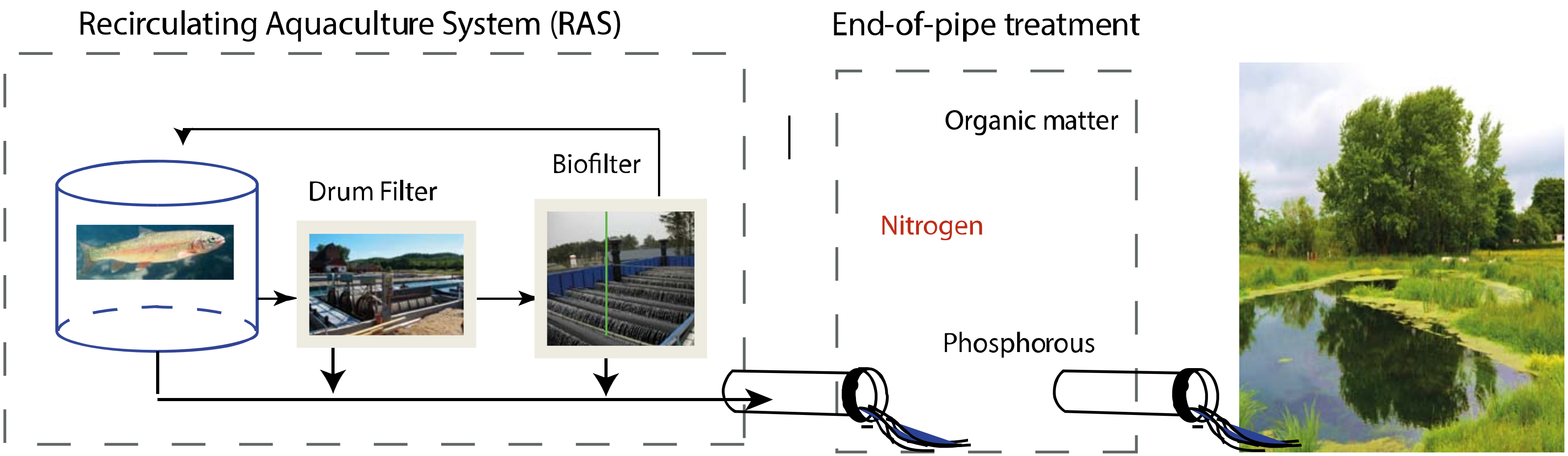
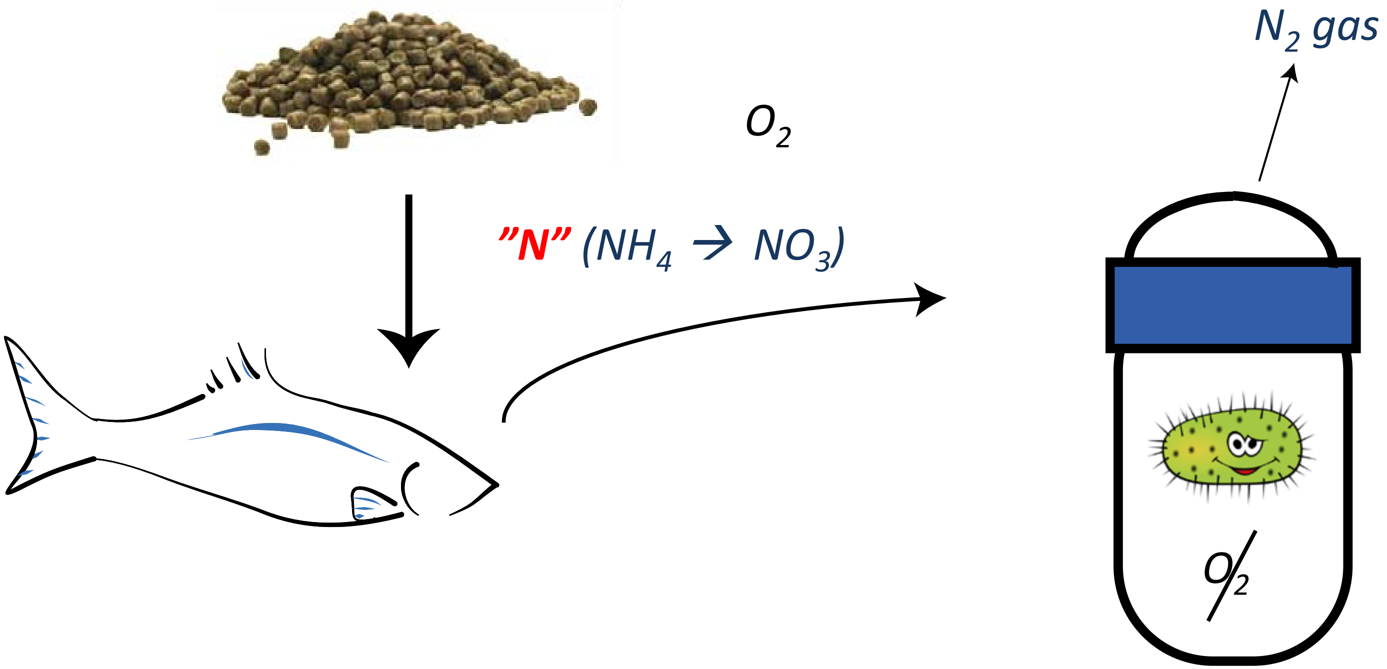
Environmental regulations has forced the improvement of water treatment technologies (End-of Pipe treatment)

Major challenge is removal of N
(15-50% best available technology)

Nitrate (NO_3) is main N compound (80%) discharged from recirculating aquaculture system (RAS)



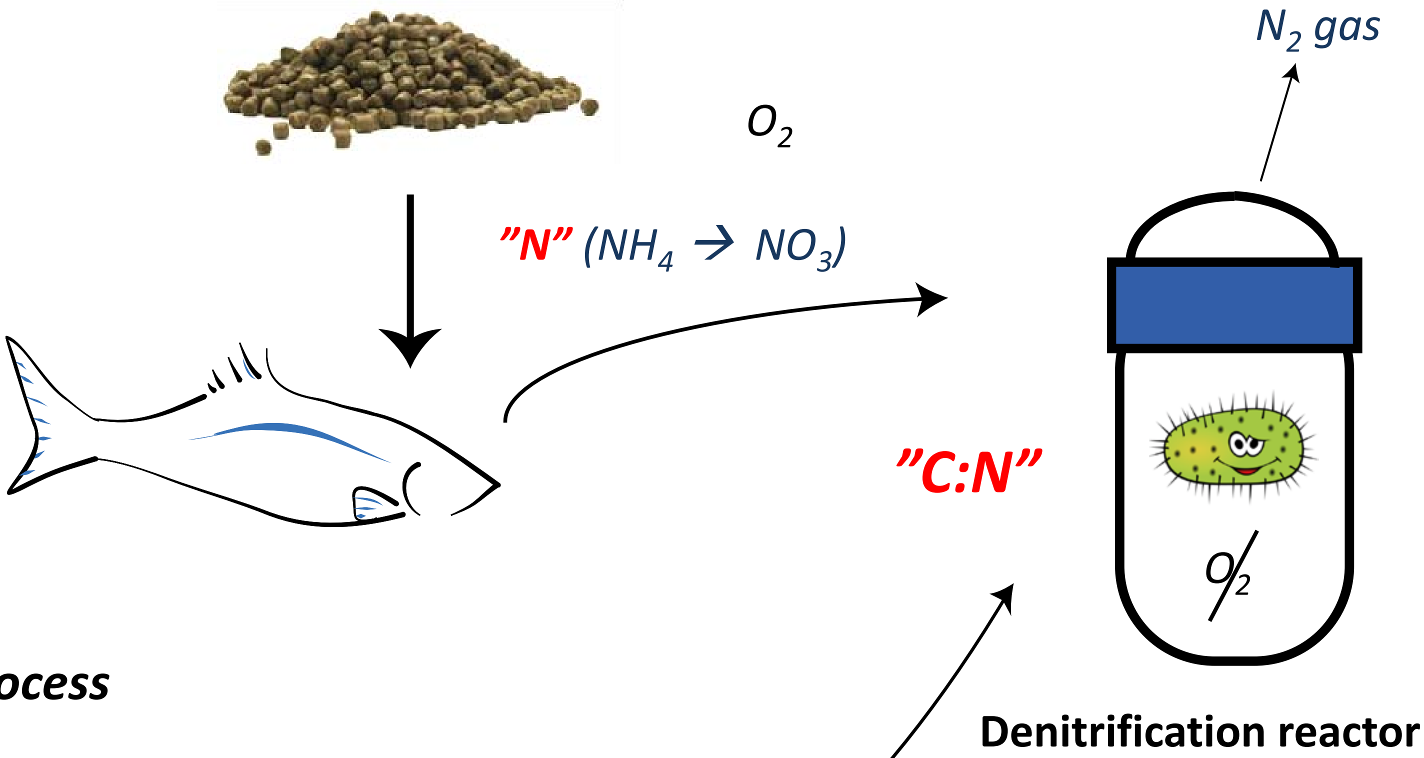


Denitrification reactor

Actual solution

Denitrification process

- Nitrate (NO_3) removed from water into N gas
- Carbon sources are required



Actual solution

Denitrification process

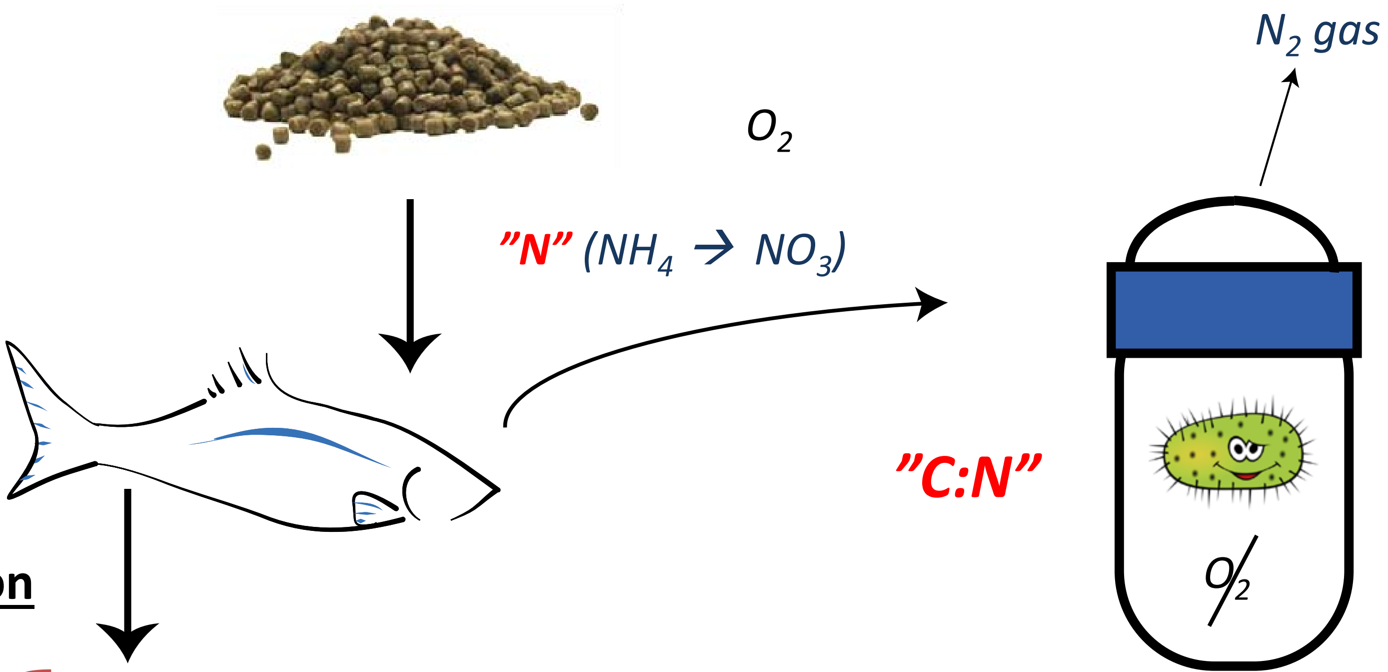
- Nitrate (NO_3) removed from water into N gas

- Carbon sources are required

400 €/MT
5-10% production costs

Purchase of external carbon sources "C"

The Industry needs cost effective solutions for removal of N



N_2 gas

O_2

"N" *(NH_4 → NO_3)*

"C:N"

$\cancel{O_2}$

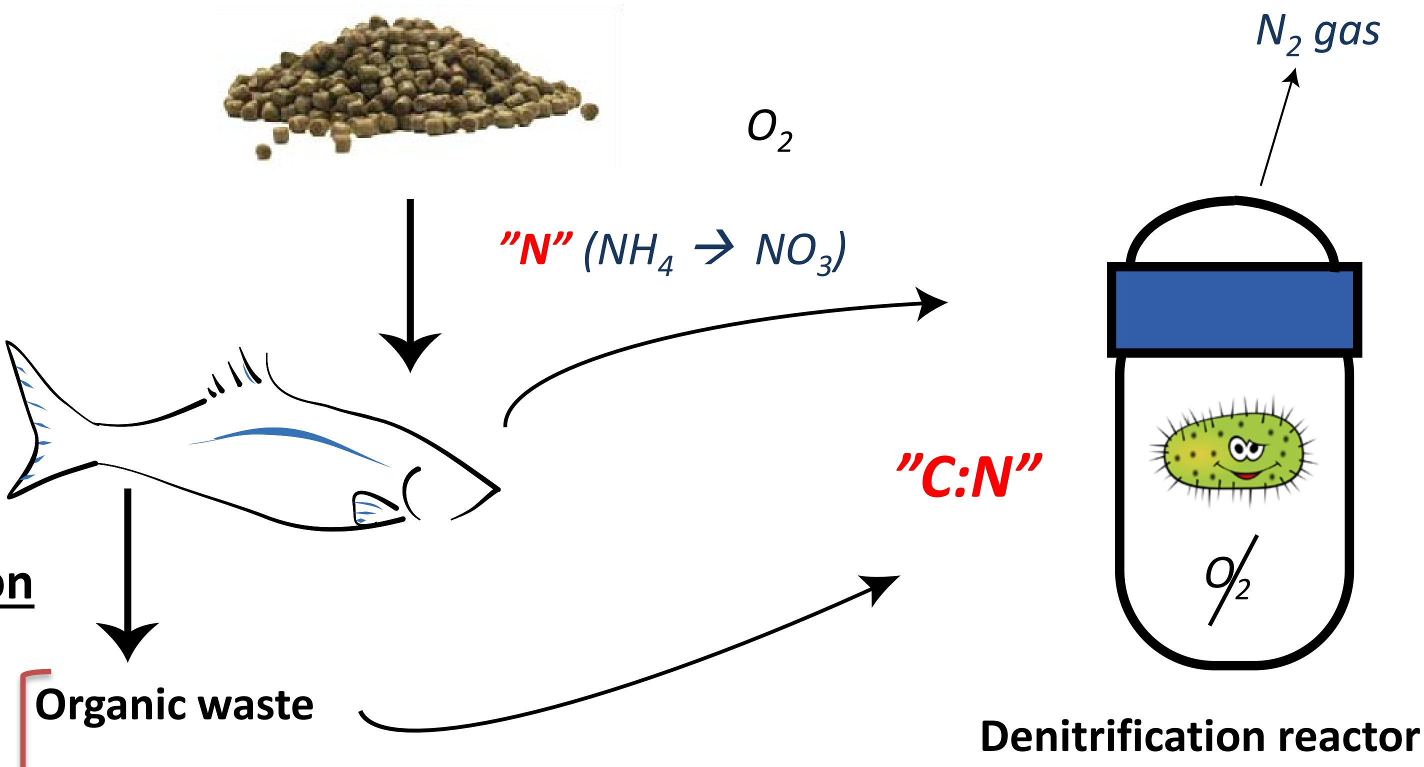
Denitrification reactor

A possible solution

Organic waste

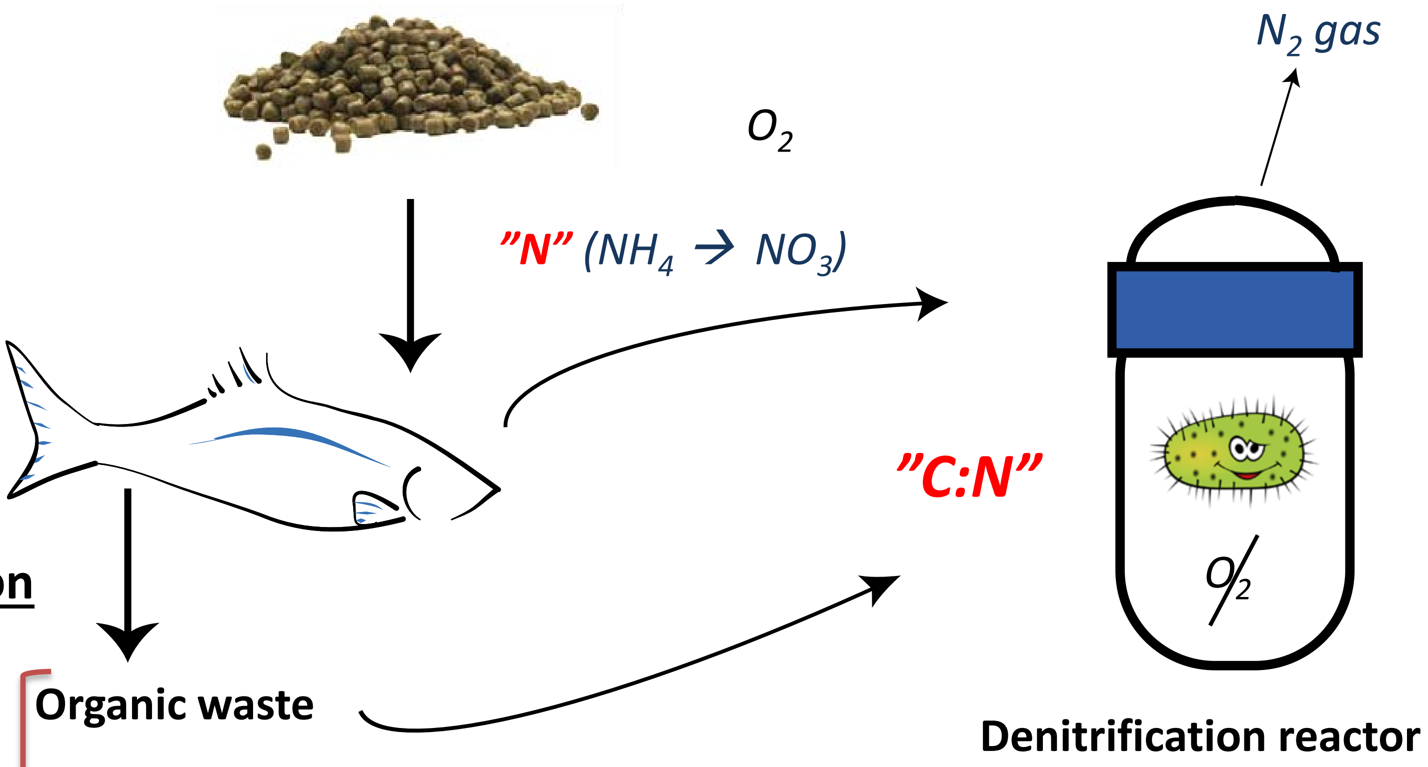
Single sludge denitrification

A possible solution



Single sludge denitrification

A possible solution



O_2

"N" ($NH_4 \rightarrow NO_3$)

N_2 *gas*

"C:N"

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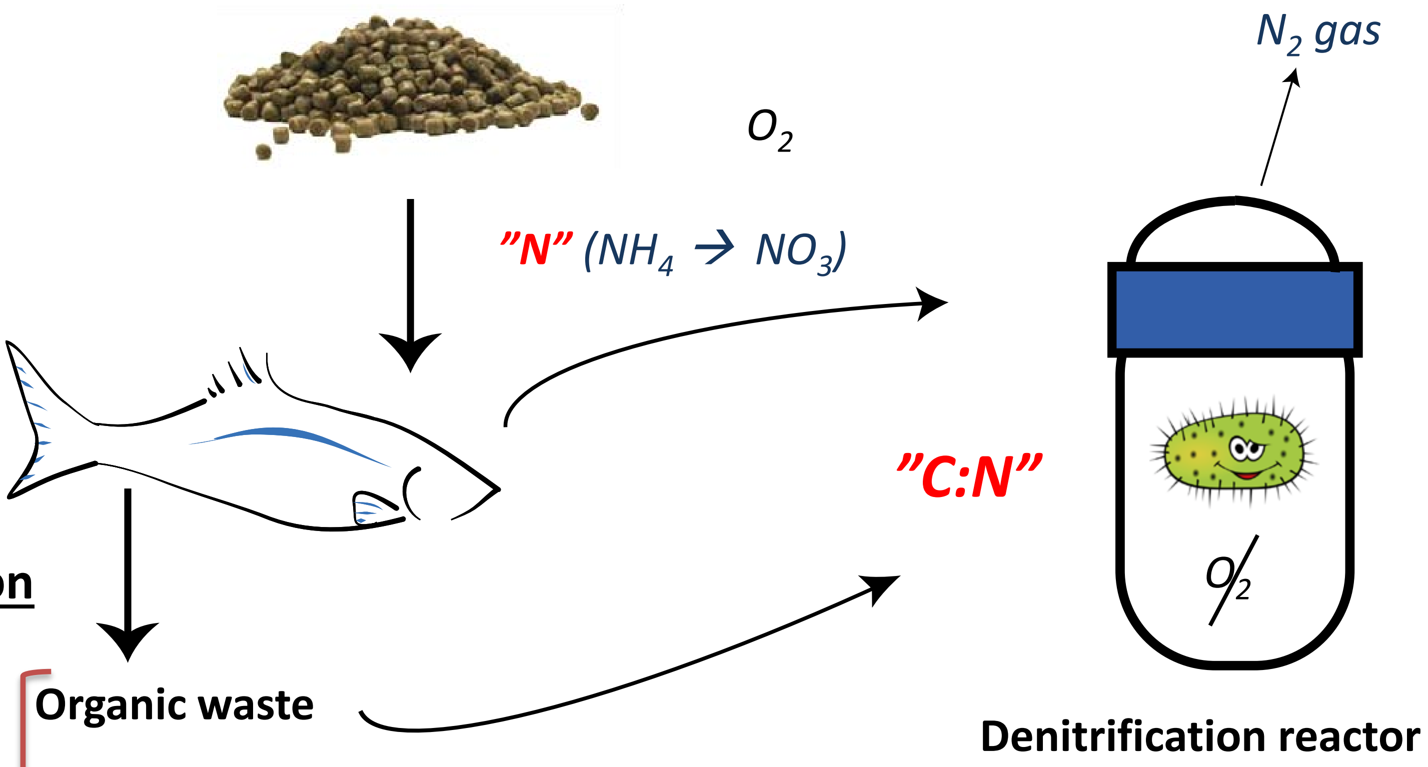
Denitrification reactor

Organic waste

- Two waste types are reduced at the same time

Single sludge denitrification

A possible solution



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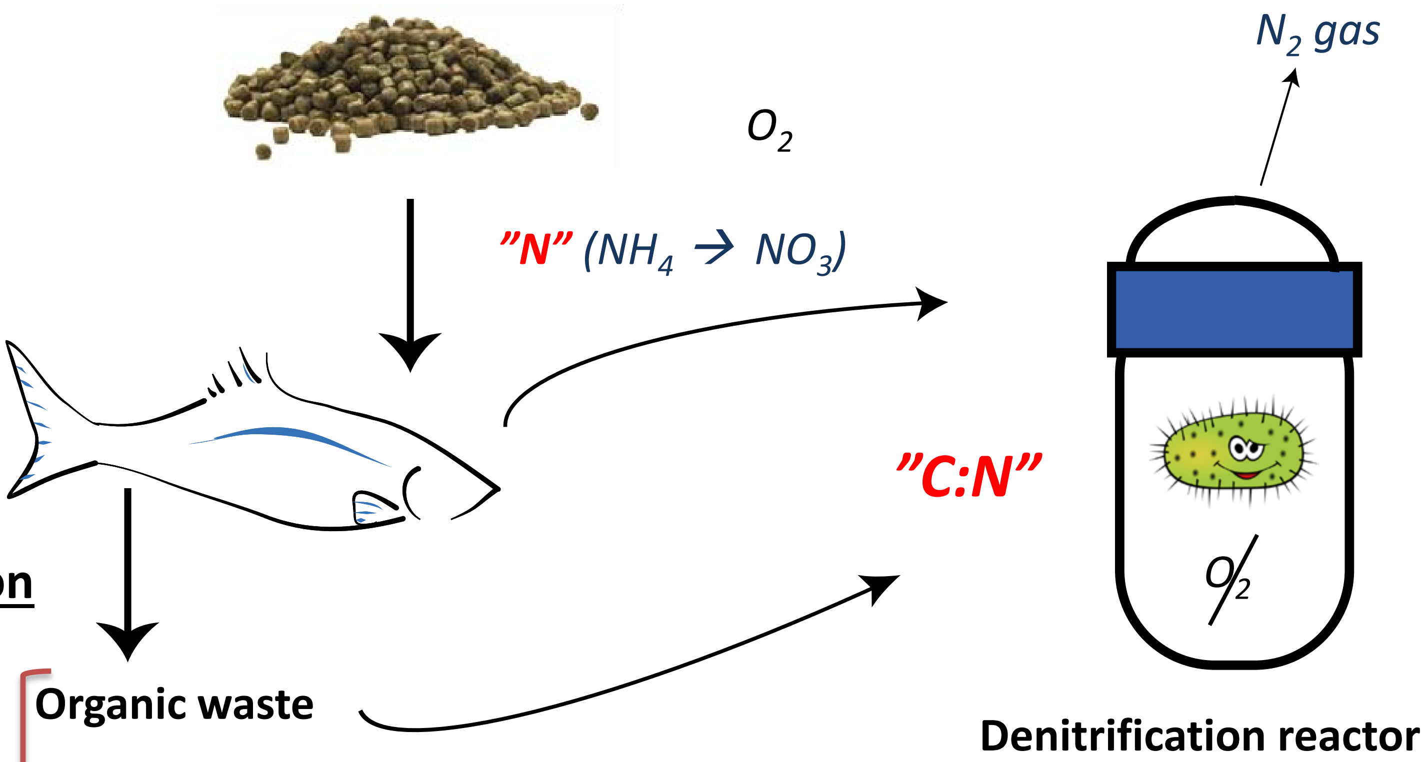
Denitrification reactor

Single sludge denitrification

- Two waste types are reduced at the same time
- The cost of buying carbon sources is reduced

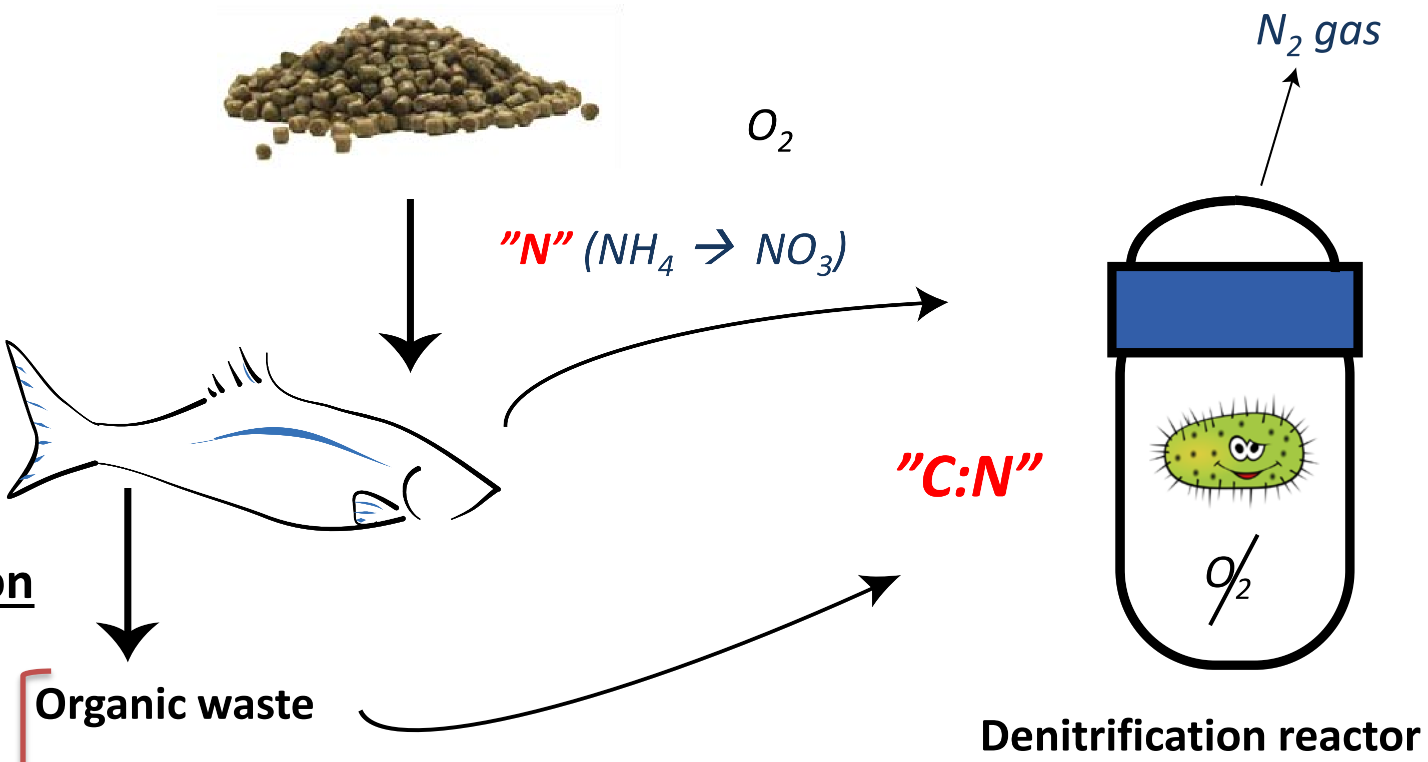


A possible solution

Single sludge denitrification

- Two waste types are reduced at the same time
- The cost of buying carbon sources is reduced
- The cost of organic waste disposal is reduced

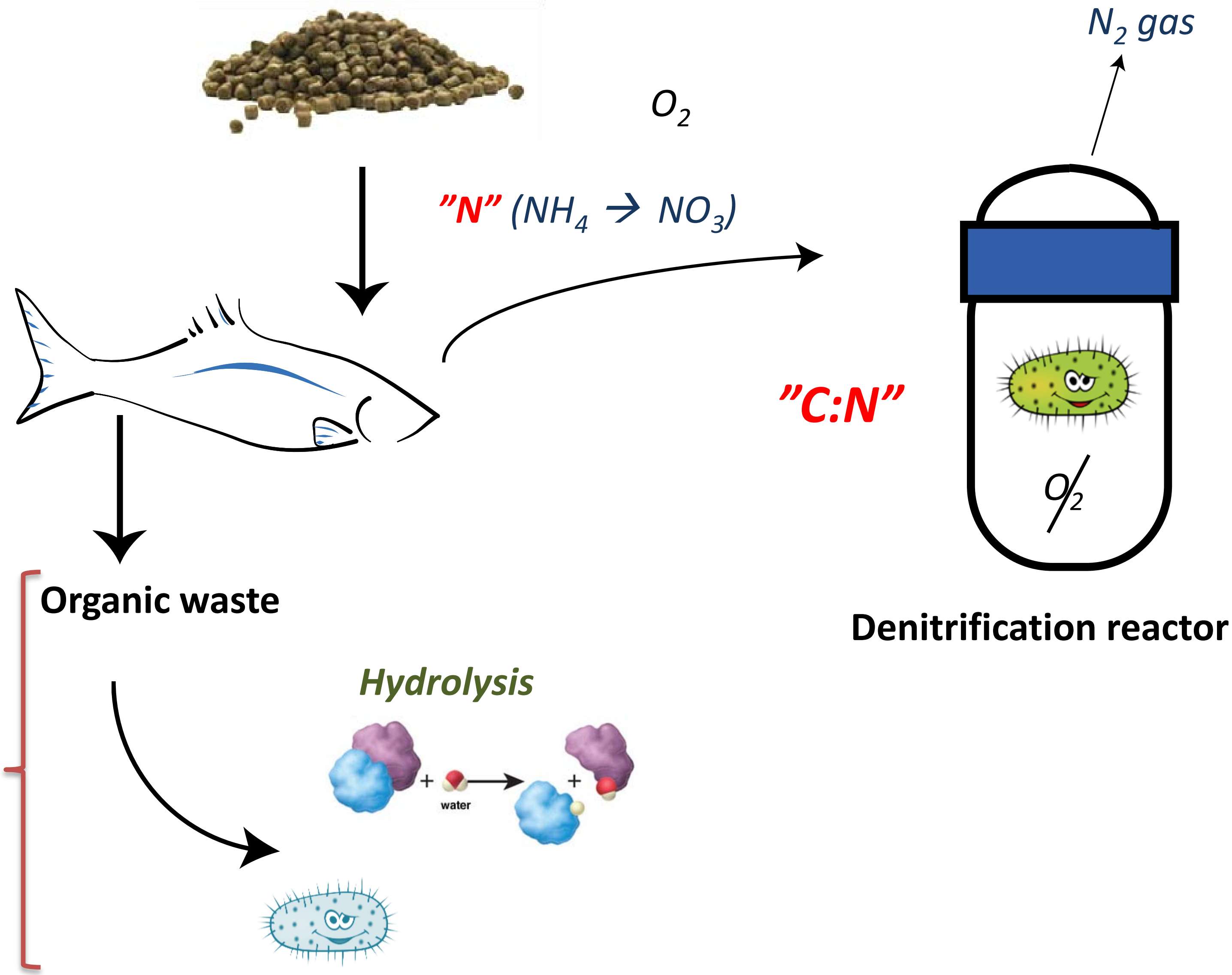
A possible solution



Single sludge denitrification

Organic waste

- Two waste types are reduced at the same time
- The cost of buying carbon sources is reduced
- The cost of organic waste disposal is reduced
- But.. the problem is that the organic waste is found majorly in particulate form so bacteria cannot use it



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Denitrification reactor

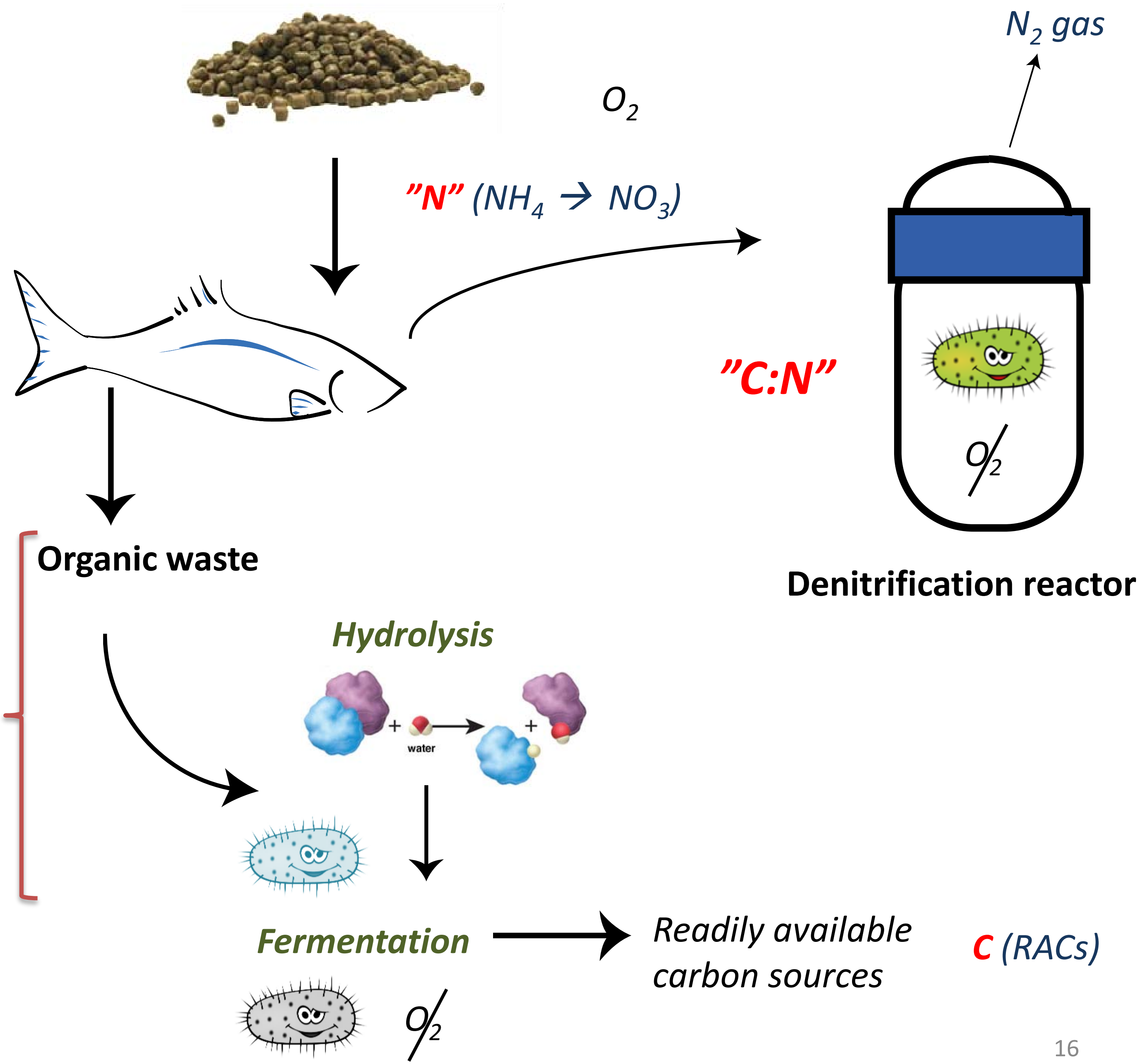
Organic waste

Hydrolysis

The approach

Single sludge denitrification

The approach



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Denitrification reactor

Single sludge denitrification

Organic waste

Hydrolysis

Fermentation

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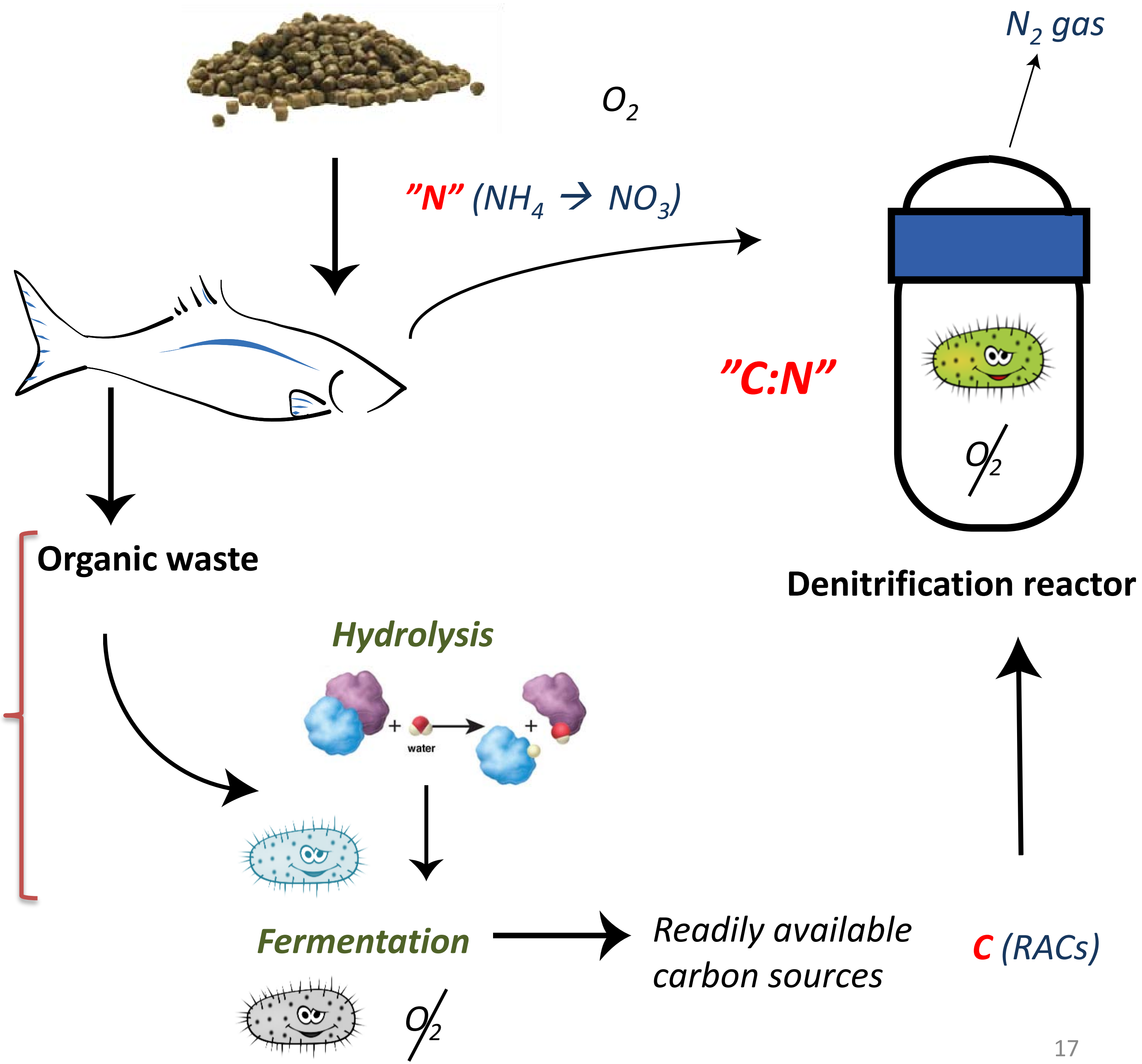
Readily available carbon sources

C (RACs)

The approach

Single sludge denitrification



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Denitrification reactor

Organic waste

Hydrolysis

Fermentation

$\cancel{O_2}$

Readily available carbon sources

C (RACs)

OVERALL OBJECTIVE

- The following PhD dissertation focuses on enhancing the use of organic waste produced by fish as an internal carbon source for on-farm denitrification

- Transforming waste into a new resource

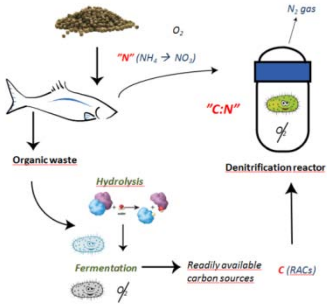
Part I: Characterizing the hydrolysis and fermentation processes of two dietary protein sources, fish meal and soybean meal

Objectives

a) Develop indicators for describing the hydrolysis and fermentation processes in order to compare dietary treatments.

b) Characterize organic waste masses and the potential for producing volatile fatty acids.

c) Estimate and compare the potential for pursuing denitrification using organic waste deriving from either fish fed fish meal based or soybean based diets.



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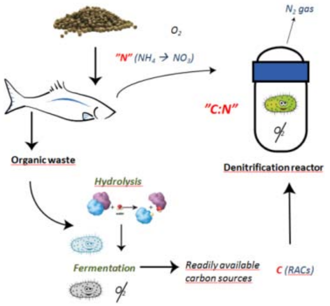
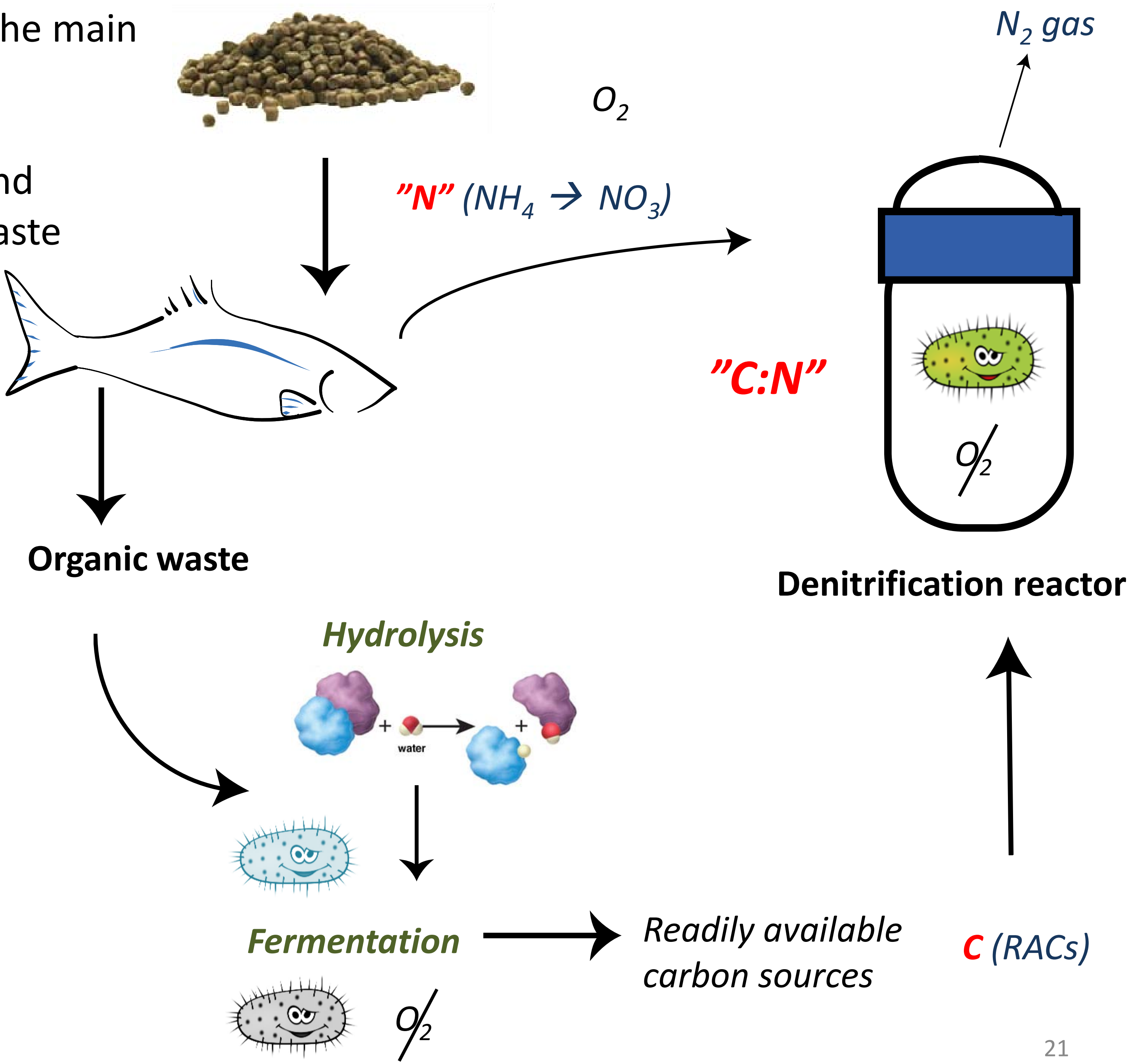


- Feed is indirectly the main source of waste
- Dictates masses and composition of waste



O_2

N_2 *gas*

"N" *(NH_4 → NO_3)*

"C:N"

$\cancel{O_2}$

Denitrification reactor

Organic waste

Hydrolysis

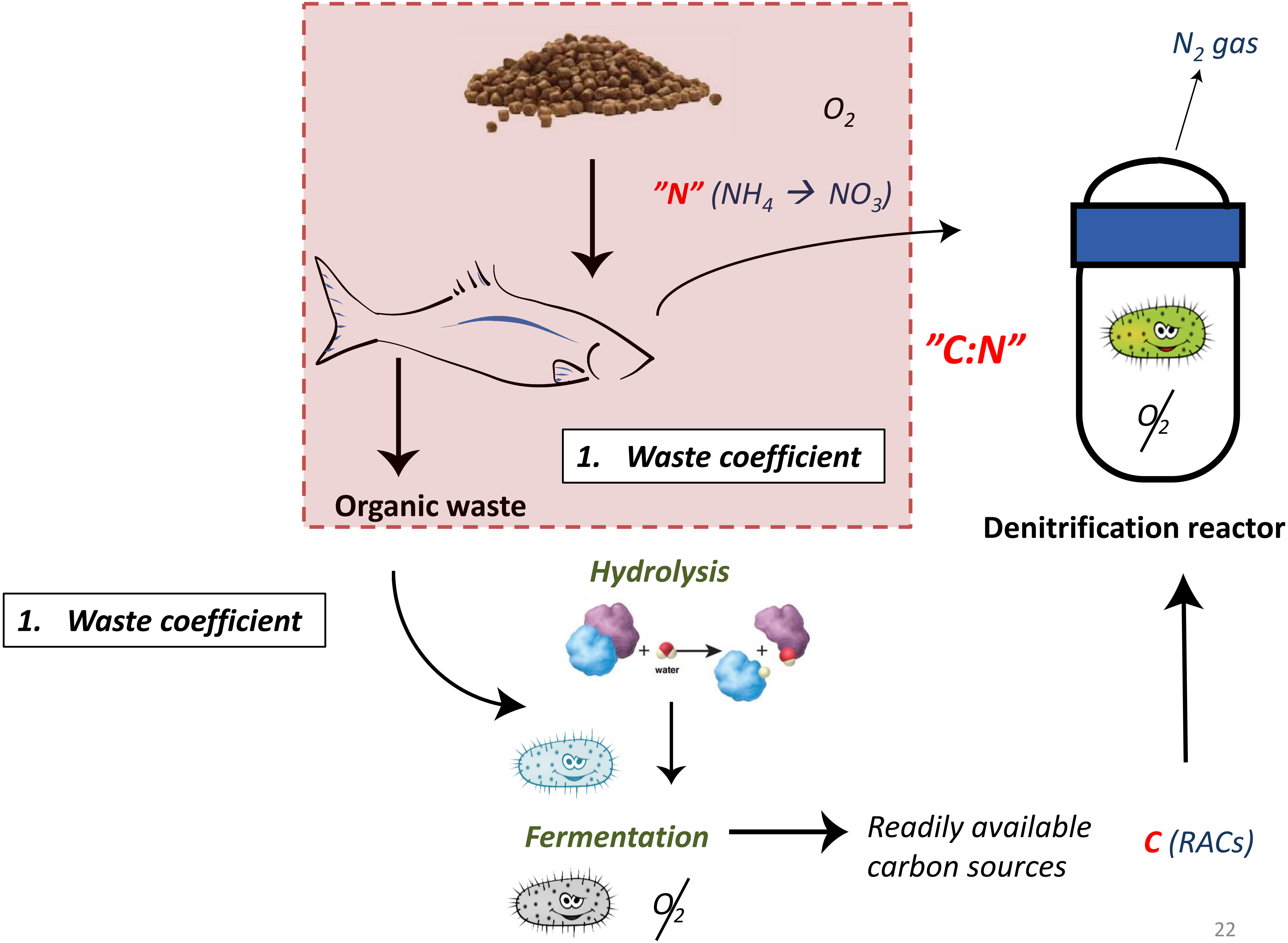
water

Fermentation

$\cancel{O_2}$

Readily available carbon sources

C *(RACs)*



O_2

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N_2 gas

"C:N"

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1. **Waste coefficient**

Organic waste

Denitrification reactor

Hydrolysis

1. **Waste coefficient**

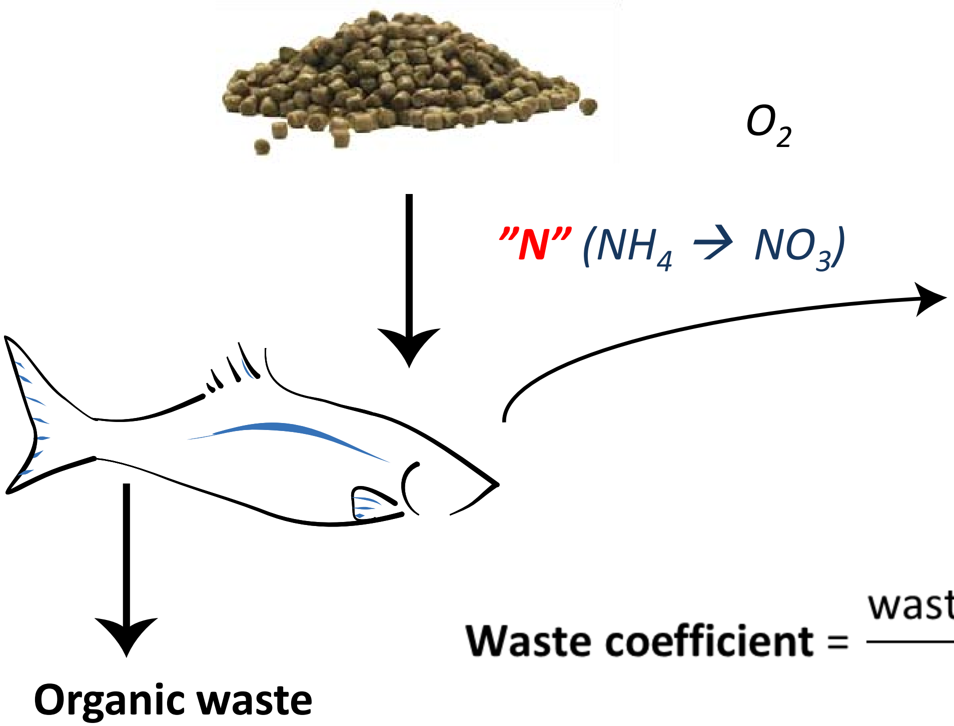
Fermentation

$\cancel{O_2}$

Readily available carbon sources

C (RACs)

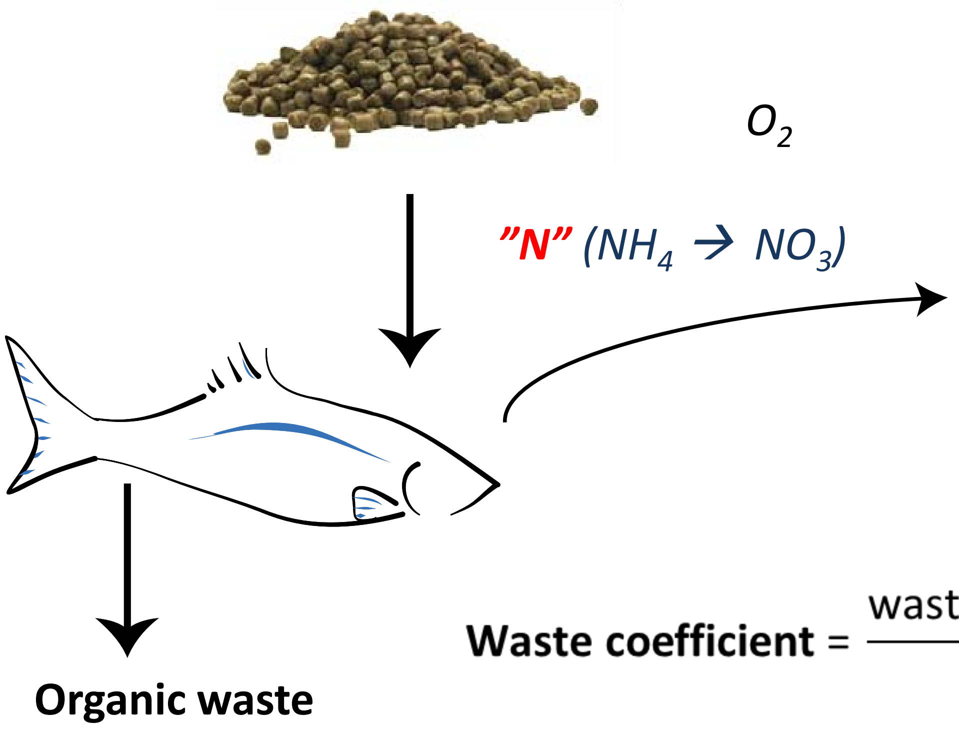
1. Waste coefficient



$$\textbf{Waste coefficient} = \frac{\text{waste produced as TS;TCOD; Protein; Lipid; TP}}{\text{feed consumed}}$$

- How much C, N and P is produced per amount of feed consumed

1. Waste coefficient



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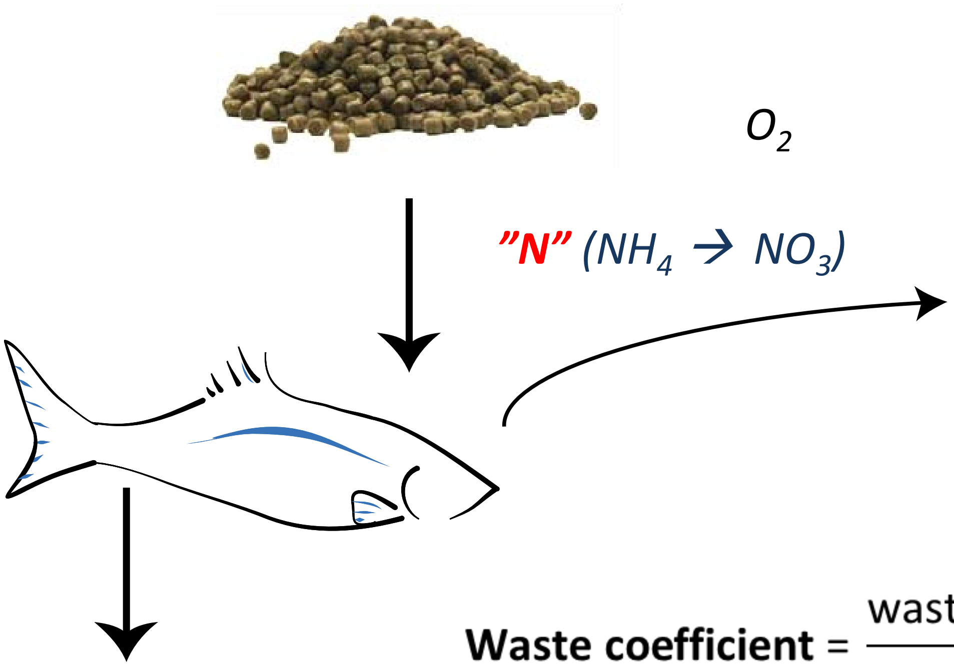
Organic waste

$$\textbf{Waste coefficient} = \frac{\text{waste produced as TS;TCOD; Protein; Lipid; TP}}{\text{feed consumed}}$$

Diet	**P:E_19**
TS (g/g)	0.17 ± 0.02
TVS (g/g)	0.10 ± 0.01
TKN (mgN/g)	7.1 ± 0.8
Protein (mg/g)	44.6 ± 4.7
Lipid (mg/g)	21.8 ± 5.6
NFE (mg/g)	73.0 ± 7.7
TP (mg/g)	9.6 ± 1.1
Ash (mg/g)	66.6 ± 5.3

- Gives C:N produced

1. Waste coefficient



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- Gives C:N produced
- How much carbon is produced in the waste

C →

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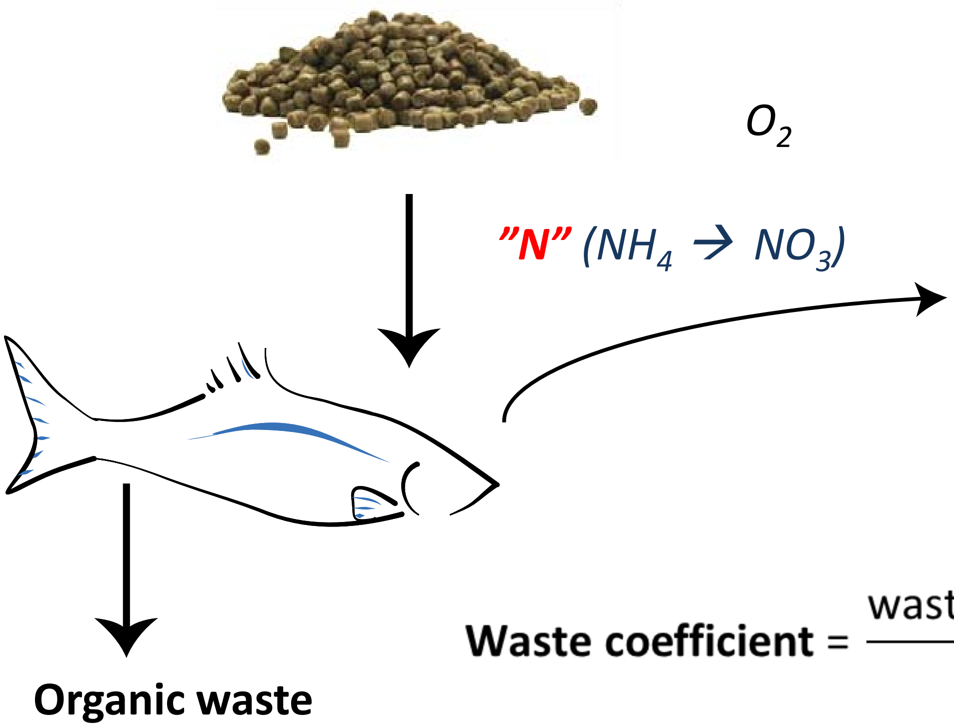

O_2

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Organic waste

$$\textbf{Waste coefficient} = \frac{\text{waste produced as TS;TCOD; Protein; Lipid; TP}}{\text{feed consumed}}$$

- Gives C:N produced
- How much carbon is produced in the waste
- How much N needs to be removed from the waste

	Diet	**P:E_19**
C →	**TS (g/g)**	0.17 ± 0.02
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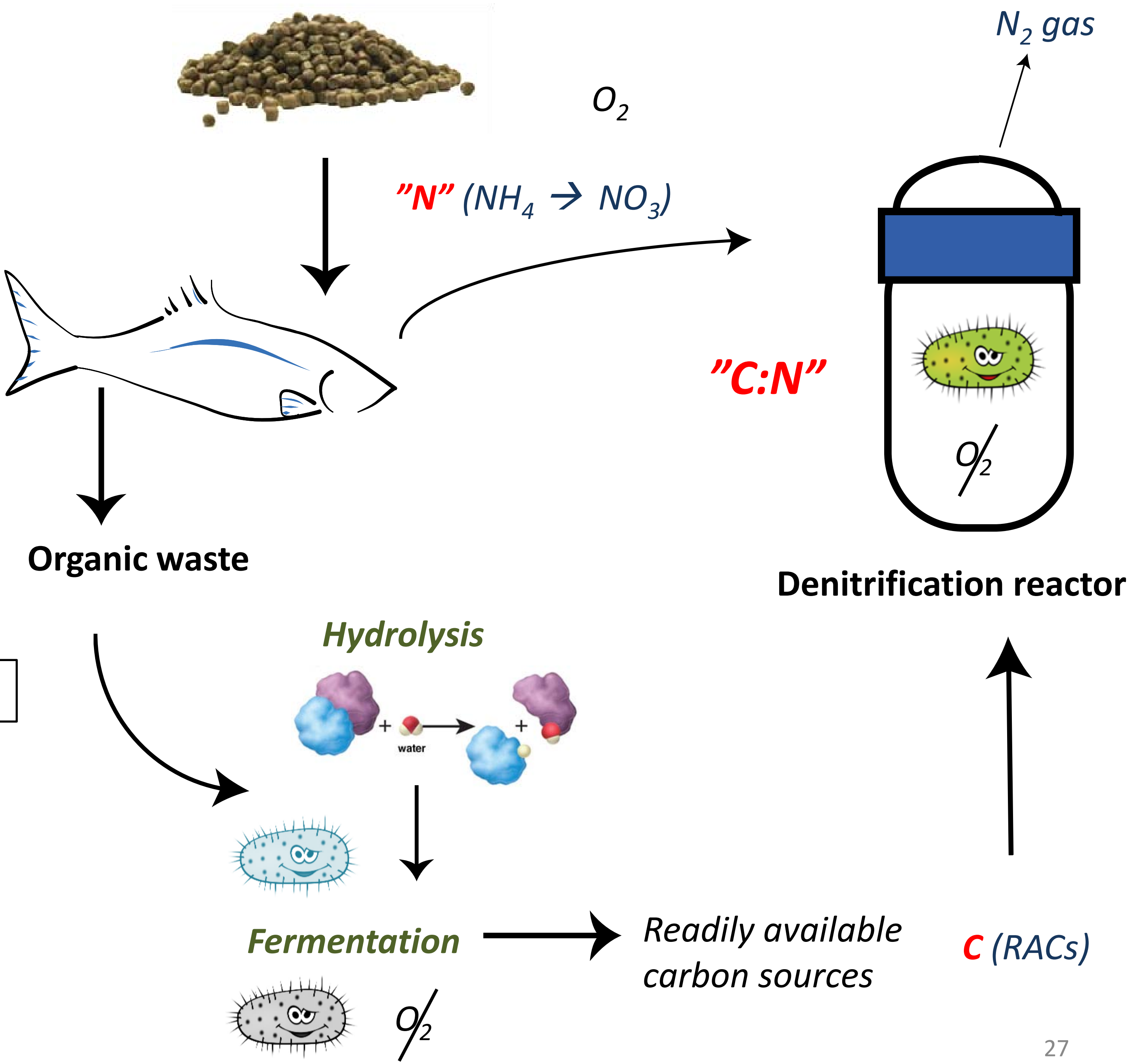

O_2

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N_2 gas

"C:N"

$\cancel{O_2}$

Denitrification reactor

Organic waste

Hydrolysis

Fermentation

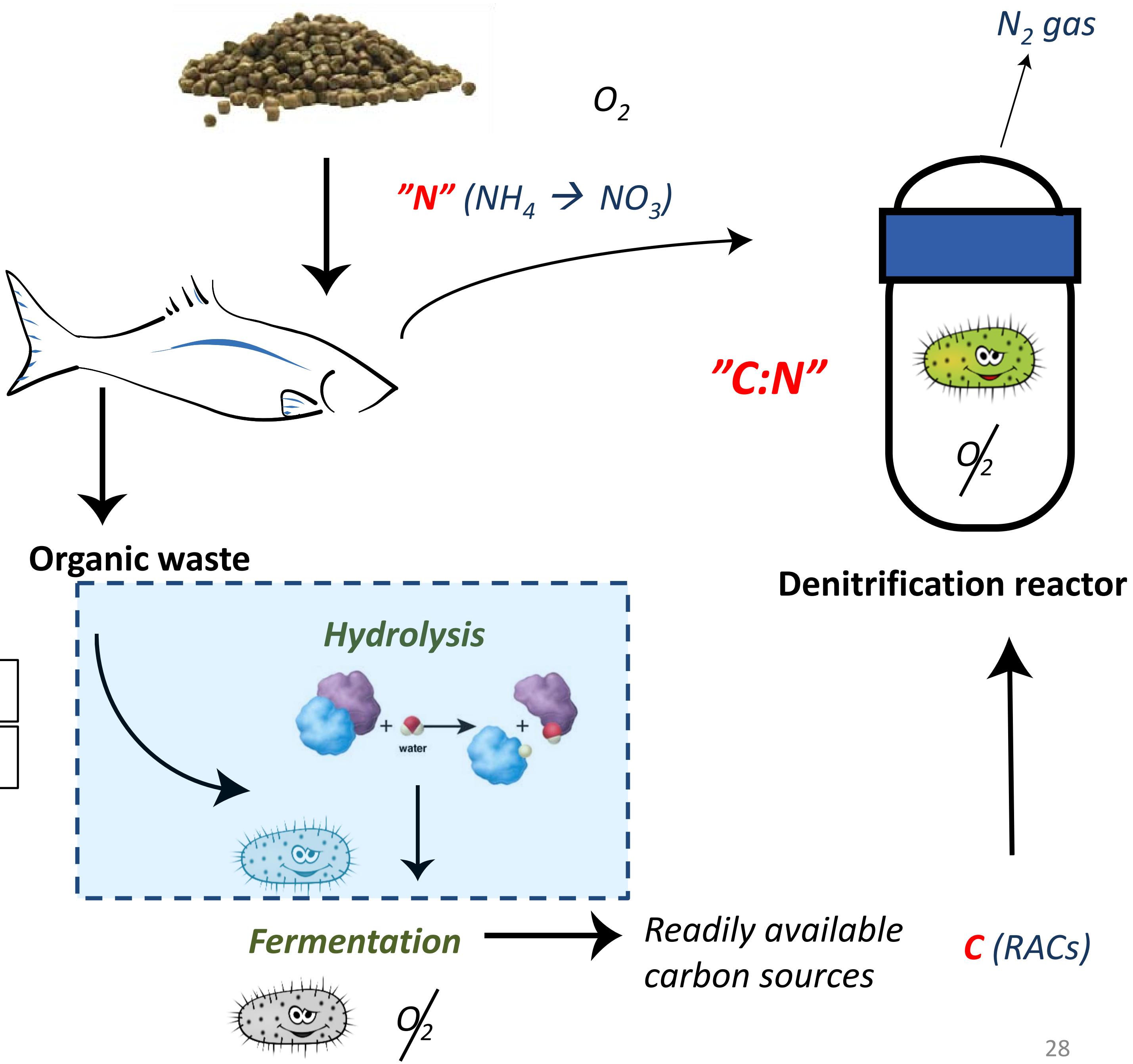
$\cancel{O_2}$

Readily available carbon sources

C (RACs)

1. *Waste coefficient*

1. **Waste coefficient**
2. **Hydrolysis**



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Hydrolysis

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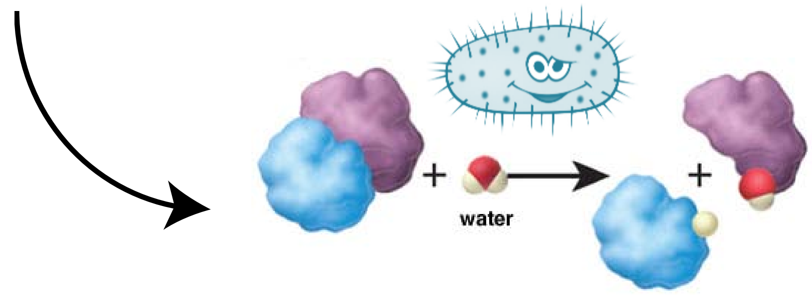


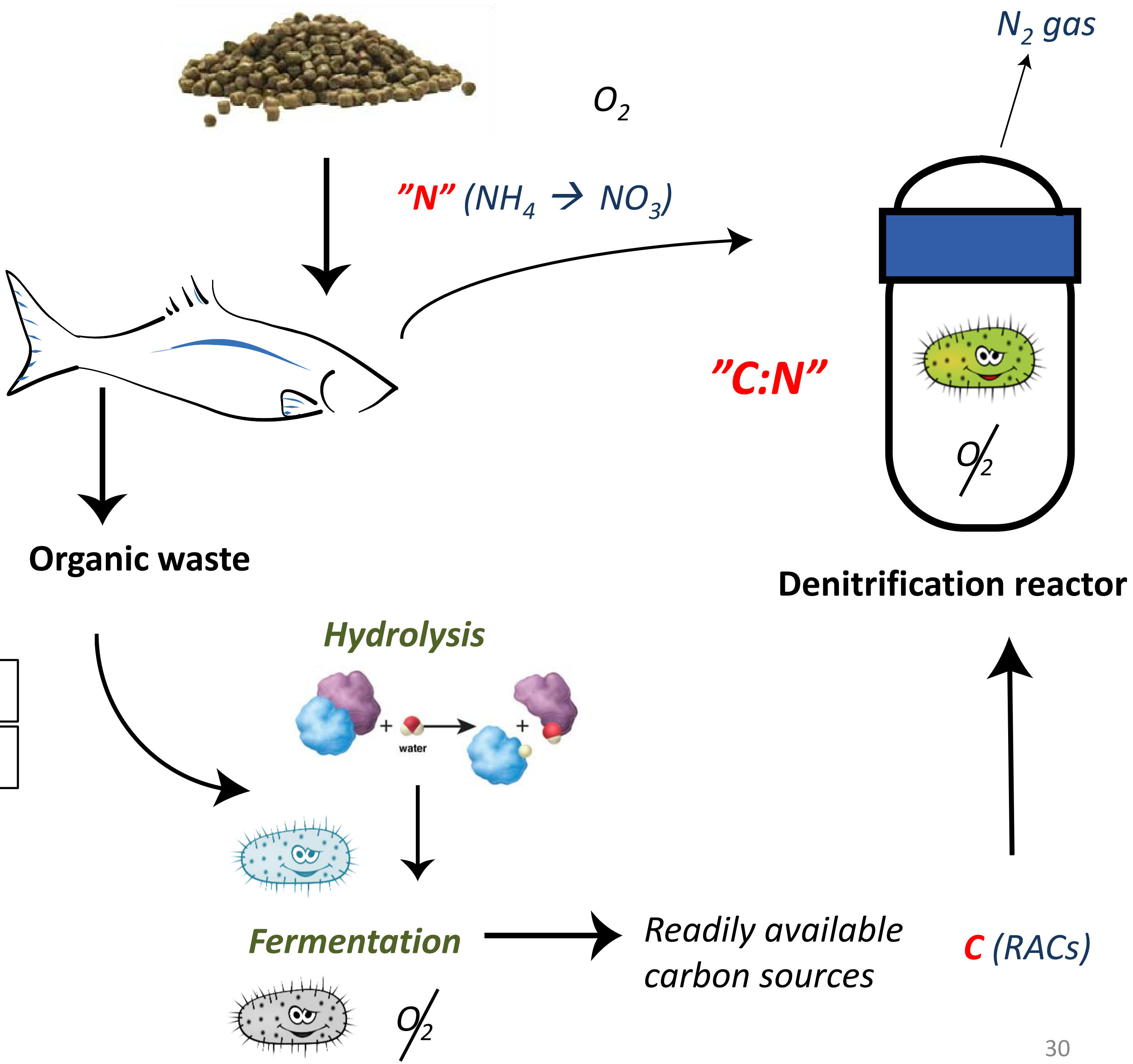
Particulate carbon

Dissolved carbon

Dissolved carbon

$$\text{Degree of solubilization} = \frac{\text{Dissolved Carbon}}{\text{Particulate Carbon}}$$

- Expresses the fraction of the particulate carbon that is solubilized (0.2 µm)



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Fermentation

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Readily available carbon sources

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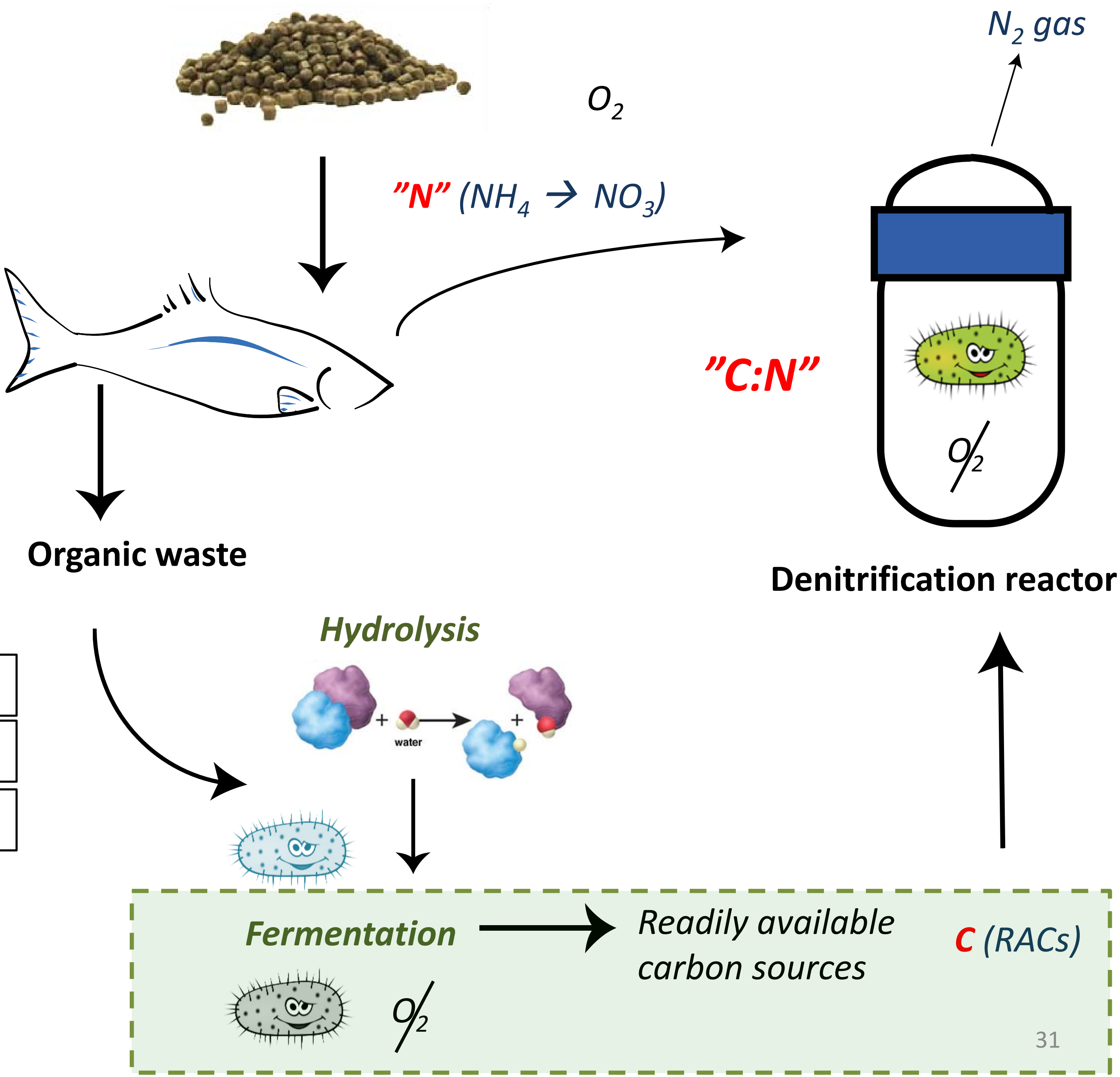
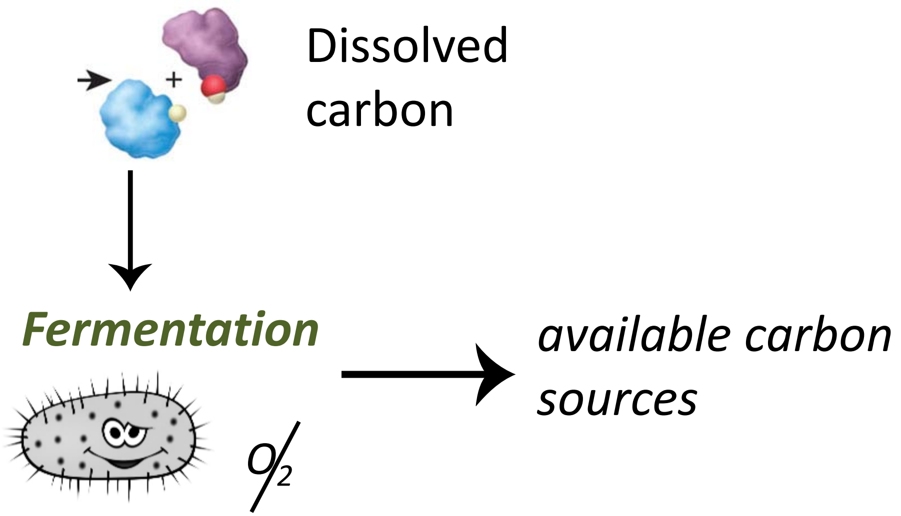


1. ***Waste coefficient***
2. ***Hydrolysis***
3. ***Fermentation***



Dissolved carbon

Fermentation

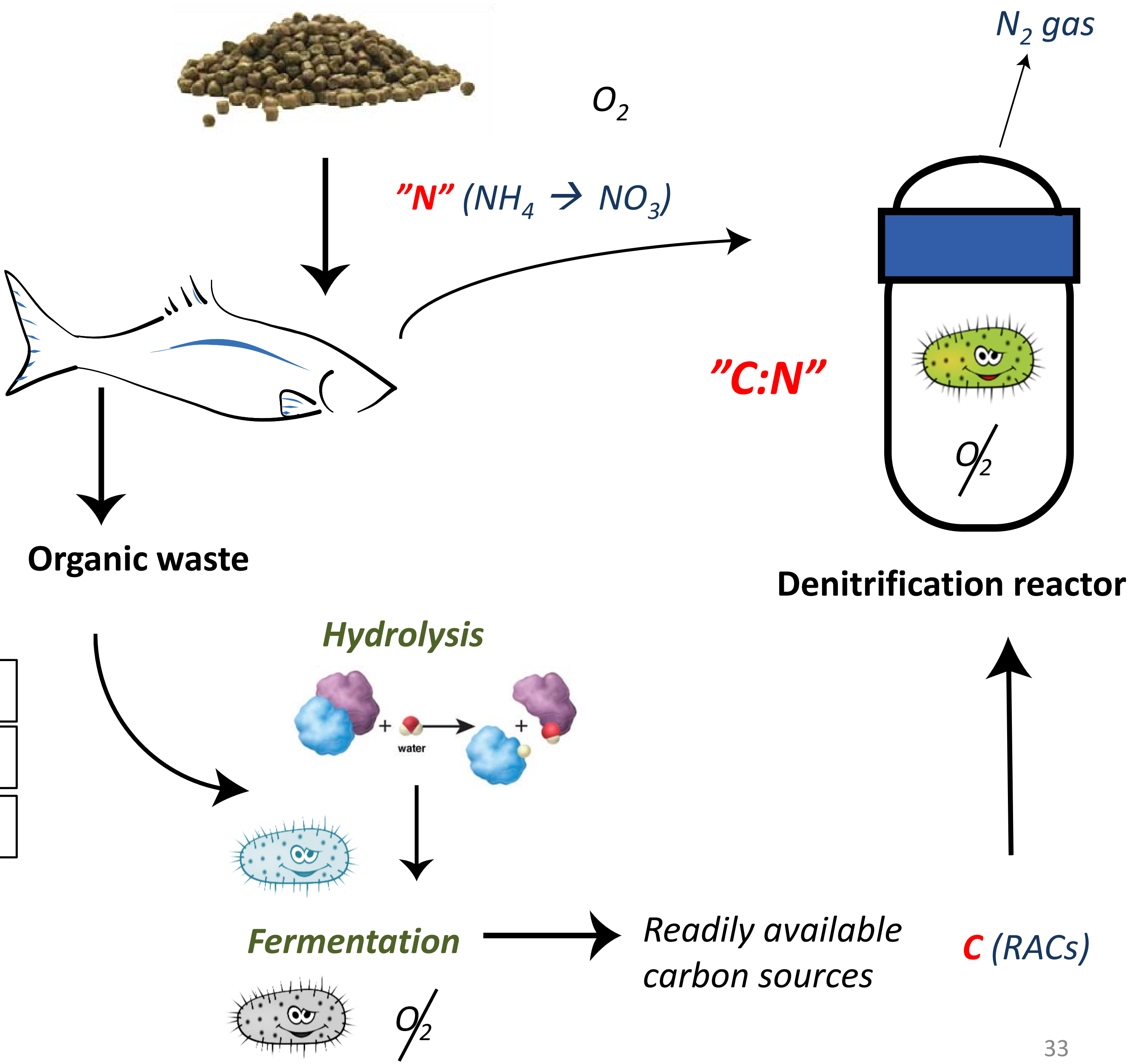
$\cancel{O_2}$

available carbon sources

C *(RACs)*

$$\textbf{Degree of fermentation}=\frac{\textit{Available Carbon (RACS)}}{\text{Dissolved } \textit{Carbon}}$$

- Expresses the fraction of already dissolved carbon (sCOD) that is fermented into available carbon (RACs) for example volatile fatty acids (VFAs)



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N_2 *gas*

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Denitrification reactor

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Hydrolysis

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Readily available carbon sources

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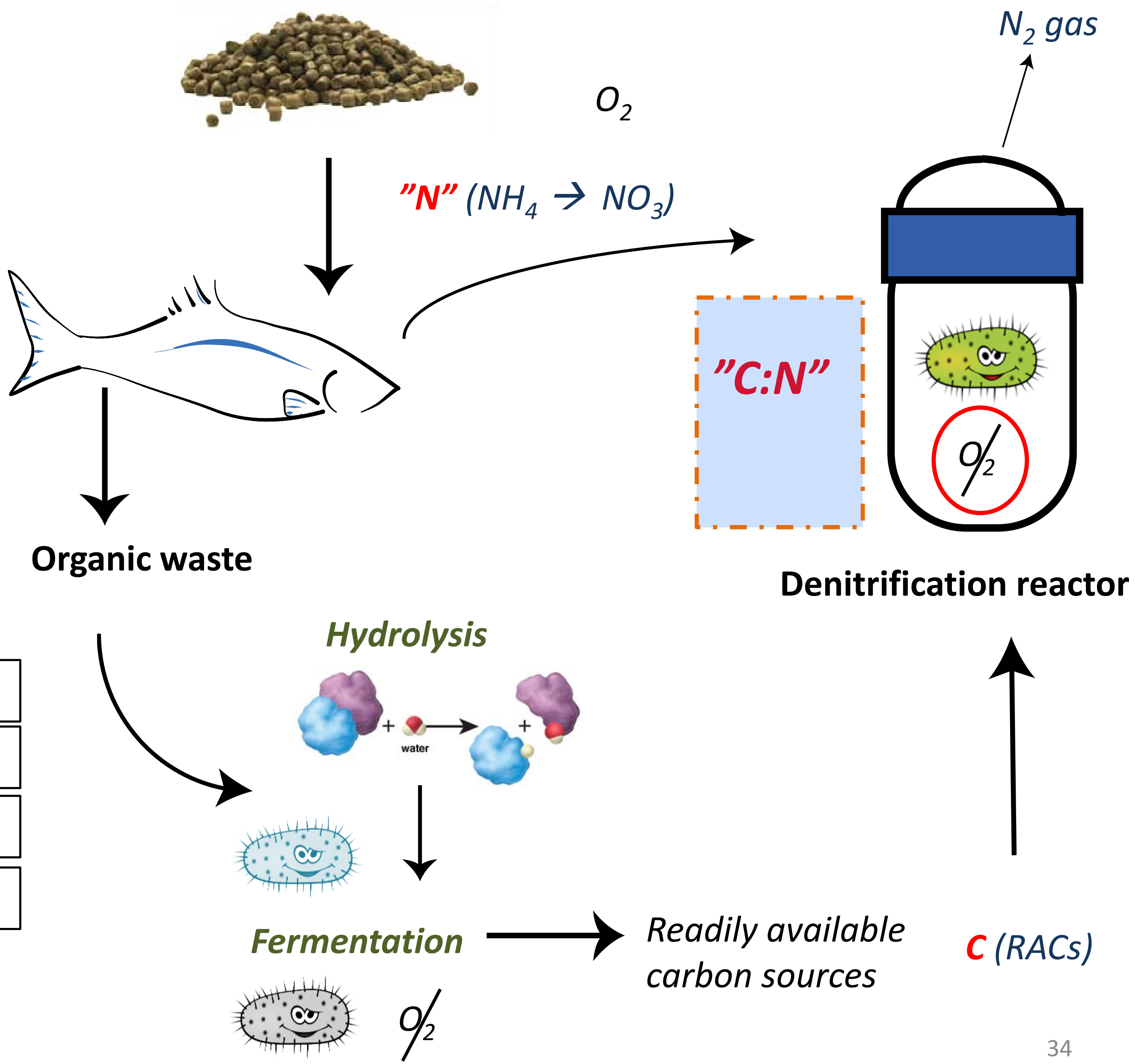

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Denitrification reactor

Organic waste

Hydrolysis

water

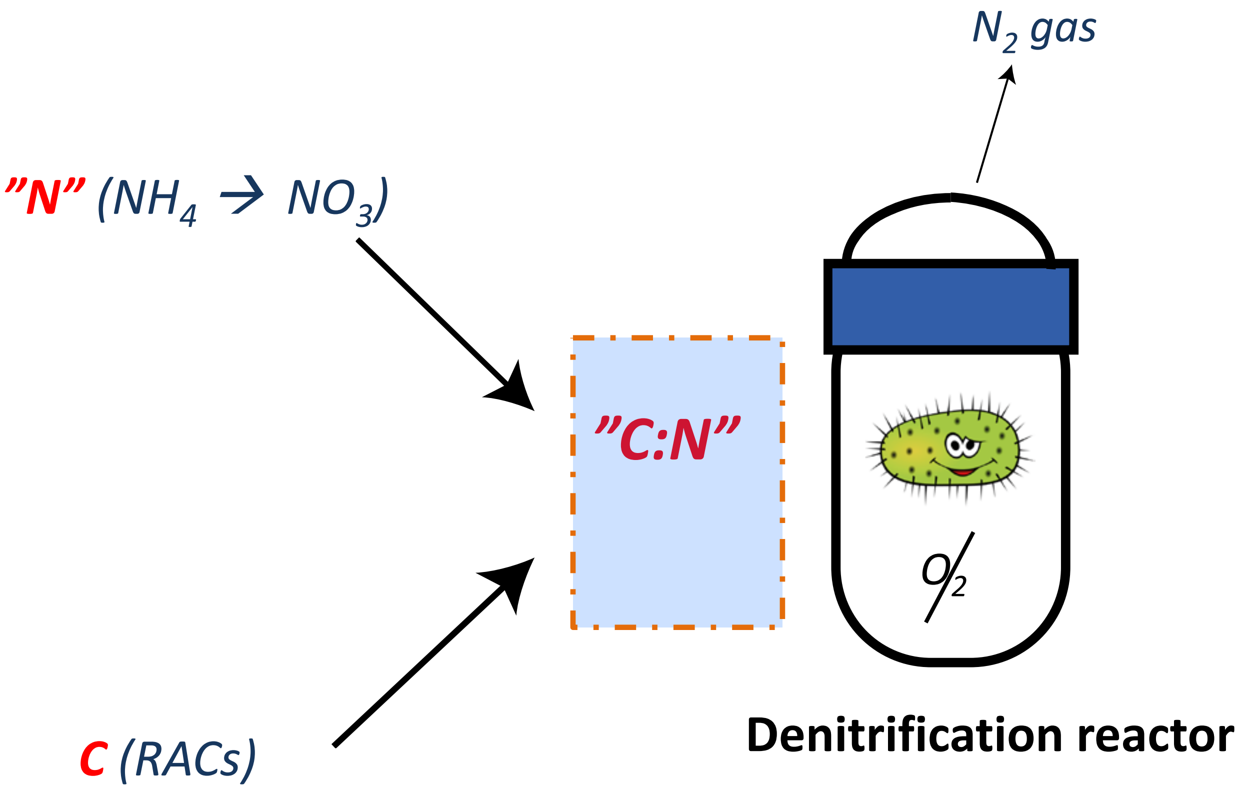
Fermentation

$\cancel{O_2}$

Readily available carbon sources

C (RACs)

1. ***Waste coefficient***
2. ***Hydrolysis***
3. ***Fermentation***
4. ***C:N Deni potential***



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C *(RACs)*

Denitrification reactor

1. ***Waste coefficient***
2. ***Hydrolysis***
3. ***Fermentation***
4. ***C:N Deni potential***

- **Knowing the amount of available carbon recovered from the waste and the N produced in the waste.**

- **The capacity for denitrification can be estimated.**

Characterizing the hydrolysis and fermentation processes of *two dietary protein sources*, fish meal and soybean meal

Fish meal

(animal protein source)

Soybean meal (SBM)

(vegetable protein source)

Trial 1: 5 isoenergetic diets with different protein levels

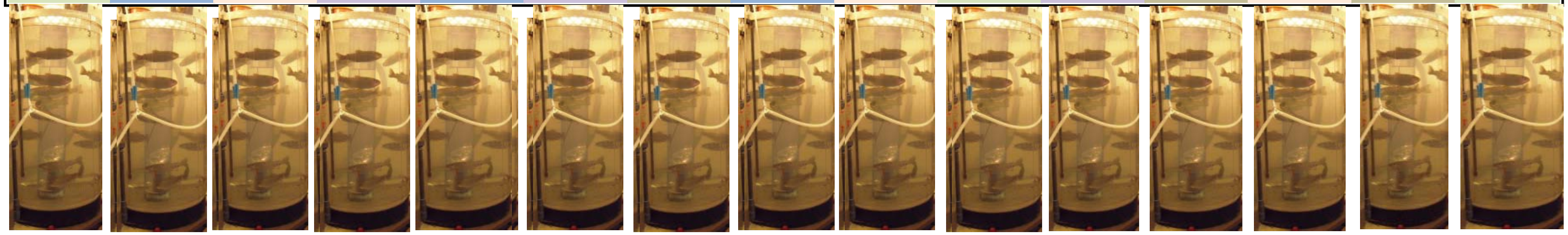
15-17-19-21-23

Experimental Setup

- Rainbow trout (mean weight 56.2 ± 6 g), fed 1.7% biomass.
- 15 Nutrient Mass Balance Systems (NMBS) at 12.3 ± 0.3 ^{0}C

  (flow through system).

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
P:E 15	P:E 17	P:E 19	P:E 21	P:E 17	P:E 21	P:E 23	P:E 17	P:E 19	P:E 15	P:E 21	P:E 23	P:E 19	P:E 23	P:E 15



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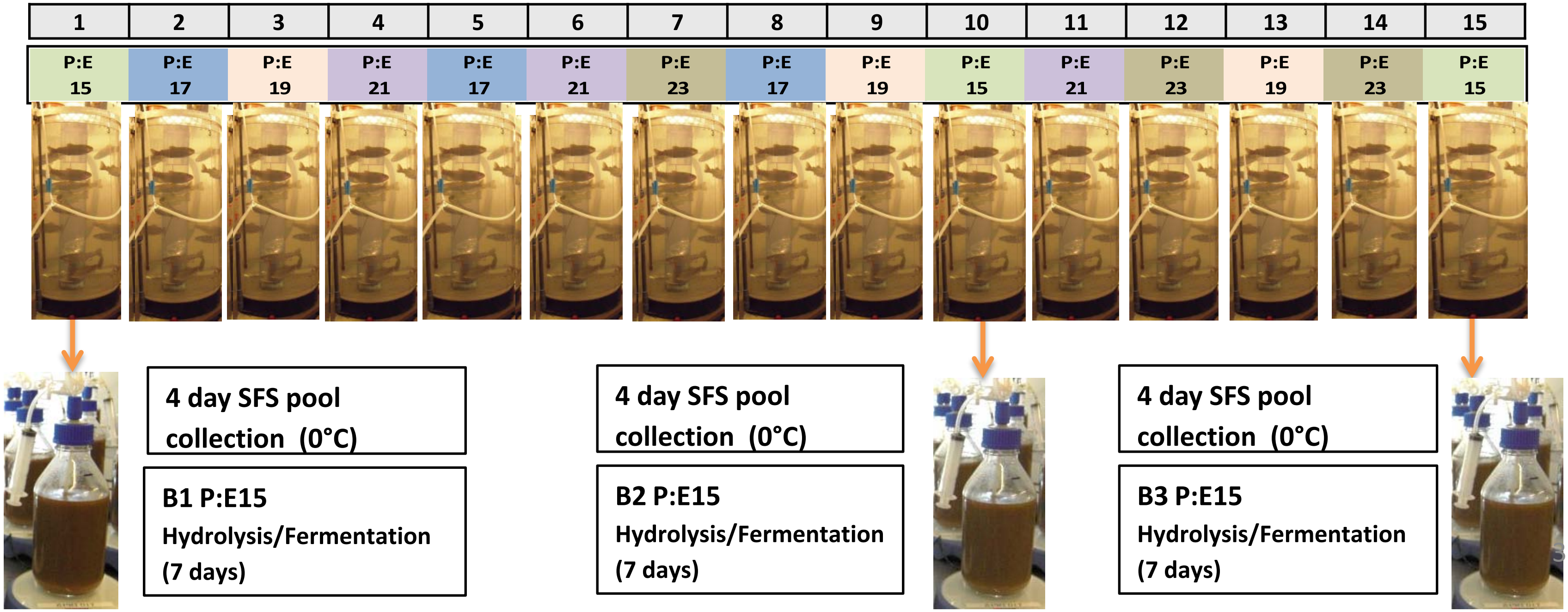


1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
P:E 15	P:E 17	P:E 19	P:E 21	P:E 17	P:E 21	P:E 23	P:E 17	P:E 19	P:E 15	P:E 21	P:E 23	P:E 19	P:E 23	P:E 15

4 day SFS pool collection (0°C)

B1 P:E15
Hydrolysis/Fermentation
(7 days)

4 day SFS pool collection (0°C)

B2 P:E15
Hydrolysis/Fermentation
(7 days)

4 day SFS pool collection (0°C)

B3 P:E15
Hydrolysis/Fermentation
(7 days)

Results Waste coefficient

Part I a): Characterizing the hydrolysis and fermentation processes

1. ***Waste coefficient***
2. ***Hydrolysis***
3. ***Fermentation***
4. ***C:N Deni potential***

From the waste coefficient we can see that.

Diet	P:E_15	P:E_17	P:E_19	P:E_21	PE:_23
TCOD (kg/kg)	0.18^a ± 0.01	0.13^b ± 0.02	0.12^b ± 0.02	0.11^b ± 0.01	0.13^b ± 0.00
Protein (g/kg) ^{2}	29.6^a ±1.8	30.6^{ab} ± 1.8	33.0^{ab} ± 3.5	34.0^{ab} ± 1.5	35.4^b ± 1.6
Lipid (g/kg)	24.0^a ± 2.7	15.8^a ± 3.6	16.1^a ± 4.1	16.0^a ± 4.1	18.5^a ± 1.89
NFE (g/kg)^3	82.6^a ± 1.1	63.1^b ± 4.8	54.0^{bc} ± 5.7	48.1^c ± 1.9	55.7^{bc} ± 2.8

NFE: Nitrogen free extract (carbohydrates)

Results Waste coefficient

Part I a): Characterizing the hydrolysis and fermentation processes

1. ***Waste coefficient***
2. *Hydrolysis*
3. *Fermentation*
4. *C:N Deni potential*

- **Lower protein in diet showed higher organic matter production (more carbon).**

Diet	P:E_15	P:E_17	P:E_19	P:E_21	PE:_23
TCOD (kg/kg)	**0.18^{a} ± 0.01**	0.13^{b} ± 0.02	0.12^{b} ± 0.02	0.11^{b} ± 0.01	0.13^{b} ± 0.00
Protein (g/kg) ^{2}	29.6^{a} ±1.8	30.6^{ab} ± 1.8	33.0^{ab} ± 3.5	34.0^{ab} ± 1.5	35.4^{b} ± 1.6
Lipid (g/kg)	24.0^{a} ± 2.7	15.8^{a} ± 3.6	16.1^{a} ± 4.1	16.0^{a} ± 4.1	18.5^{a} ± 1.89
NFE (g/kg)^{3}	82.6^{a} ± 1.1	63.1^{b} ± 4.8	54.0^{bc} ± 5.7	48.1^{c} ± 1.9	55.7^{bc} ± 2.8

NFE: Nitrogen free extract (carbohydrates)

Results Waste coefficient

Part I a): Characterizing the hydrolysis and fermentation processes

1. Waste coefficient
2. Hydrolysis
3. Fermentation
4. C:N Deni potential

- **Lower protein in diet showed higher organic matter production (more carbon).**
- **Presumably from higher amount of carbohydrates.**

Diet	P:E_15	P:E_17	P:E_19	P:E_21	PE:_23
TCOD (kg/kg)	**0.18^{a} ± 0.01**	0.13^{b} ± 0.02	0.12^{b} ± 0.02	0.11^{b} ± 0.01	0.13^{b} ± 0.00
Protein (g/kg) ^{2}	29.6^{a} ±1.8	30.6^{ab} ± 1.8	33.0^{ab} ± 3.5	34.0^{ab} ± 1.5	35.4^{b} ± 1.6
Lipid (g/kg)	24.0^{a} ± 2.7	15.8^{a} ± 3.6	16.1^{a} ± 4.1	16.0^{a} ± 4.1	18.5^{a} ± 1.89
NFE (g/kg)^{3}	**82.6^{a} ± 1.1**	63.1^{b} ± 4.8	54.0^{bc} ± 5.7	48.1^{c} ± 1.9	55.7^{bc} ± 2.8

NFE: Nitrogen free extract (carbohydrates)

Results Waste coefficient

Part I a): Characterizing the hydrolysis and fermentation processes

1. ***Waste coefficient***
2. ***Hydrolysis***
3. ***Fermentation***
4. ***C:N Deni potential***

- **Increasing protein in diet increased N found in the waste.**
- **So theres more N to be treated.**

Diet	**P:E_15**	**P:E_17**	**P:E_19**	**P:E_21**	**PE:_23**
TCOD (kg/kg)	$0.18^{a} \pm 0.01$	$0.13^{b} \pm 0.02$	$0.12^{b} \pm 0.02$	$0.11^{b} \pm 0.01$	$0.13^{b} \pm 0.00$
Protein (g/kg) [2]	**$29.6^{a} \pm 1.8$**	**$30.6^{ab} \pm 1.8$**	**$33.0^{ab} \pm 3.5$**	**$34.0^{ab} \pm 1.5$**	**$35.4^{b} \pm 1.6$**
Lipid (g/kg)	$24.0^{a} \pm 2.7$	$15.8^{a} \pm 3.6$	$16.1^{a} \pm 4.1$	$16.0^{a} \pm 4.1$	$18.5^{a} \pm 1.89$
NFE (g/kg)[3]	$82.6^{a} \pm 1.1$	$63.1^{b} \pm 4.8$	$54.0^{bc} \pm 5.7$	$48.1^{c} \pm 1.9$	$55.7^{bc} \pm 2.8$

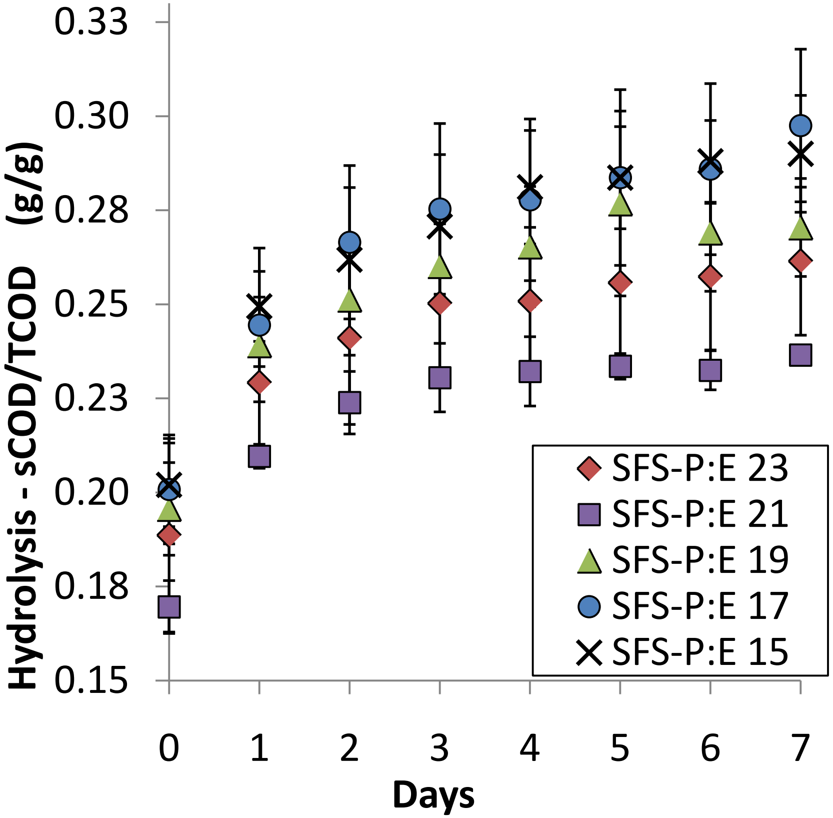
↑

Results Hydrolysis

1. ***Waste coefficient***
2. ***Hydrolysis***
3. ***Fermentation***
4. ***C:N Deni potential***

Part I a): Characterizing the hydrolysis and fermentation processes

What about the hydrolysis process?



Results Hydrolysis

1. ***Waste coefficient***
2. ***Hydrolysis***
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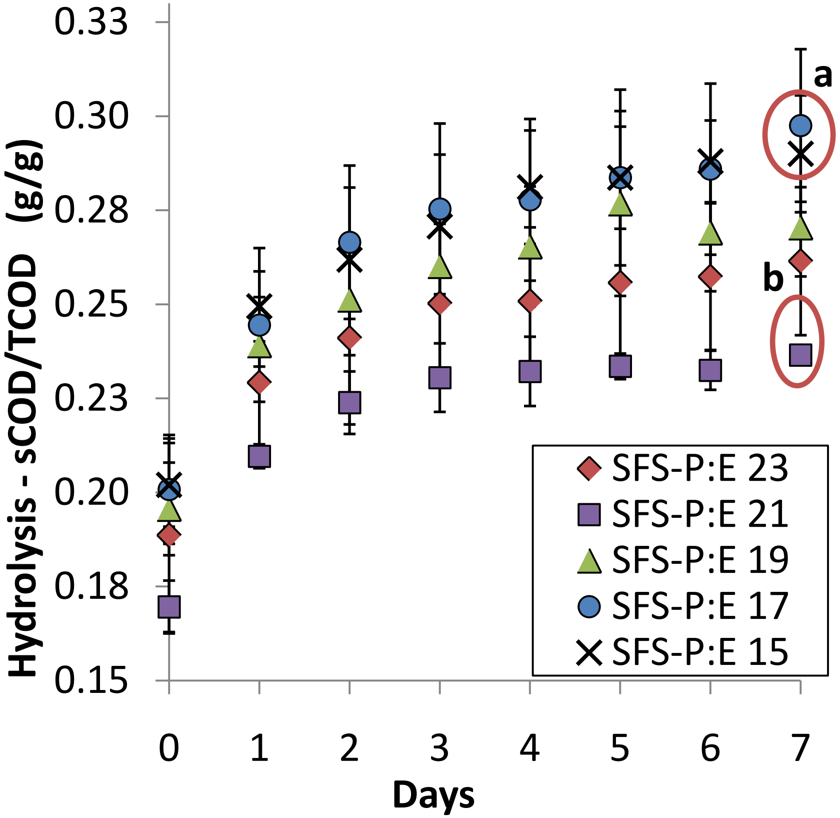
Part I a): Characterizing the hydrolysis and fermentation processes

What about the hydrolysis process?

- Lower protein content in diet the higher hydrolysis (sCOD/TCOD)



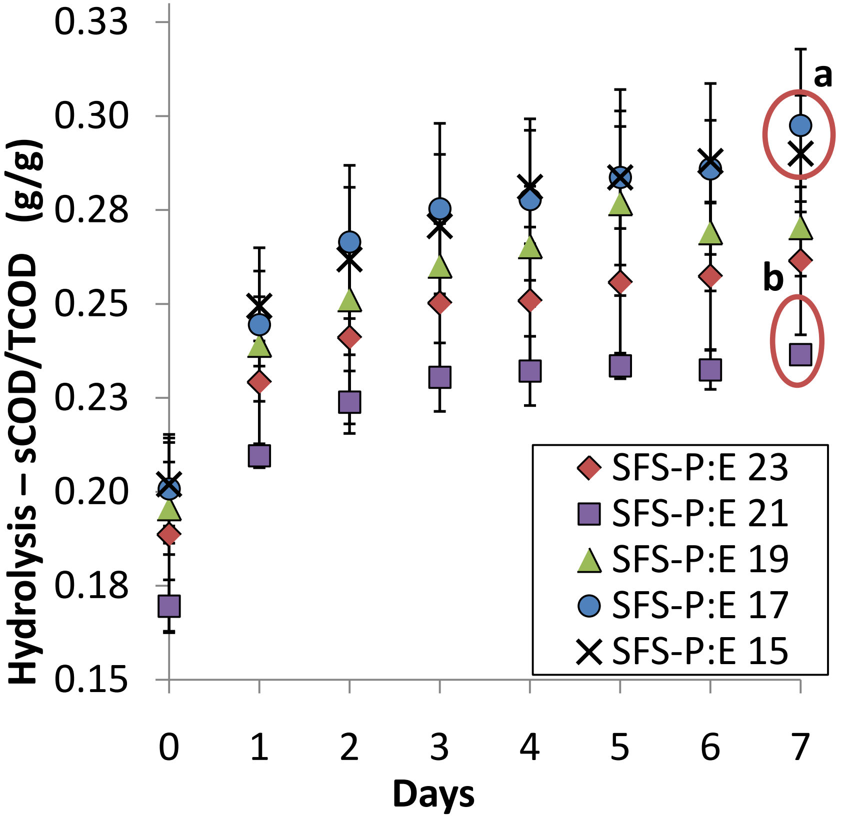
Results Hydrolysis

1. Waste coefficient
2. Hydrolysis
3. Fermentation
4. C:N Deni potential

Part I a): Characterizing the hydrolysis and fermentation processes

What about the hydrolysis process?

- Lower protein content in diet the higher hydrolysis (sCOD/TCOD)
- **Only 23-30% solubilization (sCOD/TCOD)**
- This means the only 23-30% of the recovered organic matter is solubilized
- Similar values as described before
  (Conroy and Couturier, 2010)
  (Suhr et al., 2012)



Results Fermentation

1. *Waste coefficient*
2. *Hydrolysis*
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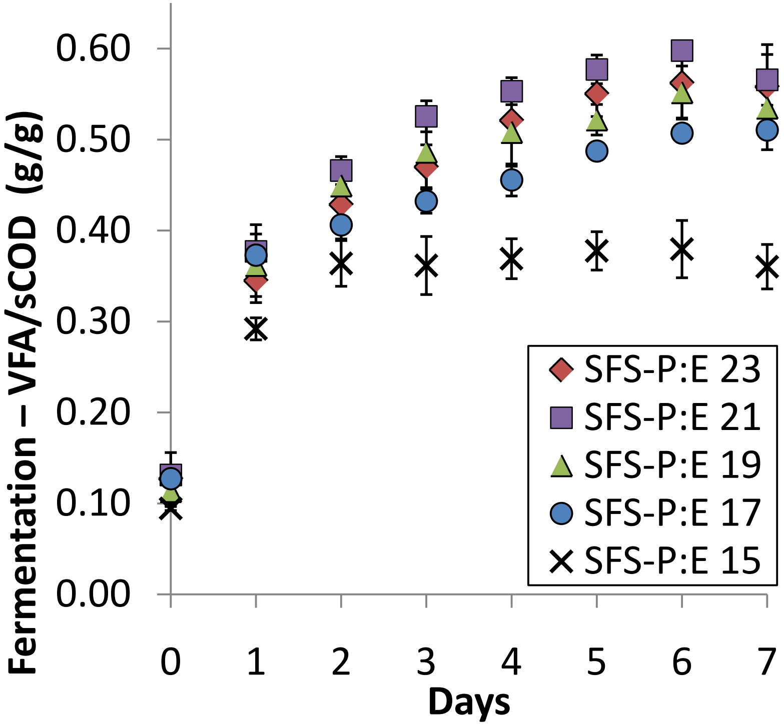
4. *C:N Deni potential*

Part I a): Characterizing the hydrolysis and fermentation processes

What about the fermentation process?



Results Fermentation

1. ***Waste coefficient***
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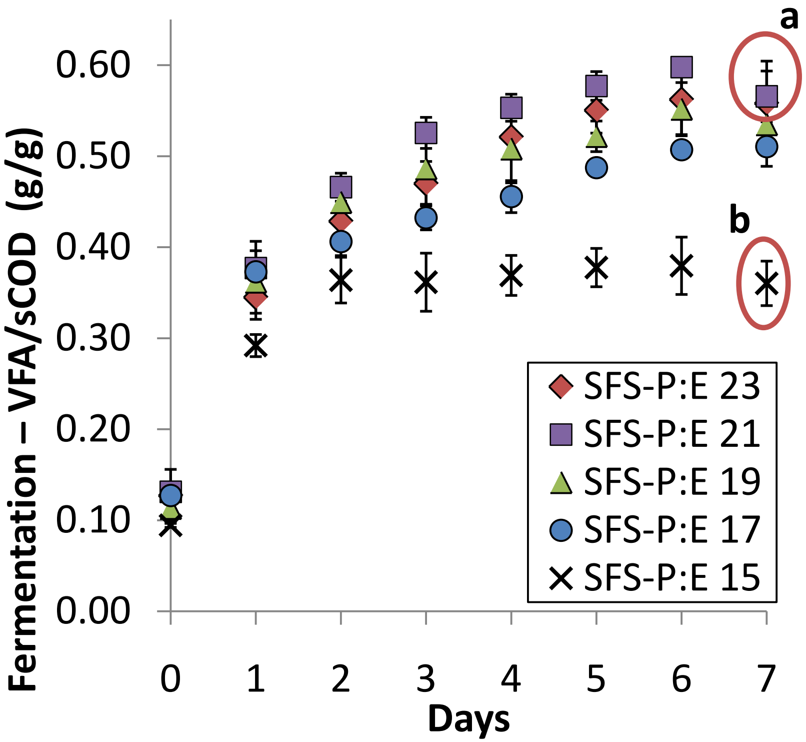
Part I a): Characterizing the hydrolysis and fermentation processes

What about the fermentation process?

- Lower protein content in diet the lower fermentation of soluble carbon (VFA/sCOD)



Results Fermentation

1. *Waste coefficient*
2. *Hydrolysis*
3. *Fermentation*
4. *C:N Deni potential*

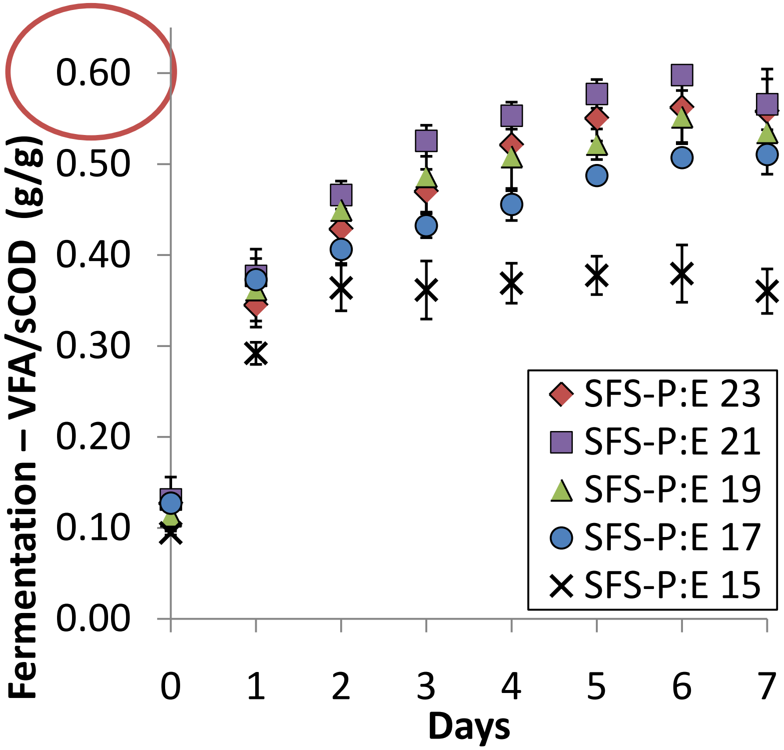
Part I a): Characterizing the hydrolysis and fermentation processes

What about the fermentation process?

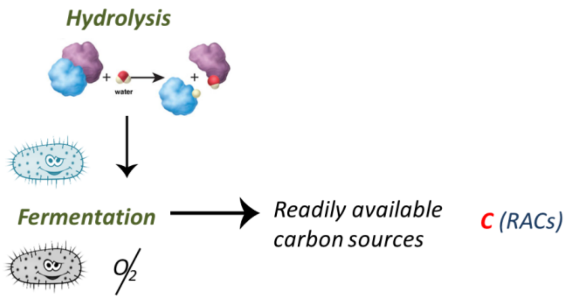
- Lower protein content in diet the lower fermentation of soluble carbon (VFA/sCOD)

- **Lower fermentation capacity than literature (60%).**

  68 – 75% RAS (Suhr et al., 2013).
  83 - 99% WWTP (Henze et al., 2000).



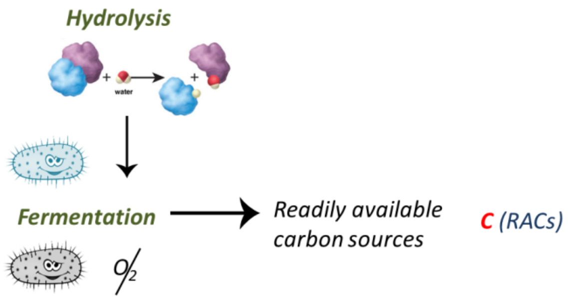
Results Hydrolysis and Fermentation



Part I a): Characterizing the hydrolysis and fermentation processes

More questions than answers…

Results Hydrolysis and Fermentation

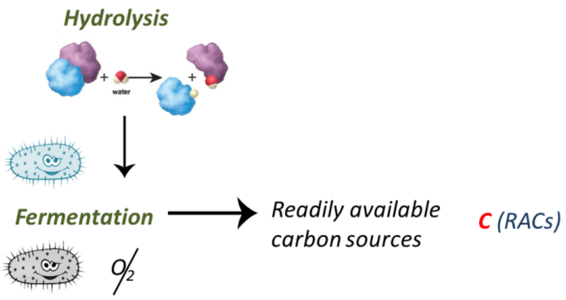


Part I a): Characterizing the hydrolysis and fermentation processes

More questions than answers…

- Why if lower protein diets produced more soluble carbon the fermentation capacity was lower?

Results Hydrolysis and Fermentation



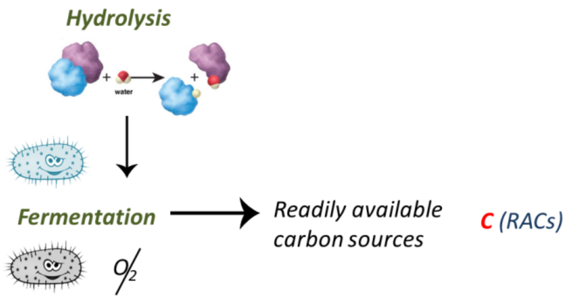
Part I a): Characterizing the hydrolysis and fermentation processes

More questions than answers…

- Why if lower protein diets produced more soluble carbon the fermentation capacity was lower?
- Is the method measuring all the carbon sources produced?

Chance of alcohols → NFE in lower protein diets

Results Hydrolysis and Fermentation



Part I a): Characterizing the hydrolysis and fermentation processes

More questions than answers…

- Why if lower protein diets produced more soluble carbon the fermentation capacity was lower?

- Is the method measuring all the carbon sources produced?

  Chance of alcohols → NFE in lower protein diets

- What to do?

  - Look into individual carbon sources (RACs)

Part I: Characterizing the hydrolysis and fermentation processes of two dietary protein sources, fish meal and soybean meal

Objectives

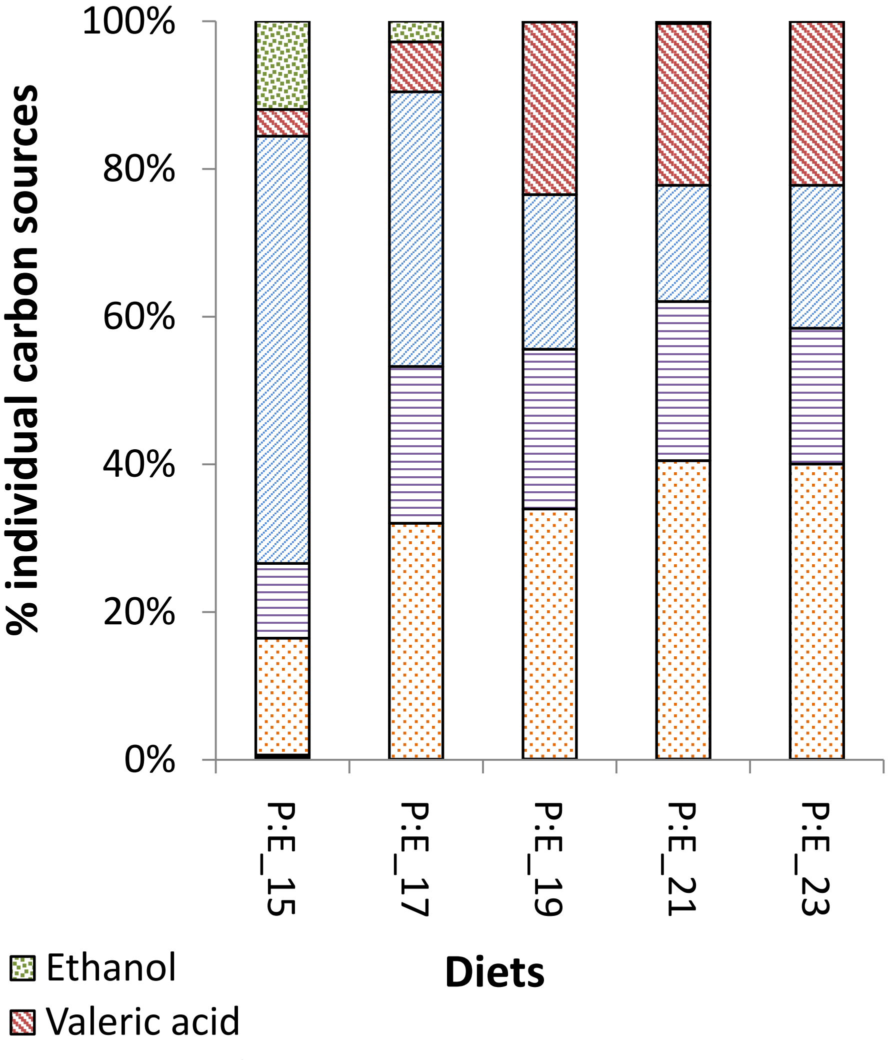
a) Develop indicators for describing the hydrolysis and fermentation processes in order to compare dietary treatments.

b) Characterize organic waste masses and the potential for producing volatile fatty acids.

c) Estimate and compare the potential for pursuing denitrification using organic waste deriving from either fish fed fish meal based or soybean based diets.

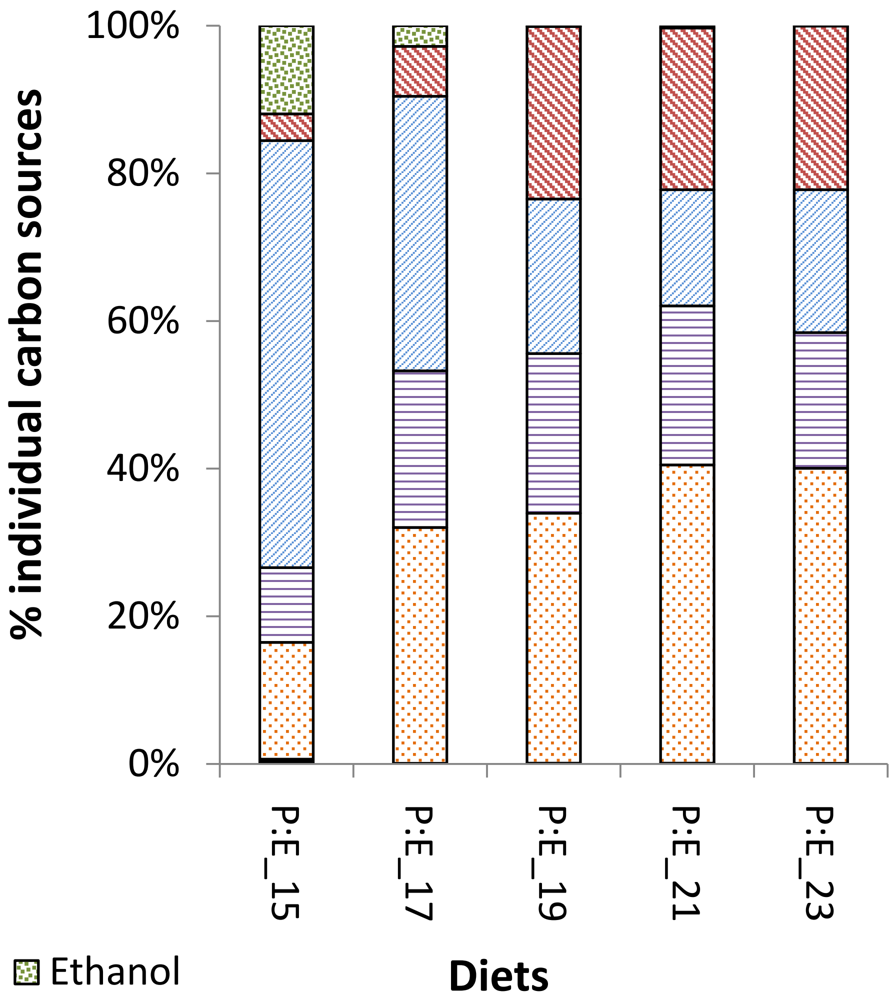
Carbon sources in Fish meal diets

- Different carbon sources were identified (Ethanol).



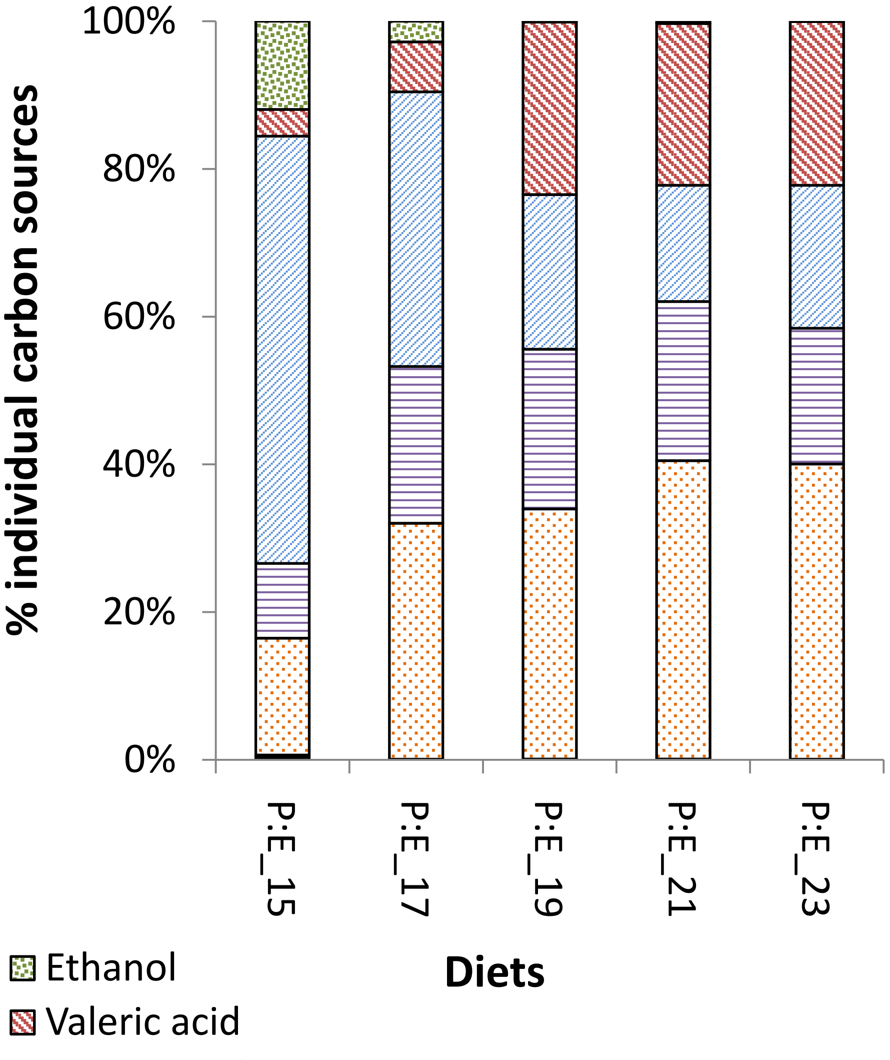
Carbon sources in Fish meal diets

- Different carbon sources were identified (Ethanol).
- Lower Protein diets produced more ethanol and butyric acid (carbohydrates).



Carbon sources in Fish meal diets

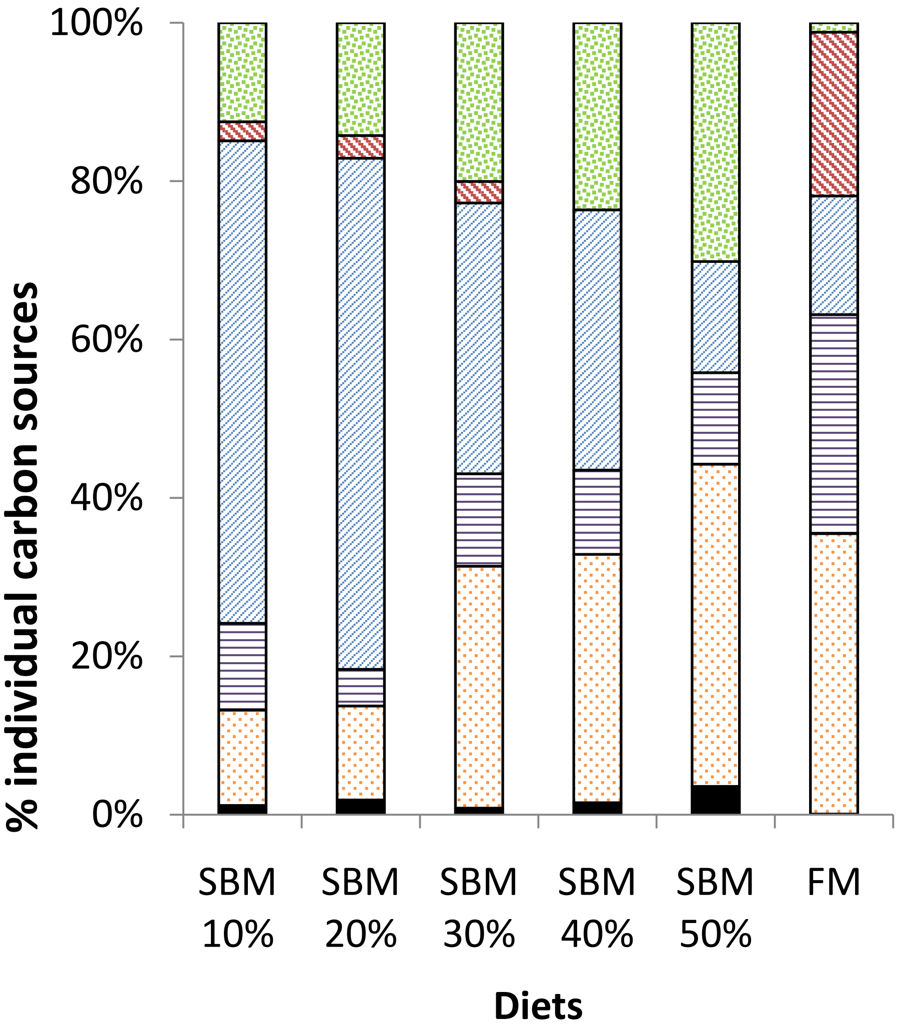
- Different carbon sources were identified (Ethanol).
- Lower Protein diets produced more ethanol and butyric acid (carbohydrates).
- Higher Protein diets produced more Valeric and Acetic acid (protein).



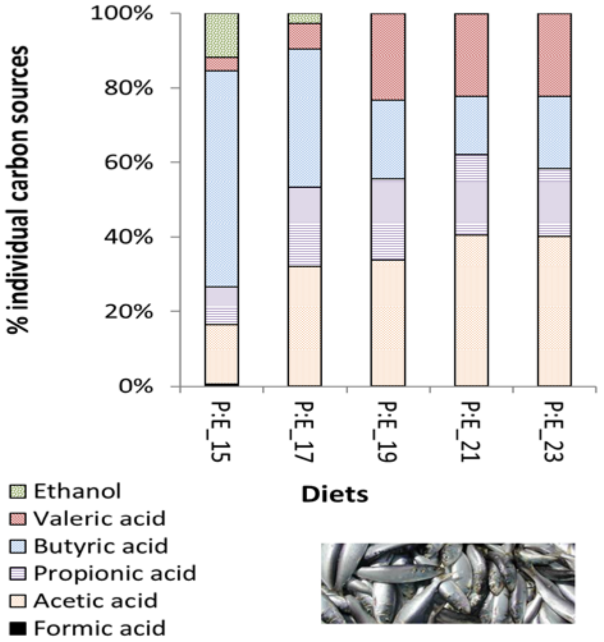
Carbon sources in Soybean meal (SBM) diets

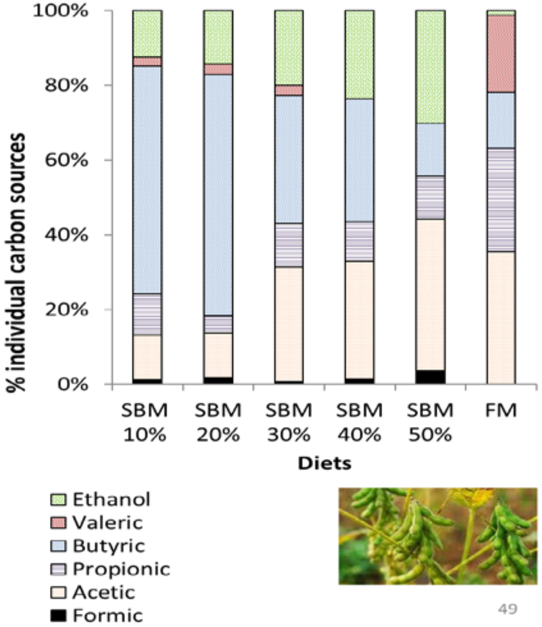
- Increasing SBM diets produced more ethanol (carbohydrates)

- Lower SBM diets produced more Butyric acid (aminoacids)

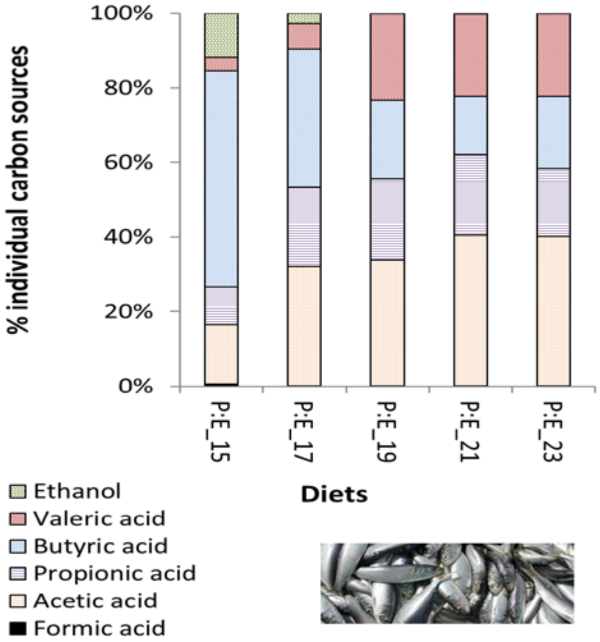


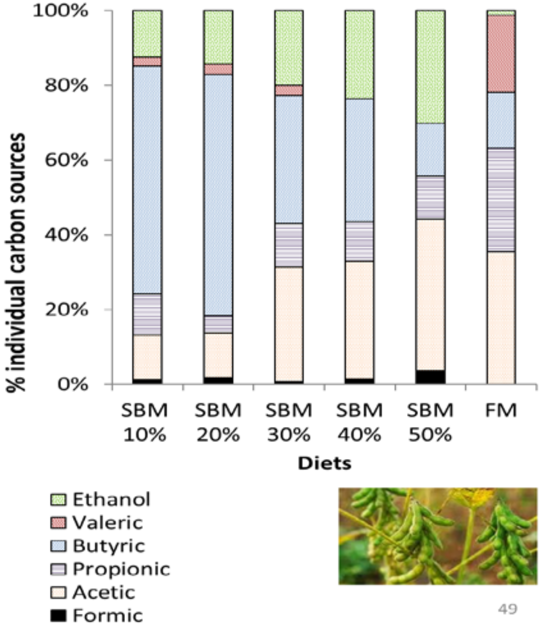
- Dietary composition affects the quantity and type of carbon source produced.





- Dietary composition affects the quantity and type of carbon source produced.
- Capacity to predict or eventually modify carbon type production through diet composition.





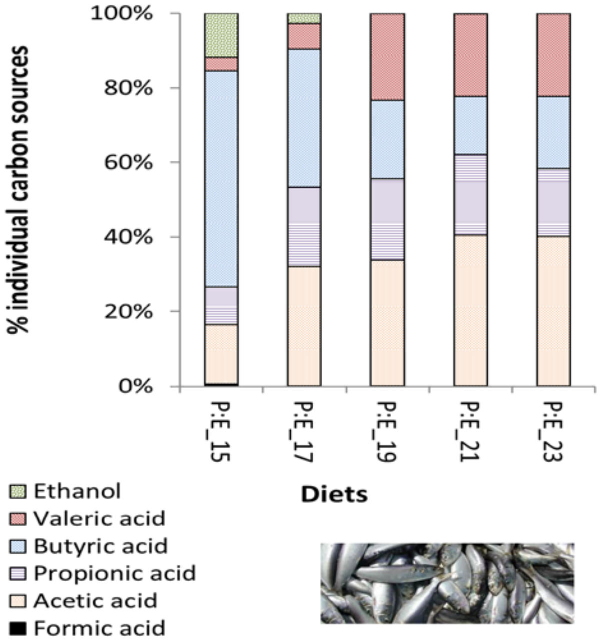
- Dietary composition affects the quantity and type of carbon source produced.

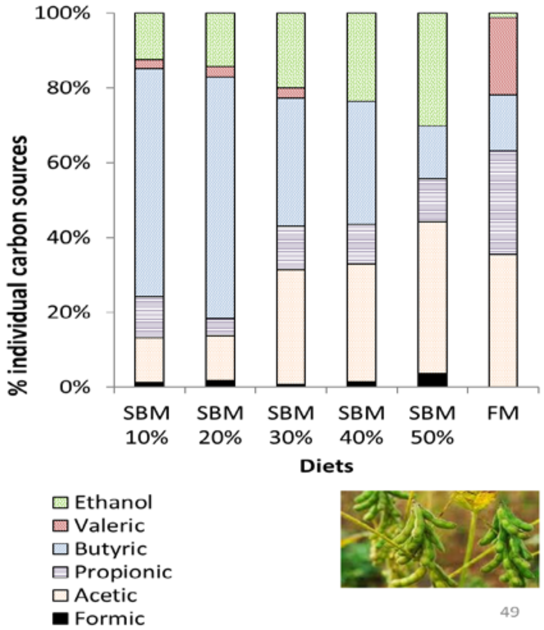
- Capacity to predict or eventually modify carbon type production through diet composition.

- Carbon sources have different:
    - C:N values.
    - Speed of process (denitrification rate).
    - Biomass production (bacteria).

	C:N	Denitrification rate (speed) (mgNO_3-N/mg VSS*d
Acetic	2.05	0.6
Butyric	1.79	0.5
Propionic	1.4	0.4
Ethanol	1.53	0.3
Valeric	1.91	0.5
Mixed VFA	2.37	0.8

(Yatong, 1996)





Part I: Characterizing the hydrolysis and fermentation processes of two dietary protein sources, fish meal and soybean meal

Objectives

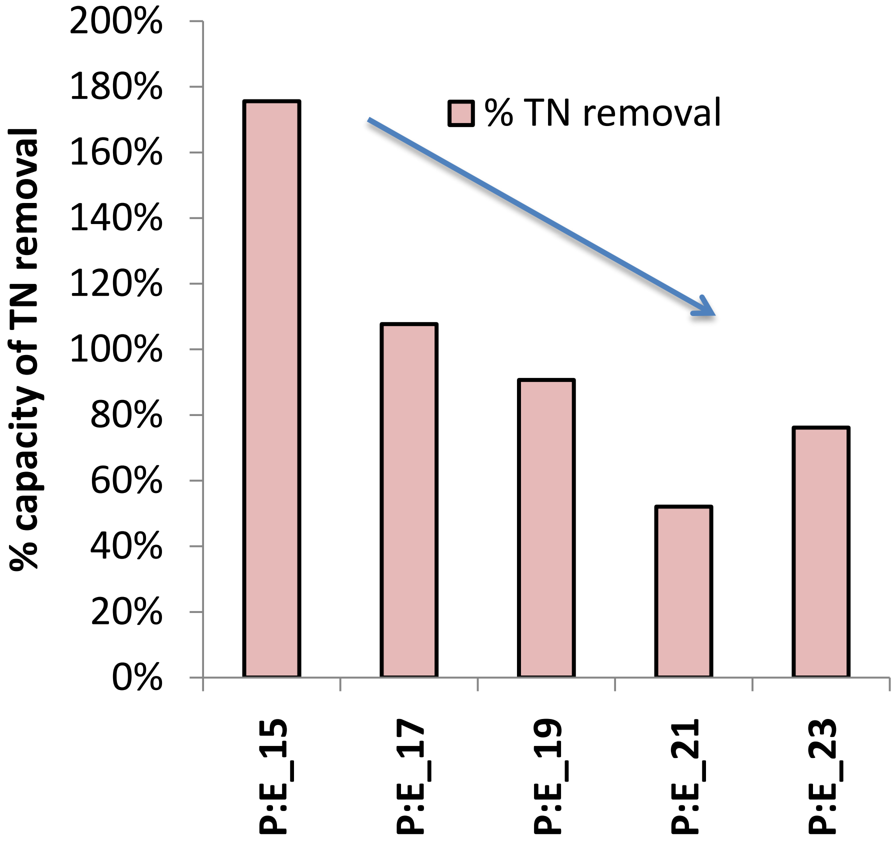
a) Develop indicators for describing the hydrolysis and fermentation processes in order to compare dietary treatments.

b) Characterize organic waste masses and the potential for producing volatile fatty acids.

c) Estimate and compare the potential for pursuing denitrification using organic waste deriving from either fish fed fish meal based or soybean based diets.

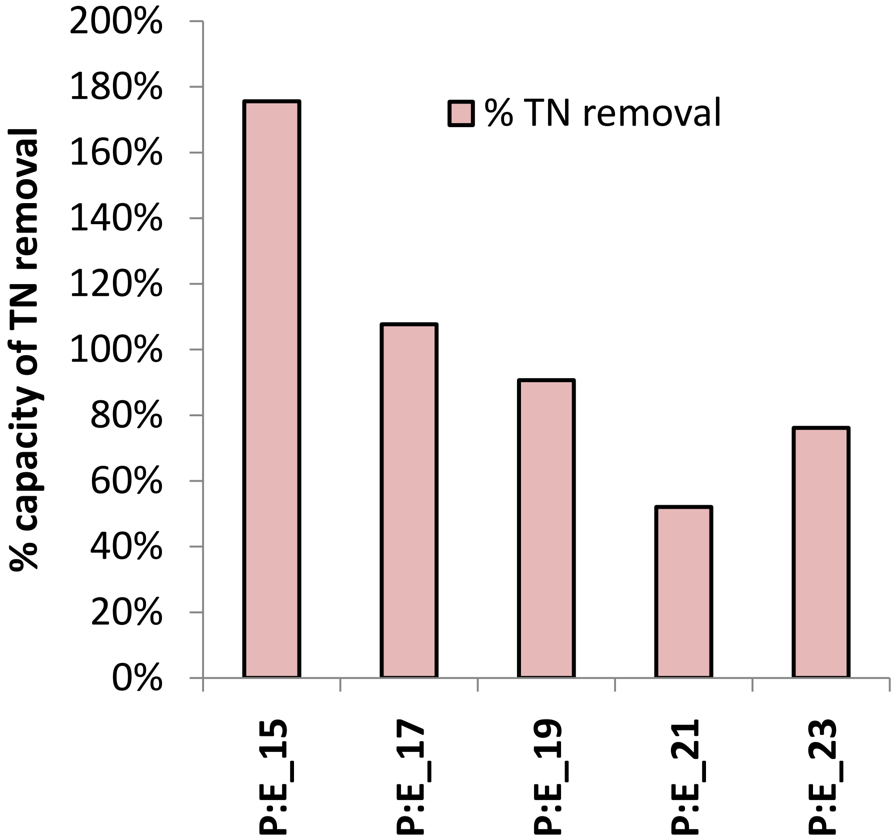
Comparison in the potential for pursuing denitrification between fish fed fish meal based or soybean based diets (C:N according to Yatong, 1996)

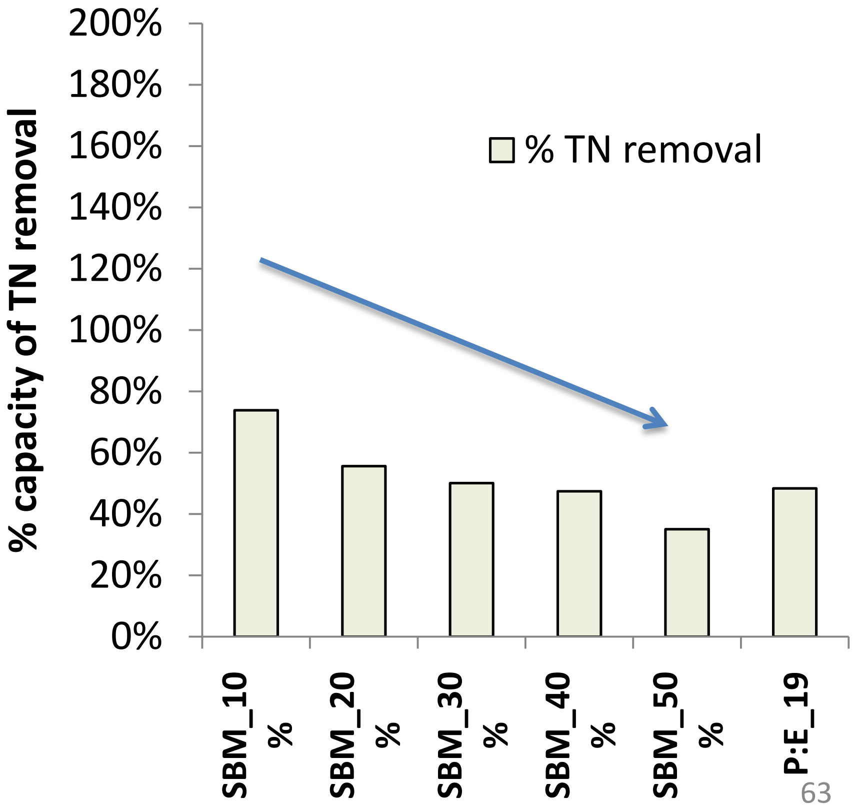
- Fish meal: denitrification capacity was reduced as protein increased (N in waste)



Comparison in the potential for pursuing denitrification between fish fed fish meal based or soybean based diets (C:N according to Yatong, 1996)

- Fish meal: denitrification capacity was reduced as protein increased (N in waste)
- Soybean: denitrification capacity was reduced as SBM inclusion increased (fermentation)





Overview of the hydrolysis fermentation process

$$C = \frac{\text{Carbons produced}}{\text{feed consumed}}$$

Overview of the hydrolysis fermentation process

$$C= \frac{\textbf{Carbons produced}}{\textbf{feed consumed}} = \overset{\boxed{1}}{\overset{\boxed{\text{Waste coefficient}}}{\underbrace{\frac{\text{TCOD produced}}{\text{Feed consumed}}}_{\textbf{20-25\%}}}}$$

Good values

Overview of the hydrolysis fermentation process

$$\boxed{1} \longrightarrow \boxed{2}$$

$$C = \frac{\textbf{Carbons produced}}{\textbf{feed consumed}} = \overset{\boxed{\text{Waste coefficient}}}{\underbrace{\frac{\text{TCOD produced}}{\text{Feed consumed}}}_{\textbf{20-25\%}}} * \overset{\boxed{\text{Hydrolysis}}}{\underbrace{\frac{\text{sCOD}}{\text{TCOD}}}_{\textbf{23-30\%}}}$$

Good values

Ok values

Overview of the hydrolysis fermentation process

$$\boxed{1} \longrightarrow \boxed{2} \longrightarrow \boxed{3}$$

Waste coefficient

Hydrolysis

Fermentation

$$C= \frac{\textbf{Carbons produced}}{\textbf{feed consumed}} = \underbrace{\frac{\text{TCOD produced}}{\text{Feed consumed}}}_{\substack{\textbf{20-25\%} \\ \text{Good} \\ \text{values}}} * \underbrace{\frac{\text{sCOD}}{\text{TCOD}}}_{\substack{\textbf{23-30\%} \\ \text{Ok} \\ \text{values}}} * \underbrace{\frac{\text{RACs}}{\text{sCOD}}}_{\substack{\textbf{85-95\%} \\ \text{Good} \\ \text{values}}}$$

- Not much to do in waste coefficient and fermentation

Overview of the hydrolysis fermentation process

$$\boxed{1} \longrightarrow \boxed{2} \longrightarrow \boxed{3}$$

Waste coefficient	Hydrolysis	Fermentation

$$C= \frac{\textbf{Carbons produced}}{\textbf{feed consumed}} = \underbrace{\frac{\text{TCOD produced}}{\text{Feed consumed}}}_{\textbf{20-25\%}} * \underbrace{\frac{\text{sCOD}}{\text{TCOD}}}_{\textbf{23-30\%}} * \underbrace{\frac{\text{RACs}}{\text{sCOD}}}_{\textbf{85-95\%}}$$

Good values	Ok values	Good values

- Not much to do in waste coefficient and fermentation

- Improvements should focus on Hydrolysis
  - **Limiting the process!!**

Summary Part I

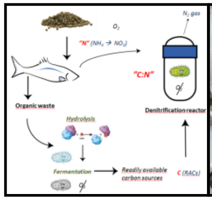
- The methodology for describing the hydrolysis and fermentation processes allows a good comparison between dietary treatments.

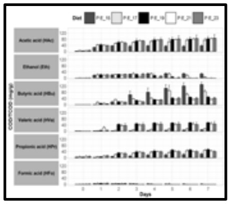
- Feed composition affects the type of carbon types (RACs) produced.

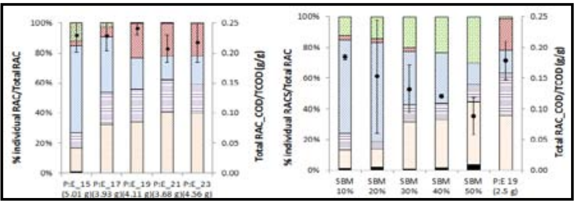
  SBM: Ethanol and Butyric acid while FM: Acetic acid and Valeric acid

- In fish meal diets increasing the amount of protein reduces the capacity for denitrification due to more N produced in the waste.

- In soybean meal diets the higher the inclusion of vegetable protein the lower the capacity for denitrification due to a lower fermentation capacity of the waste.







Part II: Optimization of the hydrolysis and fermentation processes

Objective:

- Increase the solubilization of carbon (sCOD/TCOD).

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- Increase the solubilization of carbon (sCOD/TCOD).

Different treatments were evaluated

- pH
- Temperature
- Enzyme addition (protease)
- Anaerobic sequence batch reactor
  (water was replaced under anaerobic and aerobic conditions)

Part II: Optimization of the hydrolysis and fermentation processes

Objective:

- Increase the solubilization of carbon (sCOD/TCOD).

Different treatments were evaluated
(Only the most relevant results will be shown)

- pH
- Temperature
- Enzyme addition (protease)
- Anaerobic sequence batch reactor (water was replaced under anaerobic and aerobic conditions)

Part II: Optimization of the hydrolysis and fermentation processes (pH)

Objective:

- Increase the solubilization of carbon (sCOD/TCOD).

- Fish intestine bacteria is sensitive to pH
  (30% reduction in proteolytic activity at pH 6) (Hidalgo, 1998)

- In an activated sludge system at pH 10 a higher VFA production was found and methanogenic bacteria was inhibited (compete for C sources)
  (Chen et al., 2007)

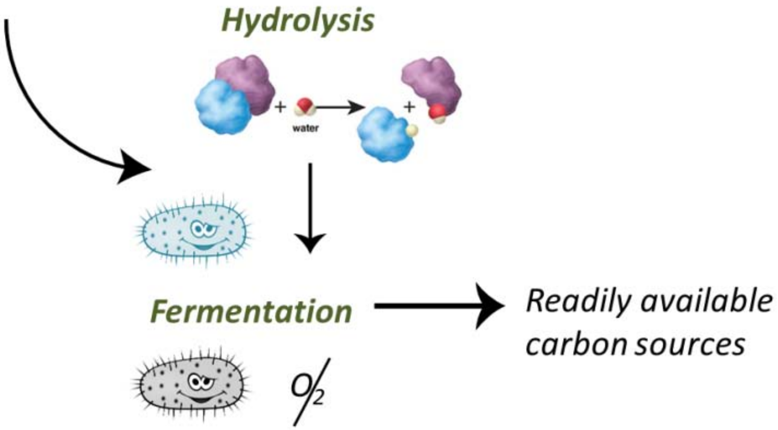
- Higher solubilization of carbon under alkaline conditions (pH 11)
  (Wu et al., 2009)

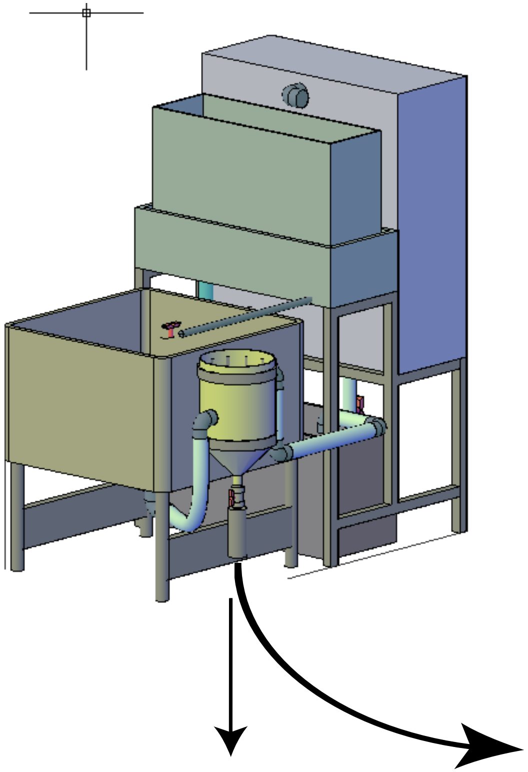
Part II: Optimization of the hydrolysis and fermentation processes (pH)

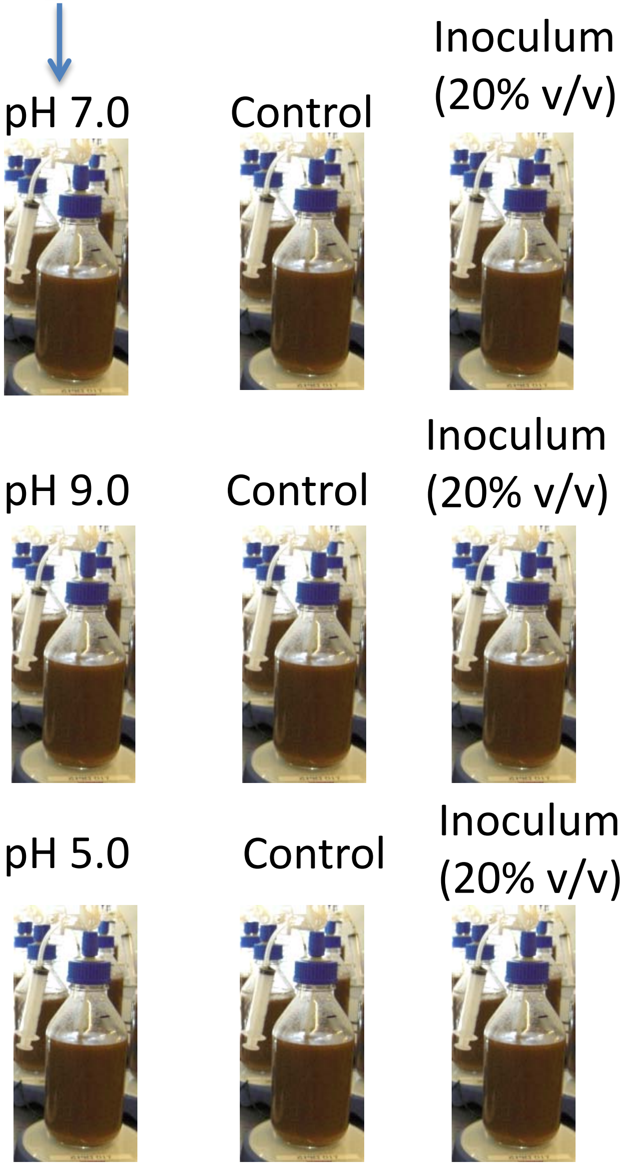
Two questions arroused:

- *How does pH affects fish waste hydrolysis/fermentation process?*

- *Can be the process enhanced with more robust bacteria?*







pH 7.0

Control

Inoculum (20% v/v)

pH 9.0

Control

Inoculum (20% v/v)

pH 5.0

Control

Inoculum (20% v/v)

Inoculum (20% v/v)

- 48 hours before
- 12 hr O_2
- 12 hr $\cancel{O_2}$

Part II: Optimization of the hydrolysis and fermentation processes (pH)

The solubilization of particulate organic matter could no be increased!! (sCOD/TCOD)

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The solubilization of particulate organic matter could no be increased!! (sCOD/TCOD)

Fermentation

pH: 9

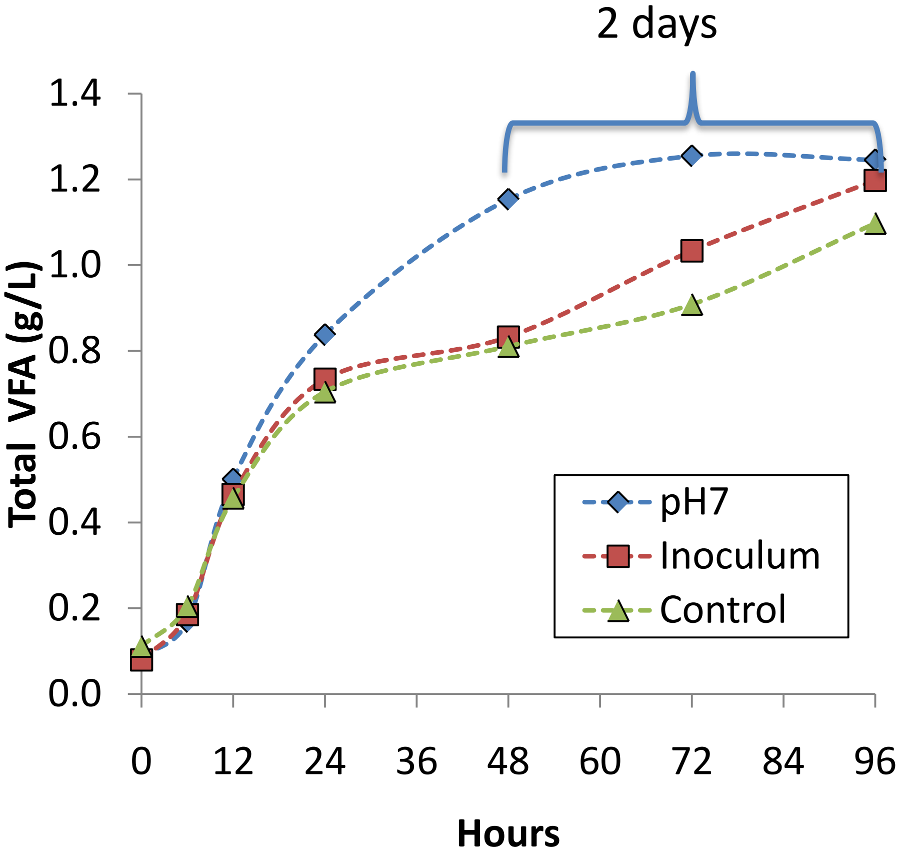
- After 48 hr consumption.

pH: 7

- Reduced the speed process 2 days (reduce size of reactor)

pH: 5

- VFA production practically inhibited.

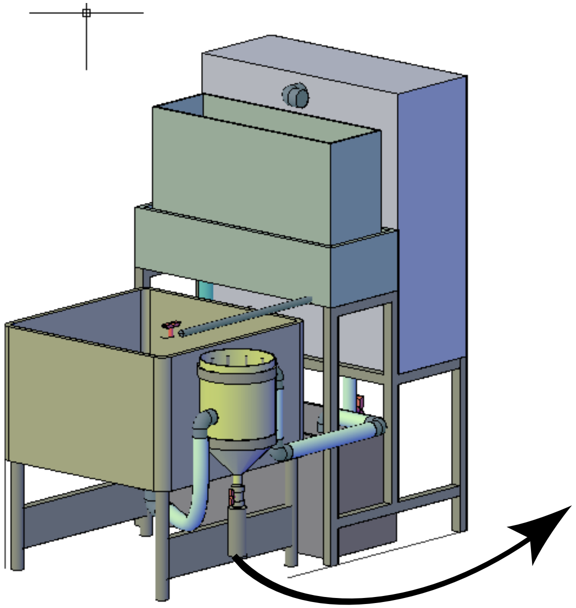


Part II: Optimization of the hydrolysis and fermentation processes (Temperature)

- Temperature is a well know parameter affecting bacterial activity
(Henze et al., 2008)

- Strong effect of temperature on hydrolysis rate (increased)
(Yuan et al., 2011)

Part II: Optimization of the hydrolysis and fermentation processes (Temperature)



20°C

20°C

20°C

40°C

40°C

40°C

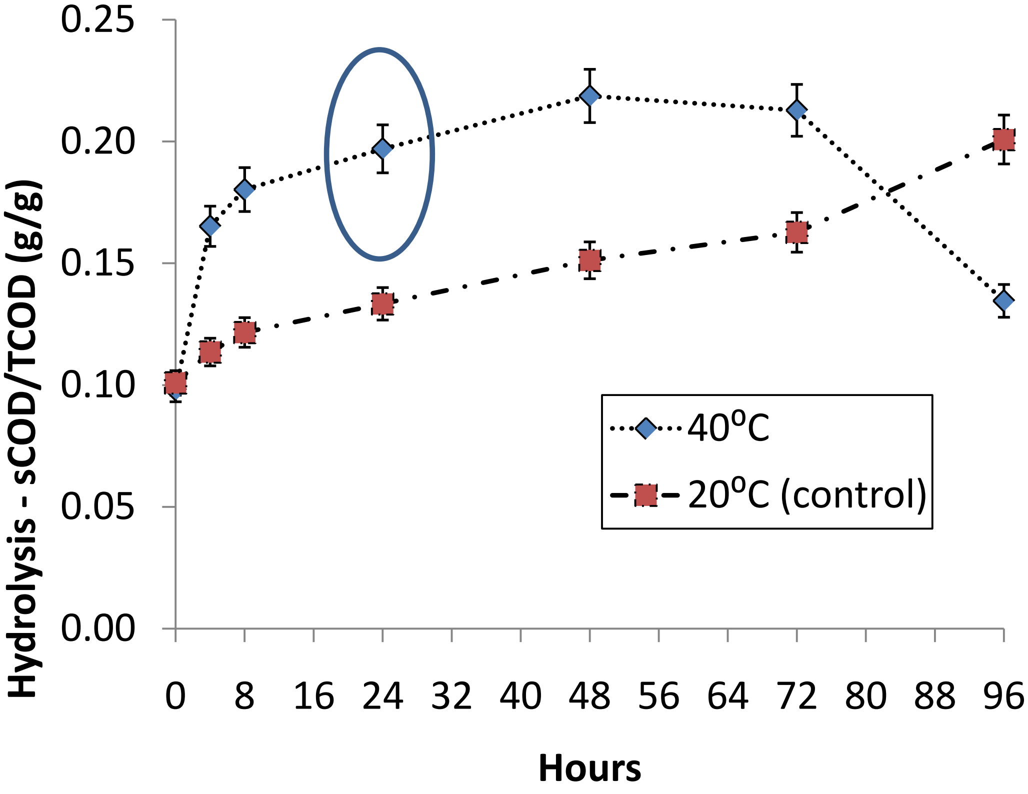
Part II: Optimization of the hydrolysis and fermentation processes (Temperature)

The solubilization of particulate organic matter could no be increased!! (sCOD/TCOD)

Part II: Optimization of the hydrolysis and fermentation processes (Temperature)

The solubilization of particulate organic matter could no be increased!! (sCOD/TCOD)

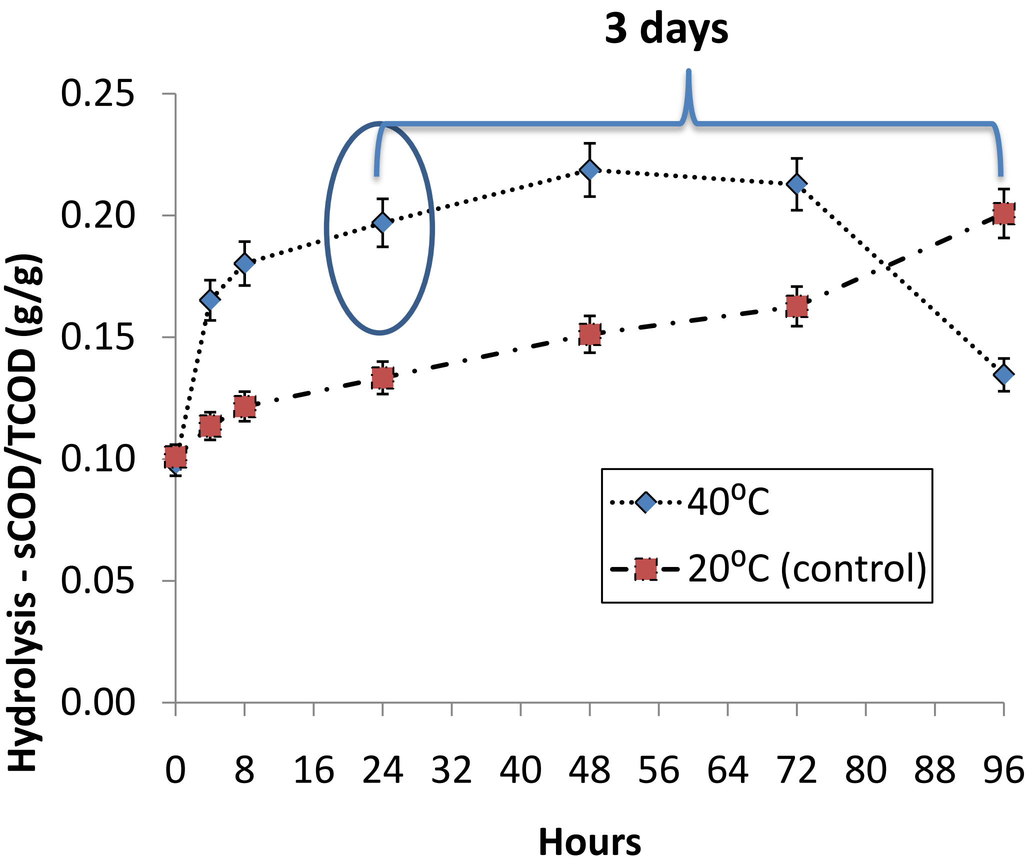
- Strong influence on hydrolysis rate
- 40°C showed 35% more sCOD at 24 hr



Part II: Optimization of the hydrolysis and fermentation processes (Temperature)

The solubilization of particulate organic matter could no be increased!! (sCOD/TCOD)

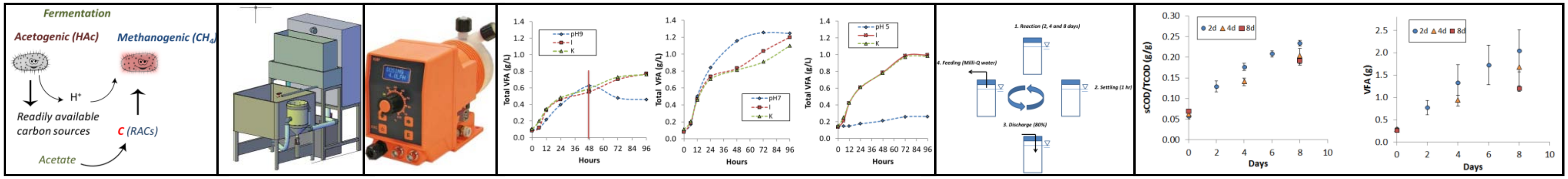
- Strong influence on hydrolysis rate
- 40°C showed 35% more sCOD at 24 hr
- Reduction of the process in 3 days (reduce size reactor)



Summary Part II

- None of the different methods applied for optimizing the degree of solubilization were able to solubilize more than 30% of the total COD.
- Temperature at 40^{o}C (mesophilic conditions) or a constant pH of 7 speed up the VFA production process

  (optimizing the required volume of the reactor).



Part III: Applicability of internal carbon sources for denitrification on a Danish brood stock farm (a mass balance approach).

Objective

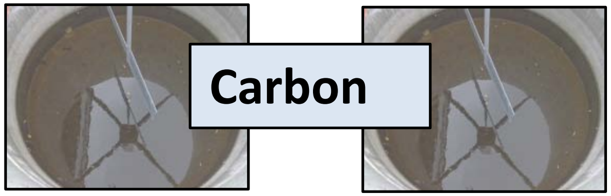
Evaluate the applicability of a side stream fermenter (SSF) for producing internal carbon sources for denitrification on a Danish trout farm.

- Hatchery and 10 earthen pond raceways
- Low intensity brood stock farm (21 m^3 make up water/Kg feed)
- 100 Kg feed/day

System design

Part III: *Applicability of internal carbon sources for denitrification on a Danish brood stock farm (real life).*

- 2 Fermenters (11.9 m^3)
- Working in parallel to deliver a constant supply ”C”



System design

Part III: *Applicability of internal carbon sources for denitrification on a Danish brood stock farm (real life).*

- 2 Fermenters (11.9 m^3)
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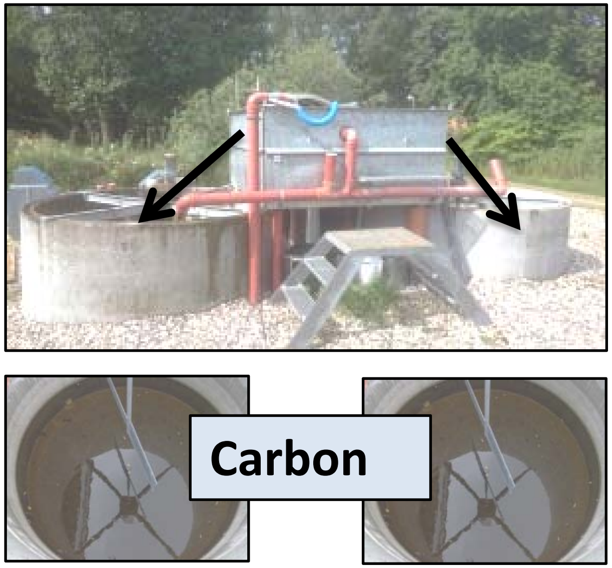

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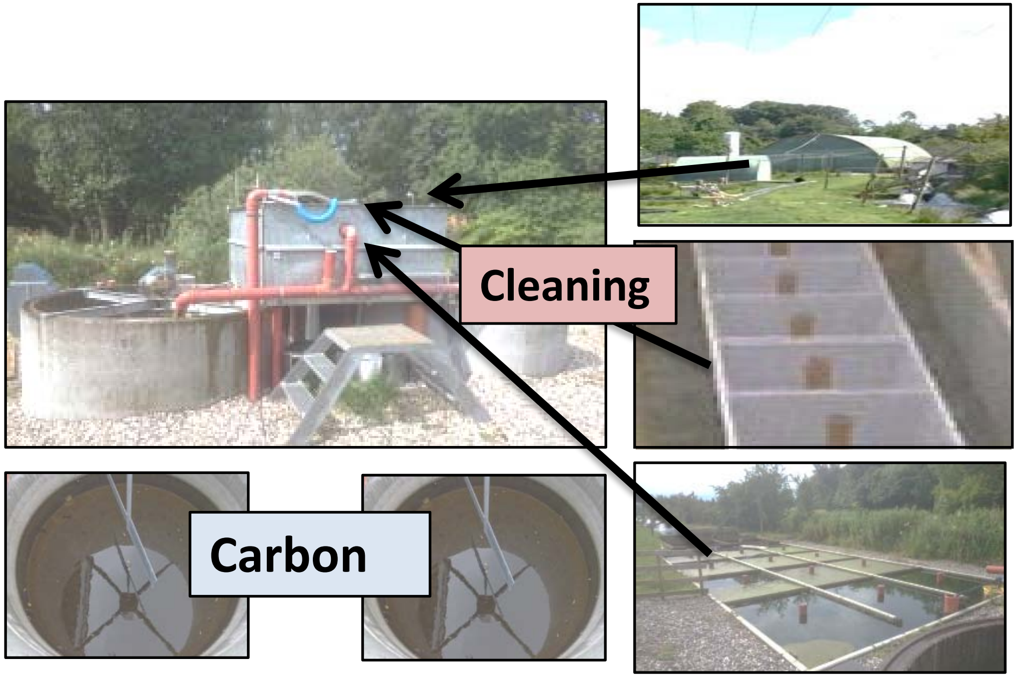

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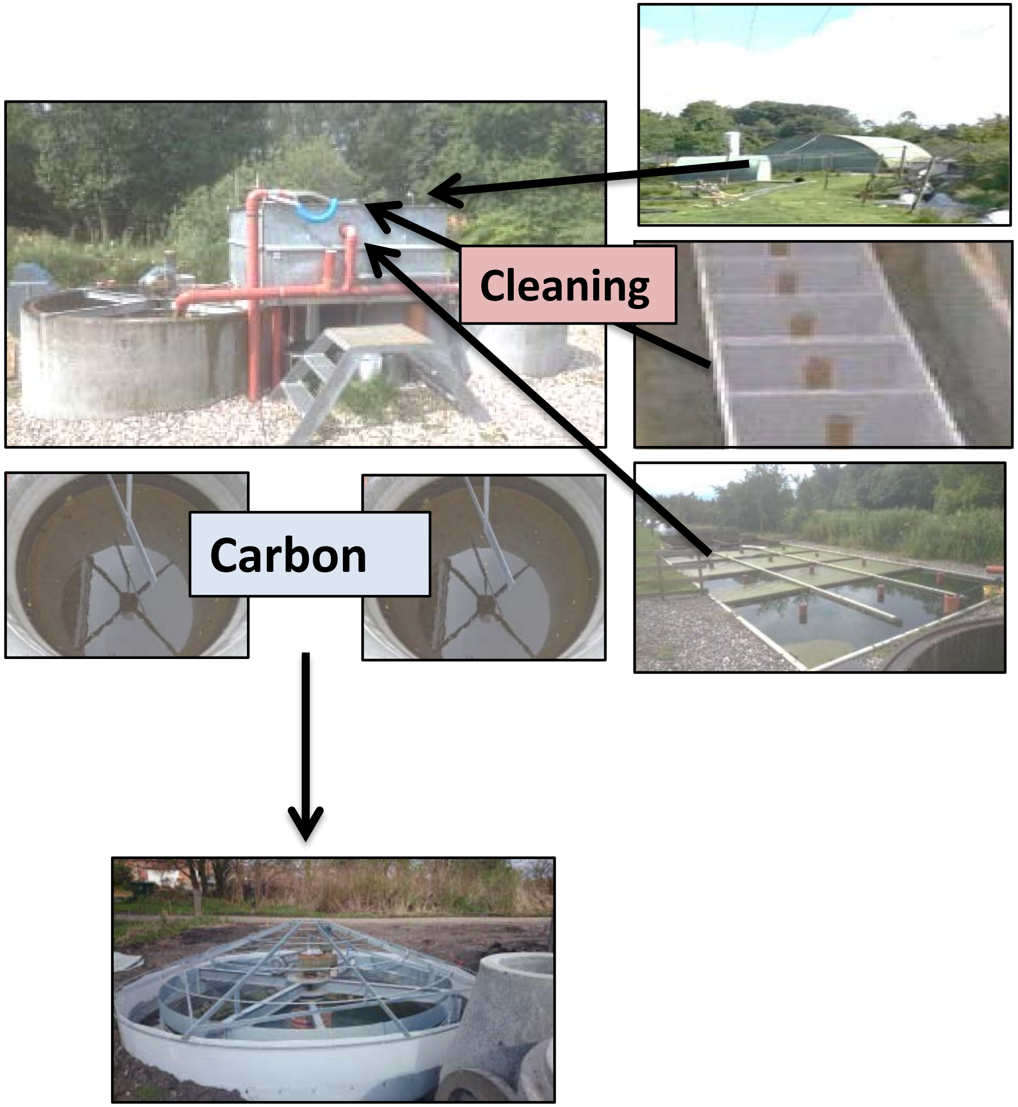

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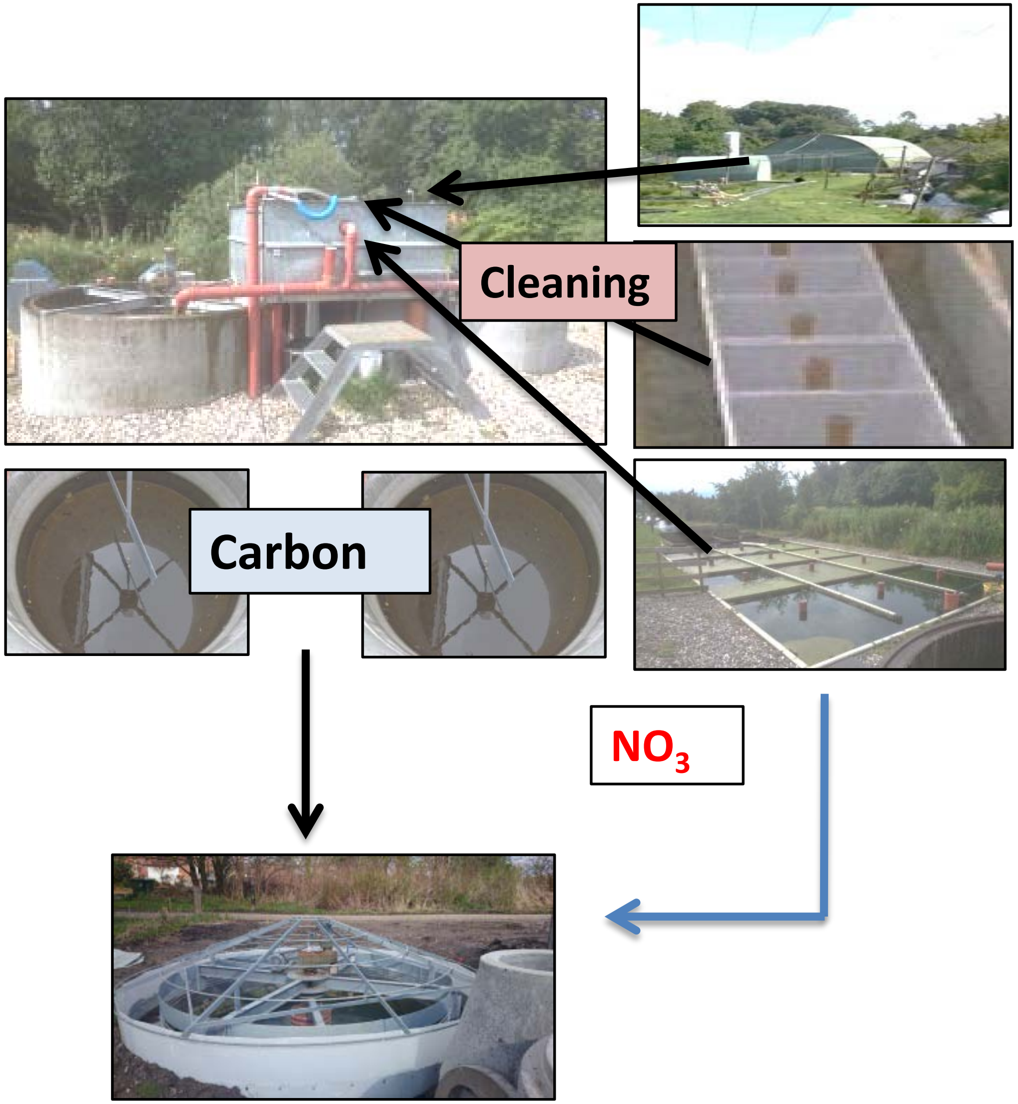

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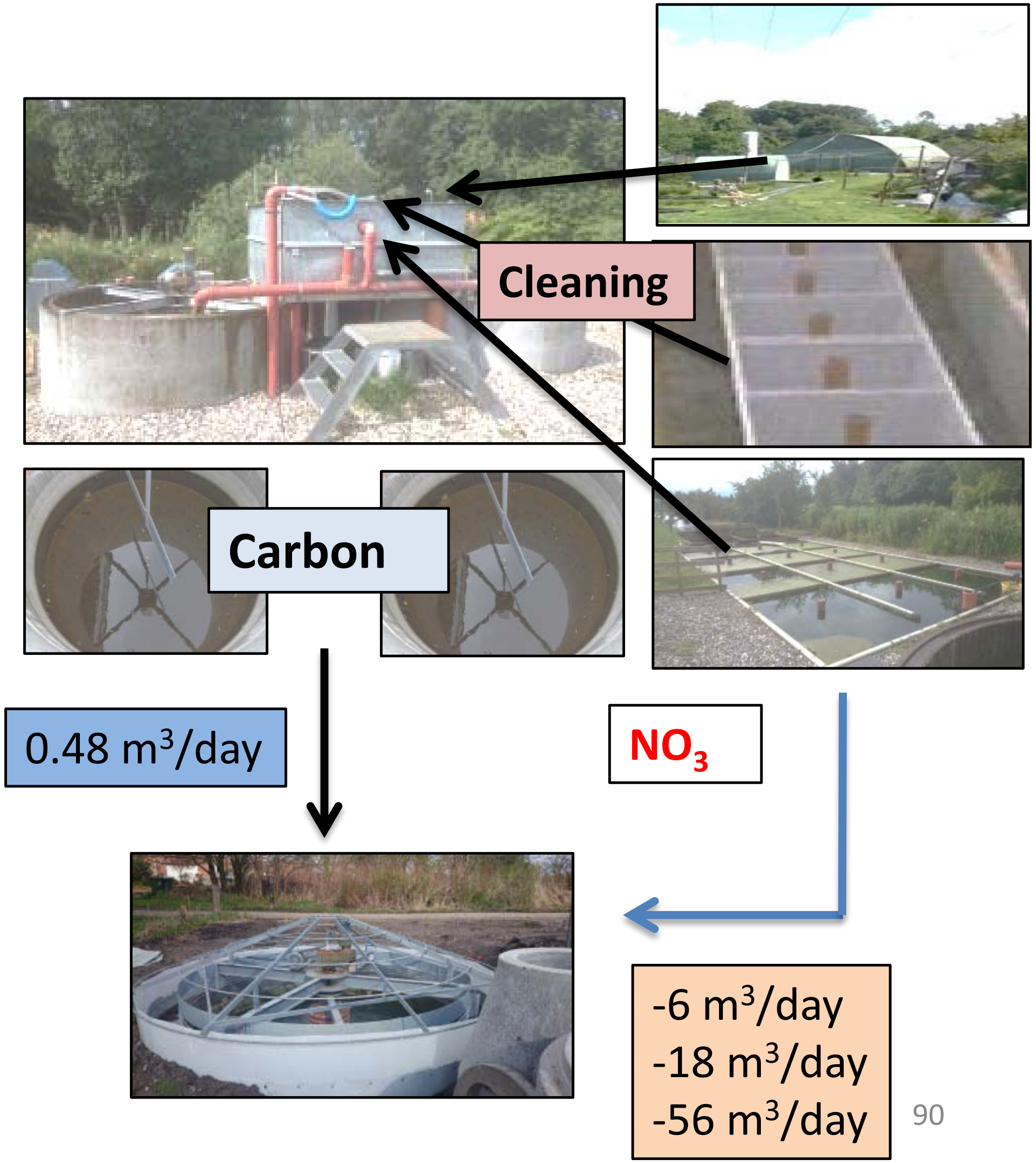


Cleaning

Carbon

0.48 m^3/day

NO_3

-6 m^3/day
-18 m^3/day
-56 m^3/day

Results/Discussion SSF

Part III: *Applicability of internal carbon sources for denitrification on a Danish brood stock farm (a mass balance approach).*

1 → 2 → 3

Waste coefficient

Hydrolysis

Fermentation

$$C= \frac{\textbf{Carbons produced}}{\textbf{feed consumed}} = \underbrace{\frac{\text{TCOD produced}}{\text{Feed consumed}}}_{\textbf{20-25\%}} * \underbrace{\frac{\text{sCOD}}{\text{TCOD}}}_{\textbf{23-30\%}} * \underbrace{\frac{\text{RACs}}{\text{sCOD}}}_{\textbf{85-95\%}}$$

Part I: Ideal conditions

Results/Discussion SSF

Part III: *Applicability of internal carbon sources for denitrification on a Danish brood stock farm (a mass balance approach).*

1 → 2 → 3

$$C = \frac{\textbf{Carbons produced}}{\textbf{feed consumed}} = \underbrace{\frac{\text{TCOD produced}}{\text{Feed consumed}}} * \underbrace{\frac{\text{sCOD}}{\text{TCOD}}} * \underbrace{\frac{\text{RACs}}{\text{sCOD}}}$$

	1	2	3
	Waste coefficient	Hydrolysis	Fermentation
Part I: Ideal conditions	**20-25%**	**23-30%**	**85-95%**
Low intensity farm	**4%**	**2.3%**	**34%**

Results/Discussion SSF

Part III: *Applicability of internal carbon sources for denitrification on a Danish brood stock farm (a mass balance approach).*

1

Waste coefficient

$$C = \frac{\text{Carbons produced}}{\text{feed consumed}} = \underbrace{\frac{\text{TCOD produced}}{\text{Feed consumed}}}$$

Part I: Ideal conditions	**20-25%**
Low intensity farm	**4%**

1

Waste coefficient

$$\underbrace{\frac{\text{TCOD produced}}{\text{Feed consumed}}}_{\mathbf{20\text{-}25\%}}$$

Part I: Ideal conditions

Low intensity farm **4%**

- Long retention time the organic matter spends in the system before collected.

(High O_2 and settling of organic matter)

Results/Discussion SSF

Part III: *Applicability of internal carbon sources for denitrification on a Danish brood stock farm (a mass balance approach).*

1 → 2

Waste coefficient

Hydrolysis

$$C= \frac{\textbf{Carbons produced}}{\textbf{feed consumed}} = \underbrace{\frac{\text{TCOD produced}}{\text{Feed consumed}}} * \underbrace{\frac{\text{sCOD}}{\text{TCOD}}}$$

	**Part I: Ideal conditions**	**20-25%**	**23-30%**
	**Low intensity farm**	**4%**	**2.3%**

2

Hydrolysis

$$\frac{sCOD}{TCOD}$$

Part I: Ideal conditions **15-30%**

- Shows the low quality of the organic matter obtained.

- Same values as Ucisik and Henze (2008) for hydrolysis of bacteria biomass discharged from activated sludge system.

Low intensity farm **2.3%**

(71% organic matter collected came from biofilter backwash).

Results/Discussion SSF

Part III: *Applicability of internal carbon sources for denitrification on a Danish brood stock farm (a mass balance approach).*

1 → 2 → 3

Waste coefficient | Hydrolysis | Fermentation

$$C = \frac{\textbf{Carbons produced}}{\textbf{feed consumed}} = \underbrace{\frac{\text{TCOD produced}}{\text{Feed consumed}}} * \underbrace{\frac{\text{sCOD}}{\text{TCOD}}} * \underbrace{\frac{\text{RACs}}{\text{sCOD}}}$$

Part I: Ideal conditions	**20-25%**	**23-30%**	**85-95%**
Low intensity farm	**4%**	**2.3%**	**34%**

3

Fermentation

$$\underbrace{\frac{\text{RACs}}{\text{sCOD}}}$$

Part I: Ideal conditions **85-95%**

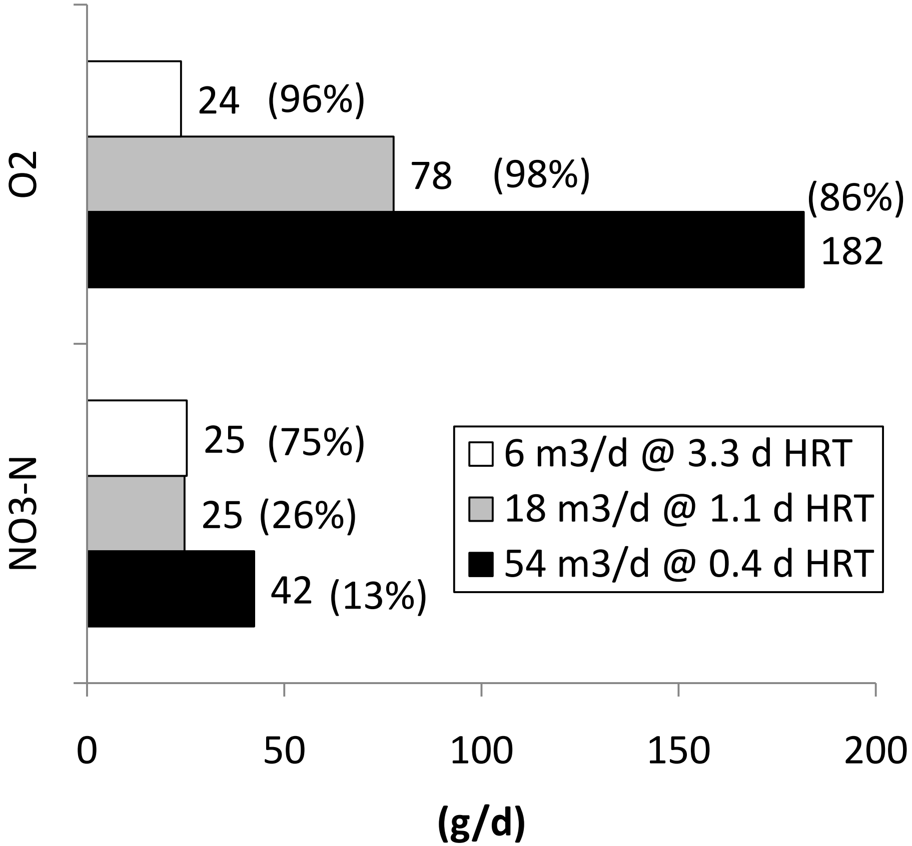
Low intensity farm **34%**

- From VFAs mainly Acetate was found (97.4%)
- Complete anaerobic digestion?
  - Competition for carbon with methanogens and sulfate reducing bacteria
- The Hydraulic retention time in the Fermenter was too long (7 days)

Results Denitrification reactor

Part III: *Applicability of internal carbon sources for denitrification on a Danish brood stock farm (a mass balance approach).*

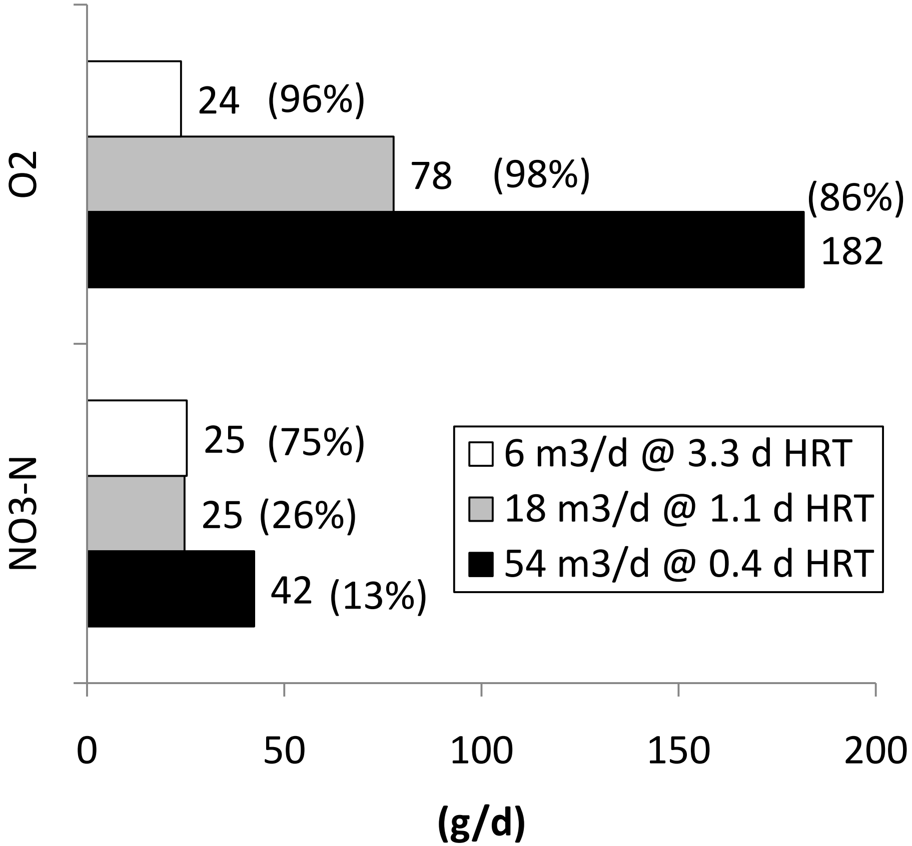
- Preference of oxygen over Nitrate (NO_3)
- Major mass removal of NO_3 and O_2 in the highest flow
- Poor mixing conditions in the reactor represented in flow 2 (18 m^3/d)



Results Denitrification reactor

***Part III:** Applicability of internal carbon sources for denitrification on a Danish brood stock farm (a mass balance approach).*

- Preference of oxygen over Nitrate (NO_3)
- Major mass removal of NO_3 and O_2 in the highest flow
- Poor mixing conditions in the reactor represented in flow 2 (18 m^3/d)
- Estimated that **1 m^3** of enhanced sludge is capable of removing **93.2 g NO_3-N plus 379 g O_2** (Anoxic conditions)



Summary Part III
Evaluation of a Fermenter in a low intensity fish farm

- The Fermenter managed to enhance the organic waste discharged (production of VFAs).
- Due to system configuration (earthen raceways) and low intensity RAS a big amount of organic waste (resource) is lost through the system before performing the hydrolysis/fermentation process.
- The farm requires 27 m^3/d of sludge to achieve environmental regulations.
- Under these conditions the carbon budgets are far beyond what is required to accomplish the environmental regulations.
- Not the best place to apply the technology.

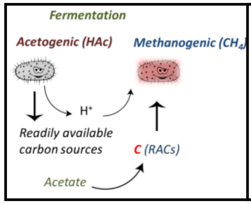
Future evaluations?

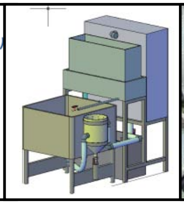
- There is interest in the industry for trying this technology.
- Following years evaluate in more intensive RAS systems (concrete tanks).
- Higher quantity and quality of organic matter will be obtained and the mass of oxygen to reduce before denitrification is lower.

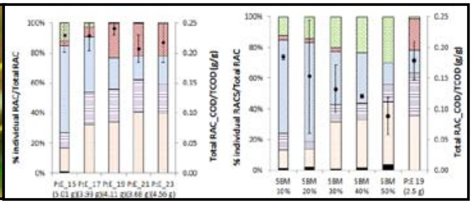
Part IV
Conclusions and future perspectives

- The method developed for describing hydrolysis/fermentation process allowed an accurate analysis on the effect dietary composition has on organic waste as a residual resource for on-farm denitrification.

- The production and type of carbon sources obtained from hydrolysis/fermentation processes are affected by dietary composition.
  - Predict the carbon types produced
  - Eventually modify diets to enhance the process
  - ***Next step***: See how carbon types affects denitrification process (C.N, denitrification rates and biomass production)







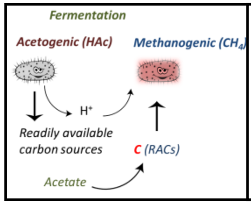
Part IV
Conclusions and future perspectives

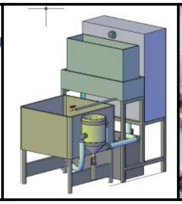
- The speed of producing carbon sources was affected by pH and temperature, optimizing the required reactor volume and associated costs.

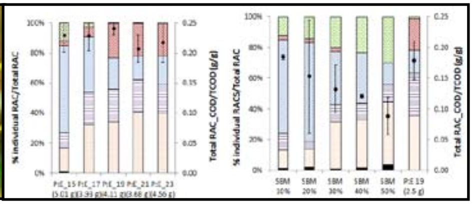
- The solubilization yield could not be optimized and reached between 23-30% (sCOD/TCOD).

- *Next Steps*

  - Identify parameters affects the process.
  - Mimic conditions complete anaerobic digestion without methanogens.
  - Nutrient media composition, bacterial consortia, reactor type and redox conditions.



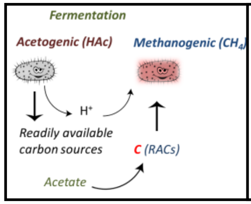


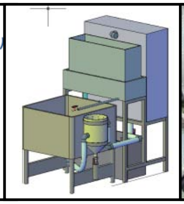


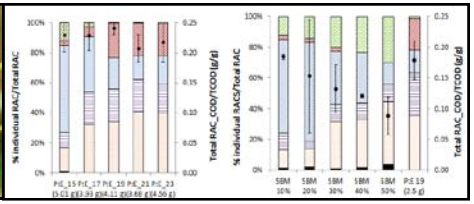
Part IV
Conclusions and future perspectives

- The use of a Fermenter enhanced the production of VFAs decoupling hydraulic retention time from the denitrification reactor.

- In low intensity systems the use of internal carbon sources limits the performance for on-farm denitrification.
  - ***Next Step***: Trials will be done in more intensive systems where more and better carbon sources can be recovered





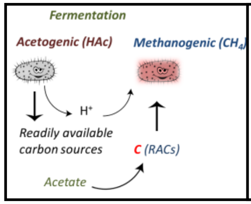


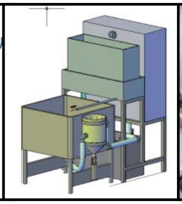
Part IV
Conclusions and future perspectives

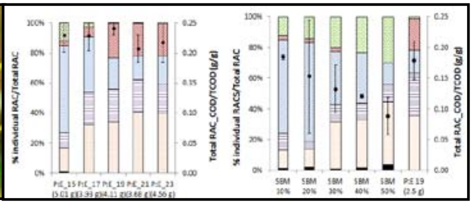
- Fish organic waste can be considered as a residual resource for producing high valuable products as VFAs as well as Ethanol (biofuel), while producing food to a constantly growing human population.

168-424 €/Ton fish produced/d → 5,000 – 12,000 €/month

- Further work is required to fully understand the process and give the industry an alternative to improve the environmental sustainability of the sector.





Thank you for your attention.

Carlos Octavio Letelier Gordo

February 2017